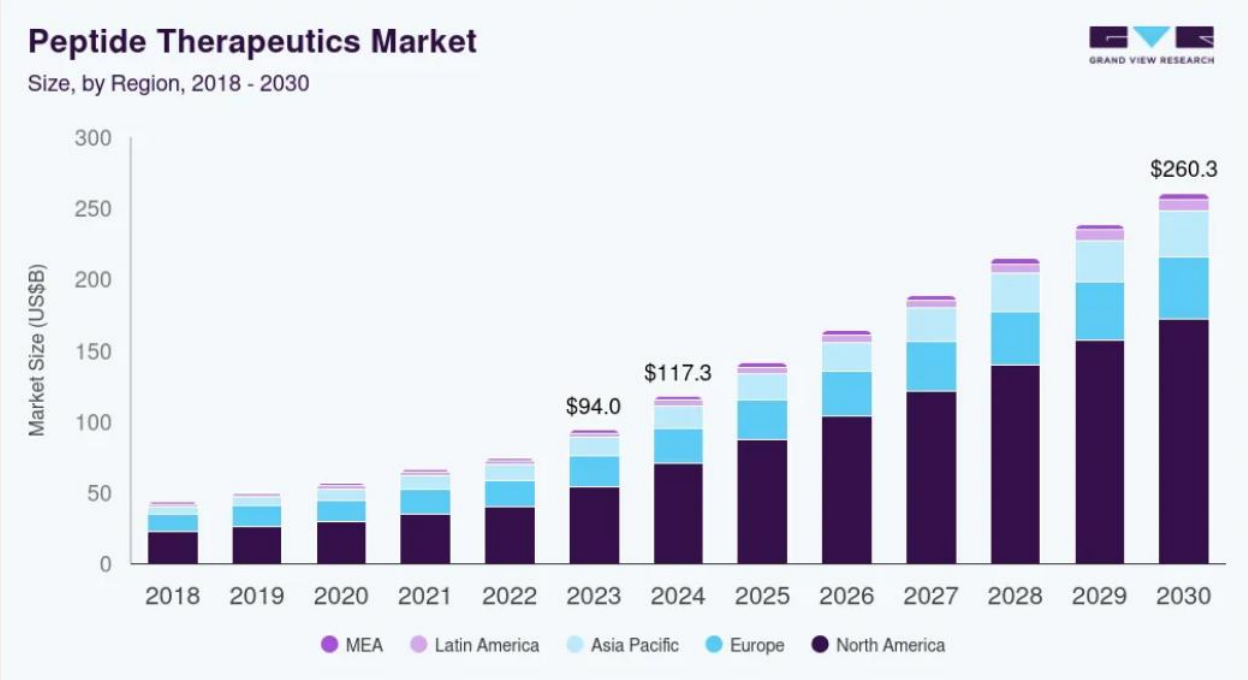
Darja Gramec Skledar, PhD, ERT
October 2026

Imunogenost terapevtskih peptidov – regulatorne zahteve

Immunogenicity of therapeutic peptides – regulatory requirements

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Growth and manufacturing diversity increase the need for peptide-specific regulatory approaches



[Peptide Therapeutics Market Size | Industry Report, 2030]

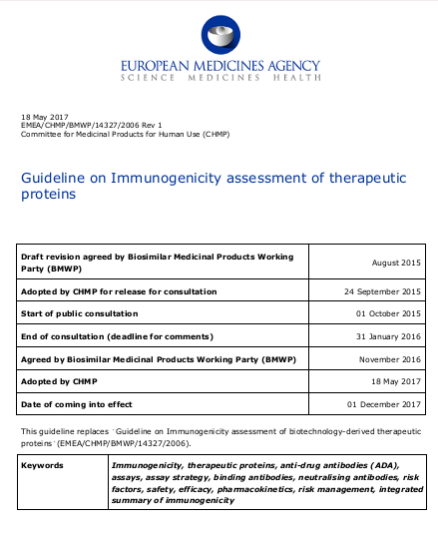
Why this matters

- The number and clinical use of peptide products continue to expand.
- Peptides are more complex than typical small molecules:
  - *Activity depends on sequence, conformation and purity.*
  - *Different manufacturing routes produce different impurity profiles.*
  - *Structural differences or impurities may affect activity, safety or immunogenicity.*

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Immunogenicity key component of peptide safety evaluation

Originator Peptides: Clinical Characterization of Immunogenicity



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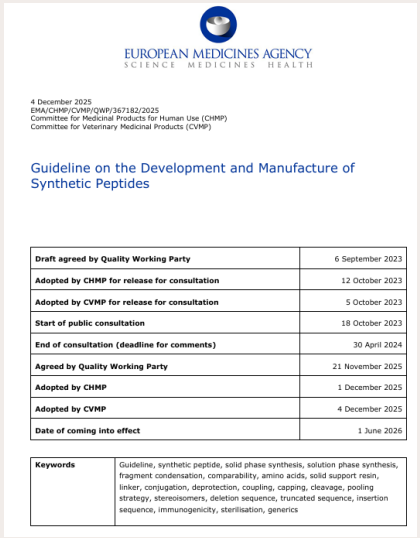
18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

Draft revision agreed by Biosimilar Medicinal Products Working Party (BMWP)	August 2015
Adopted by CHMP for release for consultation	24 September 2015
Start of public consultation	01 October 2015
End of consultation (deadline for comments)	31 January 2016
Agreed by Biosimilar Medicinal Products Working Party (BMWP)	November 2016
Adopted by CHMP	18 May 2017
Date of coming into effect	01 December 2017

This guideline replaces Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins (EMEA/CHMP/BMWP/14327/2006).

Keywords	***Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay strategy, binding antibodies, neutralising antibodies, risk factors, safety, efficacy, pharmacokinetics, risk management, integrated summary of immunogenicity***



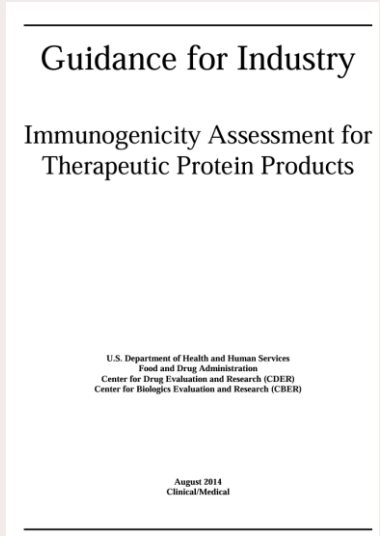
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4 December 2025
EMA/CHMP/CVMP/QWP/367182/2025
Committee for Medicinal Products for Human Use (CHMP)
Committee for Veterinary Medicinal Products (CVMP)

Guideline on the Development and Manufacture of Synthetic Peptides

Draft agreed by Quality Working Party	6 September 2023
Adopted by CHMP for release for consultation	12 October 2023
Adopted by CVMP for release for consultation	5 October 2023
Start of public consultation	18 October 2023
End of consultation (deadline for comments)	30 April 2024
Agreed by Quality Working Party	21 November 2025
Adopted by CHMP	1 December 2025
Adopted by CVMP	4 December 2025
Date of coming into effect	1 June 2026

Keywords	Guideline, synthetic peptide, solid phase synthesis, solution phase synthesis, fragment condensation, comparability, amino acids, solid support resin, linker, conjugation, deprotection, coupling, capping, cleavage, pooling strategy, stereoisomers, deletion sequence, truncated sequence, insertion sequence, immunogenicity, sterilisation, generics

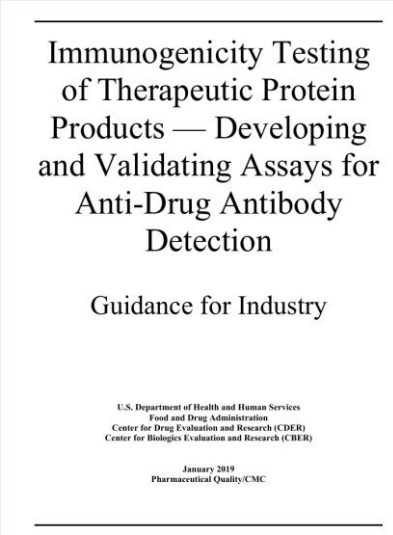


Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

August 2014
Clinical/Medical



Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

Regulatory expectations cover product development, clinical assessment, and ADA assay validation.

Regulatory expectations encompass immunogenicity risk assessment, validated ADA testing strategies and interpretation of potential clinical consequences.

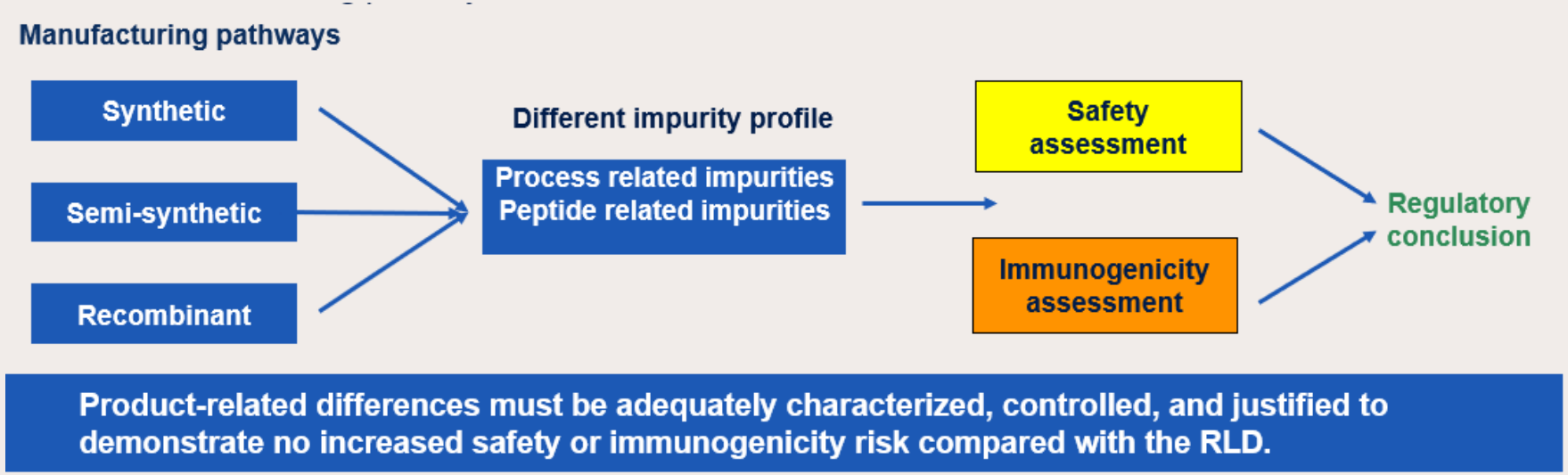
- Immunogenicity is an integral part of peptide development and is influenced by **product-, patient-, and treatment-related** factors.
- Clinically relevant effects are evaluated by monitoring **anti-drug antibodies** (ADAs) and their potential impact on **safety, exposure** and **efficacy.**

For originator peptides, clinically relevant immunogenicity is characterized during clinical development.

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Generic Peptides: Assessing Immunogenicity When Dedicated Clinical Studies Are Not Required

- The assessment builds on established knowledge of the RLD, including its safety, efficacy, and immunogenicity profile.
- Differences between the **proposed generic product and the RLD** must be characterized and evaluated for their potential impact on safety and immunogenicity.
- Particular attention should be given to **differences in impurity profiles**, which may arise from different manufacturing pathways.



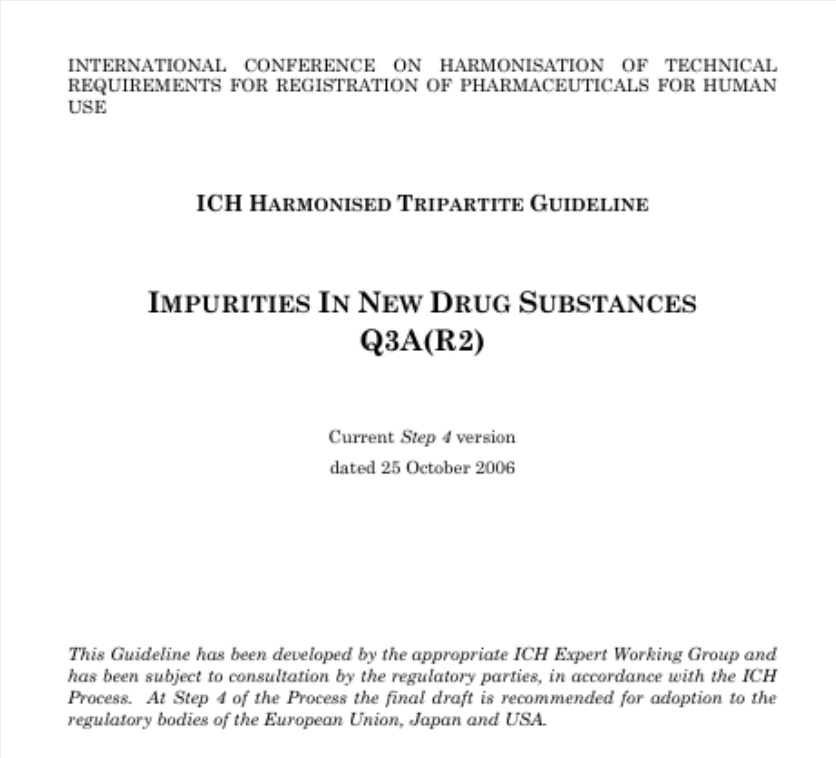
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Impurity-Related Immunogenicity Risk: Innate vs Adaptive Immune Responses

	Innate immune response *First line of defence*	**Adaptive immune response** *Specific and long lasting protection*
Primary risk drivers	**Process-related impurities** and contaminants, such as endotoxin, host-cell or other process residues, relevant product attributes, including aggregates or particles.	**Peptide-related impurities**, including deletion, insertion, substitution, truncated, modified, or other sequence-related variants that may introduce novel or stronger T-cell epitopes.
Assessment approach	**Whole-product testing** and comparison with the RLD.	Stepwise, **impurity-specific assessment** using in silico prediction and/or fit-for-purpose in vitro methods.
Type of assays	Human PBMC cytokine-release/IIRMI assay, reporter-cell assay. **PBMC=peripheral blood mononuclear cells*	In silico HLA class II-binding/epitope prediction; in vitro HLA-binding assays; MAPPS; antigen-specific T-cell proliferation or cytokine assays.

- **No standard method to evaluate immunogenicity risk, different types of in vitro studies available.**
- **Study should be fit for purpose (appropriately designed and validated for its intended application).**
- **FDA research priority for 2027: Improving and standardizing in vitro methods for assessing immunogenicity of peptide and oligonucleotide products**

Peptides require a different approach to impurity assessment



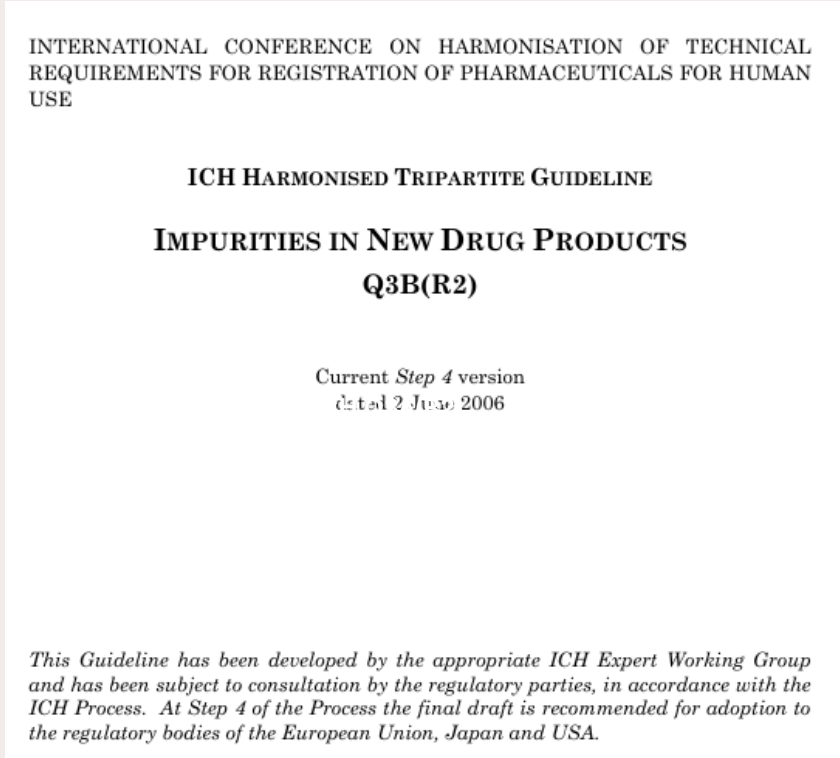
INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

IMPURITIES IN NEW DRUG SUBSTANCES
Q3A(R2)

Current *Step 4* version
dated 25 October 2006

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.



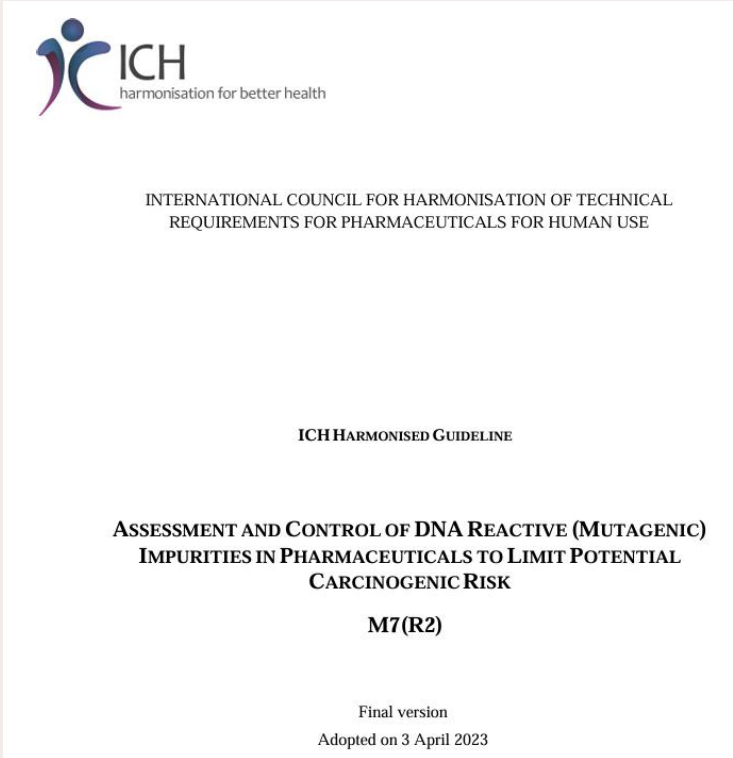
INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

IMPURITIES IN NEW DRUG PRODUCTS
Q3B(R2)

Current *Step 4* version
dated 2 June 2006

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.



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INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

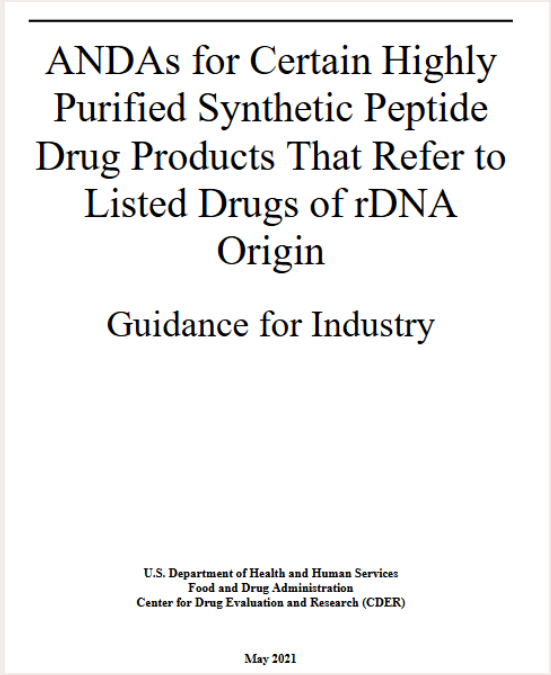
ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7(R2)

Final version
Adopted on 3 April 2023

ICH Q3A(R2) and Q3B(R2) were developed for conventional small-molecule drug substances and drug products and do not specifically address the structural complexity and immunogenicity considerations associated with peptide-related impurities. ICH M7 remains relevant to potentially DNA-reactive process-related impurities, but it does not address peptide-related impurities.

FDA Requirements for Generic Peptides



ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

May 2021

Jul 2026

- 2021 guidance was withdrawn, as it no longer reflects the Agency's current scientific thinking.
- 17 peptide PSGs revised (*Semaglutide, Teriparatide, Tirzepatide, Liraglutide, Salmon calcitonin,..) with updated recommendations*
- *A new draft guidance Considerations for Peptide Drug Products in ANDAs in planned for publication in 2026.*

Revision based on FDAs extensive experience with the review and approval of peptide and biosimilar applications.

- Minor difference in the AA sequence unlikely to have significant impact on immunogenicity risk
- Impurities that contain multiple changes in AA and are present at higher levels are rare

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Previous and revised FDA recommendations for peptides

Attribute	Previous	Revised[1]
Active ingredient source scope	Synthetic active ingredients for ANDAs referencing a limited set of recombinantly manufactured peptide RLDs	Synthetic, recombinant, and semi-synthetic active ingredients for ANDAs referencing any peptide RLD
Active ingredient-related impurity thresholds		
Reporting threshold (RT)	≥ 0.10%	> 0.1%
Identification threshold (IT)	≥ 0.10%	> 0.5%
Qualification threshold (QT)	≥ 0.10% (with a maximum allowable qualification level of 0.5% using adaptive immunogenicity in vitro assay data)	> 1.0%
Total impurities	Not defined	Total impurities in the proposed generic product should be ≤ the respective amount in the RLD
Innate immunogenicity risk assessment	Risk mitigated using comparative innate immune response modulating impurity (IIRMI) in vitro assays	Risk mitigated if product meets certain USP/ICH compendial acceptance criteria and toxicological thresholds, and aggregates are comparable to or less than the RLD
Outcome	**Adaptive and innate immunogenicity studies required**	**Reduced nonclinical requirements for generic peptide development**

EMA Approach to Peptide Impurities and Immunogenicity



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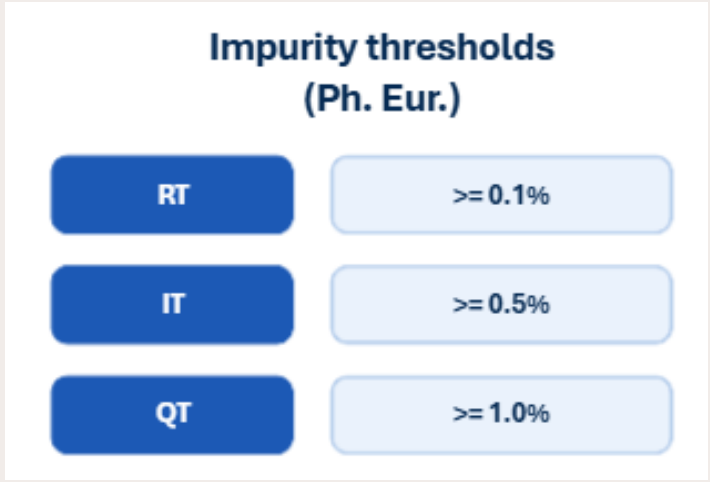
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Guideline on the Development and Manufacture of Synthetic Peptides (June 2026)



Immunogenicity of peptides is of lesser concern than that of proteins due to their size. Furthermore, changes or modifications (e.g. deamidations) of a small number of amino acids are not noticeably immunogenic. In case a novel type of impurity occurs, this novel impurity should be reduced as far as possible.

In-silico prediction of immunogenicity, e.g. based on predicted binding to T-cell receptors (TCR), or in vitro tests of T-cell activation may provide relevant information on immunogenicity potential of peptides impurities.

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Conclusions

- Peptides occupy a unique regulatory space between conventional small molecules and biologics.
- For originator peptides, clinical studies characterize ADA development and potential effects on pharmacokinetics, efficacy and safety.
- For generic peptides, the central question is whether differences from the RLD could introduce additional immunogenicity risk.
- Comparative structural characterization, impurity profiling, aggregation assessment and biological activity provide the foundation of the evaluation.
- Immunogenicity assessment should be risk-based and fit-for-purpose, addressing innate and adaptive immune responses, when required
- Regulatory expectations are evolving rapidly, with increasing convergence between FDA and EMA approaches to impurity control and immunogenicity assessment.
- Successful approval relies on demonstrating that any product-related differences do not adversely affect quality, safety, efficacy, or immunogenicity.

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