Available online at www.sciencedirect.com
ScienceDirect
Advanced Drug Delivery Reviews 59 (2007) 38–42
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Toxicological overview of impurities in pharmaceutical products ☆

David Jacobson-Kram [a,*], Timothy McGovern [b]

[a] *Office of New Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA*
[b] *Office of New Drugs, Division of Pulmonary and Allergy Products, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA*

Received 2 February 2006; accepted 25 October 2006
Available online 15 November 2006

Abstract

While the use of pharmaceuticals is always a balance of risks and benefits, the same is not true for impurities in pharmaceuticals; impurities convey only risk. A number of international guidelines and regional guidances instruct drug developers and regulatory agencies on how to evaluate and control impurities in drug substances and drug products. While impurities should always be reduced to the lowest levels that are reasonably practical, it is acknowledged that impurities cannot be reduced to zero and specifications for impurities need to be established. This chapter discusses practical and theoretical methods for qualification of different classes of impurities.

Keywords: Toxicology; Pharmaceutical impurities; European Medicines Agency (EMEA); Genotoxic impurities

Contents

1. Introduction

Decisions to approve, prescribe and consume medicines involve risk/benefit assessments by regulatory agencies, health care professionals and consumers. For serious or life threatening conditions, drugs with higher risks for adverse effects or for serious adverse effects are sometimes acceptable. For example, some life-saving cancer chemotherapies are known human carcinogens. However, if one is suffering from a life threatening tumor, a 5% risk of a secondary, treatment-related tumor is generally considered acceptable. Arguably, the same is not true for impurities found in drug substances and drug products; impurities convey only risk with no associated benefit. Drug impurities might be viewed as "pollutants" in the pharmaceutical world. Much like pollutants in the environment, few people believe that they can be entirely eliminated. The challenge for regulatory agencies is to promulgate standards that assure that unavoidable drug impurities impart no or acceptable levels of risk.

2. ICH guidelines

The pharmaceutical industry and world-wide regulatory agencies have long recognized the importance of controlling impurities in drug substances and drug products. The drafting

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Pharmaceutical Impurities: Analytical, Toxicological and Regulatory Perspectives".

* Corresponding author. Tel.: +1 301 796 0175; fax: +1 301 796 9856.
E-mail address: david.jacobsonkram@fda.hhs.gov (D. Jacobson-Kram).

0169-409X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2006.10.007

and adoption of ICH Q3A(R) [1], Q3B(R) [2], and Q3C(R) [3] represented a consensus on how impurities should be controlled in marketed products. However, a number of important issues are not addressed in the guidelines, for example, acceptable levels of impurities in drugs during development and control of genotoxic impurities.

The ICH quality guidelines note that impurities can arise from a variety of places including: starting materials, byproducts, intermediates, degradation products, reagents, ligands and catalysts. It is important to note the Q3A indicates that sponsors should "summarize the actual and *potential* impurities most likely to arise during the synthesis, purification and storage of the new drug substance. Table 1 illustrates a series of thresholds described in ICH Q3A(R) which trigger reporting, identification and qualification requirements. The thresholds are only slightly dependent on the quantity of drug consumed by the patient. Since consumption of a large quantity of drug substance would also mean exposure to higher levels of impurities, the tolerances are lower when the daily maximum exposure is greater than 2 g of API.

When an impurity reaches the level that requires "qualification", it is incumbent on the drug developer to establish the safety of the impurity. ICHQ3A(R) states that: "The level of impurity present in a new drug substance that has been adequately tested in safety and/or clinical studies would be considered qualified. Impurities that are also significant metabolites present in animal or human studies are generally considered qualified." This suggests that an impurity is qualified as long as it was present in the API used in preclinical and clinical studies at a level equal to or higher than found in the marketed product. The guideline goes on to note that an impurity can still be qualified even if the level in the marketed product is higher than what was used during development as long as the absolute amount tested in these studies is large compared to the exposure resulting from consumption of the marketed product. For example, a contaminant might be present at 0.05% in the drug substance used in development but is found at 0.1% in the marketed drug substance. If toxicology data are available where the impurity was tested at high clinical multiples such that the absolute quantity tested is high compared to the quantity consumed clinically, it may be considered qualified.

ICHQ3A(R) notes that safety testing can be avoided by lowering the level of the impurity below the qualification threshold or by providing safety data from the published scientific literature. If neither choice is an option, actual testing will have to be performed to qualify the impurity. The battery of tests generally includes an assay for gene mutations (generally a bacterial reverse mutation test, "Ames assay"), an assay for chromosomal damage (either metaphase analysis or the mouse lymphoma $TK^{+/-}$ assay) and a repeat dose general toxicity study in a single species of 14 to 90 day duration (often a 28 day rat study). Depending on the class of the impurity, additional testing may also be required. The guidance also notes that in some instances the thresholds may need to be set lower if the impurity is known to belong to a particularly toxic class of chemicals or can be set higher, if the impurity is of a chemical class generally considered to be nontoxic.

Table 1
Thresholds for APIs

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/day	0.05%	0.1% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day (whichever is lower)
>2 g/day	0.03%	0.05%	0.05%

Table 2
Thresholds for degradation products in new drug products

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤1 mg		1.0% or 5 μg TDI whichever is lower	
1 mg–10 mg		0.5% or 20 μg TDI whichever is lower	
10 mg–100 mg			0.5% or 200 μg TDI whichever is lower
<10 mg			1.0% or 50 μg TDI whichever is lower
>10 mg–2 g		0.2% or 2 mg TDI whichever is lower	
>100 mg–2 g			0.2% or 3 mg TDI whichever is lower
≤1 g	0.1%		
>1 g	0.05%		
>2 g		0.1%	
>2 g			0.15%

Perhaps the most controversial aspect of this guidance is the provision that qualification testing of impurities can be performed on the API containing the impurity. A few "back-of-the-envelope" calculations quickly show that this approach is highly insensitive, even to highly toxic chemicals. For example, drug substances are tested in the Ames assay up to doses of 5 mg/plate as long as the drug is not toxic to the bacteria. Assuming an impurity is present at 0.15%, the minimum for qualification, and the API is tested up to 5 mg/plate, powerful mutagens such as 9-aminoacridine and methyl methanesulfonate which are used as positive controls, would not be detected in the assay. If the API has any associated toxicity, this would lower the level which could be tested and thereby further reduce the sensitivity of the assay for detecting a genotoxic impurity. Many regulatory toxicologists believe that it is preferable to test the synthesized impurity alone.

ICHQ3B(R) addresses impurities in new drug products that are degradation products of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system. The guideline does not address impurities arising from excipients or from impurities extracted or leached from the closure system. Also as mentioned previously, the guideline does not apply to products used in development, i.e. in clinical trials. Also excluded is a spectrum of products including biopharmaceuticals, peptides, oligonucleotides, radiopharmaceuticals, fermentation products, and semi-synthetic products derived from herbal materials, and crude products of animal or plant origin. The guideline does not make it clear why these products are excluded. The guideline

also does not apply to polymorphic forms of drugs and enantiomeric impurities.

The thresholds for reporting, identification and qualification of impurities in new drug products are shown in Table 2. As can be seen in the table, the quantities of drug product ingested are much more stratified than the levels specified for drug substances. The qualification criteria for impurities in new drug products follow those cited above for new drug substances. A degradation product is considered qualified if it was present at comparable or higher levels during safety or clinical studies. Also as in Q3A(R), any degradation product that is present as a significant metabolite in toxicology or clinical studies is considered qualified. Similarly, higher levels can be present in the marketed product as long as the absolute amount tested in these studies is large compared to the exposure resulting from consumption of the marketed product. Impurities present at levels that exceed the qualification threshold should be reduced or qualified using data from the scientific literature or by actual experimentation. The toxicology studies mentioned in Q3B(R) are identical to the ones cited in Q3A(R).

ICHQ3C recommends acceptable amounts of residual solvents in marketed drug products and in many ways is more complex than Q3A and Q3B. As with the other impurities discussed above, solvents are used in the preparation of pharmaceuticals and it is often not possible to reduce them in the final product to levels below detection. As with other impurities, residual solvents impart no benefit, only risk. The approach taken in Q3C is to list the most commonly used solvents and to classify them into one of three groups. Class 1 solvents are thought to cause "unacceptable" toxicities and, if at all possible, should be avoided in the preparation of drug substances, excipients and drug products. This class includes chemicals known or thought to be human carcinogens or that are known to be significant environmental hazards, for example, benzene and carbon tetrachloride. Class 2 solvents are described as chemicals that cause "less severe toxicity"; examples include chloroform, dichloromethane and methanol. This class includes solvents which have been found to be nongenotoxic animal carcinogens, teratogens or neurotoxins. Also included are solvents "suspected of other significant but reversible toxicities." Class 3 solvents are considered to be the least toxic and should be used whenever practical. The level of contaminating solvents can be established in one of two ways: the drug product can be tested directly or the cumulative amount can be derived from the sum of the levels in each constituent. As with the other guidelines, Q3C only addresses marketed products, not materials used in clinical trials.

Q3C coined the new term "permitted daily exposure", or PDE. This refers to a "pharmaceutically acceptable intake of residual solvents" and describes the methods for establishing these exposure limits. Class 3 solvents have PDEs of 50 mg or more per day. Limits for class 2 solvents can set in one of two ways. The guideline provides a table with concentration limits (ppm) for each solvent. As long as all components in a formulation meet this concentration they can be used in any proportion as long as the total daily dose of the drug is 10 g or less. In the second option, the solvent contributions of each component in the formulation are summed. An acceptable PDE is achieved if the sum of the contributions from each component is equal to or less than the PDEs given in the guidance for that particular solvent.

Five solvents are listed as belonging to class 1: benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene and 1,1,1-trichloroethane. The guideline specifies concentration limits for each of these solvents. A group of commonly used solvents is also described for which inadequate data exist with which to calculate PDEs.

While the PDEs described in the guidance are listed with a fair amount of precision, for example, the PDE for xylene is 21.7 mg/day, it is instructive to note how these numbers are derived. For example the PDEs for class 2 solvents were calculated using the following formula:

$$\mathrm{PDE} = \frac{\mathrm{NOEL} \times \text{weight adjustment}}{F1 \times F2 \times F3 \times F4 \times F5}$$

The NOEL is derived from "the most relevant animal study." The modifying factors relate to species extrapolation (from a low of F1 = 2 for extrapolation from dogs to humans to F1 = 12 for extrapolation from mice to humans). This takes into account body surface area: weight ratios for individual species and man. F2 = 10 and accounts for inter individual variation. F3 is a variable that takes into account the duration of the study used in calculating the NOEL. The number can vary from 1 to 10 depending on the species studied and the duration of the study. F4 is a variable related to the severity of toxicity and can vary from 1 to 10. F5 is a variable factor applied when a NOEL was not established and the LOEL is used; in this case F5 = 10. The "take home message" from this discussion is that while the PDEs appear precise, the standard errors around these numbers are likely to be very large.

Calculating PDEs for genotoxic solvents is perhaps the biggest challenge. Unlike other toxicological endpoints which are thought to have thresholds i.e. doses at which there is no risk, no thresholds are assumed for genotoxic chemicals, all exposures are assumed to present some risk. Data used to calculate PDEs are most often derived from rodent carcinogenicity studies. The methods used to extrapolate from these types of data to PDEs for humans will be discussed in a subsequent section on genotoxic impurities.

3. EMEA guideline on limits of genotoxic impurities

The European Medicines Agency's (EMEA) Committee for Medicinal Products for Human Use (CHMP) published a guideline on the limits of genotoxic impurities [4]. This guideline recommends dichotomizing genotoxic impurities into those for which there is "sufficient (experimental) evidence for a threshold-related mechanism" and those "without sufficient (experimental) evidence for a threshold-related mechanism." Those genotoxic compounds with sufficient evidence would be regulated using methods outlined in ICH Q3C, for class 2 solvents. This approach calculates a "permitted daily exposure" (PDE) which is calculated using the NOEL or LOEL from the

most relevant animal study plus incorporation of safety factors. Examples of genotoxins that may fall into this class include chemicals that induce aneuploidy by interfering with the mitotic spindle, chemicals interfering with activity of topoisomerase or chemicals that inhibit DNA synthesis.

For genotoxic compounds without sufficient evidence for a threshold-related mechanism, the guideline proposes a policy of controlling levels to "as low as reasonably practicable" (ALARP principle). This approach specifies that every effort should be made to prevent the formation of such compounds during drug substance synthesis and, if not possible, efforts should be made to reduce such impurities through technical efforts (e.g. purification steps). Compounds falling into this class are generally those that interact with DNA either directly or indirectly such as alkylating agents, intercalating agents or agents generating free radicals. Since all exposures to such agents theoretically convey some level of carcinogenic risk, regulatory agencies generally perform quantitative risk assessments to calculate the increased levels of adverse events, such as cancers, that result from particular exposures and set exposure levels which result in "acceptable" risks; often 1 in 10^5 or 1 in 10^6 additional cancers from lifetime exposures. Methods for these quantitative risk assessments are referenced in ICH guidance Q3C, Appendix 3, in reference to Class 1 carcinogenic solvents.

While the approach described above has sound scientific support, in most instances sufficient mechanistic data will be lacking with which to decide whether a threshold mechanism is applicable for genotoxic impurities. Furthermore, it is also unlikely that data will exist on which quantitative risk assessments can be performed. The guideline recognizes these limitations and therefore proposes the use of a "threshold of toxicological concern" (TTC) for genotoxic impurities. The TTC refers to a threshold exposure level to compounds that will not pose a significant risk of carcinogenicity or other toxic effects and was originally developed as a "threshold of regulation" for food contact materials by the FDA [5]. The draft guideline proposes a TTC of 1.5 μg/day. This threshold corresponds to a 10^{-5} lifetime risk of cancer, a risk level that the EMEA considers justified due to the benefits derived from pharmaceuticals. Importantly, however, this draft guideline only addresses levels of genotoxic impurities in marketed products; the guideline is silent on what might constitute acceptable TTCs for drugs during development, especially for trials of short duration.

4. PhRMA genotoxic impurity task force white paper

The Pharmaceutical Research and Manufacturing Association (PhRMA) established a Genotoxic Impurity Task Force which developed a White Paper (document outlining the proposal is currently in press) and presented their proposal at various public meetings [6]. The document outlines a procedure for testing, classification, qualification and toxicological risk assessment of potentially genotoxic impurities in pharmaceutical products. The Task Force proposed that all identified or predicted impurities should be classified into one of five classes: those known to be genotoxic (mutagenic) and carcinogenic, those known to be genotoxic (mutagenic) but with unknown carcinogenic potential, those with a unique alerting structure and of unknown genotoxic (mutagenic) potential, those with an alerting structure related to the parent active pharmaceutical ingredient, and those with no structural alert.

Table 3
PhRMA genotoxic impurity task force proposal — allowable daily intake (μg/day) for genotoxic impurities during clinical development using staged TTC approach

	Duration of clinical trial exposure				
	≤1 month	>1–3 months	>3–6 months	>6–12 months	>12 months
Allowable daily intake (μg/day) for all phases of development	120	60	20	10	1.5
Alternative maximum level of allowable impurity based on percentage of impurity in API	0.5%	0.5%	0.5%	0.5%	

The Task Force proposal for addressing genotoxic impurities for marketing applications is similar to that of the previously described EMEA draft guideline. However, the Task Force recognizes that the TTC established a limit for daily human exposure to genotoxic impurities for lifelong treatment while most medicines are given for limited time spans, especially in early clinical development. Therefore, the Task Force proposes a *staged* TTC approach with adjusted limits for shorter duration clinical trials. The adjusted limits are derived from a linear extrapolation of the TTC for a lifetime daily exposure to short-term daily exposures as described by Bos et al. [7]. The proposed limits for short-term exposures (<12 months) are based on a 10^{-6} risk because of the common inclusion in clinical studies of normal volunteers, for whom there is assumed to be no pharmacological benefit. The proposed limit for exposures greater than 12 months in duration is based on a 10^{-5} risk, because individuals in longer-term clinical studies have the target indication and may benefit from treatment, an approach consistent with the EMEA guideline. The limits associated with the staged TTC approach are shown in Table 3.

5. US FDA Center for Drug Evaluation and Research guidance on genotoxic impurities

As noted earlier, ICH guidances do not provide clear recommendations for handling these types of impurities. The Center for Drug Evaluation and Research (CDER) of the US FDA is developing guidance to address issues with genotoxic impurities in pharmaceutical products. The CDER is considering the proposals of the EMEA and PhRMA in developing its guidance.

The presence of genotoxic impurities should be avoided if possible. However, it is recognized that complete removal is often not possible. In these cases, the amounts of genotoxic

impurity present should be limited to a level that represents an insignificant increase in risk to clinical trial subjects or patients. This level may be based on adequate compound-specific data to calculate an acceptable risk-specific dose or may be based on a toxicological threshold derived from a robust carcinogenicity database. A staged implementation of the threshold approach is considered acceptable for products that are under development. In applying qualification thresholds, consideration should be given to the product's stage of clinical development, the maximum duration of drug administration at that stage, and the proposed indication. In some cases, increases in the recommended thresholds may be supported in the presence of a potential pharmacological benefit to patients.

References

[1] ICH Q3 Q3A(R): impurities in new drug substances (revised guideline), Federal Register 68 (68) (2003) 6924–6925.

[2] Q3B(R): impurities in new drug products (revised guideline), Federal Register 68 (220) (2003) 64628–64629.

[3] Q3C: impurities: guideline for residual solvents, Federal Register 68 (219) (2003) 64352–64353.

[4] European Medicines Evaluation Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on the limits of genotoxic impurities. CPMP/SWP/5199/02, June 28, 2006 London.

[5] U.S. Food and Drug Administration (FDA), food additives: threshold of regulation for substances used in food-contact articles (final rule), Federal Register 60 (1995) 36582–36596.

[6] L. Müller, R.J. Mauthe, C.M. Riley, M.M. Andino, D. De Antonis, C. Beels, J. DeGeorge, A.G.M. De Knaep, D. Ellison, J.A. Fagerland, et al., A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regul. Toxicol. and Pharmacol. 44 (2006) 198–211.

[7] P.M.J. Bos, B. Baars, T.M. Marcel, M.T.M. van Raaij, Risk assessment of peak exposure to genotoxic carcinogens: a pragmatic approach, Toxicology Letters 151 (2004) 43–50.