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# Mutagenicity assessment strategy for pharmaceutical intermediates to aid limit setting for occupational exposure

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ABSTRACT

Pharmaceutical intermediates (IM) are used in the synthesis of active pharmaceutical ingredients. They are not intended for human administration, yet employees may be exposed to IM during the manufacturing process. In the context of occupational health, hazard assessment of IM is needed to identify potential intrinsic hazards which could cause unwanted adverse effects. In particular, a carcinogenic potential influences the protection strategy in the workplace. DNA reactive substances may, even if present at very low levels, lead to mutations and therefore, potentially cause cancer.

The use of *in silico* methods to predict mutagenicity is increasingly acknowledged and implemented in the recently released ICH M7 guideline for the limitation of DNA reactive impurities. In this study we investigate the possibility to apply (quantitative) structure–activity-relationships ((Q)SARs) during hazard identification to reduce the number of Ames tests needed for a hazard assessment of IM while maintaining high standards of protection of employees. Ames test outcomes for 188 substances used in the pharmaceutical production were compared with their *in silico* predictions using two different (Q)SAR methodologies (knowledge based and statistical) complemented by expert knowledge. The results of the analysis showed that a negative prediction for mutagenicity provides a high confidence that the IM is not mutagenic in the Ames test with the negative predictive value of 97%. On the other hand the positive predictive value was only 57% and therefore considered too low to reliably consider positive predicted IM to be mutagenic. In order to avoid any unnecessary burden for occupational health purposes caused by falsely positive predicted IM, all positive predicted IM and those with insufficient coverage by the *in silico* systems are submitted to an Ames test to verify or reject the prediction. It is shown that the described *in silico* prediction approach ensures appropriate protection strategy of the employees. Resources for performing Ames tests which do not add additional or new information for the purpose of hazard assessment could be reduced.



## 1. Introduction

Occupational exposure limits (OELs) are health hazard assessment values that are determined from the extrapolation of all available data from preclinical and clinical studies to health effects in humans. The OEL is defined as the time weighted average (TWA) for an eight hour inhalative exposure for five days per week during working lifetime. It is accepted that workers can be exposed to the OEL of a given compound repeatedly without any adverse events (Sargent and Kirk, 1988). Industrial hygiene monitors these levels by using results of airborne sampling. The OEL depends on the pharmacological activity and critical hazard effects of the substance and in general, can only be based on effects which show dose–response relationships (Zielhuis and Notten, 1979). However during pharmaceutical production employees may be handling pharmaceutical intermediates (IM) for which usually such data are not available. IMs typically have only a minimum dataset of studies available which do not allow quantitative evaluation and determination of OELs. Therefore, IM are assigned to occupational hazard bands (OHB) and performance-based exposure limits associated with these bands are applied. The hazard band defines the exposure level still acceptable to assure employee safety. It is achieved through the use of engineering controls and safe handling practices (Naumann et al., 1996). In case of IM the band assignment provide

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guidance for assessing health hazard since no calculation of occupational exposure limit is possible.

Currently, no international guidance describing testing strategy for IMs for occupational health purposes exists. Therefore, pharmaceutical companies apply different testing strategies to obtain sufficient data for health hazard determination. This is done to assure appropriate and consistent engineering, process design and personal protective equipment and is intended to reduce the exposure to IMs. A representative set of hazard evaluation criteria is used to assess IMs similar to what is done for drug substances. Important information includes physico-chemical properties, acute toxicity, local effects such as dermal and eye irritation, dermal sensitization and mutagenicity *in vitro*. These properties are considered to be indicators for the potential to cause chronic adverse effects and, when eventually combined with exposure factors, are influencing the risk of exposure. In particular, substance mass, its physical form, and facilities engineering variables help to define the likelihood of occupational exposure (Olson et al., 1997).

The evaluation of the mutagenic potential of IMs is already done at an early stage of drug substance development, usually when the first-in-human batch is produced. This process is required according to the ICH M7 guideline since IMs stemming from the synthetic route could potentially remain as impurities in the drug substance and exposure of healthy volunteers and patients in Phase I clinical trial needs to be reduced to the acceptable levels. For IMs that are isolated and therefore handled by employees, this early stage mutagenicity evaluation can also be used for occupational health purposes in Phase IIa/IIb and thus, become part of the handling evaluation (Fig. 1).

Mutagenicity assessment is usually a first step in the OHB determination of IM as the stricter OHB is applied for mutagenic substances compared to other adverse effects. Ames test is generally the only genotoxicity test performed for low tonnage IMs.

Mutagens have the potential to directly cause DNA damage when present at low levels, leading to mutations and therefore, potentially causing cancer. This type of mutagenic carcinogens are usually detected in a bacterial reverse mutation (mutagenicity) assay (Ames test) (Ames, 1973; Ames et al., 1973; Mortelmans and Zeiger, 2000; OECD, 1997) which is seen as an indicator test for rodent carcinogenicity (correlation of 60–70%) (Kirkland et al., 2005; Matthews et al., 2006; McCann and Ames, 1976). Hence, IM with positive Ames test should be considered potential occupational carcinogens. It should, however, be noted that according to the Globally Harmonized System of classification and labelling one positive *in vitro* mutagenicity test is considered insufficient to warrant classification (EC, 2011).

Structural alerts to support the prediction of mutagenic activity have been available for more than 30 years (Ashby and Tennant, 1991). Structure-based assessments that include the use of (Q) SAR ((quantitative) structure–activity relationship) *in silico* tools are increasingly used by pharmaceutical industry for potentially mutagenic impurities (OECD, 2007; Sutter et al., 2013). The ICH M7 guideline for limitation of mutagenic impurities (ICH, 2014) (currently step 5) indicates that a structural assessment is sufficient to assess the potential for mutagenicity. The guideline states that in the absence of a structural concern, it is appropriate to conclude that an impurity has no mutagenic potential.

In pharmaceutical companies structural assessment for identification of potentially mutagenic impurities is usually generated from early development stage onwards and before first-in-human studies. Following the recommendations of the ICH M7 for potential impurities in the drug substance an Ames test is only performed if a structural alert for mutagenicity is identified. However, for occupational health purposes IM have been historically tested in the Ames test regardless of a previous negative in *silico* prediction

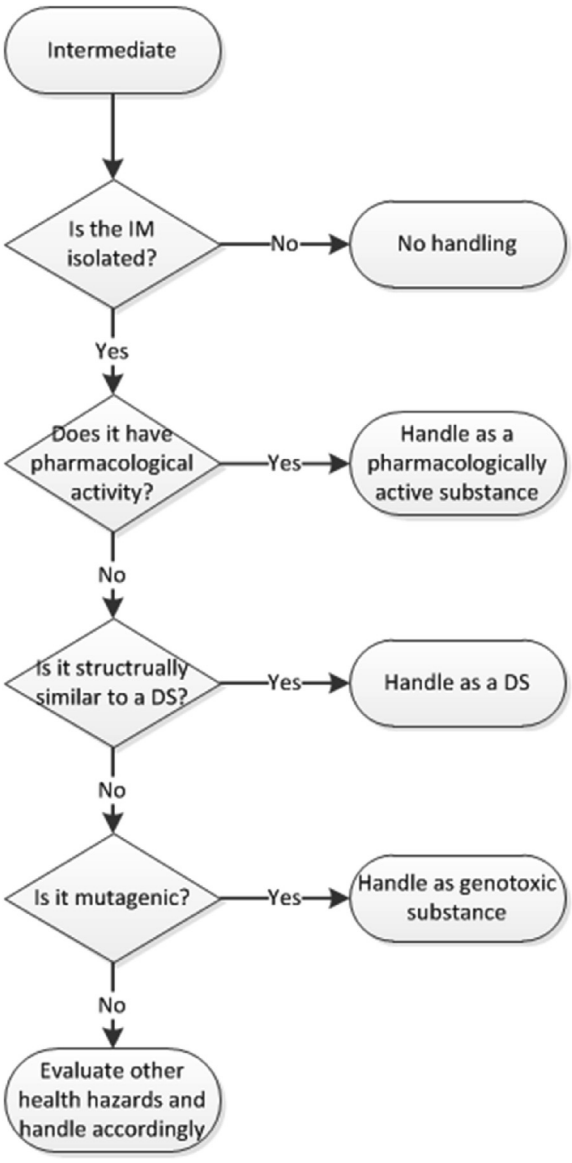


**Fig. 1.** Decision tree determine for handling of IMs. DS: Drug substance.

(usually in Phase II a/b of the drug development process).

In this paper we investigate whether the use of *in silico* predictions is a sufficiently sensitive approach to detect potential mutagenicity of IM and thus, provide the basis of their hazard assessment for occupational health. All IM used for this validation exercise have Ames test data that has been generated for occupational health purposes.

## 2. Methods

### 2.1. Dataset

The data set consisted of 188 IM which are unique to the synthesis schemes of Novartis products. The results of the Ames tests yielded 30 positive substances (i.e. substance that showed a mutagenic response in at least one tester strain) and 158 negative (non mutagenic) substances. The rate of Ames positive substances of only 16% is in the range of what has been published and thus, biased towards Ames negative substances (Sutter et al., 2013). For the IM used for this study, no Ames test data were available in the