Protecting Public Health Using (Quantitative) Structure-Activity Relationships and Expert Knowledge: Considerations for ICH M7 Class 4 Impurities

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Introduction

Drug impurities are regulated as either non-mutagenic or mutagenic according to ICH Q3A/B or ICH M7 guidelines, respectively. Impurity classification relies on a computational toxicology assessment using two complementary (quantitative) structure-activity relationship [(Q)SAR] model predictions and/or experimental data (ICH, 2023). (Q)SAR models make a prediction of mutagenic potential based on an impurity's chemical structure by identifying structural alerts, which are substructures associated with mutagenicity. There are two scenarios where shared structural alerts with an experimentally negative comparator are assessed:

	Description	Impurity Assignment
Scenario 1	An impurity is considered non-mutagenic when it contains a structural alert which is shared with an experimentally negative (GLP compliant 5 strain Ames test [OECD, 1997]) drug substance or closely-related intermediate and no other alerts are present	Class 4: Non-mutagenic Impurity
Scenario 2	An impurity has more than one structural alert present. If one alert is shared with an Ames negative comparator, the 'class 4 argument' may be used to downgrade the shared alert. However, the non-shared alert would need to be addressed separately, leading to either a mutagenic or non-mutagenic overall assignment [Jayasekara et al., 2021]	Class 3: Mutagenic Impurity or Class 5: Non-mutagenic Impurity

Table 1: *Scenarios for Class 4*

In both scenarios, expert knowledge is applied to evaluate the similarity of the shared structural alert type, position, and surrounding chemical environment to downgrade the alert and support the overall classification of the impurity. This poster presents case studies for both Class 4 scenarios and expert knowledge considerations for each.

Materials and Methods

A data set of 109 (Q)SAR consults submitted to the FDA/CDER Computational Toxicology Consultation Service (CTCS) was analyzed for Class 4 assignments to 1) determine the frequency of Class 4 arguments as a percentage of total consults to CTCS and 2) to identify areas where the similarity-based arguments for dismissing a shared structural alert are not acceptable. Three commercial (Q)SAR modeling software were used by CTCS to generate predictions for bacterial mutagenicity: Lhasa Limited, Leadscope Model Applier, and CASE Ultra.

Results and Discussion

Of 109 consults evaluated, 14 (13%) were found to contain Class 4 impurity classifications or arguments. Commonly observed issues with the expert review were: 1) insufficient structural similarity between the impurity and negative comparator; 2) an experimentally positive API containing the alert was used as a comparator, 3) limited, insufficient/non-robust, or no experimental data was provided for the negative comparator.

Figure 1: Percentage of Class 4 references

Expert Knowledge Considerations: Availability and Reliability of the Experimental Data of Comparator

- ✓ Bacterial mutagenicity data (Ames test) must be negative for the comparator molecule.
- ✓ De novo Ames data should be consistent with OECD 471 guidelines [ICH, 2023; OECD, 1997] e.g. adequate controls, use of 5 bacterial strains, 37°C, with and without S9 metabolic activation, and testing at 5 concentrations up to 5 µl/plate.
- ✓ FDA drug labels are considered reliable and robust sources for mutagenicity data, e.g. section 13 Nonclinical Toxicology

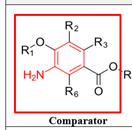
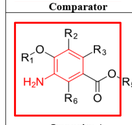
Case Study: Experimental Data for Comparator	API is Negative Y/N	Quality of Ames Neg Data	Environment around the Alert	Are there other non-shared alerts?	Non-shared alerts can be dismissed?	Comments
 **Comparator**	Unknown	No information per drug product label	Di-substituted aniline with an ether and carboxylic ester group at the ortho and meta positions, respectively and where R4 and R5 are different. All other R groups are identical.	No "R" groups have other structural alerts associated with them	n/a	Impurity A cannot be considered a Class 4 impurity. **Reason:** There are no experimental data available for the comparator, so even though the shared alert is in the same chemical environment, Class 4 criteria are not met.
 **Impurity A**	n/a	Not tested				

Table 2: *Evaluation of experimental data for comparator where (Q)SAR has identified a shared structural alert (aromatic amine)*

Expert Knowledge Considerations: (Q)SAR Alert Environment

Scenario 1: Shared Alert Assessment Class 4

- ✓ Comparator molecule and impurity have the **same alert** per regulatory (Q)SAR models
- ✓ The presenting alert is in the same chemical environment in both the comparator and the impurity
  - ➢ Evaluate deactivating features in the comparator that may not be present in the impurity---these could be the reason for the Ames negative result
  - ➢ Evaluate activating features in the impurity that are not present in the comparator—these could change the environment enough that applying structural similarity is not appropriate
- ✓ There are no other **non-shared alerts** in the impurity

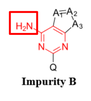
Case Study: Scenario 1	API is Negative Y/N	Quality of Ames Neg Data	Environment around the Alert	Are there other non-shared alerts?	Non-shared alerts can be dismissed?	Comments
 **API**	Yes	Per RLD label	Secondary aromatic amine/API is deactivated due to steric hindrance (R1) around the alert which may prevent metabolic activation and mutagenic activity	No	n/a	Impurity B cannot be considered a Class 4 impurity. **Reason:** The aromatic amine alert in Impurity B is not in the same chemical environment as the experimentally negative API.
 **Impurity B**	n/a	Not tested	Primary aromatic amine with scaffold found in the experimentally Ames negative API/ metabolic activation to form the nitrenium ion is not hindered			

Table 3: *Evaluation of deactivating features in comparator that are not present in the Impurity B for the (Q)SAR identified shared aromatic amine alert*

Scenario 2: Shared and Non-shared Alert Assessment Class 3 or Class 5

- ✓ If the shared alert is dismissed based on Class 4 arguments and the non-shared alert is also dismissed by expert review, the overall impurity classification is non-mutagenic, Class 5
- ✓ If the shared alert is dismissed based on Class 4 arguments and the non-shared alert is not dismissed by expert review, the overall impurity classification is mutagenic, Class 3

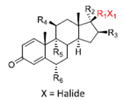
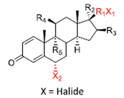
Case Study: [Jayasekara et al., 2021]	API is Negative Y/N	Quality of Ames Neg Data	Environment around the Alert(s)	Are there other non-shared alerts?	Non-shared alerts can be dismissed?	Comments
 X = Halide **API**	Yes	Per OECD	Steroidal backbone	Yes, X_2 is a non-shared alert	Yes, based on the application of expert knowledge for non-primary alkyl halides and supporting training set analogs with steroid backbone	Overall, Impurity is considered non-mutagenic (Class 5) using a "Class 4 argument" to dismiss the shared alert and after separate evaluation of the non-shared alert which was ultimately also dismissed. **NOTE:** If the non-shared alert could not be dismissed, this impurity would be considered mutagenic (Class 3)
 X = Halide **Impurity**	n/a	Not tested	Same steroidal environment for shared alert R_1X_1; Non-shared alert X_2 requires assessment			

Table 4: *Comparison of chemical environment between shared and non-shared Structural alerts*

Conclusion

It was found that approximately 13% (n=109) of expert reviews contained reference to "Class 4." This poster details the difference between a "Class 4 assignment" (impurity with a shared structural alert with an experimentally negative comparator where no other alerts are present) and a "Class 4 argument" (impurity that has both a shared alert with an experimentally negative comparator and also a non-shared alert). Expert review is required to determine: 1) robustness of the comparator's negative experimental data 2) sameness of the structural alert and 3) similarity of the environment around the alert. Applying expert knowledge, as outlined in this poster and consistent with ICH M7(R2) Q&A Q9.2 (ICH, 2023), can promote the submission of well-documented (Q)SAR analyses for impurities regulated under ICH M7, potentially enhancing review efficiency and ensuring patient safety is protected.

References:

ICH (2023) M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk Guidance for Industry. https://ich.org/page/multidisciplinary-guidelines

Jayasekara et al., Regulatory Toxicology and Pharmacology, Volume 125, 2021, 105006, https://doi.org/10.1016/j.yrtph.2021.105006

OECD (1997) Guideline for Testing of Chemicals Section 4 Test 471. Bacterial Reverse Mutation Test.