SEQENS 'Lab

Investigating Genotoxic Impurities (GTIs)

To secure active pharmaceutical ingredients (APIs) and comply with regulatory requirements

WHY CHOOSE SEQENS' LAB

- **We provide a comprehensive solution to support genotoxic impurities requirements and secure your development.**
- **We have built a « Safety by Design® » approach placing safety at the core of the process of synthesis of APIs.**
- **We have a long term expertise combining toxicology in-silico prediction with in-vitro testing.**
- **We provide state of the art equipment's with analytical expertise allowing high capacity and fast turn-around.**
- **We supply you with consistent data in line with a regulatory frame, with a experienced team always available to answer any questions and coordinate the project.**

Integrated solution

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Identification of GTIs along the synthesis of APIs

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In silico analysis from chemical structure of GTIs : Level 1

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No alert about a mutagenic potential

Alert about a potential mutagenic toxicity

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In Vitro Arms test: level 2 in vitro tests Ames type with 5 strains OECD – regular or GLP compliant (ICH) and formulation analysis

ICH M7 guideline

- The synthesis of drug substances involves the use of reactive chemicals, reagents, solvents, catalysts, and other processing aids.
- As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated drug products.
- While ICH Q3A(R2): Impurities in New Drug Substances and Q3B(R2): Impurities in New Drug Products provides guidance for qualification and control for the majority of the impurities, limited guidance is provided for those impurities that are DNA reactive.
- The ICH M7 guideline is to provide a practical framework that is applicable to the identification, categorization, qualification, and control of these mutagenic impurities to limit potential carcinogenic risk.

Comprehensive strategy

1. Impurities evaluation

- **Analytical determination of impurities:**
  - Generated during the synthesis
  - Generated during storage:
    - Prediction in-silico (Zeneth)
    - Forced degradation study
- **Confirmation stability study**
- **Analytical identification when the content is higher**
- **ICH Q3A and/or ICH Q3B**

2. Risk assesment

- **QSAR analysis (level 1) with 2 predicitve models:**
  - Harmonic Pharma
  - OECD
- **AMES test (level 2) including formulation analysis:**
  - GLP Testing
  - Non-GLP testing with NanoAMESTM

3. Control strategy

- **Control strategy implementation:**
  - Analytical method
  - In-silico Purge Factor assessment (Mirabilis)

4. Risk characterization

- **Determining acceptable intakes**
- **Classification of impurities (Class 1 to 5)**

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GTI's sources

Starting material
• Genotoxic impurities
• Starting material and its impurities

Intermediate product
Catalysts, reagent, solvents, byproduct and intermediates carry over

Drug substances
Degradation on storage and shipment

GTI's classification

Class	Technology	Proposed action for control
Class 1	Known mutagenic carcinogens	≤ compound-specific limit
Class 2	Known mutagens with unknown carcinogenic potential	≤ appropriate TTC
Class 3	Alerting structure, unrelated to structure of DS, no mutagenicity data	≤ appropriate TTC or conduct Ames test (non-mutagenic = Class 5 ; mutagenic = Class 2)
Class 4	Alerting structure, same alert in DS or compounds related to DS which have been tested and are non-mutagenic	Non-mutagenic impurity (ICH Q3A/B)
Class 5	No structural alerts, or alterting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Non-mutagenic impurity (ICH Q3A/B)

In silico Approaches
Statistical classification and investigation of mechanism of action regarding GTIs

A – Statistical classification

• Set of mutagenic compounds
• Set of non mutagenic compounds

QSAR* method based on a selection of molecular descriptors

Checking applicability of the methods
Study GTIs

Generating alerts according to the ranking

• Mutagenic potential
• No mutagenic potential

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Classification of GTIs

B – Analysis of mechanisms of action

Structure of GTI

Comparisons of GTI with chemical compounds in the Harmonic format

Polypharmacology profile

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Deciphering diverse mutagenic potentials with regard to associated biomolecular targets

The method « QSAR mutagen »

• « QSAR mutagen » is a statistical model to predict a rank of a GTI among an ensemble of compounds having or not a mutagenic potential

• Process used for any GTI:
  • Molecular descriptors are calculated and compared with those of reference molecules,
  • The model generates a score value ranging from 0 to 1 reflecting the GTI position in the ensemble of reference molecules,
  • An alert is generated depending on the obtained ranking

• Set of mutagenic compounds 3878 molecules
• Set of non mutagenic compounds 1953 molecules

Molecular descriptor
• Geometric • Electronic,
• Hydrophobicity, ...
Study GTIs

Algorithm Analysis Statistics
« Generalized Linear Model »

• Potential mutagenic character
• No mutagenic potential

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Classification of GTIs

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*QSAR: « Quantitative Structure Activity Relationship »

In vitro Assesment
When to perform Ames test?

Parameters	Class				
	1	2	3	4	5
In silico system 1	**Positive**	**Positive**	**Positive alterting structure**	**Negative**	**Known as non-mutagen**
In silico system 2	**Positive**	**Positive**	Negative or out of domain	Out of domain or equivocal	**Known as non-mutagen**
Experimental (Ames test) result	**Known as mutagen carcinogen**	**Known mutagen unknown carcinogen**	To be performed	**Negative**	**Negative**
Compounds	⬟	⬟	⬟	⬟	⬟

Based on M7 guideline, testing is Performed for class 3 alert.

In-vitro testing – GLP testing

Address to Class 3 products, or test item that cannot be said to be clearly negative or positive

Perform Nano Ames test when a new and "unknown" is present according to OECD 471 (Salmonella typhimurium and/or E. Coli: 5 strains, +/- metabolic activation) wit only **35-100 µg**:

- If the result is negative, the impurity is considered non-genotoxic, and should be confirmed in GLP Ames test
- If positive the impurity is considered genotoxic

Perform Mini/Micro Ames test when a few amount (35 mg) of impurities is available according to OECD 471 (Salmonella typhimurium and/or E. Coli: 5 strains, +/- metabolic activation):

- If the result is negative, the impurity is considered non-genotoxic, and should be confirmed in GLP Ames test
- If positive the impurity is considered genotoxic

Perform GLP Ames test test with formulation analysis (formulation analysis may be non-GLP) 1 to 3 g for according to OECD 471 (Salmonella typhimurium and/or E. Coli: 5 strains, +/- metabolic activation):

- If the result is negative, the impurity is considered non-genotoxic
- If the result is positive the impurity is considered genotoxic

In-vitro testing – Non GLP testing

Strategy to test very early with very low quantities of test article (formed impurities, starting material etc.):

- Classical approach, when alert structure is clearly identify, in few amount of synthesis of impurity, a quick results are obtained Mini Ames with 35 mg of Test article allow testing impurity
- Nano Ames a minimum of 40 µg (micrograms) of test item, or impurities collected (chromatography) lead to obtain a results in OECD strains with and without metabolic activation, with unknown structure

In both cases if the results are positive consider impurity to be genotoxic

Managing impurity at its TTC level depending on the status of the clinic saves time on the project. It is always very tricky to manage impurities at the end of the project (mistake made by many start-ups)

Save time and money, assess GTI in early phase!

Control strategy
Analytical testing & Purge factor determination

Option 1
Quantify the impurity content in the active ingredient and then assess compliance with the toxicological impact threshold (TTC)

Option 2
Quantify impurity content in intermediates, starting materials and processes and then assess compliance with toxicological impact threshold (TTC)

Option 3
Quantify the impurity content in intermediates, starting materials and processes and then demonstrate by a Purge study that any content above the toxicological impact threshold (TTC) will be corrected by the analytical process

Option 4
Calculate Purge Factors to demonstrate that impurities will be reduced to negligible content during Process by in-silico assessment

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Analytical challenges (option 1, 2 & 3)

The analysis of genotoxic impurities can be very complex as they must be controlled at levels well below 0.01-0.03%

Thus, the analytical procedure should allow detection limits between 1 and 5 ppm (0.0001-0.0005% w/w)

Such low levels require:

- More sensitive analytical instruments
- Higher selectivity requirements (higher number of other organic impurities potentially present at different concentrations)

Analytical solutions (option 1, 2 & 3)

Hyphenated chromatography and mass spectrometry technologies are available to meet sensitivity and specificity requirements for ultra traces analysis, such as:

- GC-MS, HS-GC-MS, GC-MS/MS, HS-GC-MSMS
- UPLC-MS, UPLC-MS/MS, UPLC-MSQTOF

Purge factor (option 4), *in-silico* evaluation

PMI requiring management

Purge Ratio (PR)
*Predicted Purge factor
**Required Purge factor

- **PR > 1000**: **Option 4 supported**
  Provide purge ratio
- **1000 > PR > 100**: **Option 4 supported**
  Full purge calculation and supporting literature
- **100 > PR > 1**: **Option 4 supported**
  Only with strong supporting data
- **PR < 1**: **Option 4 not supported**
  Option 3, 2 or 1 required

*Predicted Purge factor : this value is calculated in Mirabilis and is made up of reactivity, solubility and volatility purge factors.
**Required Purge factor : this is calculated in Mirabilis once the user has entered an API dose, PMI initial concentration and PMI control limit.

Impurity requires management as (P)MI

Determine Purge Ratio (PR) in current API route for (P)MI

Purge Ratio =
Predicted Purge factor for (P)MI Required Purge factor to achieve TTC or PDE for (P)MI

Select initial ICH M control strategy for (P)MI during development based on Purge Ratio. Implement ecommanded experimental data collection and regulatory reporting strategies based on upon Purge Ratio

Does final data package support commercial ICH M7 Option 4 strategy ?

Yes
Select ICH M7 Option 4 Commercial Strategy

No
Select ICH M7 Option 1, 2 or 3 Commercial Strategy, as appropriate

Regulatory overview

EMA

- EMA, ICH guideline M7(R1) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (25 August 2015)
- EMA, Application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes (23 July 2015)
- EMA, Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal product (24 February 2017)
- EMA, ICH M7 Guideline: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk — Questions and answers (2 July 2020)
- EMA, Overview of comments received on ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk — Questions and answers (13 October 2020)

FDA

- FDA, Guidance for industrie, M7(R1) Assessment and Control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (March 2018)
- FDA, M7 Assessment and Control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk — Questions and answers (June 2020)

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