Controlling the quality of medicines is just as important as demonstrating efficacy. The International Conference on Harmonisation has published general guidance on the quality and safety assessment of impurities in pharmaceutical drug substances and drug products. More recently, the European Medicines Evaluation Agency has published a guideline focusing on limits for genotoxic impurities. This is based on a Threshold of Toxicological Concern (TTC) derived from animal carcinogenicity data using multiple worst case assumptions to estimate a daily dose (1.5 mg/day) associated with a lifetime cancer risk of 1 in 100,000, a risk level considered acceptable for genotoxic impurities in human medicines. Based on these assumptions, presentation of the TTC as a single figure infers an unwarranted level of precision and supports the adoption of a more flexible approach by regulatory authorities when evaluating new drug products; a range within fivefold of the TTC limit would seem sensible. Furthermore, the limit is based on 70 years continuous daily exposure, a scenario that is uncommon for most medicines and irrelevant to the preregistration clinical development phase. To address this latter point, a staged TTC has been developed that proposes limits based on shorter durations of treatment, e.g., up to 1 year. Based on recent history, this approach has been acceptable to some authorities but not to others, and it is imperative that steps are taken to reach a common agreement between the pharmaceutical industry and regulatory authorities globally in order that new medicines can continue to be developed and delivered to benefit patients in a safe and timely manner.

Key Words: genotoxic impurities; qualification; risk assessment; pharmaceuticals; threshold of toxicological concern.

INTRODUCTION

Have you ever wondered about the quality of the prescription medicines you take? Similarly, do you ever think about the levels of impurities in these medicines and whether they are safe? Probably not, as your main concern is likely to be focused on whether it will be effective in treating the condition you have taken it for. Nevertheless, the reality is that safety, quality, and control of the quality of medicines are just as important to pharmaceutical companies and regulatory authorities as is the efficacy of a drug product.

Such is the importance of the chemical manufacturing and control aspects of a drug product that internationally agreed guidance has been developed over the last decade under the auspices of the International Conference on Harmonisation (ICH). There are three ICH documents that provide general guidance on organic and inorganic impurities: one covers impurities in drug substances [ICH Q3A(R2), 2006], another deals mainly with degradation products [ICH Q3B(R2), 2006], and the third gives limits for residual solvents [ICH Q3C(R3), 2005]. The guidelines apply to new drugs intended to be marketed. It is important to note that the “Q” highlights that these are primarily quality guidelines, although it is recognized that the safety of impurities is intrinsic to the development of quality drug substances and products.

GUIDANCE ON GENERAL IMPURITIES

In the guidelines covering drug substances and products, threshold levels for impurities are listed at which they must be identified, reported, and qualified. Qualified here means that the impurities have to be established as safe at or above the levels present in a drug substance or product. For drug substances, this is normally done by testing batches of drug containing the impurities at appropriate levels in preclinical toxicology studies during drug development so that assessment of the effects of the drug substance includes any contribution resulting from the presence of the impurities. As an example, ICH Q3A states that for a drug substance taken at a daily dose of up to 2 g, the threshold for qualification for an impurity is the lower of either 0.15% (equal to 1500 ppm) or 1 mg/day. In circumstances where different batches are used in the preclinical and clinical studies and where one or more new impurities are present above the qualification threshold in the clinical batch or product to be marketed, specific qualification
studies may be required. According to ICH, these include as a minimum an assessment of general toxicity (a study of between 14 days and 3 months usually in rats) and genotoxicity, the latter involving tests for point mutations and chromosomal aberrations.

Until relatively recently, the acceptable default way in which to qualify impurities in these studies, including those for genotoxicity, was to test them as part of the drug substance, as indicated above. This sometimes entailed spiking one or more impurities at higher levels than normal in the drug substance in order to provide a higher specification limit in a drug substance or, for degradation products, by testing material subject to accelerated degradation to provide an extended shelf life. Drug products marketed before and since the introduction of the ICH Q3 guidelines are likely to have had their impurities qualified in this way. However, there is a dilemma or “Catch-22” in relation to the thresholds; Q3A(R2) states that identifying impurities at levels below the identification threshold (the lower of 0.10% or 1 mg/day for a drug dose of up to 2 g/day) is generally not necessary. It also states “however, analytical procedures should be developed for those potential impurities that are expected to be unusually potent, producing toxic or pharmacological effects at a level less than or equal to the identification threshold.” The difficulty is this: unless there were something obvious from an assessment of the chemical route of synthesis, how would one know that an impurity was “unusually potent” if its structure was not already identified? Conversely, what would be the trigger to identify impurities at levels below the identification threshold unless there was a particular cause for concern? Nevertheless, from the final version of the guideline, it is clear that the ICH committee agreed that a limit of just under 0.1% was acceptable even for unidentified impurities that may possess genotoxic activity. What then has changed to require a more conservative approach?

GUIDANCE ON GENOTOXIC IMPURITIES—THE THRESHOLD OF TOXICOLOGICAL CONCERN

At the end of 2002 and at least partly as a result of this lack of clarity on unusually potent impurities, the Committee for Proprietary Medicinal Products of the European Medicines Evaluation Agency (EMEA) released a draft position paper on the limits of genotoxic impurities (subsequently finalized as EMEA, 2006). It highlighted that the ICH Q3 guidelines were insufficiently detailed on the subject of acceptable limits for genotoxic impurities and used the often cited but frequently challenged regulatory assumption that in vivo DNA-reactive compounds have the potential to damage DNA at any level and thus there is no discernible threshold. A consequence of this assumption is that any level of exposure carries a risk, and thus, it is impossible to define a “safe” level of exposure. As a result, a different approach is required to define an acceptable exposure level for these compounds. The guideline then describes the “Threshold of Toxicological Concern” (TTC) originally established by the U.S. Food and Drug Administration (FDA) as a “Threshold of Regulation” for chemicals migrating from food packaging materials. The limit for the latter was set at 0.5 ppb and, assuming consumption of 3000 g/day food, equates to 1.5 μg/day. This dose was intended to be low enough to be of negligible risk, even in the event that a substance exempted from regulation was later found to be carcinogenic.

As a slight but important aside, this approach in fact represented a legal way in which the presence of trace potentially carcinogenic contaminants could be justified without having to repeal or revise the Delaney Clause of 1958, an amendment to the Food, Drugs, and Cosmetic Act of 1938 which specified that no food or color additive could be deemed safe (or given approval) if found to cause cancer in man or animals. With the advent in the 1970’s and 1980’s of significant improvements in analytical technology, it became clear that trace levels of numerous substances shown to be animal carcinogens such as pesticides or residues from food contact materials that had previously been unquantifiable were now in fact detectable at low levels.

The TTC is based on an analysis of the carcinogenic potency in rodents of over 700 genotoxic and nongenotoxic carcinogens from which has been estimated that daily exposure to less than or equal to 1.5 μg/day for most genotoxic carcinogens is not likely to exceed a lifetime cancer risk of one in a million. This is defined as meaning that consumption of up to 1.5 μg of a carcinogen every day over a lifetime (considered to be 70 years) should give rise to no more than one additional case of cancer in a population of 1 million people, essentially an insignificant level of risk sometimes also described as a “virtually safe dose” (Rulis, 1986). A more recent analysis by Kroes et al. (2004) estimated a TTC of 0.15 μg/day for all but the highest potency genotoxic compounds in the so-called Cohort category (aflatoxin-like, N-nitroso, and azoxy compounds). For pharmaceuticals, it is argued in the EMEA guideline that a one in 100,000 lifetime risk, equating to a TTC of 1.5 μg/day, is acceptable based on the expected benefit of medicinal products. For a drug taken at a dose of 100 mg/day to 1 g/day, this equates to concentration limits of 1.5–15ppm for each impurity (see Figure 1), levels many hundreds of times lower than the ICH identification threshold.

It is worth briefly expanding on the derivation of the TTC in order to understand the level of conservatism in the approach. First, regarding animal carcinogenicity data for any chemical in the database, the dose giving rise to a 50% incidence of tumors at the most sensitive site and in the most sensitive species is calculated. This is then used to extrapolate in a linear manner through zero to a dose estimated to give a cancer incidence of one in 1 million. Interestingly, because of the extrapolation of the line through zero, it has been suggested that the single most important factor for determining the slope, the major quantitative parameter derived from these studies, is the maximum...
dose of the chemical tested in the cancer bioassay—the more toxic the substance is, the steeper the resulting slope. This observation raises serious questions about the scientific credibility of this approach with regard to meaningful estimations of expected cancer risk at low doses (Hrudey, 1998).

The no threshold assumption for DNA-reactive carcinogens inferring that “one molecule can cause cancer” was originally a science policy decision intended as a cautious approach to public health protection. However, it has been the subject of repeated challenges over many years, including, for example, that it takes no account of biological homeostatic or repair processes and ignores the concept of hormesis, a widely recognized observation in chemical and radiation carcinogenicity that refers to beneficial effects at low (subtoxic) levels of exposure (Calabrese and Baldwin, 2003). It also ignores our emerging knowledge of cancer as a mechanistically complex disease, much more so than assuming that a single molecule of a carcinogen causes a mutation that ultimately develops into a tumor in a random process. It also assumes humans to be similarly susceptible to cancer as rodents, another factor that is open to challenge. For example, in an analysis comparing rodent bioassay data with the human epidemiological evidence for cancer for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a chemical that the U.S. Environmental Protection Agency qualifies as the “most potent carcinogen” (Gough, 2003), Aylward et al. (1996) demonstrated that humans may be up to 90 times less responsive to TCDD-induced cancer than rats.

The discussion above supports the contention that the TTC is based on multiple assumptions, often worst case, each of which is subject to a significant level of variability. To illustrate the impact such compounded conservatism can have, McKone and Bogen (1991) used mathematical simulations in a case study of perchloroethylene in Californian water supplies to demonstrate that use of worst case estimates at each step of the risk assessment process in this example led to an overestimation of the actual calculated risk by almost 400-fold. If nothing more, this example serves to illustrate that the presentation of the TTC as a single limit of 1.5 μg/day infers an inappropriate and unwarranted level of accuracy that, regarding the application of such a limit for potentially genotoxic impurities in new pharmaceutical drug substances, fully supports the adoption of a flexible approach by regulatory authorities. A suggested acceptable range for the TTC of fivefold the current limit is likely to be toxicologically indistinguishable in terms of cancer risk.

In spite of these and other shortcomings to the cancer risk assessment model from which the TTC is derived, no other viable alternative approach acceptable to regulatory authorities has emerged, and this is reflected in the number of areas of regulation in which the concept has been used, including food flavoring substances and indirect food additives. Therefore, while accepting this as the only model currently acceptable, recognition of its assumptions, limitations, and overconservatism is imperative in its application.

A STAGED TTC BASED ON DURATION OF TREATMENT

Following release of the original draft EMEA position paper, a generic limit of 1.5 μg/day for a genotoxic impurity was the expectation for new drug substances. However, it was unclear if this limit was also intended for application to drug substances in the clinical development phase. Largely, based on this lack of clarity and the significant adverse consequences to drug development if the TTC of 1.5 μg/day were to be applied during this phase, a genotoxicity taskforce was established under the auspices of the Pharmaceutical Research and Manufacturers of America to look specifically at appropriate levels of genotoxic impurities in drug substances where the duration of treatment is often limited such as in clinical development, bearing in mind that the TTC limit is based on daily dosing for 70 years. The taskforce produced a position paper, subsequently published as Müller et al. (2006), in which a “staged” TTC approach was proposed for impurities known to be genotoxic based on concepts put forward by Bos et al. (2004), with dose limits varying dependent on the duration of clinical dosing. The paper recognises that, early in drug development, routes of synthesis for drug substances are not optimized, are poorly understood in terms of process control, and are likely to change later in development. It also highlights that the main concern regarding drug impurities is the presence of DNA-reactive carcinogens, the overwhelming majority of which can be identified using computer-based structure-activity relationship tools such as DEREK (Deductive Estimation of Risk from Existing Knowledge, http://www.chem.leeds.ac.uk/luk/derek/) or MCASE (Multi Computer Automated Structure Evaluation, http://www.multicase.com/products/prod01.htm) together with an in vitro assay for point mutations such as the Ames test.

As examples of the staged TTC, a limit of 120 μg/day is proposed for genotoxic impurities in a drug substance taken for up to 28 days (e.g., relevant to early clinical trials), while for studies of 1–3 months a limit of 40 μg/day is recommended (see Table 1). Importantly, by using a lower level risk estimate (one in a million rather than one in 100,000), these limits take into account the frequent participation of healthy volunteers in early clinical trials that derive no benefit from the drug substance and for whom, therefore, a lower level of risk would be expected and appropriate. Figure 1 shows the relationship between daily dose of drug substance and impurity concentration limit based on the TTC and staged TTC dose limits. Thus, for a drug substance intended for use for up to 3 months duration and having a maximum dose of 1 g/day, the concentration limit for a genotoxic impurity would be 40 ppm. Since the publication of the Müller paper and in the absence of specific regulatory guidance on acceptable limits of genotoxic impurities for drug substances in the clinical development phase, several pharmaceutical companies have based such limits on a staged TTC approach, be it the limits proposed by Müller et al. (2006) or other limits. Initial responses to this
approach both within and between various competent authorities have been variable, with some even invoking the EMEA guideline TTC levels of 1.5 \( \text{mg} / \text{day} \) for drug substances at the phase I Investigational New Drug stage. This lack of consistent approach provides significant difficulties for pharmaceutical companies particularly when clinical studies are often international, involving volunteers and/or patients from many different countries and therefore requiring interaction with several authorities. Clarification of acceptable limits for genotoxic impurities in drug development with regulatory authorities would be a major step forward; within Europe, potentially, the current EMEA guideline could be extended to bring drug substances in clinical development in scope and to ratify the staged approach outlined by Müller et al. (2006) or provide some other identified acceptable limits. As this article goes to press, the EMEA guideline remains the only official regulatory authority view on genotoxic impurities that is different to existing ICH Q3 guidance. Although the U.S. FDA is known to have been working on a draft guidance document for at least 12 months, this has yet to be published. However, it is considered likely to concur largely with the EMEA guideline, based on a recent article by key FDA contributors to the debate (McGovern and Jacobson-Kram, 2006). The published views of the Japanese authorities on acceptable limits for genotoxic impurities are not yet known. Given the impact of the European guideline on pharmaceutical companies worldwide, development of a globally harmonised approach under the auspices of the ICH would be helpful.

EXCIPIENTS AND HERBAL MEDICINES

Following finalization of the EMEA guideline in late 2006, concerns have been expressed by other industries involved in drug product manufacture, most notably excipient manufacturers concerned about the impact on sale and use of excipients in the European market. If the concern over genotoxic impurities from a regulatory viewpoint is based on human safety, there is no rational reason why the guidance should not be applied to excipients as well as active pharmaceutical substances. If this is the case, it may not be possible to use certain excipients in Europe, creating serious issues for many existing drug products. The International Pharmaceutical Excipients Council of the Americas is understood to be considering a white paper on this topic highlighting reasons why the guideline should not be applied to excipients as well as active pharmaceutical substances. If this is the case, it may not be possible to use certain excipients in Europe, creating serious issues for many existing drug products. The International Pharmaceutical Excipients Council of the Americas is understood to be considering a white paper on this topic highlighting reasons why the guideline should not be applied to excipients (Van Amum, 2006). It is of great interest to note from this article that FDA guidance, when available, is unlikely to address excipients in the same manner as drug substances in the United States. If this turns out to be true, it would be difficult to rationalize a requirement for control of genotoxic impurities in drug substances to exquisitely low levels in many cases with no such requirement for other components of a drug product, particularly when the latter often constitute the bulk of the product by mass.

In another interesting turn of events on this topic, the EMEA’s Committee on Herbal Medicinal Products has released a concept paper proposing the development of a guideline for the assessment of genotoxic constituents in herbal substances and preparations (HMPC, 2006). The paper

### TABLE 1

Proposed Allowable Daily Intakes (µg/day) for genotoxic impurities—A Staged TTC Approach Depending on Duration of Exposure (from Müller et al., 2006)

<table>
<thead>
<tr>
<th>Duration of Exposure</th>
<th>≤ 1 month</th>
<th>&gt; 1–3 months</th>
<th>&gt; 3–6 months</th>
<th>&gt; 6–12 months</th>
<th>&gt; 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allowable daily intake (µg/day) for different durations of exposure</td>
<td>120’ or 0.5(^{bc}) whichever is lower</td>
<td>40’ or 0.5(^{bc}) whichever is lower</td>
<td>20’ or 0.5(^{bc}) whichever is lower</td>
<td>10’ or 0.5(^{bc}) whichever is lower</td>
<td>1.5(^{bc}) whichever is lower</td>
</tr>
</tbody>
</table>

\(^a\)Probability of not exceeding a 10\(^{-6}\) risk is 93%.

\(^b\)Other limits (higher or lower) may be appropriate.

\(^c\)Probability of not exceeding a 10\(^{-5}\) risk is 93%, which considers a 70-year exposure.
raises several important points of direct relevance to pharmaceutical drug substances, including the fact that herbal medicinal products (HMPs) have been used for many years by a significant proportion of the population and that, notwithstanding occasional signals of adverse effects, clinical experience regarding the most apparent adverse reactions points to their relative safety. This brings us back to the scope of the EMEA guideline in relation to existing marketed drug products; it clearly states that these are out of scope unless there is a “cause for concern.” If this relates to clinical safety concerns, as the author believes it should, then the previous statement regarding HMPs and pharmaceuticals would infer that except in rare circumstances, there would be no cause for concern. However, a definition is not given in the EMEA genotoxic impurities guideline, leaving this term open to multiple interpretations, most of which are unlikely to have any demonstrable impact on the benefit/risk assessment of existing drug products to patients. Indeed, the scope also covers new applications for existing drug substances where “assessment of the route of synthesis, process control, and impurity profile does not provide reasonable assurance that no new or higher levels of genotoxic impurities are introduced as compared to products currently authorized in the European Union containing the same active substance.” The inference from this is that current accepted specifications for existing marketed drug substances, e.g., in the Pharmacopoeial monographs, would continue to be acceptable. Further clarification of this point within the guideline is required.

SUMMARY

The EMEA guideline on limits of genotoxic impurities came into force on 1st January 2007 and is being applied by European authorities for new drug products and in some cases also to drug substances in drug development despite these being currently out of scope. The compounded conservative assumptions underlying the TTC limit of 1.5 μg/day fully supports the adoption of a flexible approach by regulatory authorities in the application of this limit to genotoxic impurities in new pharmaceutical drug products. For clinical drug development, the staged TTC as outlined in Müller et al. (2006) represents a pragmatic and safety focused approach to the control of genotoxic impurities during this phase and it has been accepted by some regulatory authorities. In light of this, it is imperative that steps are taken to develop an approach mutually acceptable to both the pharmaceutical industry and regulatory authorities worldwide so that new drug products can continue to be delivered to benefit patients in a safe and timely fashion; an ICH guideline would satisfy this requirement. Finally, unless data emerge to suggest currently marketed drug products represent a clinical safety concern attributable to their impurity content, there should be no requirement to change existing approved specifications for these products.

REFERENCES


