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Use of *in silico* systems and expert knowledge for structure-based assessment of potentially mutagenic impurities

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ABSTRACT

Genotoxicity hazard identification is part of the impurity qualification process for drug substances and products, the first step of which being the prediction of their potential DNA reactivity using *in silico* (quantitative) structure–activity relationship (Q)SAR models/systems. This white paper provides information relevant to the development of the draft harmonized tripartite guideline ICH M7 on potentially DNA-reactive/mutagenic impurities in pharmaceuticals and their application in practice. It explains relevant (Q)SAR methodologies as well as the added value of expert knowledge. Moreover, the predictive value of the different methodologies analyzed in two surveys conveyed in the US and European pharmaceutical industry is compared: most pharmaceutical companies used a rule-based expert system as their primary methodology, yielding negative predictivity values of $\geqslant$78% in all participating companies. A further increase (>90%) was often achieved by an additional expert review and/or a second QSAR methodology. Also in the latter case, an expert review was mandatory, especially when conflicting results were obtained. Based on the available data, we concluded that a rule-based expert system complemented by either expert knowledge or a second (Q)SAR model is appropriate. A maximal transparency of the assessment process (e.g. methods, results, arguments of weight-of-evidence approach) achieved by e.g. data sharing initiatives and the use of standards for reporting will enable regulators to fully understand the results of the analysis. Overall, the procedures presented here for structure-based assessment are considered appropriate for regulatory submissions in the scope of ICH M7.

1. Introduction

Genotoxicity hazard identification is part of the impurity qualification process for drug substances and products. Genotoxicity evaluation should be considered for impurities above the qualification limits according to ICH Q3 A/B guidelines. More recently the European Medicines Agency (EMA) released a guideline and a series of follow-up questions and answers documents on the Limits of Genotoxic Impurities in active pharmaceutical ingredients

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(European Medicines Agency, 2006, 2007). In this context, a draft guideline was also issued by the US Food and Drug Administration (FDA), and an ICH guideline M7 draft consensus document (step 2) has been published (2013).

Structural alerts to support the prediction of genotoxic activity have been available for more than 30 years (Ashby and Tennant, 1991). For impurities expected to be present at low levels, i.e., below the qualification limits, genotoxicity evaluation is generally limited to DNA-reactive mutagenic compounds considered to have a linear dose–response relationship potentially bearing a carcinogenic potential that are usually detected in the Ames bacterial reverse mutation assay (McCann et al., 1975; Zeiger, 1987, 1998). The rationale to focus on bacterial mutation as described in ICH M7 is based on analyses showing good correlation of Ames test data with carcinogenicity data for genotoxic carcinogens and non-carcinogens (Kirkland et al., 2005; Matthews et al., 2005; McCann and Ames, 1976). Genotoxicants with non-linear dose–response relationships and a negative outcome of the Ames test typically do not pose an increased cancer risk at levels present as impurities (Müller et al., 2006). Consequently, structural alerts for genotoxicity prediction other than bacterial mutation are not applied for impurity hazard assessment. This approach follows the ICH M7 guidance on Ames positivity for sensitivity. Carcinogenicity alerts that might also identify non-genotoxic carcinogens are generally not used for the evaluation of mutagenic impurities as DNA reactive mutagenic carcinogens are covered by the prediction of Ames mutagenicity while other classes of carcinogens (non-DNA reactive genotoxic carcinogens and non-genotoxic carcinogens) are not included in ICH M7. For these reasons, the first step in the identification of mutagenic impurities is to assess the structures of all compounds used and formed during the synthesis process that may be present in the drug substance and degradants that may be formed in the drug product. This includes starting materials, reagents, intermediates, impurities in starting materials and intermediates, and likely or plausible reaction by-products.

Computational, so-called *in silico* methods that make use of the known relationships between chemical structure and mutagenicity have been developed based on the findings by Ashby and Tennant, 1991 and have been continuously improved. The present paper describes different processes of mutagenicity structure-based assessment and focuses on the most commonly *in silico* (quantitative) structure–activity relationship ((Q)SAR) systems used in the pharmaceutical industry. Moreover, it discusses the advantages and limitations of the different *in silico* systems in the context of the evaluation of mutagenic impurities. In order to illustrate the predictive value of the different processes in place, the results of two surveys are presented. They compared the predictive value of the different approaches with the data obtained in the Ames test, and illustrated how expert knowledge can be used to complement *in silico* systems.

Overall, this white paper should help (1) clarify the place/use of (Q)SAR models in the structure-based prediction of mutagenicity, (2) highlight the quality criteria for (Q)SAR models to be used for the evaluation of impurities and possibly reach a consensus in pharmaceutical industry on recommendations for users, (3) enhance the transparency of the whole process, and (4) discuss the important contribution of expert knowledge to interpret the data from *in silico* systems.

2. Structural alerts for mutagenicity and DNA reactivity

2.1. Well-known structural alerts for DNA reactive, mutagenic carcinogens

Structural alerts for DNA reactivity and mutagenicity were first proposed by Ashby and Tennant, 1991 based on mechanistic information available for well-known rodent carcinogens that are also positive in the Ames test. These structural classes have formed the basis of alerts for mutagenicity that have been extensively studied and evaluated against both public domain and proprietary data sets from the pharmaceutical industry. These structural alerts are typically well understood and can be linked to a specific chemical reaction with DNA either without or with metabolic activation, leading to mutations. Therefore those alerts are referred to as "validated" alerts. However, not all chemicals bearing those alerts and belonging to each of these chemical classes react with DNA since other influencing factors such as their activation by enzymes and steric hindrance interfering with DNA interaction may play a role. *In silico* tools have generally been optimized to consider these influencing factors in predicting whether a chemical has the potential to react with DNA and exhibit mutagenic activity.

2.2. Recommendations for the validation and application of new structural alerts for DNA reactive mutagens with unknown carcinogenic potential

The highest level of confidence regarding DNA reactivity has been typically developed not only based on Ames test data but derived from structural groups with mechanistic evidence (e.g. chemical mechanism of biochemical reactions) for an interaction with the genetic material, together with evidence for carcinogenicity. In lieu of carcinogenicity data, relevant *in vivo* mutagenicity data would also provide a high level of confidence in an alert – the recommendations for follow-up testing given in the ICH M7 draft consensus document (2013) should be considered for the assessment of confidence in a specific structural alert. Further supportive material, e.g. Kirkland and Speit (2008), can be referred to, where appropriate. However, relevant alerts may also be derived from training sets with Ames assay data alone. Even when data from carcinogenicity studies is absent, the assumption that Ames positive compounds are potential mutagenic carcinogens is by default applicable. Therefore beyond Ashby-Tennant alerts for mutagenic carcinogens, there are additional alerts that should be considered when assessing the DNA-reactive potential of impurities. These are so-called "exploratory" or in-house alerts based on a few examples that are Ames positive within a chemical series, sometimes without obvious or well-understood DNA-reactive chemical mechanism or supporting carcinogenicity data. These exploratory alerts may have a high false positive rate (i.e., not many compounds confirmed as mutagenic in the Ames test) even when broadly defined within the context of the chemical series from which they were derived as they are often based on limited data. As they are often unique to a particular chemical series, they are not applicable outside of the chemical space they were derived from. Exploratory alerts are typically used by pharmaceutical companies to flag other compounds from the same chemical series so that they can be evaluated early in the development of a drug program. However because of the structural similarity of some synthesis intermediates with the active pharmaceutical ingredient (API), exploratory alerts might also be appropriate for the evaluation of mutagenic impurities.

Thus, due to the quite heavy consequences of identifying a potentially mutagenic impurity under ICH M7, the degree of confidence in a structural alert must be reasonable to justify testing in the Ames test to verify the mutagenic activity or controlling impurity levels to the threshold of toxicological concern (TTC) considering that the impurity is potentially a mutagenic carcinogen. The rules to define the TTC concept were derived from a database of known mutagenic carcinogens. A dose of 1.5 μg/patient/day would be justified based on a theoretical maximum 10^{-5} lifetime cancer risk. Some groups of high potency mutagenic carcinogens ('cohort of concern'; Cheeseman et al., 1999; Kroes et al., 2004) are