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Potentially mutagenic impurities: Analysis of structural classes and carcinogenic potencies of chemical intermediates in pharmaceutical syntheses supports alternative methods to the default TTC for calculating safe levels of impurities

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ABSTRACT

Potentially mutagenic impurities in new pharmaceuticals are controlled to levels with negligible risk, the TTC (threshold of toxicological concern, 1.5 μg/day for a lifetime). The TTC was based on the more potent rodent carcinogens, excluding the highly potent "cohort of concern" (COC; for mutagenic carcinogens these are N-nitroso, Aflatoxin-like, and azoxy structures). We compared molecules with DEREK "structural alerts" for mutagenicity used in drug syntheses with the mutagenic carcinogens in the Gold Carcinogenicity Potency Database. Data from 108 diverse synthetic routes from 13 companies confirm that many "alerting" or mutagenic chemicals are in structural classes with lower carcinogenic potency than those used to derive the TTC. Acceptable daily intakes can be established that are higher than the default TTC for many structural classes (e.g., mono-functional alkyl halides and certain aromatic amines). Examples of ADIs for lifetime and shorter-term exposure are given for chemicals of various potencies. The percentage of chemicals with DEREK alerts that proved mutagenic in the Ames test ranged from 36% to 83%, depending on structural class, demonstrating that such SAR analysis to "flag" potential mutagens is conservative. We also note that aromatic azoxy compounds need not be classed as COC, which was based on alkyl azoxy chemicals.

1. Introduction

Over the past 10 years, there have been increased efforts to minimize exposure of patients to impurities in pharmaceuticals which may be genotoxic and potentially carcinogenic. The EMA developed a guideline on genotoxic impurities (finalized as EMA, 2006), and in parallel the pharmaceutical industry in Europe and the US also developed a strategy for control of such impurities (Müller et al., 2006). Both the final EMA guideline and the industry strategy adopted the use of the TTC, i.e., the threshold of toxicological concern, to address control of mutagens or potentially mutagenic structures that had insufficient data to carry out quantitative risk assessment. Lifetime daily doses of 1.5 μg/day were considered to represent negligible risk; based on principles used for contaminants in foods (Cheeseman, 2005; Cheeseman et al., 1999; Kroes et al., 2004; Munro, 1990; Munro et al., 1999), the default TTC for pharmaceuticals was estimated to have a risk of 1 in 100,000 (1 in 10^5) excess cancer cases in humans. Similar use of the TTC was proposed in the draft FDA guidance on genotoxic impurities (USFDA, 2008). The International Conference on Harmonization (ICH) is developing a new guidance on DNA Reactive (Mutagenic) Impurities in Pharmaceuticals known as ICH M7 (signed as Step 2 and released for consultation in 2013).

Many pharmaceutical companies have developed processes for systematic examination of the chemicals used in synthetic pathways, assessment of their structures for potential genotoxicity, and control of mutagens or potential mutagens to low levels in the active pharmaceutical ingredient (API), to ensure that patient exposure to mutagens is at or below the TTC (Brigo and Müller, 2010; Callis et al., 2010; Cimarosti et al., 2009; Dobo et al., 2006; Looker et al., 2010; Pierson et al., 2008; Robinson, 2010; Sun et al., 2010). Since the TTC of 1.5 μg/day is calculated to pose negligible risk even for a lifetime (70 years) of treatment, a "staged" TTC (EMA, 2008; Müller et al., 2006) was developed, to maintain the negligible risk while allowing higher levels of impurities in compounds given to people for short periods, for example during clinical development. The staged TTC was based on the concept that carcinogenic risk is dependent on both dose and duration of

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exposure (Bos et al., 2004; Felter et al., 2011). It is important to remember that the TTC is a conservative default used when the carcinogenic potency of the mutagen is not known. The TTC was derived to protect from potent compounds; in many cases, the calculated risk is much <1 in 10^5.

While the use of the TTC has improved consistency in regulation of levels for potentially genotoxic impurities in pharmaceuticals, the spectrum of chemical structural classes used in drug syntheses differs in some important respects from the dataset of oral rodent carcinogens used to derive the TTC. This prompts questions about the suitability for general application of a default TTC that was developed based on potent chemicals. (For an illustration of the derivation of the TTC from the more potent chemicals in the dataset, see Fig. 3 in Munro et al., 1999). Scientists in the pharmaceutical industry repeatedly comment that most of the materials encountered in synthetic routes, although reactive, are not usually in the structural classes of potent carcinogens from which the TTC was derived. Delaney (2007) pointed this out in his detailed comparison of the kinds of chemicals encountered in pharmaceutical syntheses with the kinds of carcinogens that compose a large proportion of the data sets used by to derive the TTC for materials in foods (Barlow et al., 2001; Cheeseman, 2005; Cheeseman et al., 1999; Kroes et al., 2000, 2004; Munro, 1990; Munro et al., 1999; Rulis, 1986). These data are contained in the widely used Gold (Berkeley; CPDB) database of rodent carcinogens that we have used here (Gold et al., 1984, 1989, 1999; Peto et al., 1984). Excluded from the TTC was the cohort of concern (COC), certain structural classes that were considered extremely potent. (The COC classes of mutagens/carcinogens were noted as N-nitroso, azoxy and Aflatoxin-like). Nonetheless, in this data set, the potencies of carcinogens cover a range of many orders of magnitude. Thus, application of a default TTC based on potent carcinogens (even after exclusion of the COC) means that in many cases the risk from trace impurities is being greatly overestimated, and the efforts to control impurities with structures in the less potent classes are disproportionate to their lower risk. Knowledge of the carcinogenic potency of the structural classes may allow use of more knowledge-based calculations of acceptable exposure, in preference to the default TTC.

Here we collected data from 13 pharmaceutical companies on a diverse series of 108 synthetic routes, to determine which potentially mutagenic structures are commonly used in pharmaceutical syntheses, and analyzed the carcinogenic potencies of these types of structural classes published in the Gold CPDB. Structural alerts for potential mutagenicity are often identified by the predictive software known as DEREK (Greene et al., 1999; Dobo et al., 2012), so the information collected centered on the "DEREK alerts" for mutagenicity. Since many of the structural classes used in pharmaceutical syntheses are not potent mutagenic carcinogens, the data presented here support broader use of structural class information in calculating acceptable daily intakes of potentially mutagenic impurities, as an alternative to the default TTC of 1.5 μg/day for a lifetime, or the default staged TTC for shorter term exposures.

2. Methods

2.1. Data collected on synthetic schemes and structural alert analysis

Each pharmaceutical company was asked to provide information from as diverse as possible a set of up to 10 synthetic schemes from a wide variety of therapeutic indications. Synthetic pathways for intentionally genotoxic pharmaceuticals such as oncology "cytotoxic" drugs were excluded. For each pathway, information supplied was as follows:

- A list of the "alerting" materials identified, giving not structures but the names of the alert.
- Alerts were for mutagenicity in bacterial reverse mutation tests, known popularly as the Ames test.
- The version of DEREK used.
- The results of the Ames test on each alerting chemical, if done.

For any company that used a different classification scheme for structural alerts for mutagenicity and did not have access to DEREK, the classes of alerts they assigned were requested. In collating the data these alerts, often Ashby–Tennant alerts (Ashby and Tennant, 1988, 1991), were classified into the DEREK (version 12.5) mutagenicity alerts. The lists of DEREK alerts and corresponding commonly used Ashby–Tennant alerts are in Appendix A.

2.2. Carcinogenic potency database: structural alert analysis and potency evaluation

Carcinogens have been assigned a potency measure in the Gold carcinogenic potency database (CPDB), the estimated TD_{50}, a number extrapolated from the tumor study data to describe a dose rate (in mg/kg/day) which would induce tumors in half the animals at the end of their lifetime. Since tumors do occur in control animals, the TD_{50} is more precisely described as the dose to halve the probability of remaining tumor-free throughout the lifetime of the species. A low value for the TD_{50} indicates a potent carcinogen, and a higher value a less potent carcinogen.

2.2.1. DEREK alerts

All the rat and mouse carcinogens with a TD_{50} value in the CPDB were analyzed in the DEREK mutagenicity bacterial model (version 12.5) to enable direct comparison of the classes of DEREK alerting compounds in the CPDB with the set of chemicals use in pharmaceutical syntheses. Of the carcinogens with DEREK alerts, there were 212 mouse and 282 rat carcinogens. Some DEREK alerting carcinogens had no Ames outcomes (not tested) in the database, and some were reported as negative in the Ames test. Of the DEREK alerts in the CPDB, the carcinogens that were positive in the Ames test were used for further comparison; there were 199 of these with a corresponding TD_{50}. (86 were carcinogenic in both species.) The Ames result (i.e., positive or negative) was extracted from the CPDB without additional interpretation. These 199 were selected for our comparison with the synthetic chemicals, because the TTC is used for mutagens and potential mutagens in pharmaceuticals. (They are listed in the Supplementary Table).

2.2.2. Most sensitive TD_{50}

To assess the potency of these chemicals, we used the lowest available potency value (TD_{50}) from the more sensitive rodent species and the most sensitive tumor site, because this method was used in derivation of the TTC (Cheeseman et al., 1999; Kroes et al., 2004; Munro, 1990; Munro et al., 1999; Rulis, 1986). For individual chemicals for which multiple carcinogenicity studies were done, this most sensitive TD_{50} can be much lower than the harmonic mean of potencies, but choice of the harmonic means or most sensitive TD_{50} does not greatly affect the overall distribution of hundreds of chemicals in the CPDB (Fiori and Meyerhoff, 2002). For our calculations based on the CPDB we also used the most sensitive route of administration, including the inhalation route, although inhalation data exist only for a small set of compounds. The TTC was originally developed for foods, so that the lowest TD_{50} developed from studies by the oral route was used in prior databases (Cheeseman et al., 1999; Kroes et al., 2004; Rulis, 1986).