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RISK ASSESSMENT - GENOTOXIC IMPURITIES IN CELECOXIB [CLASS - COX-2 SELECTIVE INHIBITOR]

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ABSTRACT

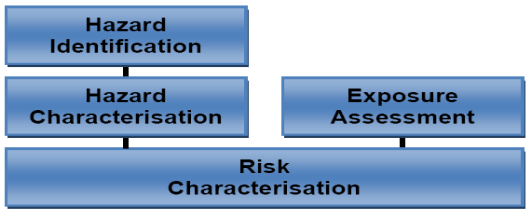
Genotoxic impurities are distinguished class of impurities that can cause genetic mutations and/or chromosomal rearrangements at very low levels. Control of genotoxic impurities is a crucial activity that is required for any pharmaceutical entity. The key element in this is the risk assessment of associated genotoxic concern. Based on the outcome of risk assessment, appropriate strategy for control and monitoring is decided. This article reveals a risk assessment case study carried out on Celecoxib, an active pharmaceutical ingredient, to evaluate the associated genotoxic concern.

KEYWORDS:

INTRODUCTION

Celecoxib being a COX-2 selective inhibitor is a non-steroidal anti-inflammatory drug (NSAID) that targets cyclooxygenase-2 [COX-2], an enzyme responsible for inflammation and pain. Selectively targeting COX-2 reduces risk of peptic ulceration and contributes as main feature of this drug class. It basically works by reducing hormones that cause inflammation and pain in the body. Celecoxib is used to treat pain or inflammation caused by many conditions such as arthritis, ankylosing spondylitis and menstrual pain. It is also used in treatment of hereditary polyps in colon. As per available literature maximum daily dose recommended is 400 mg.[1]

Control of genotoxic impurities is a crucial activity that is required for any pharmaceutical entity. The key element of this is the risk assessment of associated concern for genotoxic. As per approved regulations reported in ICH Q9[2], the risk assessment has been therefore intended with a four stage approach as confirmed after US National Academy of Sciences, 1983[3]



Brief discussion for each phase is discussed in the subsequence for ease of understanding.

Hazard Identification

In order to identify the potential hazard causing compounds, it is recommended that all available information from the manufacturing process is gathered and reviewed. This review should also consider available evidence of genotoxicity and any other toxicity that may be relevant to understanding the mechanism by which the substance poses concerns for genotoxicity. Genotoxicity hazard identification is part of the impurity qualification process for drug substances, the first step of which being the prediction of their potential DNA reactivity using in silico (quantitative) structure-activity relationship (Q)SAR models/systems.

Hazard Characterization

Evaluates potential adverse health effects attributable to specific genotoxic agents, the mechanisms by which agents exert their toxic effects and the associated dose, route, duration and timing of exposure.

Exposure Assessment

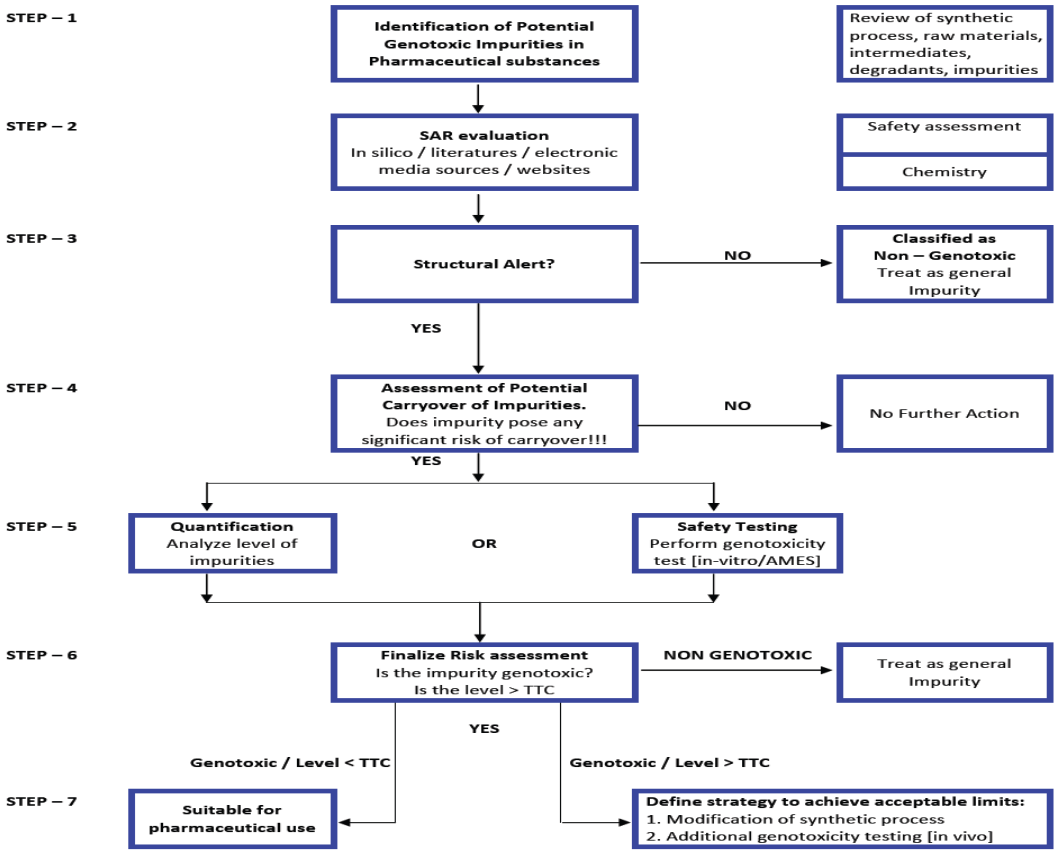
Exposure assessment estimates probable exposure by determining source, magnitude, frequency and duration of exposure to the suspected impurities by which it may enter the body. Exposure assessment is an increasingly important aspect of carcinogen risk assessment, given the increasing use of approaches such as the Threshold of Toxicological Concern.

Risk Characterization

Risk characterization is the final phase of risk assessment process. It integrates three phases: Hazard Identification, Hazard Characterization and Exposure Assessment.

The above risk assessment strategy has been further simplified to demonstrate a pragmatic approach for evaluating of the risk associated with the API without compromising on the safety aspects. This article emphasizes on primary components, focusing on effective use of in-silico assessment tools along with available literature data to augment this process. Therefore to simplify the risk assessment for genotoxic concern in Celecoxib active pharmaceutical ingredient the 'four stage approach' has been transformed to a 'seven step risk assessment procedure' detailed in subsequent page. Correlation between transformations of the 'four stage approach' to simplified 'seven step risk assessment procedure' is demonstrated in the subsequent table.

Sr. No.	Four Stages – Risk Assessment Approach Stage Name And Number	Seven Step Logical Path Resembling Step Numbers
1	Stage 1 – Hazard Identification	Step – 1, Step – 2
2	Stage 2 – Hazard Characterization	Step – 3
3	Stage 3 – Exposure Assessment	Step – 4, Step – 5, Step – 6
4	Stage 4 – Risk Characterization	Step – 7

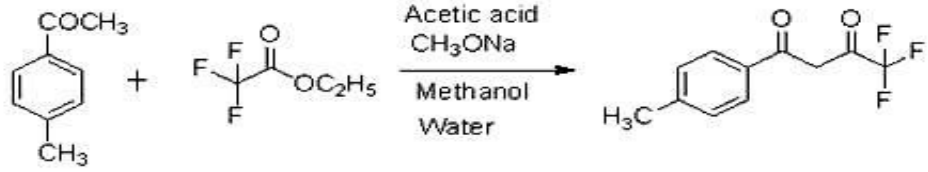


The risk assessment for genotoxic concern in Celecoxib active pharmaceutical ingredient was performed with meticulous consideration of synthesis, raw materials, intermediates, derivatives, by-products and degradation products. The evaluation has been extended to determine possible impurities arising from starting materials.

The simplified seven step logical path was employed for Risk Assessment in Celecoxib. All steps described in the logical path have been reported in the following to draw practical conclusion from risk assessment study.

Step 1 – Review of Synthetic Process, Raw Material, Intermediates, Degradants and Impurities Synthesis route used for manufacturing of Celecoxib Active Pharmaceutical Ingredient is described in subsequence; Celecoxib is synthesized in two stages.

Stage-I: Preparation of 4,4,4-trifluoro-1-p-tolylbutane-1,3-dione

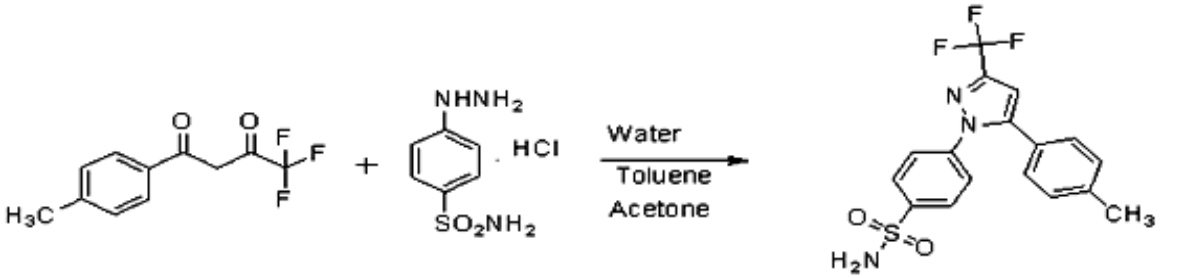


$C_9H_{10}O$: 134.18 — 4-Methyl acetophenone

$C_4H_5F_3O_2$: 142.08 — Ethyl trifluoro acetate

$C_{11}H_9F_3O_2$: 230.18 — 4,4,4-trifluoro-1-p-toluylbutane-1,3-dione

Stage-II: Preparation of Celecoxib



$C_{11}H_9F_3O_2$: 230.18 — 4,4,4-trifluoro-1-p-toluylbutane-1,3-dione

$C_6H_{10}ClN_3O_2S$: 223.68 — 4-hydrazino benzene sulfonamide Hydrochloride

$C_{17}H_{14}F_3N_3O_2S$: 381.37 — Celecoxib

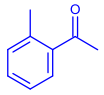
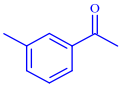
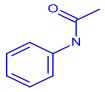
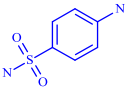
Step 2 – SAR[1] evaluation

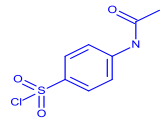
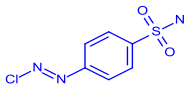
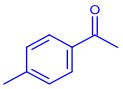
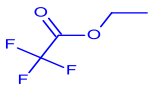
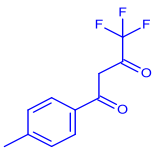
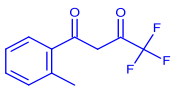
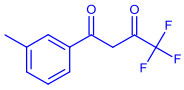
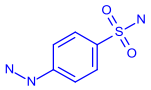
The above evaluation was carried out with listing of all compounds assumed under theoretical impurities. Theoretical impurities are potentially genotoxic impurities that are based on theoretical considerations but not found in practice as demonstrated by studies during development or manufacture. Possibility for genotoxic concern associated with each theoretical impurity was studied with in-silico analysis based on structural alerts. Interpretation from in-silico analysis was drawn from regulatory accepted software.[4] To strengthen the postulation the evaluation is further extended with the study of available literatures, electronic media sources and websites.

Step 3 – Segregating theoretical impurities for presence of Structural Alert

Basing on the outcome from Step 2, theoretical impurities with presence of structural alerts and findings from the experimental studies in research literatures were further classified for associated genotoxic concern. The summary of observations for theoretical impurities, their origin in the process, chemical structure and possibility for genotoxic concern has been provided in subsequence for ready reference.

Identified theoretical impurities in Celecoxib API.

S. No.	Theoretical impurities	Origin	Structure	Genotoxic concern
4-Methyl acetophenone – Starting Material – 1				
1.	2-Methyl acetophenone	Possible impurity in 4-methyl acetophenone		NO
2.	3-Methyl acetophenone	Possible impurity in 4-methyl acetophenone		NO
4-Hydrazino benzenesulfonamide HCl – Starting Material – 2				
3.	Acetanilide	Used in synthesis of 4-hydrazino benzene sulfonamide HCl		NO
4.	Sulfanilamide	Intermediate of 4-hydrazino benzene sulfonamide HCl		NO

5.	Acetanilide sulfonyl chloride	Intermediate of 4-hydrazino benzene sulfonamide HCl		YES
6.	4-[(E)-chloroazo] benzene sulfonamide [Diazo salt]	In-situ intermediate of 4-hydrazino benzene sulfonamide HCl		YES
Celecoxib – Final API				
7.	4-Methyl acetophenone	Starting Material – 1		NO
8.	Acetic acid	Used in API synthesis		NO
9.	Ethyl trifluoro acetate	Used in API synthesis		NO
10.	Trifluoroacetic acid	Possible impurity in ethyl trifluoroacetate		NO
11.	4,4,4-trifluoro-1-p-toluyl butane-1,3-dione	Intermediate of Celecoxib		NO
12.	4,4,4-trifluoro-1-o-toluyl butane-1,3-dione	Possible impurity in Celecoxib Stage-I		NO
13.	4,4,4-trifluoro-1-m-toluyl butane-1,3-dione	Possible impurity in Celecoxib Stage-I		NO
14.	4-Hydrazino benzene sulfonamide HCl[1]	Starting Material – 2		NO

Above impurities were further studied for presence of structural alerts in reported literature data for the possibility of associated genotoxic concern. The impurities found without genotoxicity concern were proposed for monitoring under general impurities recommended in the ICH 3A guidance and have been kept out of scope for this study.

The theoretical impurities observed with genotoxic concern were further evaluated for potentiality for carryover in downstream synthesis in subsequent step.

Step 4 – Assessment of Potential Carryover of Impurities

Theoretical impurities found with potential genotoxic concerns but posing no risk for carryover in downstream synthesis requires no further action.

Theoretical impurities found with potential genotoxic concerns, having likelihood for carryover in subsequent step is progressed with quantification study.

Step 5 – Quantification or Safety Testing

The quantification is primarily performed to ensure the observed level of any residual carryover of suspected genotoxic impurities arrived from earlier steps.

Theoretical or suspected genotoxic impurities with adequate experimental evidence from toxicological studies for safe intake level, is regulated using methods based on evaluation of Permissible Daily Intake.

Suspected genotoxic impurities with inadequate experimental evidence, the existing research studies propose a limit called as "threshold of toxicological concern (TTC)." A TTC value of 1.5 g/day intake of a genotoxic impurity is considered to be associated with an acceptable risk. The concentration limit in parts per million (ppm) of genotoxic impurity permitted in a API is the ratio of TTC in µg/day and daily dose in g/day. Risk assessment for identified genotoxic impurity in API can be concluded with establishing a TTC with respect to the API.

TTC for Celecoxib against a maximum daily dose of 400 mg confirms to 3.75 ppm. However as an additional precaution content of identified genotoxic impurities were tested in API with a far lower limit for '30 percent of TTC' i.e. 1.12 ppm.

Safety studies can be conducted for potential impurities containing structural alerts. Safety studies can be defined as in vitro tests designed to detect compounds that induce genetic damage by various mechanisms as discussed under earlier section of this article. However the safety studies are essentially approached to ensure the confirmatory interpretation for the suspected genotoxic impurities.

Evaluation infers, 'acetanilide sulfonyl chloride' and '4-[(E)-chloroazo] benzene sulfonamide [diazonium salt]' involved in the starting material synthesis and 4-hydrazino benzenesulfonamide HCl are impurities suspected with genotoxic concern.

Step 6 – Finalizing Risk Assessment

With final categorization of identified impurities with genotoxic concern, scopes for carryovers were demonstrated in final API with suitable TTC limit.

Acetanilide sulfonyl chloride is an intermediate formed during synthesis of 4-hydrazino benzenesulfonamide HCl starting material. As added precaution carry over analysis in final API is performed with suitable limit of 1.12 ppm [30% of TTC].

4-[(E)-chloroazo] benzene sulfonamide [Diazo salt] is an in-situ intermediate produced during synthesis of 4-hydrazino benzenesulfonamide HCl starting material. In aqueous solution diazonium salts are highly unstable at elevated temperatures, the -N+≡N group tends to be lost as nitrogen gas. Thus confirming to reaction conditions during synthesis of 4-hydrazino benzene sulfonamide HCl, diazonium compound cannot retain in reaction as un-reacted content.

4-hydrazino benzenesulfonamide HCl; study of structural alerts through in-silico analysis does not fully establish the genotoxic concern associated with 4-hydrazino benzenesulfonamide HCl, however due to presence of potential structural alerts in the structure, this compound has been considered under suspected genotoxic impurities.

Summary of analysis result for all impurities tested in six consecutive commercial batches of final API is provided in the following for ready reference.

S. No.	Impurities	Limit [ppm]/ Method	Celecoxib API – Batch Numbers						LOD [ppm]	LOQ [ppm]
			0001	0002	0003	0004	0005	0006		
1	Acetanilide sulfonyl chloride	1.12 / LCMS	ND	ND	ND	ND	ND	ND	0.066	0.199
2	4-[(E)-chloroazo] benzenesulfonamide [Diazo salt]	1.12 / LCMS	Analysis not required; highly unstable in nature.							
3	4-Hydrazino benzenesulfonamide HCl	1.12 / LCMS	ND	ND	ND	ND	ND	ND	0.067	0.204

ND: Not Detected.

As per above analysis data Acetanilide sulfonyl chloride and 4-hydrazino benzenesulfonamide HCl is not detected in any of the API batches hence the results confirm insignificant possibility for their presence in final API.

Step 7 – Risk Characterization for Evaluation of suitability for pharmaceutical use or defining strategy to achieve acceptable limits

Risk characterization is the final phase of the health risk assessment process. It integrates the three phases: Hazard Identification, Hazard Characterization and Exposure Assessment. Risk assessment for identified potential genotoxic impurity in API is concluded with risk characterization through any one of the approach of combination thereof for reducing potential cancer risk with patient exposed to genotoxic impurities.[5]

1. Modify synthesis or purification to minimize formation or removal of impurity.
2. Allowing maximum daily exposure target of 1.5 µg per day of relevant impurity.
3. Characterize genotoxic and carcinogenic risk to support appropriate impurity specifications, either for higher or lower values.

The inference on risk characterization is drawn based on the below risk quantification mechanism derived from the EU guidance on genotoxic impurities.[6]

Case	Observation from Batch Analysis Trend Results	Recommendation
1	< 30% of TTC/safe limit when tested in intermediate	Testing not required
2	< 30 % of TTC/safe limit when tested in API	Non routine/Skip testing
3	< TTC/safe limit but >30% of TTC/safe limit when tested in API	Routine testing in API
4	> TTC/safe limit	Process change

CONCLUSION

The risk characterization study infers the following.

- Impurities are observed at extremely insignificant level in API batches; this confirms possibility for carryover in final API is highly unfeasible.
- Therefore routine monitoring for these impurities in API is not required.
- 4-hydrazino benzene sulfonamide HCl is used in manufacturing process at final stage therefore, monitoring this impurity with skip testing in API specification with a limit of 30% of TTC [i.e. by 1.12ppm] is recommended.

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