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Determination of compound-specific acceptable daily intakes for 11 mutagenic carcinogens used in pharmaceutical synthesis

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ABSTRACT

The synthesis of pharmaceutical products often involves the use of reactive starting materials and intermediates. Low levels may be present in the final product as impurities and of particular concern are impurities that have mutagenic and carcinogenic potential. Regulatory guidance documents provide a general framework to minimise human exposure to these impurities; however, compound-specific recommendations are limited. Our practical experience with 11 pharmaceutical impurities is presented. The genotoxicity and carcinogenicity data are summarised and the approach used to derive an acceptable daily intake (ADI) is described for each chemical. We have highlighted the considerations and challenges associated with calculating ADIs based on available carcinogenicity data. This may provide a useful reference to others in the pharmaceutical industry regarding impurity control, where the weight of evidence indicates the chemical is a mutagenic carcinogen.

1. Introduction

Starting materials and intermediates used to synthesise pharmaceuticals may be intrinsically reactive. It is this attribute that often means they may also react with cellular components such as DNA and as a consequence may be mutagenic and carcinogenic. Following the synthetic process, starting materials and intermediates may reside as impurities, often at low levels, in the final active pharmaceutical ingredient (API). It is widely accepted that their presence offers no benefit to the patient and, as such, diligence is required by pharmaceutical companies to limit human exposure to such impurities during clinical trials and from commercial products. Regulatory authorities recognise the presence of impurities in the final API is unavoidable and consequently guidance related to the control of mutagenic and/or carcinogenic impurities has evolved considerably over the last decade. Historically, guidance was limited to the International Conference on Harmonisation (ICH) who adopted a number of quality documents intended to minimise the presence of impurities whilst maintaining patient safety (ICH Q3A(R2), 2006; ICH Q3B(R2), 2006; ICH Q3C(R4), 2009). However, none of these documents specifically addresses acceptable exposure limits for impurities that are known mutagens or carcinogens. Q3C recommends avoidance of extremely toxic or known carcinogenic solvents and describes levels considered to be toxicologically acceptable for some common residual solvents. Also, mathematical risk assessment models are presented for setting exposure limits in cases where reliable carcinogenicity data are available. A concentration limit for a known human carcinogen, benzene, is provided, but further compound-specific recommendations are limited. More recently, the European Medicine Agency's Committee for Medicinal Products for Human Use (CHMP) acknowledged this regulatory deficiency and published a guideline describing a general framework to manage the control of mutagenic impurities in new drug products (CHMP, 2006; CHMP Q&A(R3), 2010). The US Food and Drug Administration (FDA) followed shortly and issued draft guidance for industry on recommended approaches for control of mutagenic and carcinogenic impurities (FDA, 2008). Most recently, this topic has been adopted for development of an ICH guideline.

Both guidance documents rely on several common principles to provide the basis for establishing appropriate exposure limits. First, both acknowledge that to determine acceptable exposure levels to mutagenic carcinogens, considerations of the dose–response relationship and possible mechanisms of action are important. Based on this, mutagenic impurities may be distinguished into two classes:

(1) DNA-reactive (mutagenic) compounds with sufficient experimental evidence for a threshold-related mechanism and
(2) DNA-reactive (mutagenic) without sufficient experimental evidence for a threshold-related mechanism.

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This manuscript is focused on DNA-reactive compounds without sufficient experimental evidence for a threshold-related mechanism. Therefore, DNA-reactive compounds with sufficient experimental evidence for a threshold-related mechanism are not specifically addressed. This topic is explored in another manuscript currently in preparation. However, it is important to note that there is compelling experimental evidence which indicates threshold or sub-linear dose–response relationships exist for some mutagenic carcinogens (Dobo et al., 2011; Elhajouji et al., 2011; Gocke et al., 2009; Johnson et al., 2009; Pottenger and Gollapudi, 2010).

For mutagenic chemicals where the carcinogenic potential is known, a compound-specific risk assessment should be the first consideration for determining an ADI (CHMP, 2006; FDA, 2008; Müller et al., 2006). There are numerous methods available to calculate compound-specific ADI values for mutagens with no threshold. Neither the CHMP (2006) nor FDA (2008) provides guidance on a particular technique. One cautious approach considers the tumorigenic dose evaluated in long-term cancer bioassays from the most sensitive species and sex. Linear extrapolation is made from this dose to a dose level which attains an acceptable excess cancer risk in humans.

Based on a similar principle, the Threshold of Toxicological Concern (TTC) was proposed as an acceptable daily intake for compounds with unknown carcinogenic potential (Kroes et al., 2004). The TTC is recognised to be a very conservative limit, as numerous 'worst case' assumptions were applied to >700 carcinogens in the Carcinogenic Potency Database (CPDB) to establish the limit (Delaney, 2007; Kroes et al., 2004). The assumptions were as follows:

(i) Establishment of dose giving 50% tumour incidence in carcinogenicity studies (TD_{50}) using data from the most sensitive species and most sensitive site (Cheeseman et al., 1999).
(ii) Use of a select subset of the CPDB which had adequate estimates of TD_{50} following oral dosages.
(iii) Simple linear extrapolation from TD_{50} to a one in 1,000,000 incidence (daily human exposure level below which there is considered negligible risk to human health).
(iv) All biological processes involved in the generation of tumours at high dosages are linear over a 500,000-fold range of extrapolation.
(v) Possible effects of cytoprotective, DNA repair, apoptotic and cell cycle control processes are not taken into account.

As pharmaceuticals offer a benefit to patients, an acceptable cancer risk level in humans associated with exposure to a mutagenic impurity is defined as an exposure resulting in a maximum excess cancer risk of one in 100,000 in a 70 year lifetime and is pragmatically considered as 'virtually no risk' to humans (CHMP, 2006; Müller et al., 2006). Where the carcinogenic potential is unknown, both the EMEA and FDA recommend a TTC limit of 1.5 μg/day for lifetime exposure to a mutagenic impurity residing in API, for all but a highly potent subset of chemical classes.

During clinical trials, a key concept termed 'staged TTC' establishes higher ADIs for impurities based upon duration of exposure (CHMP, 2006; FDA, 2008; Müller et al., 2006). Although higher limits for shorter exposure durations is important, this current publication focuses on establishing control limits for mutagenic carcinogens in marketing applications for new chronic use drug products. Therefore, lifetime exposure is assumed, and consideration is not given to control of impurities during the clinical stage of drug development.

In this manuscript, the approach and practical experience from calculating compound-specific ADIs for 11 pharmaceutical impurities is presented. In addition, the limitations and challenges associated with relying on the available carcinogenicity data is discussed. Given that the compounds evaluated are likely to be commonly used for drug substance synthesis across pharmaceutical companies, these cases may serve as a useful reference for industry.

2. Methods

The genotoxicity and carcinogenicity data for each chemical was reviewed and is summarised in Tables 1 and 2, respectively. Complete and consistent data sets were not available for all chemicals. Where conflicting genotoxicity data existed, assessments conducted by organisations such as International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), United States Environmental Protection Agency (US EPA), Scientific Committee on Occupational Exposure Limits (SCOEL), and the World Health Organisation (WHO) International Programme on Chemical Safety (IPCS) and Concise International Chemical Assessment Documents (CICAD) were relied upon.

The Carcinogenic Potency Database (Gold and Zeiger, 1997) was the primary resource for carcinogenicity data. However, other databases (INCHEM, http://www.inchem.org/; TOXNET, http://toxnet.nlm.nih.gov/; Expub, http://www.expub.com/) and literature were searched for more recent or supplementary information. In addition, experimental evidence available in the literature on potential mode of action was considered and is included in the "Mechanism" column of Table 2.

Our approach was to evaluate existing genotoxicity and carcinogenicity data to determine whether each chemical was a mutagenic carcinogen or a non-genotoxic carcinogen. Next, based on a review of scientific literature available on the mechanism of action of the chemical or structurally-related chemicals, the dose response curve was classified as threshold or non-threshold. If the carcinogenic mechanism was unknown, or there was insufficient data to support a threshold dose–response relationship, then compounds were assumed to demonstrate a linear dose response.

Within the context of the regulatory guidance on genotoxic impurities in pharmaceuticals, DNA-reactive compounds are regarded to be potentially trans-species and multi-organ carcinogens. Since direct DNA reactivity is of high concern, the primary endpoint for defining an impurity as genotoxic is mutagenicity (CHMP, 2006, 2010; FDA, 2008). The Ames assay is a sensitive assay for mutagen detection and is one of the most common tests used for identifying DNA-reactivity of pharmaceutical impurities (Kenyon et al., 2007; Müller et al., 2006). The authors acknowledge that no single mutagenicity test is able to detect the entire spectrum of induced mutagenic events (US EPA, 2007) and for alternative human health assessments other genotoxicity endpoints may be more relevant. Nevertheless, the result of the Ames assay is generally considered acceptable to determine the DNA-reactivity of impurities (CHMP, 2006, 2010; FDA, 2008). Therefore, for the purposes of determining whether a compound was to be classified as genotoxic, mutagenicity results were generally utilised unless there was a reason to consider a compound to be DNA-reactive in the absence of a positive Ames test (e.g. the test system does not have appropriate metabolic components to generate a mutagenic metabolite).

ADI values were calculated using linear extrapolation from the TD_{50} of the most sensitive species or the harmonic mean TD_{50} for the most sensitive species when there was more than one positive study. The TD_{50} provides a standard quantitative measure for comparisons and analyses of carcinogenicity studies (Peto et al., 1984). It is a numerical description of carcinogenic potency and is estimated for each set of tumour incidence data reported in the CPDB (Gold and Zeiger, 1997). The TD_{50} is defined as dose-rate in mg/kg body weight/day which, if administered chronically for the standard lifespan of the species, will halve the probability of