



soluzioni informatiche

# **Role of *in silico* Genotoxicity Tools in the Regulatory Assessment of Pharmaceutical Impurities**

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## knowledge and solutions in chemistry and life sciences

- Molecular Modeling and (Q)SAR
- Computational Toxicology
- Information Management
- Quality by Design

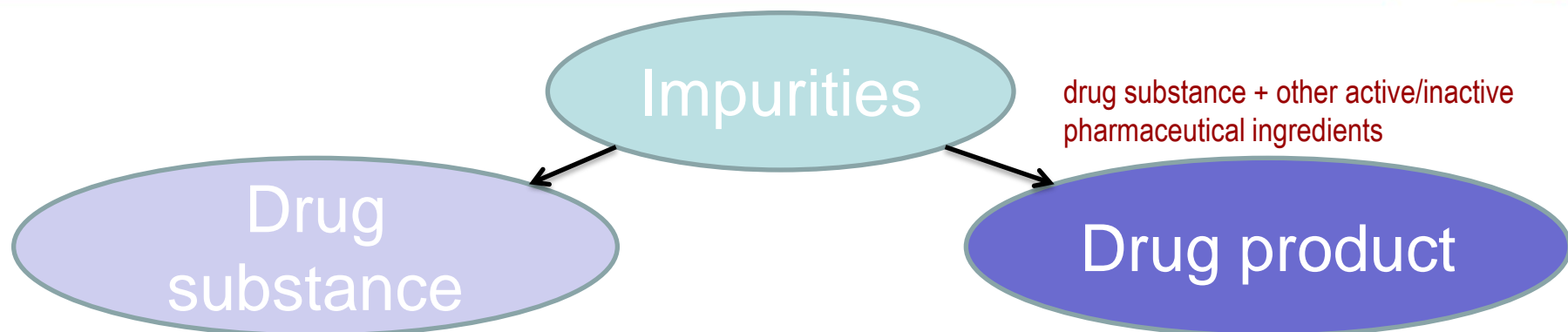
- consulting services
- training activities
- *in silico* predictions
- contracted research
- software solutions



Vicenza

- Background information
- Regulatory framework
- *In silico* tools
- Conclusions

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**any component that is not the chemical entity defined as the drug substance**

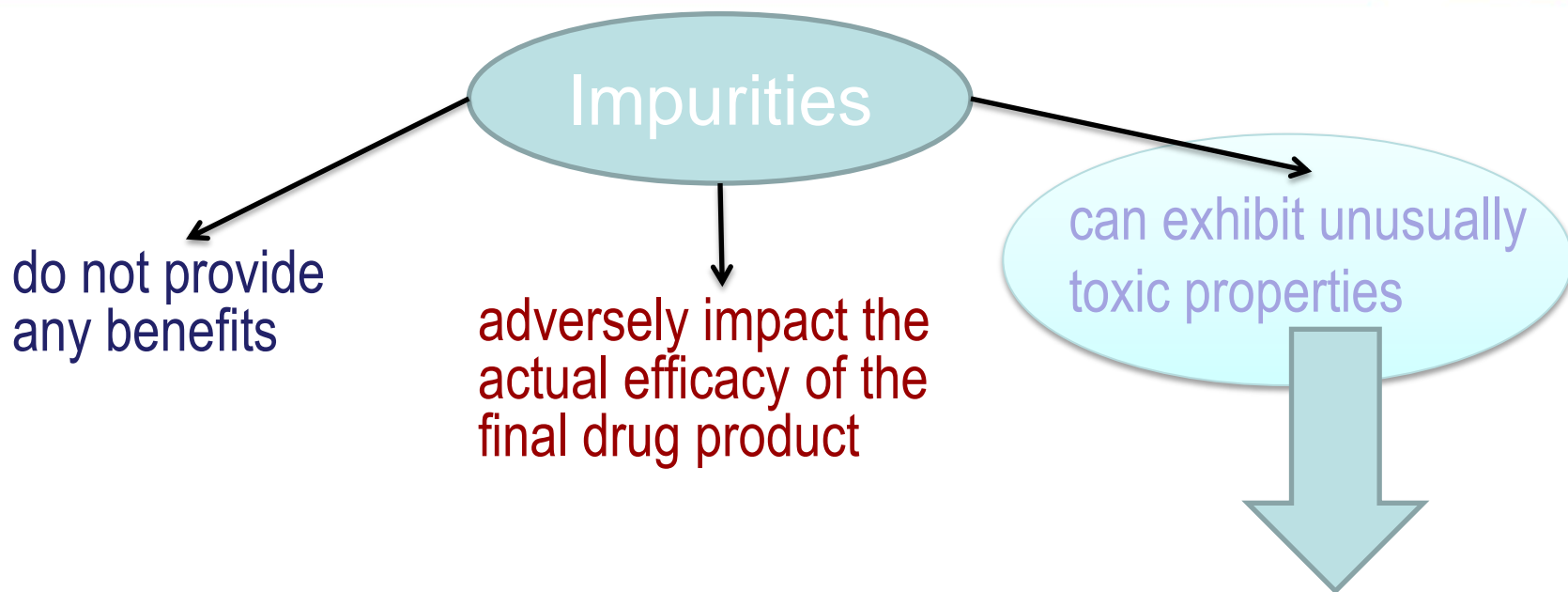
from the manufacturing processes and/or storage of the drug substance

- organic impurities
- inorganic impurities
- residual solvents

**any component that is neither a drug substance nor an excipient in the drug product**

resulting from

- the degradation of the drug substance or from a reaction of the drug substance with an excipient
- external contamination as well as from the packaging materials



## Genotoxic impurities, GTIs:

- capable of inducing direct or indirect damage to DNA
- giving the “positive” answers in established *in vitro* or *in vivo* genotoxicity tests

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Impurities of drug substances: guidance **Q3A(R2), 2006** and **Q3C(R4), 2009**

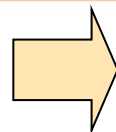
Impurities of drug products: guidance **Q3B(R2), 2006**

**ICH**  
**Guidance Documents**  
Technical Requirements for Registration of Pharmaceuticals for Human Use

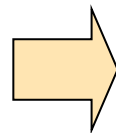
Basic concepts and principles on how to control the impurities

address only non-genotoxic impurities!

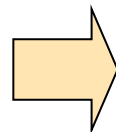
“unusually toxic” (e.g. genotoxic) compounds may require tighter thresholds or control limits



Reporting threshold: “A limit above which an impurity should be reported” \*



Identification threshold: “A limit above which an impurity should be identified”, i.e. its structure should be determined \*



Impurity qualification: “The process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified” \*

\* with respect to the maximum daily dose of a drug

For **potentially genotoxic (PGT) impurities** (i.e. those exhibiting a potential safety concern for genotoxicity, on the basis of existing structural alerts, for instance), the routine identification thresholds are not applicable!

**ICH Draft Consensus  
Guidance S2(R1)  
(2008)**

dealing with the

identification of genotoxic impurities

The impurity is classified as genotoxic (GTI) when there are “positive” answers in *in vitro* or *in vivo* genotoxicity tests.

## STANDARD TESTING BATTERY

### OPTION 1

#### STEP 1:

A test for gene mutation in bacteria

#### STEP 2:

A cytogenetic test for chromosomal damage (the **in vitro** metaphase chromosome aberration test or *in vitro* micronucleus test), or an *in vitro* mouse lymphoma tk gene mutation assay

#### STEP 3:

An **in vivo** test for genotoxicity, generally a test for chromosomal damage using rodent hematopoietic cells, either for micronuclei or for chromosomal aberrations in metaphase cells

### OPTION 2

#### STEP 1:

A test for gene mutation in bacteria

#### STEP 2:

An **in vivo** assessment of genotoxicity with two tissues, usually an assay for micronuclei using rodent hematopoietic cells and a second *in vivo* assay

- Criticisms to the standard testing battery\*
  - The strategy is outdated and not sufficiently accurate
  - Leads to results of low specificity
  - The tests require high concentration of the chemical compound
  - The lack of detoxification enzyme systems in rat liver S9 (used to mimic the metabolic activation processes), may result in inappropriate metabolism.

\* D. Tweats, *Future approaches for genotoxicity testing of drugs and impurities*, Toxicol. Lett. 196(1) (2010), pp. S11-4.

„Thresholded”  
impurities

## EMA (CHMP) Guideline on the Limits of Genotoxic Impurities (2006)

„Non-thresholded”  
impurities

Genotoxic impurities with a “sufficient  
(experimental) evidence for

**a threshold-related mechanism” of action:**

- interfering with the mitotic spindle apparatus leading to aneuploidy
  - modifying the activity of topoisomerase
    - inhibiting of DNA synthesis
- overloading of defence mechanisms or metabolism
  - causing physiological perturbations

Genotoxic impurities with **no** “sufficient  
(experimental) evidence for

**a threshold-related mechanism” of action:**

- directly target DNA
- any exposure to them is associated with certain level of carcinogenic risk

- ICH Q3C(R4) guideline (2009) for residual solvents of class 2 (to be limited)
- **Permitted Daily Exposure (PDE)** value
- NOEL/LOEL doses in the most relevant animal *in vivo* study after incorporating appropriate safety (uncertainty) factors (UF)

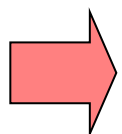
- **Pharmaceutical assessment** of acceptable limits (formulation and manufacturing strategy): the amount of impurities should be decreased to “as low as reasonably practicable” limit (**ALARP principle**)
- **Toxicological assessment:** Threshold of Toxicological Concern (**TTC approach**) (1.5 µg/day)

## PhRMA

White paper introducing a concept of a staged TTC approach  
(Müller et al., 2006)

### Classification scheme for (genotoxic) impurities

- (i) class 1: impurities known to be genotoxic (mutagenic) and carcinogenic;
- (ii) class 2: impurities known to be genotoxic (mutagenic) but with unknown carcinogenic potential;
- (iii) class 3: impurities with a unique alerting structure and of unknown genotoxic (mutagenic) potential;
- (iv) class 4: impurities with an alerting structure related to the parent active pharmaceutical ingredient;
- (v) class 5: impurities with no structural alert, which can be successfully covered by existing ICH Q3A(R), Q3B(R), and Q3C guidelines.



**The class of genotoxic impurities without structural alerts omitted!**

EMA (CHMP)  
(2006)

Guideline on the Limits of Genotoxic Impurities

**Clarifications!**

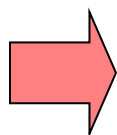
EMA (CHMP)  
(2008)

Question & Answers on the CHMP Guideline on the Limits of Genotoxic Impurities

! when a potential impurity **contains structural alerts**, its **additional genotoxicity testing**, typically in a bacterial reverse mutation assay, should be considered

! the **absence of a structural alert** based on a well-performed assessment (e.g. through application of commonly used QSAR assessment software such as DEREK or MCASE) is **sufficient to conclude that the impurity is of no concern** with respect to genotoxicity → no further 'qualification' studies or justification required

! **A negative Ames test** (conducted to regulatory acceptable standards) **overrules a structural alert** → no further studies required providing the level remains below ICH Q3A/B limits

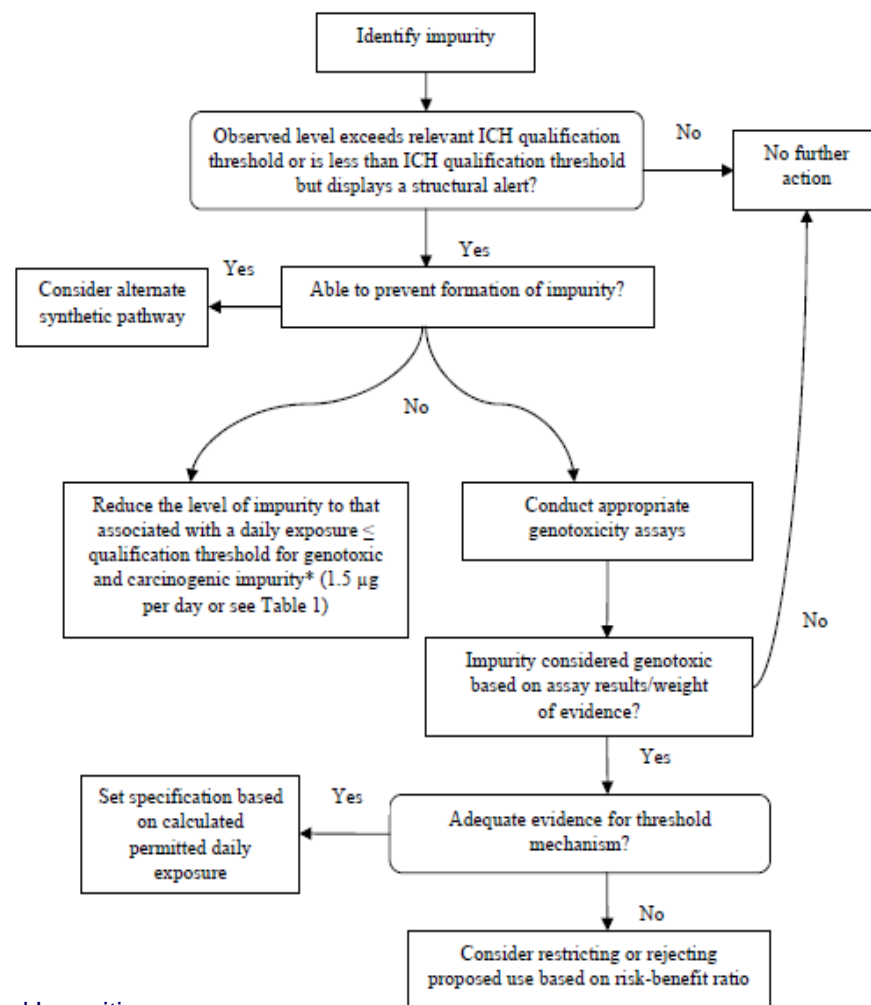


**Danger of missing information and improper qualification of genotoxic impurities without structural alert!**

## U.S. FDA (2008)

### Genotoxic and Carcinogenic Impurities in Drug Substances and Products – Recommended Approaches

- Agrees with the former EMEA guidance
- Necessity to evaluate each impurity at the levels below the ICH qualification thresholds
- Evaluation according to the structural activity relationship (SAR) assessments → *in silico* tools
- For impurities with an identified alert → *in vitro* mutation assay (Ames test) should be conducted
- For impurities with the functional groups not well recognized by bacterial assay (e.g. carbamates) → mammalian cell assay
- The negative results of initial (SAR) investigation → sufficient to conclude that the impurity is not associated with genotoxic activity



- Background information
- Regulatory framework
- ***In silico* tools**
- Conclusions



## Rule-based systems

- “if-then-else” rules
- combine toxicological knowledge, expert judgment and fuzzy logic

## Hybrid models

- combination of knowledge-based rules and statistically-derived models
- within the structural space of a single SA, statistically derived models can quantitatively predict the variation in the reactivity of the alert conditioned by the rest of the molecular structure

## Statistically-based systems

- statistical, rule-induction, artificial intelligence and pattern recognition techniques
- build models from non-congeneric databases



## Rule-based systems

### Advantages

- Provide hints on the mechanism of action
- Provide a reasoning

### Disadvantages

- usually cannot explain differences of the activity within a chemical class
- applicability domain often restricted

## Hybrid models

- combines adv/disadv of both approaches

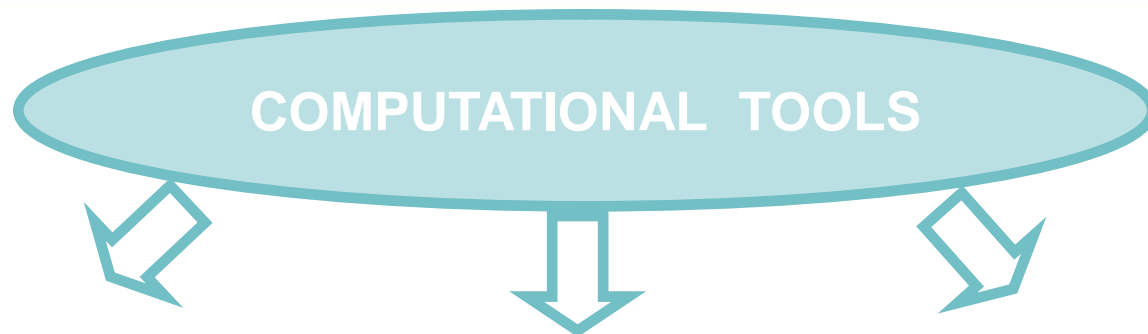
## Statistically-based systems

### Advantages

- usually higher accuracy
- can be use when mechanism of action is unknown

### Disadvantages

- usually difficult to interpret
- often non-transparent



## Rule-based systems

- **DEREK for Windows**
- SAR-based system; toxicophore-based alerts for various toxicological endpoints
- **Hazard Expert**
- toxic fragments-based predictions for various toxicological endpoints
- **ONCOLOGIC**

## Hybrid models

- **TOXTREE**
- Classification schemes: extended Cramer scheme, Benigni-Bossa rulebase, ToxMic rulebase & 3 QSAR models
- **LEADSCOPE**
- **ACD/Labs**
- Knowledge base and NN algorithm
- **OASIS TIMES**

## Statistically-based systems

- **BIOEpisteme (QSAR)**
- **CAESAR**
- CP-ANN for carcinogenicity & SVM for mutagenicity
- **LAZAR**
- **MDL QSAR**
- **MOLCODE TOOLBOX**
- **MULTICASE**
- **TOPKAT**
- **PASS**

## RECOMMENDED IN THE GUIDANCE DOCUMENTS

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 CMTPI 2011 - 03 - 07 September 2011 Maribor

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- From the regulatory perspective *in silico* approaches ....
  - SA identification
  - Assistance in the process of providing required rationale for establishing certain acceptance criteria for a given impurity on the basis of the relevant safety considerations
  - High-throughput, animals-, time- and costs-saving tools for preliminary assessment of genotoxic/carcinogenic potential
  - In case of ambivalent results obtained from experimental test, *in silico* tools can be used for clarification purposes
  - Under extreme conditions in which genotoxicity tests cannot be employed (e.g. for technical reasons), relevant, validated *in silico* tools can be particularly valuable

As far as regulatory purposes are considered, genotox potential prediction should be based on

## **A battery of models**

combining

**high sensitivity models** (low rate of false negatives)

with **high specificity ones** (low rate of false positives)

and ***in vitro* assays**

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# AND YOU ALL