EMA
EUROPEAN MEDICINES AGENCY

Relevant guidance documents, frameworks and on-going activities at other EU agencies – EMA's view

Workshop on EFSA's Genotoxicity Guidance Revision

Brussels, 3-4 November 2025

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Two guidances on genotoxicity testing in human/ veterinary products

Human

Directive 2001/83/EC, Regulations (EU) 2004/726 and (EU) 2014/536 mandate the use of ICH guidelines and OECD TGs

- ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use
    - Revision 1 adopted in December 2011, coming into effect June 2012

Veterinary

Regulations (EU) 2019/6 and (EU) 2018/782 (MRL) mandate the use of VICH guidelines and OECD TGs

- VICH GL23: Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing
    - New update adopted in August 2025, for implementation by August 2026

Scope of (V)ICH guidances

Provide Standard Battery of Tests that can be used for the evaluation of the genotoxicity of veterinary drug residues (VICH GL23) or for predicting of potential human risks of new “small molecule” drug substances being developed as human pharmaceuticals (ICH S2(R1)).

Human pharmaceuticals

Timing of genotoxicity studies (ICH M3(R2))

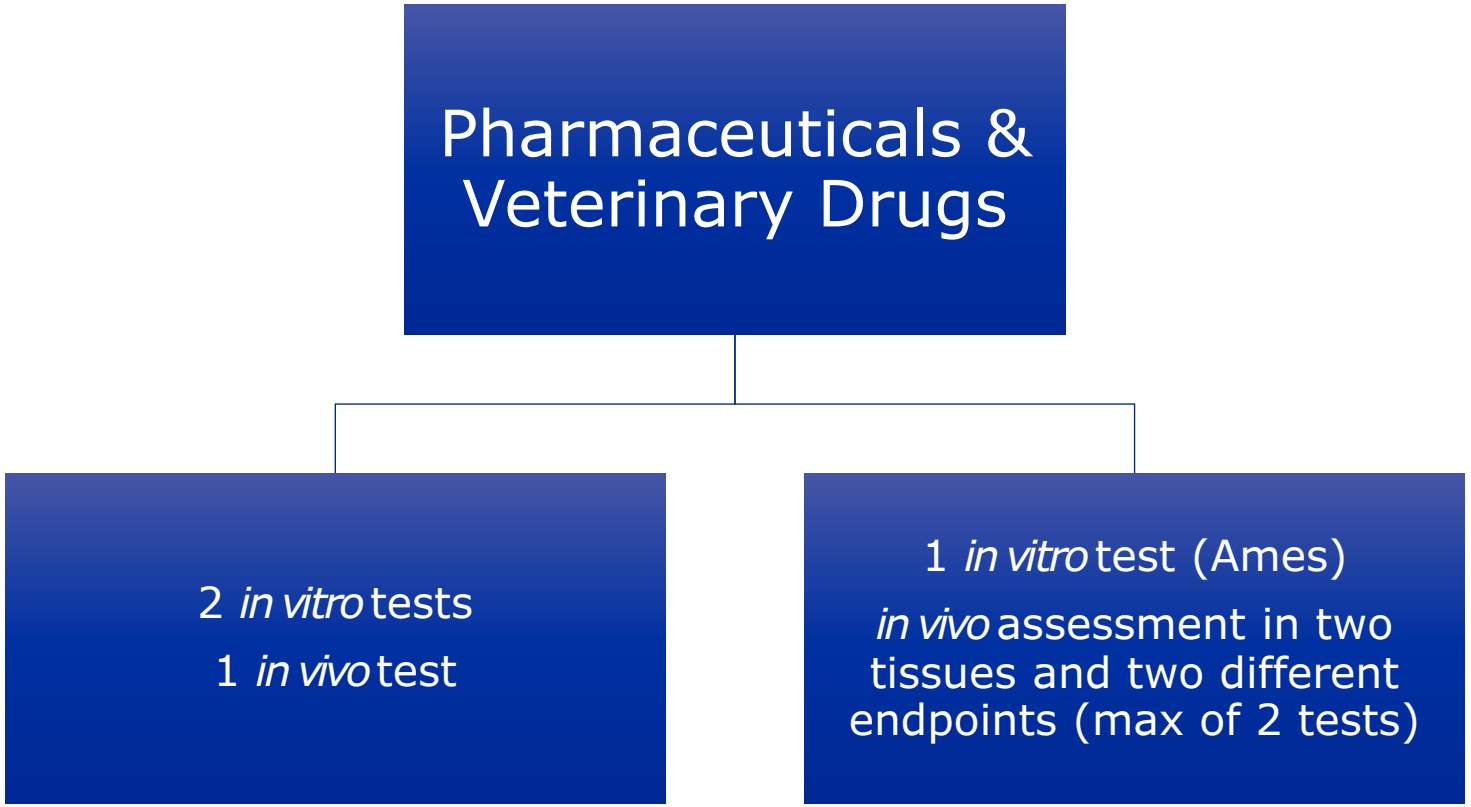
- Single dose clinical development trials: assay for gene mutation
- Multiple dose clinical development trials: an additional assessment capable of detecting chromosomal damage in a mammalian system(s)
- Before initiation of Phase II efficacy trials: Complete battery of tests for genotoxicity

Options provided

2 options

Battery with *in vitro* mammalian cell assay

Battery with two *in vivo* assays in two tissues



Human pharmaceuticals (ICH S2(R1))

- Option 1

  i. gene mutation test in bacteria (OECD471)

  ii. *in vitro* mammalian cell test

  - mammalian cell micronucleus test (OECD487),
  - chromosome aberration test (OECD473),
  - mouse lymphoma Tk gene mutation assay (OECD490)

  iii. *in vivo* test for genotoxicity, generally a test for chromosomal damage using rodent hematopoietic cells (metaphase chromosome aberration (OECD475) or micronucleus (OECD474)).

- Option 2

  i. gene mutation test in bacteria (OECD471)

  ii. *in vivo* assessment of genotoxicity with two different tissues, usually an assay for micronuclei using rodent hematopoietic cells (OECD474)

  ii. second *in vivo* assay, usually DNA strand breakage assay in liver (OECD489), unless otherwise justified.

Veterinary drugs (VICH GL23)

- Option 1

  i. A test for gene mutation in bacteria (OECD471)

  ii. *in vitro* tests for chromosomal effects in mammalian cells, either

  - *in vitro* mammalian cell micronucleus test (OECD487),
  - *in vitro* chromosome aberration test (OECD473),
  - *in vitro* mammalian cell gene mutation test using Hprt and xprt genes (OECD476)
  - *in vitro* thymidine kinase gene (OECD490)

  iii. An *in vivo* test for chromosomal effects using rodent hematopoietic cells, either *in vivo* mammalian erythrocyte micronucleus test (OECD474) or *in vivo* mammalian bone marrow chromosome aberration test (OECD475)

- Option 2

  i. A test for gene mutation in bacteria (OECD471)

  ii. An *in vivo* test for chromosomal effects using rodent hematopoietic cells, either

  - *in vivo* mammalian erythrocyte micronucleus test (OECD474)
  - *in vivo* mammalian bone marrow chromosome aberration test (OECD475)

  ii. Second *in vivo* test, either *in vivo* mammalian alkaline comet assay (OECD489), or the *in vivo* transgenic mouse/rat mutation assay (OECD488).

Other genotoxicity guidelines/guidance documents – mutagenic impurities

- ICH M7(R2) Guideline on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
  - to provide a practical framework that is applicable to the identification, categorisation, qualification, and control of these mutagenic impurities to limit potential carcinogenic risk. This guideline is intended to complement ICH Q3A(R2), Q3B(R2) (Note 1), and ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorizations for Pharmaceuticals
- EMA/CVMP/SWP/377245/2016: Veterinary-specific guidance on mutagenic impurities
  - provide a practical framework that is applicable to the identification, categorisation, qualification, and control of these mutagenic impurities, to limit potential carcinogenic risk associated with the exposure to potentially mutagenic impurities. This guideline is intended to complement VICH GL10 and VICH GL11.

Other genotoxicity guidelines/guidance documents – maximum residue limit (MRL) for veterinary drugs

To establish maximum residue limit (MRL)

- TTC approach can be used to justify presence of genotoxic impurities using EMA/CVMP/SWP/377245/2016: Veterinary-specific guidance on mutagenic impurities.
- Active substances that are genotoxic carcinogens are not allowed for use in food producing animals. No safe threshold can be established, and **MRLs are generally not set**

Cohort of concern: Nitrosamine impurities in human pharmaceuticals EMA/409815/2020 Rev.23

- Threshold of Toxicological Concern (TTC) <1500 ng/day
  - Acceptable intake based on Carcinogenic Potency Categorization Approach (CPCA) based on potency classes (1, 18 ng/day; 2, 100 ng/day; 3, 400 ng/day; 4&5 1500 ng/day) that **uses the chemical structure of a nitrosamine impurity** to recommend AI limits.
  - Enhanced Ames test (organic solvent <4.4% and 30% rat & hamster S9)
    - Negative -> TTC of 1500 ng/day
    - Positive -> AI based on CPCA, or in vivo TGR study
  - *In vivo* TGR study (OECD488)
    - Negative -> not mutagenic
    - Positive -> AI based on CPCA
- ICH M7 subgroup is installed to develop an addendum to the M7 Guideline on Assessment and Control of DNA Reactive (mutagenic) Impurities to address safety assessment and establishment of appropriate controls for nitrosamine impurities.

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Thank you

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