European Food Safety Authority

Statistical evaluation of field trials for food and feed safety

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Objective of New Approach

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Current 2006 Guidance:

- Description of general principle
- No strict rules for design of experiment + statistical analysis

New approach delivers:

- Minimum requirements for experimental design of field trials (replications, inclusion of commercial varieties)
- Criteria for appropriate evaluation of ‘background variation’
- New statistical methodology for data evaluation: maximum efficiency and statistical power

New approach allows:

- Harmonization of approach across dossiers
- Allow better interpretation of differences (or lack of equivalence) within a risk assessment framework

Exp. design for field trials within site: one GM event

GM	C	CV1	CV2	CV3	CV4
CV3	CV2	CV1	GM	C	CV4
CV4	CV3	C	CV2	CV1	GM
C	CV2	GM	CV3	CV4	CV1

C = Non-GM comparator

CVs are different commercial varieties

GM, non-GM comparator & commercial varieties are all randomised and replicated

replication must be *at least* 4 if there are only three commercial varieties then the replication must be *at least* 5

must be *at least* three commercial varieties at each site

Exp. design for field trials within site: multiple events, same crop

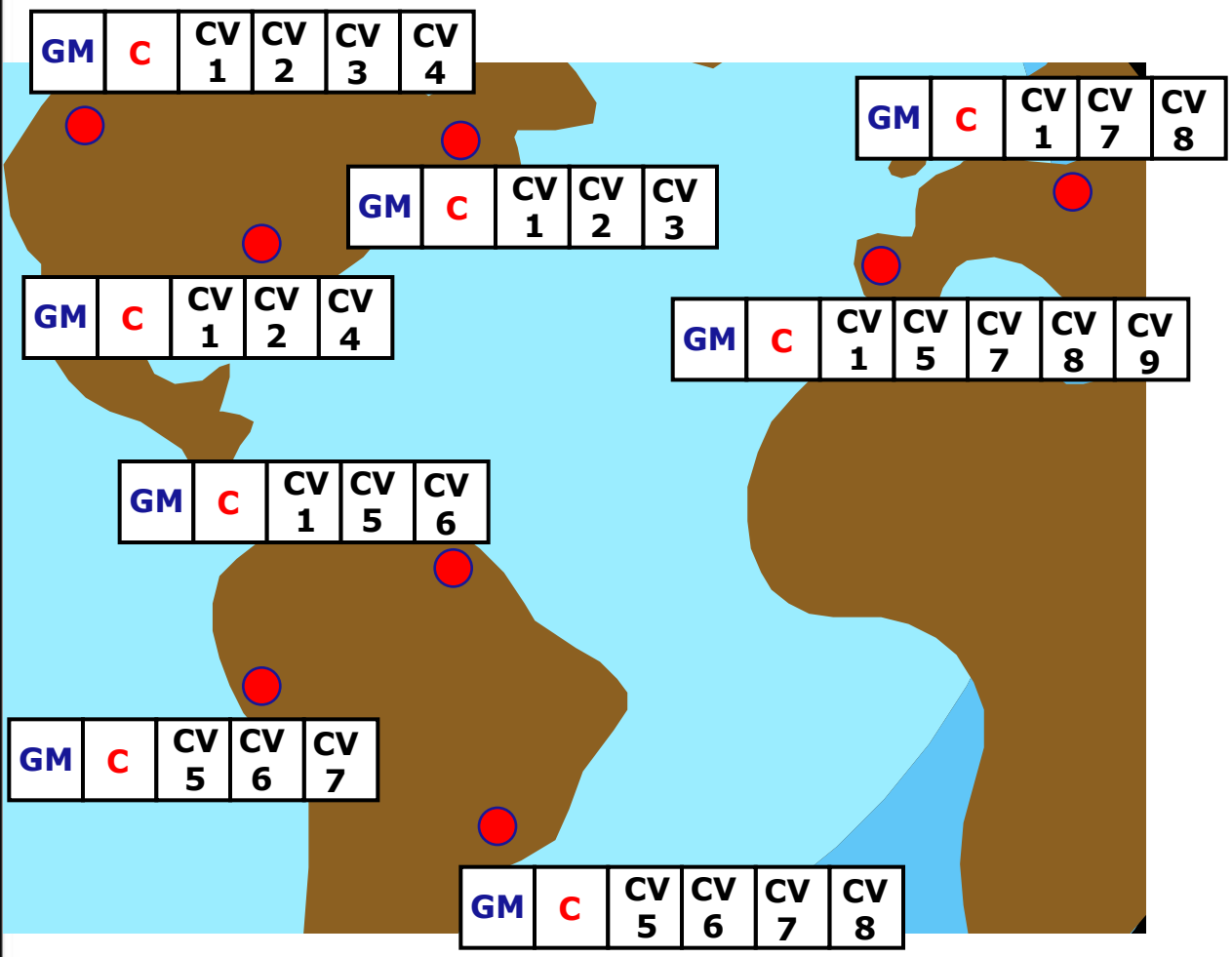
Example for 1 site:
GM1, GM2 and GM3 = 3 different GM maize events
NIC1, NIC2 and NIC3 = 3 respective conventional counterparts
CV1, CV2, CV3 and CV4 = 4 commercial varieties

Block	Plot									
	1	2	3	4	5	6	7	8	9	
1	GM2	CV2	CV1	GM3	NIC3	NIC1	CV3	GM1	NIC2	
2	CV2	GM2	CV3	NIC3	NIC2	GM1	NIC1	CV4	CV1	
3	NIC1	NIC3	GM1	CV1	GM3	NIC2	CV2	CV4	CV3	
4	GM3	GM2	CV1	NIC1	CV2	NIC2	NIC3	CV3	CV4	

- Each counterpart occurs together with its GMO in the same block
- All GMOs, their counterparts, comm. varieties: randomized in each block
- GMOs are assessed separately

(e.g. for GM1: only plots 2,3,6,7,8,10 in block 1 enter the analysis)

Exp. design for field trials between sites



must be at least 8 sites, over one or more years

must be the same GM, non-GM comparator at each site

may be different commercial varieties at each site

must be at least 6 commercial varieties over all the sites

Two tests: Difference & Equivalence

Test of Difference:

To verify whether the GMO is different from the non-GM comparator (identification of possible hazard)

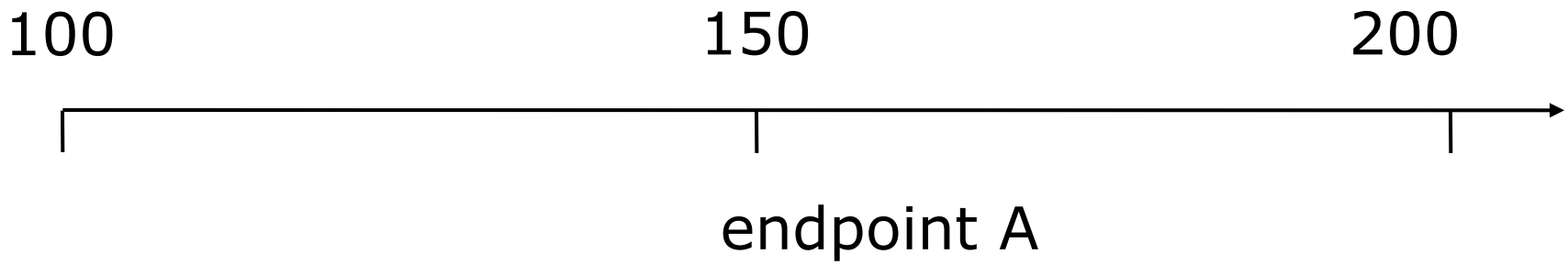
Test of Equivalence:

To verify whether the GMO is equivalent to appropriate reference variety/varieties (need to define equivalence limits)

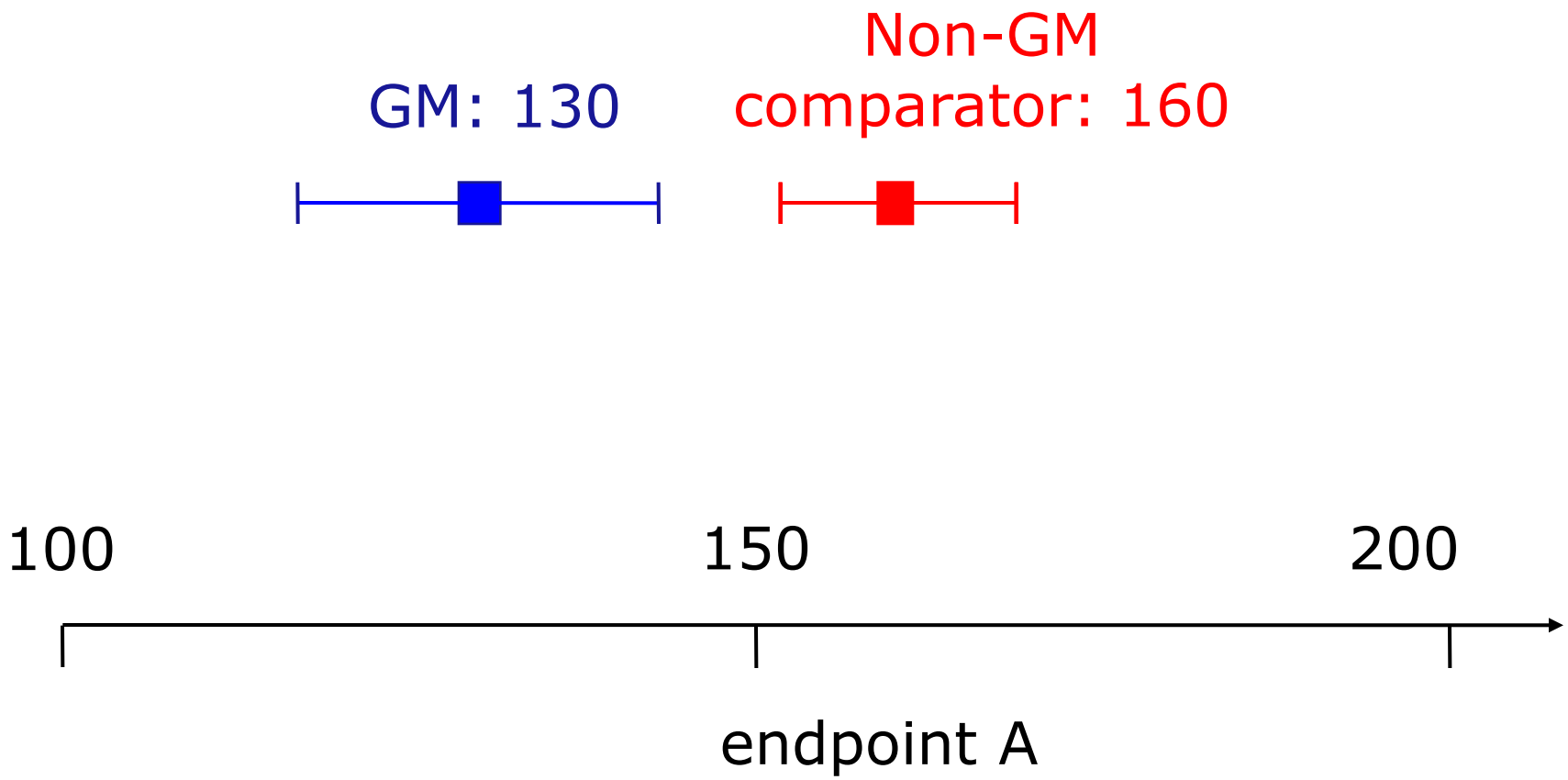
Results of both tests are displayed on a single graph simultaneously for **comprehensive** evaluation

simple, informative, transparent evaluation…

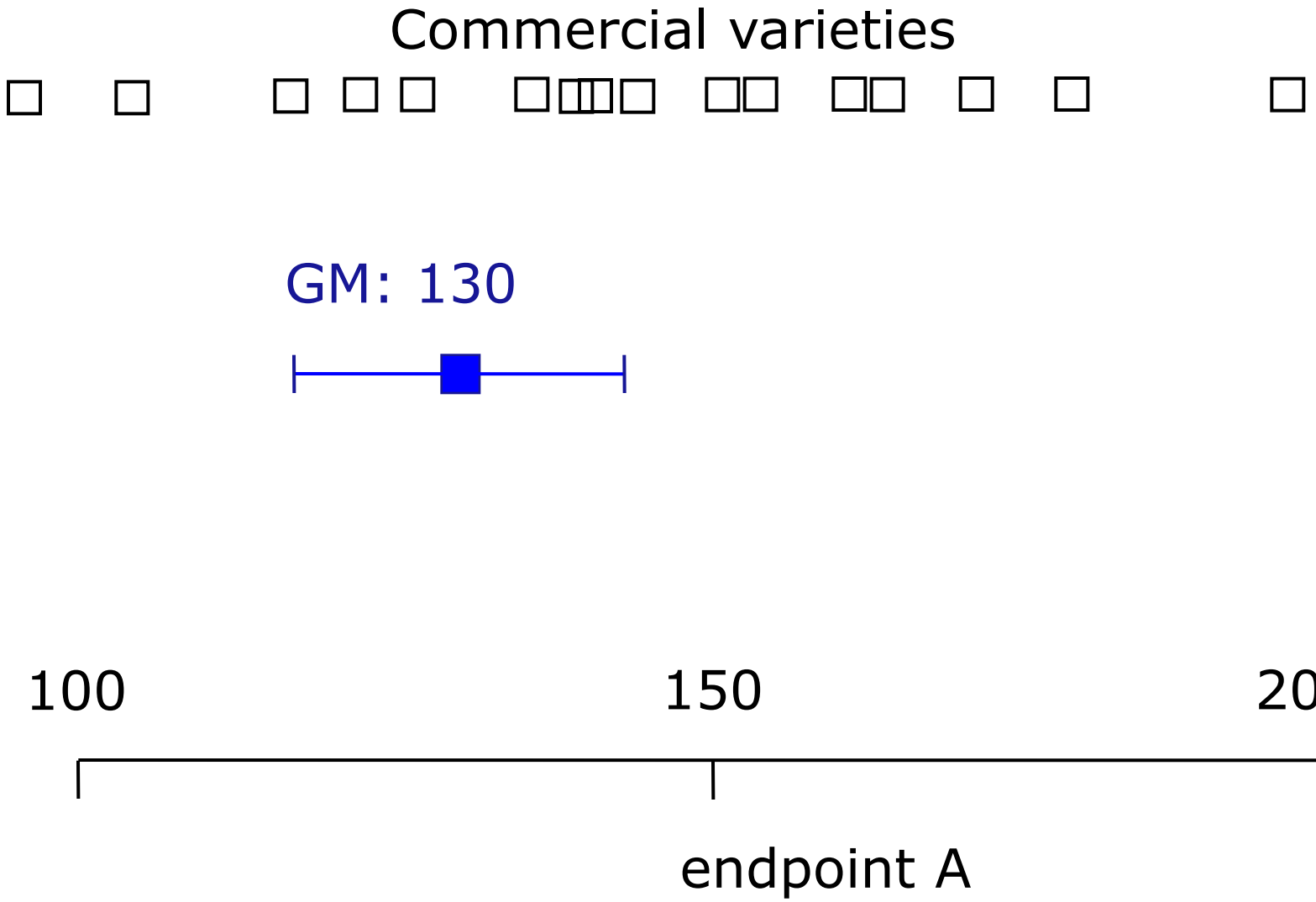
Example: single endpoint



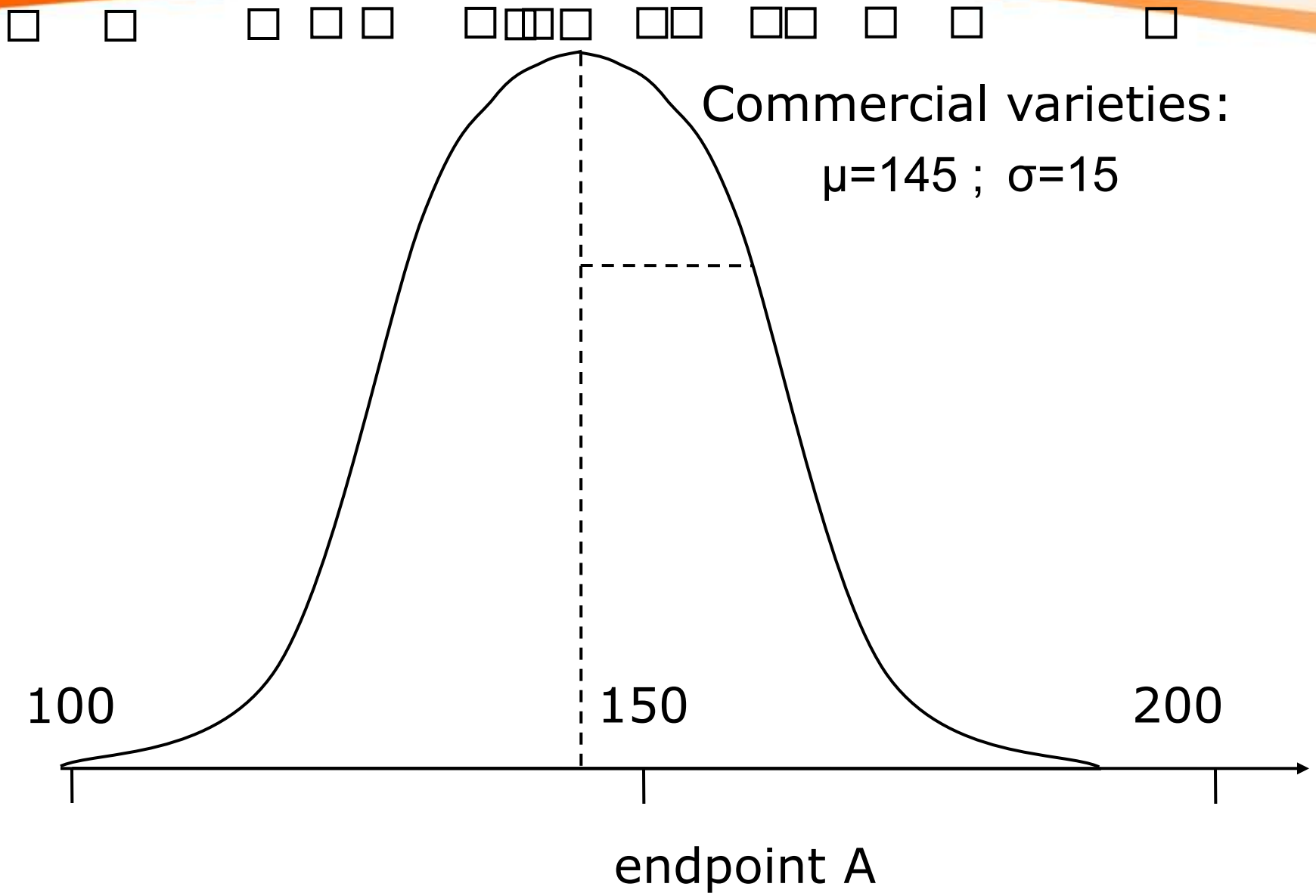
A test of difference: GMO vs comparator



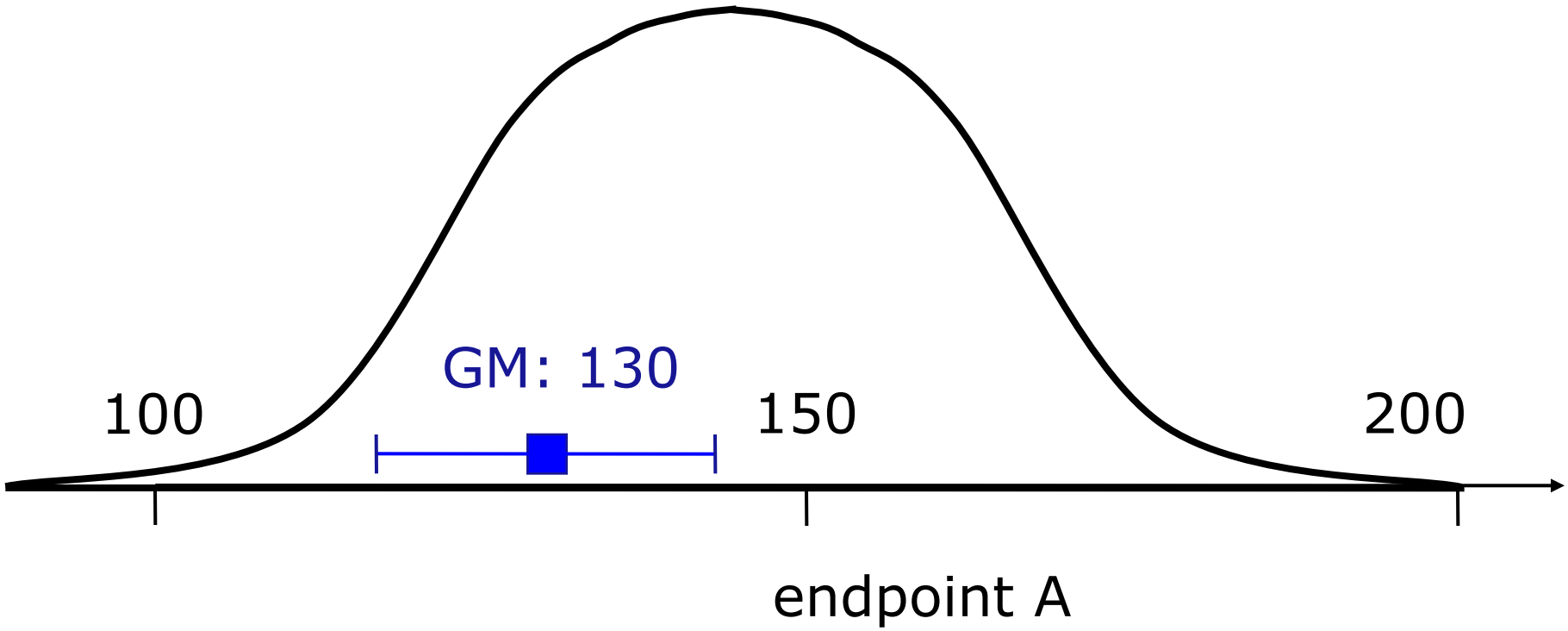
The principle of substantial equivalence



Commercial varieties form a distribution

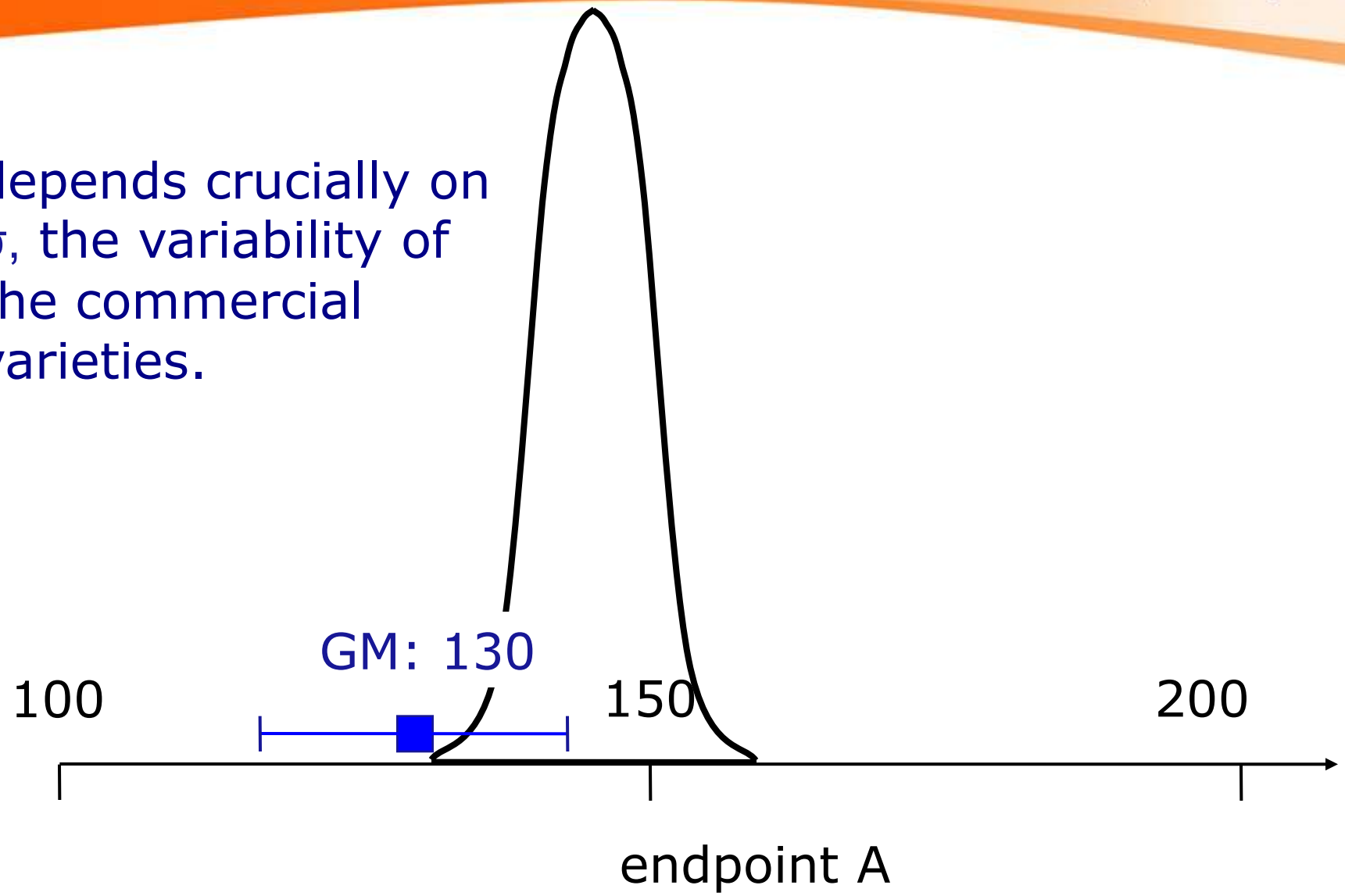


Assessing whether the GMO is equivalent



or not equivalent…

depends crucially on σ, the variability of the commercial varieties.

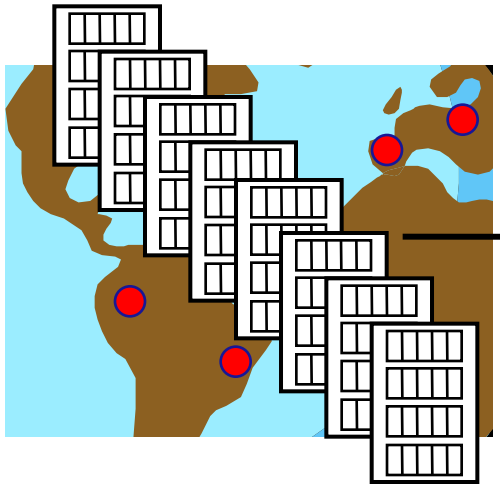


A statistical mixed model

All of the parameters that represent the variability in the field trials are estimated simultaneously from the **full set of field trial data**, including:

- sites
- years (if applicable)
- the GM
- the non-GM comparator
- the commercial varieties
- randomized blocks within sites, etc

using what is technically termed a '**statistical mixed model**'

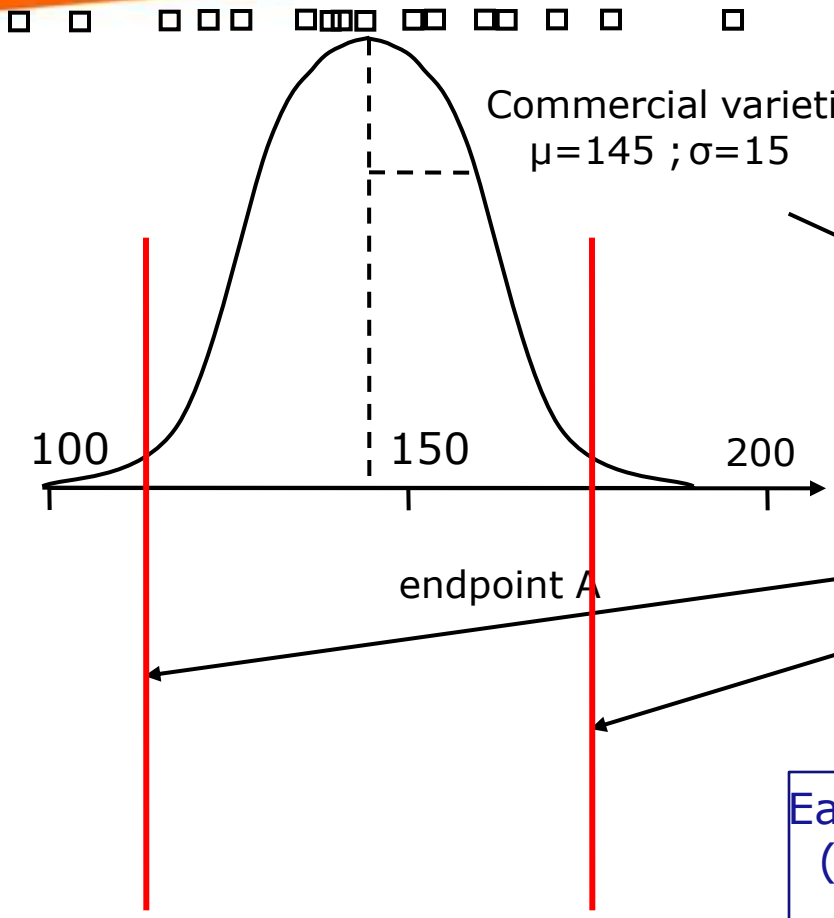


Data
+
fixed &
random
effects

mixed model

estimates of variability

A formal test of equivalence requires *equivalence limits*



estimates of variability from mixed model

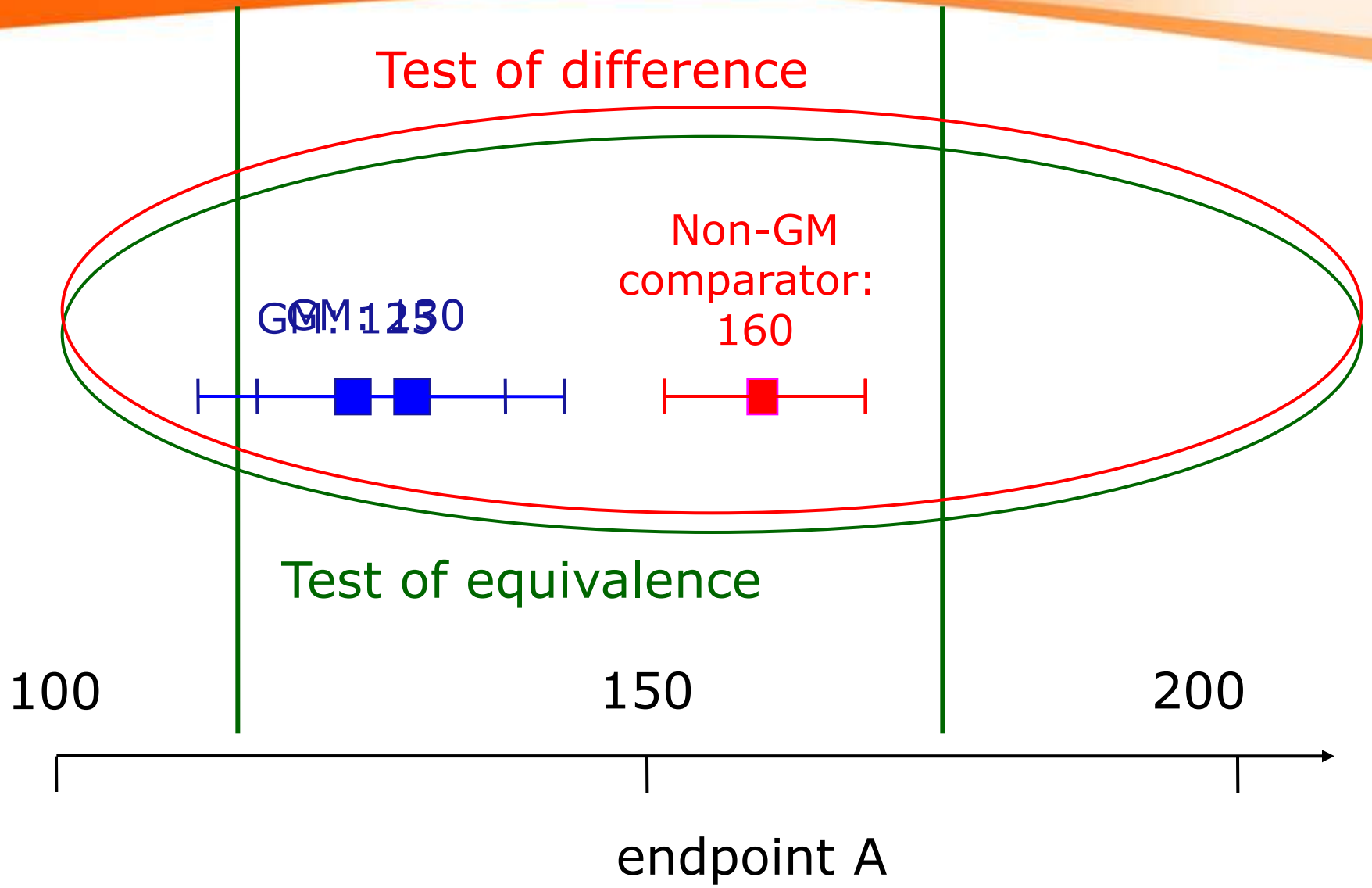
lower equivalence limit

upper equivalence limit

Each equivalence limit shall be calculated as μ (the estimated mean of all comm. varieties)
+/-
t * s.e.d. [mean GM & μ]

t distribution (standard error difference)

The two tests displayed on one graph

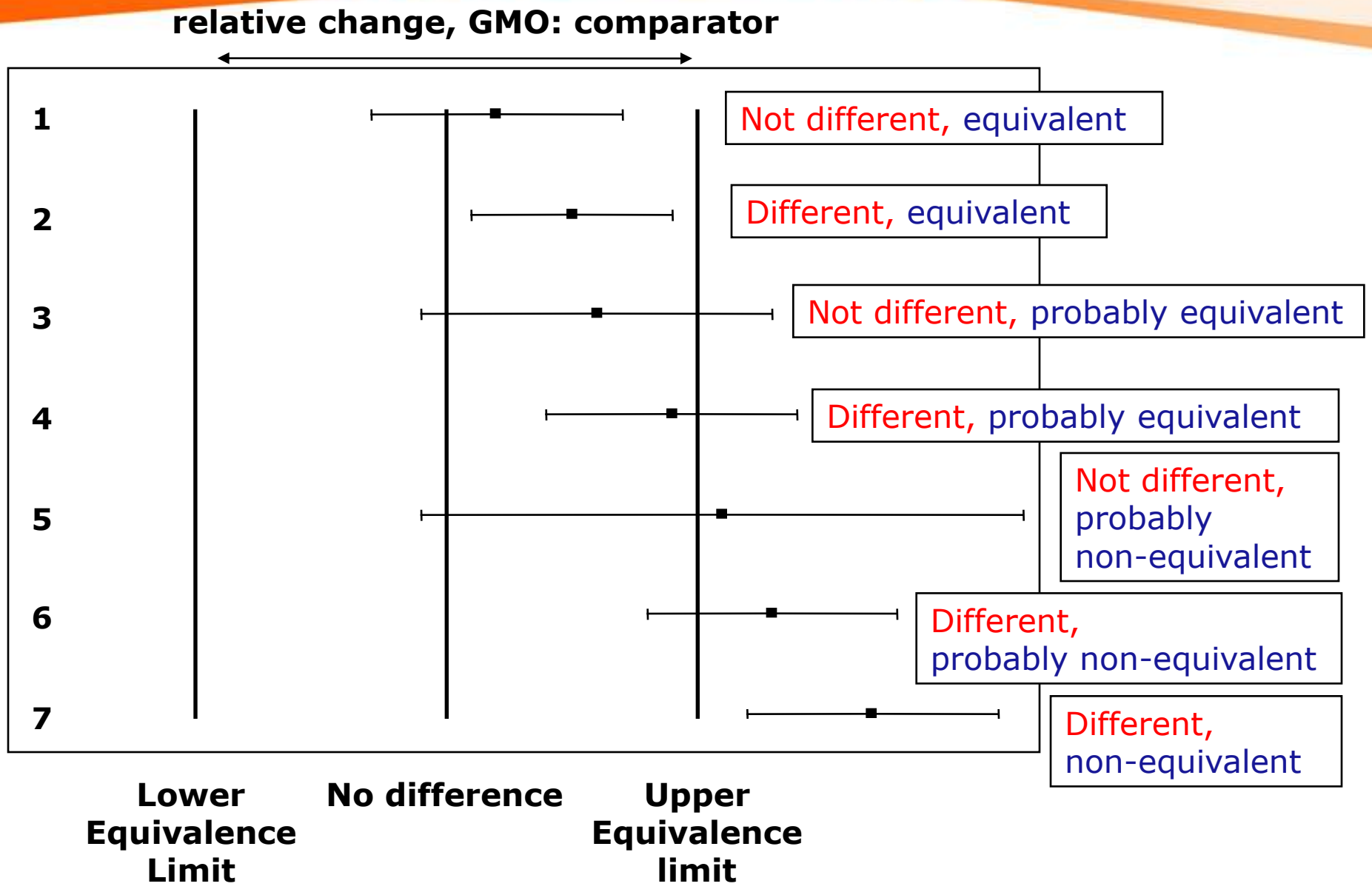


The two tests: null hypotheses

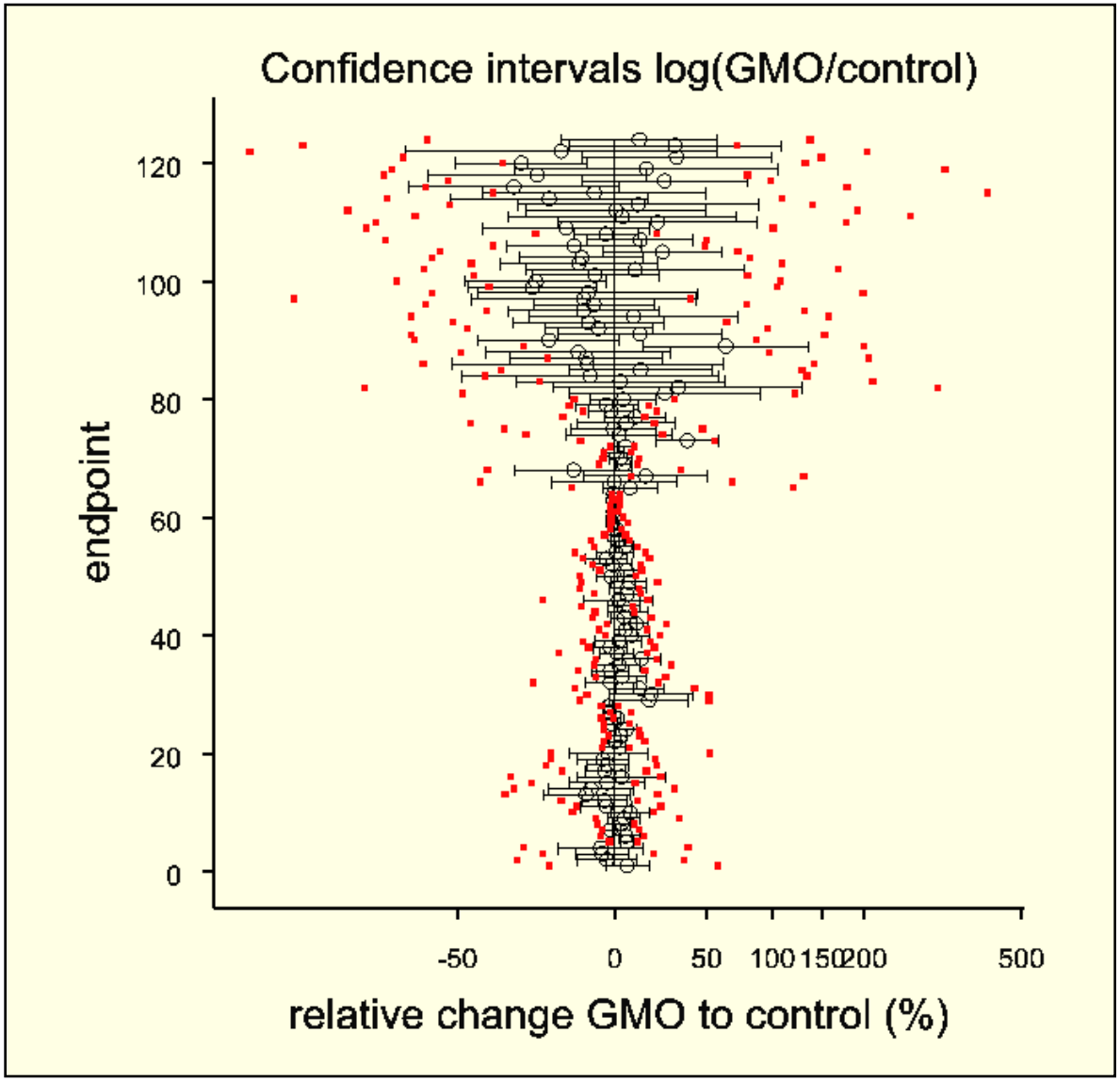
Test of Difference		Verdict	
		Not different	Different
Truth	H_0: Mean of GM and comparator the same	OK	**Type I error** 'false positive' Risk to Producer
	H_1: Mean of GM and comparator NOT the same	**Type II error** 'false negative' Risk to Consumer	OK

Test of Equivalence		Verdict	
		Not Equivalent	Equivalent
Truth	H_0: non-equivalent (GM mean outside lower or upper equiv. limit)	OK	**Type I error** 'false positive' Risk to Consumer
	H_1: equivalent (GM mean **strictly** within equiv. limits)	**Type II error** 'false positive' Risk to Producer	OK

Seven possible outcomes



Example for many endpoints



Take home message

The two tests are complementary:

Difference → changes → biologically relevant

Difference → changes → biologically NOT relevant

Equivalence → changes → within natural range of variation

Equivalence → changes → outside natural range of variation

A procedure combining both approaches (test of difference and test of equivalence) provides a richer and more objective framework for GMO risk assessment

Where to find more details

This presentation relates to the experimental design and statistical analysis of field trials to collect data for compositional assessment. Methods may also prove useful for the analysis of appropriate data, but do not relate to the design of animal feeding trials.

- **July 2008**: Report of the EFSA Statistics Working Group
- **July – September 2008**: Public consultation
- **April 2009**: Adoption of GMO Panel Opinion (including: experimental design in case of multiple events of the same crop; a worked example; software code SAS + Genestat)
- **August 2009**: publication of Opinion + summarised answers to public comments

Future - Annex II Scientific Requirements for Risk Assessment concerning Food and Feed Safety Aspects

EFSA GMO Statistics Working Group

Panel members:

- Hans Christer Andersen
- Salvatore Arpaia
- Gijs Kleter
- Harry Kuiper (formal chair)
- Joe Perry
- Willem Seinen

Ad hoc experts:

- Marco Acutis
- Ludwig Hothorn
- Jim McNicol
- Hilko van der Voet (acting chair)

EFSA scientists:

- Claudia Paoletti
- Billy Amzal

Thanks for your attention!

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