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A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity

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Abstract

The synthesis of pharmaceutical products frequently involves the use of reactive reagents and the formation of intermediates and by-products. Low levels of some of these may be present in the final drug substance and drug product as impurities. Such chemically reactive impurities may have at the same time the potential for unwanted toxicities including genotoxicity and carcinogenicity and hence can have an impact on product risk assessment. This paper outlines a procedure for testing, classification, qualification, toxicological risk assessment, and control of impurities possessing genotoxic potential in pharmaceutical products. Referencing accepted principles of cancer risk assessment, this document proposes a *staged* threshold of toxicological concern (TTC) approach for the intake of genotoxic impurities over various periods of exposure. This staged TTC is based on knowledge about tumorigenic potency of a wide range of genotoxic carcinogens and can be used for genotoxic compounds, for which cancer data are limited or not available. The delineated acceptable daily intake values of between ~1.5 μg/day for ~ lifetime intake and ~120 μg/day for ⩽1 month are virtually safe doses. Based on sound scientific reasoning, these virtually safe intake values do not pose an unacceptable risk to either human volunteers or patients at any stage of clinical development and marketing of a pharmaceutical product. The intake levels are estimated to give an excess cancer risk of 1 in 100,000 to 1 in a million over a lifetime, and are extremely conservative given the current lifetime cancer risk in the population of over 1 in 4 (http://seer.cancer.gov/

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statfacts/html/all.html). The proposals in this document apply to all clinical routes of administration and to compounds at all stages of clinical development. It is important to note that certain types of products, such as those for life-threatening indications for which there are no safer alternatives, allow for special considerations using adaptations of the principles outlined in this paper.

Keywords: Pharmaceuticals; Impurities; Genotoxic; Carcinogenic; Risk assessment; Regulation; Staged TTC concept

1. Introduction

Residual impurities resulting from manufacturing and formulation, or from degradation of the active pharmaceutical ingredient (API)[1] and excipients, may be present in pharmaceutical products. A subset of these impurities may present a potential for genotoxicity and therefore pose an additional safety concern to clinical subjects and patients.

The pharmaceutical industry and those that regulate it recognize their respective obligation to limit genotoxic impurities. Therefore, substantial efforts are made during development to control all impurities at safe concentrations. However, the effort made to limit impurities must be commensurate with the risk assessed at each phase of clinical development, taking into account the extent of the hazard, the disease indication, the size and characteristics of the exposed population, and the duration of that exposure, as well as the likely delay in the availability of beneficial medicines if the burden of limiting or controlling impurity levels is disproportionate. A balance of these considerations can be described best as the "as low as reasonably practicable" (ALARP)[2] principle.

It follows that the presence of impurities with genotoxic (mutagenic[3]) potential may be unavoidable in clinical trial and ultimately in approved and marketed materials. Control of impurities in the drug substance and degradants in drug product are addressed in ICH Quality Guidelines Q3A(R) and Q3B(R), respectively, and the Q3C guideline that deals with residual solvents. However, no specific guidance for determining acceptable levels for genotoxic impurities is provided in these documents other than to recognize the fact that unusually toxic impurities may require tighter limits of control. Toxicological assessment and justifications of limits per these ICH guidelines are normally based on the qualification of representative batches of the API including its impurities in pivotal toxicity studies that include genetic toxicology tests. The European Medicines Agency Committee for Medicinal Products for Human Use (CHMP) has issued a Draft Guideline on the Limits of Genotoxic Impurities, which describes an approach for assessing genotoxic impurities of unknown carcinogenic potential or potency based on the TTC[4] concept (CHMP, 2004). The proposals detailed in this paper extend the CHMP approach to include the concept of a *staged* TTC that establishes allowable daily intakes of impurities based upon duration of exposure. It should be noted, however, that the CHMP draft document attempts to provide guidance to industry on how to address specifications for impurities possessing genotoxic potential in marketing applications for new drug products and does not consider how such impurities should be handled in the exploratory stage of drug development, i.e., for clinical trial materials.

This paper describes a process for testing, classifying, and controlling of such impurities in a way that balances therapeutic benefit with the potential risks associated with a medicinal product and concomitant levels of potentially mutagenic impurities. The process seeks to establish rational acceptance criteria that take into account the stage of clinical development, the duration of a clinical trial, subject safety, and the feasibility of adequately sensitive analytical methods. In the early stages of clinical development process and impurity information is limited and hence the emphasis is placed on known reagents, intermediates, and reaction products in the synthetic process. Both *structurally identified* impurities (those for which the chemical structure is known) and *readily predicted* impurities (those that a technical review of the synthetic process

[1] *Abbreviations used:* ADI, allowable daily intake; ALARA, as low as reasonably achievable; ALARP, as low as reasonably practicable; API, active pharmaceutical ingredient (note: API and DS—drug substance are synonymous and often used interchangeably); COC, cohort of concern; chemical groups requiring control to levels lower than the TTC due to potent carcinogenic potential; MTD, maximum tolerated dose; PDE, permitted daily exposure; Qualification—process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at specified levels (Draft CHMP Guideline); TTC, Threshold of Toxicological Concern; VSD, virtually safe dose.

[2] Often, the As Low As Reasonably Achievable (ALARA) principle is referred to in product quality. This concept indicates that the detection and limitation of impurities at low levels is technically feasible with state of the art process and analytical technologies. However, the investment required to develop these capabilities for each candidate, particularly when the attrition rate of candidate molecules in early clinical trials is high, must be balanced with the need to control impurities to levels that are considered safe, pragmatic and practical. Thus, a distinction is made between ALARA levels that imply controlling levels to the lowest detectable level and ALARP levels that imply controlling levels to a practicable and safe level using the methods described in this paper.

[3] The idioms "genotoxic" and "mutagenic" are used interchangeably in this publication. However, it is noted that they are not synonyms. While "genotoxic" very generally refers to any measurable DNA damage effects and includes, e.g., indirect DNA damage measurements such as DNA strand breakage and DNA repair, the term "mutagenic" more specifically refers to heritable changes in DNA sequence or information content in somatic or germ cells. Such heritable changes are known to be important for critical steps in the process of carcinogenesis.

[4] The Threshold of Toxicological Concern (TTC) was originally introduced by the FDA Center of Food Safety and Nutrition as a threshold of regulatory concern (i.e., 0.5 ppb in the diet) as a level low enough to ensure that if an untested substance is later shown to be a potent carcinogen, the use of the substance would pose negligible concerns provided the regulatory criteria were met (Cheeseman et al., 1999).

suggests might be present) are assessed and classified. After impurities are classified, acceptance criteria for impurity levels are set based on structural analysis, data from genotoxicity testing, and by using a conservative risk-based approach based on the staged TTC. Since genotoxicity data are normally not suitable for a quantitative risk assessment, the (*staged*) TTC is based on animal carcinogenicity data and the knowledge about correlations between genotoxic processes and carcinogenesis for a substantial number of carcinogens.

2. Classification of potential genotoxic impurities

2.1. Considerations on testing of impurities for genotoxic potential

The general framework for genotoxicity testing of pharmaceuticals is given in two internationally agreed ICH safety guidelines (ICH S2A, 1995; ICH S2B, 1997). One of these guidelines (ICH S2B, 1997) describes the standard battery of tests for genotoxicity for drug substance, which consists of:

(i) A test for gene mutation in bacteria.
(ii) An in vitro test with cytogenetic evaluation of chromosomal damage in mammalian cells *or* an in vitro mouse lymphoma *tk* assay.
(iii) An in vivo test for chromosomal damage in rodent hematopoietic cells.

The ICH safety guidelines (S2A and S2B) state: "For compounds giving negative results, the completion of this 3-test battery, performed and evaluated in accordance with current recommendations, will usually provide a sufficient level of safety to demonstrate the absence of genotoxic activity." In this context, genotoxicity is a broad term encompassing effects from mutagenicity through DNA reactivity, DNA damage, and chromosomal damage, both structural chromosome breakage and aneuploidy. Any compound that produces a positive result in one or more assays in the standard battery has historically been regarded as genotoxic, which may require further testing for risk assessment. Thus, the standard battery of genotoxicity assays used for testing the API provides important information about a diversity of mechanisms of genotoxicity, both directly and indirectly associated with effects on DNA (Müller et al., 1999). Genotoxicants that do not act directly on DNA are typically associated with threshold-related mechanisms, while those that directly target DNA (typically detected in assays measuring the reverse or forward mutations in a specific gene with a selection agent) are considered by regulatory authorities not to have threshold-related mechanisms. Requirements for control of genotoxic impurities in pharmaceutical products are different depending upon whether or not there is evidence for a threshold-related mechanism. DNA-reactive genotoxic impurities for which there is no evidence of a threshold-related mechanism are regarded to be potentially trans-species and multi-organ carcinogens that may require control at relatively low levels. In contrast, it is accepted that impurities acting via threshold-related mechanisms do not require control at similarly low levels. Since the main concern that should drive control of impurities to relatively low levels is direct DNA reactivity, the primary endpoint of relevance for genotoxic impurities is mutagenicity.

Extensive knowledge about chemical functional groups that can react with DNA causing mutagenicity and concern regarding initiation of tumor processes is available in the scientific literature (Ashby and Tennant, 1988, 1991; Ashby and Paton, 1993; Beningi, 2004; Munro et al., 1996). Such knowledge has been used to develop rule-based computer programs such as DEREK (http://www.chem.leeds.ac.uk/luk/derek/), MCase (http://www.multicase.com/products/prod01.htm), or TOPKAT (http://www.accelrys.com/products/topkat/) and others. In addition, a recent analysis of the performance of various in vitro genotoxicity assays against the Carcinogenic Potency Database (CPDB) implies that a single mutation assay possesses the necessary sensitivity and specificity for detection of non-thresholded genotoxic carcinogenic chemicals (Kirkland et al., 2005). This has been confirmed using a larger database of carcinogens that includes proprietary data submitted to the US EPA and US FDA (Matthews et al., 2005). Hence, DNA-reactive carcinogens can be identified with a low incidence of false negative results by a procedure that combines the assessment of chemical structural features that infer DNA reactivity (such as electrophilicity) with a single biological hazard identification test such as a bacterial reverse mutation test, known as the "Ames test" (Bailey et al., 2005; Fetterman et al., 1997). A flexible use of this approach is sometimes advisable since genotoxicity assessment of impurities in mammalian cells may be needed for specific structural groups, such as carbamates, which are known carcinogens and that are known to be inefficiently detected in bacterial genotoxicity tests (Allen et al., 1982).

A clearly negative result in an appropriate genotoxicity test (i.e., a bacterial reverse mutation test or mammalian cell assay) usually indicates a sufficient level of safety to conclude the absence of genotoxicity for the purpose of controlling impurities.

2.2. Impurity classification with respect to genotoxic potential

It is proposed here that impurities be classified into one of five classes using data (either published in the literature or from genotoxicity testing) and comparative structural analysis to identify chemical functional moieties correlated with mutagenicity. The five classes are:

2.2.1. Class 1—Impurities known to be both genotoxic (mutagenic) and carcinogenic

This group includes known animal carcinogens with reliable data for a genotoxic mechanism and human

carcinogens. Published data on the chemical structure exist demonstrating the genotoxic nature of the impurity.

2.2.2. Class 2—Impurities known to be genotoxic (mutagenic), but with unknown carcinogenic potential

This group includes impurities with demonstrated mutagenicity based on testing of the impurity in conventional genotoxicity tests, but with unknown carcinogenic potential.

2.2.3. Class 3—Alerting structure, unrelated to the structure of the API and of unknown genotoxic (mutagenic) potential

This group includes impurities with functional moieties that can be linked to genotoxicity based on structure, but which have not been tested as isolated compounds. They are identified based on chemistry and using knowledge-based expert systems for structure–activity relationships. The alerting functional moiety is not present in the structure of the parent API. Some widely recognized alerts for DNA reactivity, i.e., mutagenic activity, are depicted in Fig. 1.

Some generic rule-based alerts may be quite unspecific (e.g., the general alerts for aromatic amines; Cash et al., 2005); and further consideration must be given to chemical structural constraints, chemical environment, or experimental data in the assessment of potential genotoxicity. Due to the uncertain relevance of structural alerts, regulatory action should not be based solely on the presence of a particular functional group; rather the accuracy for predicted genotoxicity should be evaluated case-by-case based on the available scientific literature, additional unpublished (proprietary) data on the chemical class and further available (genotoxicity) test results on closely related structures.

2.2.4. Class 4—Alerting structure, related to the API

This group includes impurities that contain an alerting functional moiety that is shared with the parent structure. The genotoxicity of the isolated impurity is unknown, but the genotoxicity of the active principle has been characterized through conventional genotoxicity testing. Similar chemical constraints and chemical environment exist for the alerting substructure in the impurity and the API.

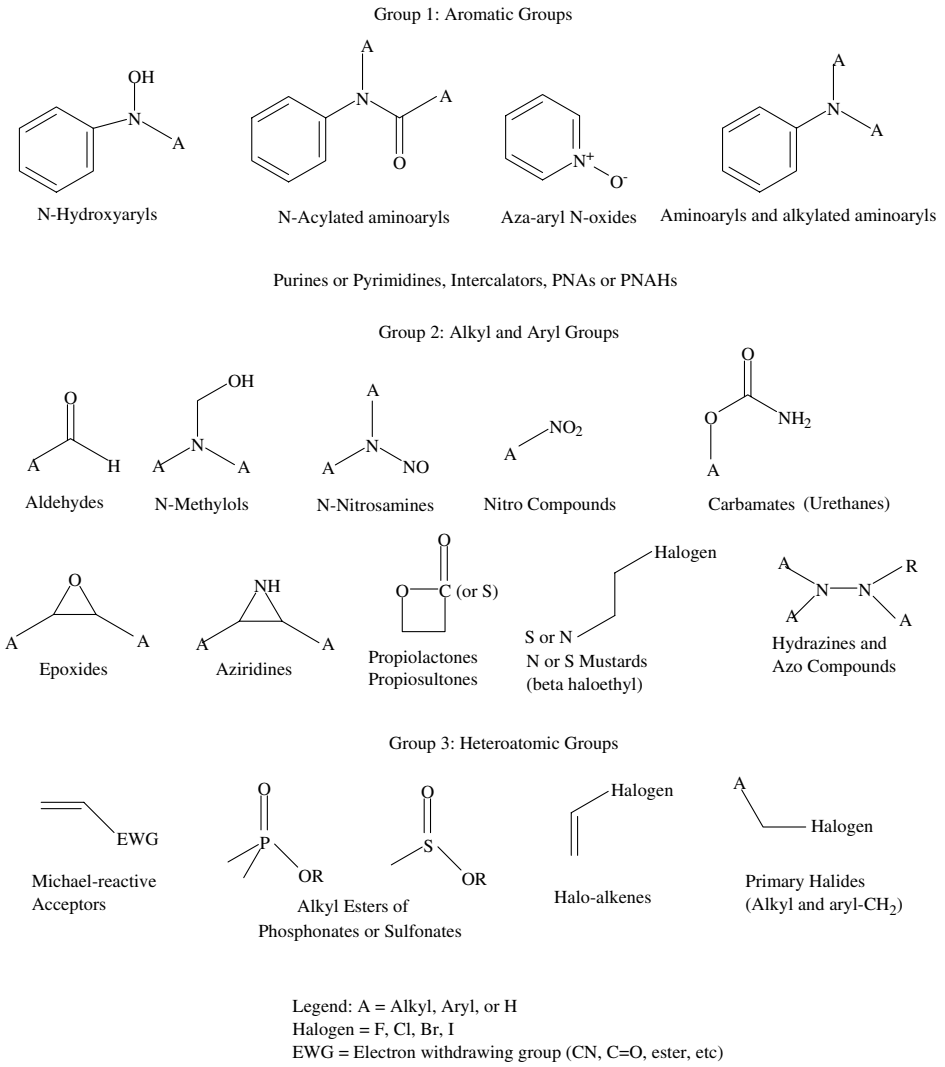

Fig. 1. Some examples of structurally alerting functional groups that are known to be involved in reactions with DNA (this list is not exhaustive).

2.2.5. Class 5—No alerting structure or sufficient evidence for absence of genotoxicity

This group would be adequately covered by existing ICH Q3A(R), Q3B(R), and Q3C guidelines.

It has to be emphasized that this classification system would be used solely for the purpose to decide whether an impurity possesses a high level of risk and is therefore to be controlled at very low levels of daily intake. Hence, this classification is not a general classification of genotoxicity.

3. Qualification of impurities

The relevant ICH guidelines concerning the qualification of impurities in commercial manufacture are Q3A(R) and Q3B(R) that focus on impurities in drug substances and drug products, respectively, while Q3C recommends limits for residual solvents in the drug product. The guidance given in these regulatory documents is considered to be applicable at the time of registration of a new pharmaceutical entity. The first two guidelines describe threshold levels above which impurities are required to be reported, identified, and qualified either in toxicological investigations or in the clinic. The threshold levels vary according to the maximum daily dose of a drug. For drug substance, the identification thresholds are within the range of 500 and 1000 ppm (i.e., 0.05 and 0.1%). ICH Guidelines Q3A(R) and Q3B(R) state that although identification of impurities is not generally necessary at levels less than or equal to the identification threshold, "analytical procedures should be developed for those potential impurities that are expected to be unusually potent, producing toxic or pharmacological effects at a level not more than ($\leqslant$) the identification threshold." Thus in the case of impurities where a potential safety concern for genotoxicity exists, the guidelines imply that the routine identification threshold is not considered to be applicable. With regard to qualification, the requirements for qualifying potential genotoxic impurities are not specifically addressed in the guidelines and hence have been left to a case-by-case assessment.

This case-by-case assessment is now proposed to be replaced by a general concept that is based on the knowledge and approaches as defined by the Threshold of Toxicological Concern (TTC). In agreement with the CHMP Draft Guideline on Genotoxic Impurities, the TTC concept (Barlow et al., 2001; Kroes et al., 2000; Kroes and Kozianowski, 2002; Munro et al., 1999) is used to establish a limit of 1.5 μg/day as a virtually safe dose for most genotoxic compounds, while recognizing that some highly potent genotoxic compounds (specifically *N*-nitroso compounds, azoxy-compounds, and aflatoxin-like compounds; see Fig. 1 for structural moieties) may require even lower levels (Kroes et al., 2004). Based on the conservative approach of linear back-extrapolation from animal cancer data, the TTC concept, despite some limitations, establishes pragmatic limits for daily human exposure to genotoxic impurities assuming lifelong treatment (or intake as food contact materials; Bailey et al., 2005). Yet many medicines are given for limited time spans and to limited numbers of patients. Further, exploratory drugs are given in clinical development phases prior to marketing for limited duration under well controlled conditions. Hence, a pragmatic approach should be appropriate for determining acceptable exposures to genotoxic impurities throughout clinical trials and for shorter-than-lifetime exposure. Based on the stochastic mode of action (dependency on total cumulative dose; Bos et al., 2004), the staged TTC approach outlined in Table 1 should be used to determine allowable daily limits for shorter-than-lifetime duration clinical studies. The conservative approach outlined in this paper regards all exposures >12 months as potential lifetime exposures, unless specific arguments are given not to assume this. It is acknowledged that regulatory guidances for pharmaceuticals usually require rodent lifetime carcinogenicity tests when the clinical use (continuous or cumulative) of a pharmaceutical exceeds six months as this is referred to as "chronic" for clinical use and thus requiring a lifetime animal model for cancer risk assessment. However, it was felt that the use of the >6 month intake criterion for ultimate control of genotoxic impurities at a calculatory lifetime cancer risk level would still be quite disconnected to the

Table 1
Proposed allowable daily intake (μg/day) for genotoxic impurities of unknown carcinogenic potential during clinical development, a staged TTC approach depending on duration of exposure (ADIs for shorter durations than 12 months are based on linear extrapolation (Bos et al., 2004) from TTC value of 0.15 μg/day (Cheeseman et al., 1999; Kroes et al., 2004))

	Duration of exposure				
	⩽1 month	>1–3 month	>3–6 month	>6–12 month	>12 month
Allowable Daily Intake (μg/day) for different duration of exposure (as normally used in clinical development)	120[a] or 0.5%[c] whichever is lower	40[a] or 0.5%[c] whichever is lower	20[a] or 0.5%[c] whichever is lower	10[a] or 0.5%[c] whichever is lower	1.5[b] [c]

Known carcinogens should have compound-specific risk calculated (see text and Fig. 1).

[a] Probability of not exceeding a 10^{-6} risk is 93%.

[b] Probability of not exceeding a 10^{-5} risk is 93%, which considers a 70-year exposure.

[c] Other limits (higher or lower) may be appropriate and the approaches used to identify, qualify, and control ordinary impurities during developed should be applied. In particular, approaches that foresee a very low dose of the API ("microdoses") may facilitate higher limits than 0.5%.

basis for the cancer risk calculation, i.e., a lifetime exposure of 60–70 years.

Detection, quantitation, and control of potentially genotoxic impurities to very low levels below the above mentioned identification threshold presents considerable challenges for the synthetic and analytical chemist for the development, manufacture, and control of API, impurities in the API and the drug product. These challenges are most acute during the initial stages of development (pre-clinical, phase I and II) where manufacturing changes occur often so that evaluation of genotoxic impurities would likely have to be repeated, but still pertain later throughout development. Structural identification and characterization as well as robust control of impurities at low levels are generally not achieved until the efficacy of the drug is established, a commercial route of synthesis is selected and a high level of process understanding is obtained. In particular, for control of an impurity to a very low level, an understanding of the functional relationship between process parameters and quality attributes learned through the synthesis of multiple lots is essential.

Structural identification of a potential genotoxic impurity at a level above the identification threshold as per ICH guidelines, while sometimes still challenging, enables hazard identification and classification to be performed, followed by risk assessment and any necessary follow-up action. However, if such an impurity were present at a level below the identification threshold, unless there was a reason to be concerned a priori, the impurity is unlikely to be identified or quantitated, thus ruling out a formal risk assessment and the opportunity to apply subsequent control measures. Also, it is not possible to identify the structures of all possible impurities. Hence, for the purpose of controlling genotoxic impurities down to the level of the TTC concept, the ICH qualification of impurities needs to be adapted as laid out in the following proposal:

Step 1: Identify structural alerts in parent compound and expected impurities (both *structurally identified* and *readily predicted*), and classify the impurities into one of the five classes defined above.
Step 2: Establish a qualification strategy for the impurities based upon the classification, as detailed below.
Step 3: Establish acceptable limits of the impurity in the API, based upon the Allowable Daily Intake and the TTC concept.

3.1. Step 1: determination of structural alerts for parent compound and impurity

Prior to the development of a qualification strategy, a scientific review of the synthetic route should be conducted to identify compounds of potential concern, including process impurities, reagents, or intermediates. All identified or readily predicted impurities are then classified into one of the five classes described earlier.

3.2. Step 2: qualification strategy for impurities

Following classification of the impurities, a qualification strategy defines the genotoxic potential of the impurity or establishes permitted specification limits for the impurity in the drug product. A summary of the qualification strategies based upon impurity classification is shown in Fig. 2 and detailed below.

3.2.1. Class 1—Impurities known to be both genotoxic (mutagenic) and carcinogenic

An impurity may be identified as carcinogenic based on literature information and internal testing data. The presence of a carcinogenic impurity is of highest concern to the safety of clinical populations. The goal in such situations is to avoid these impurities by modification of formulation options or technology, synthetic route, starting materials, reactants, or purification steps. When it is not practical or realistic to avoid these impurities, specifications should be determined as described below.

If sufficient 2-year rodent data are available to determine the carcinogenic potency of the impurity itself, a compound-specific calculation of risk should be conducted. Different risk calculation methods are available, including the use of cancer slope factors, TD_{50} values (the average daily dose estimated to halve the probability of remaining tumor-free throughout a 2-year study), or maximum tolerated dose (MTD) information (Gaylor and Gold, 1995).

If insufficient information is available to calculate a compound-specific risk, a general risk assessment based on the TTC concept can be employed. For genotoxic carcinogens, this may be an exceptional case. Since the compound is in principle an established genotoxic carcinogen, Munro et al. (1999) derived 0.3 μg/day as the appropriate TTC, which is 5-fold lower than the TTC for genotoxic compounds of unknown carcinogenic potential. Nevertheless, the principles of the *staged* TTC should be used at outlined in this paper.

3.2.2. Class 2—Impurities known to be genotoxic (mutagenic), but with unknown carcinogenic potential

An impurity may be identified as genotoxic based upon the literature or by testing positive in one or more genotoxicity tests. Conventional genotoxicity tests are designed to identify genetic hazard, and the data generated in these tests are not suitable for quantitative characterization of risk. Thus, data from genotoxicity testing must be evaluated for biological relevance, as some assays, i.e., in particular assays using mammalian cells in culture, are known to have a high rate of false positive results or poor correlation with rodent carcinogenicity (Kirkland et al., 2005; Matthews et al., 2005; Müller and Kasper, 2000; Snyder and Green, 2001).

Impurities for which there is strong evidence of mutagenicity, either from testing or from the literature, should be assessed on the basis of whether or not there is evidence for a threshold-mediated mechanism, as detailed below.

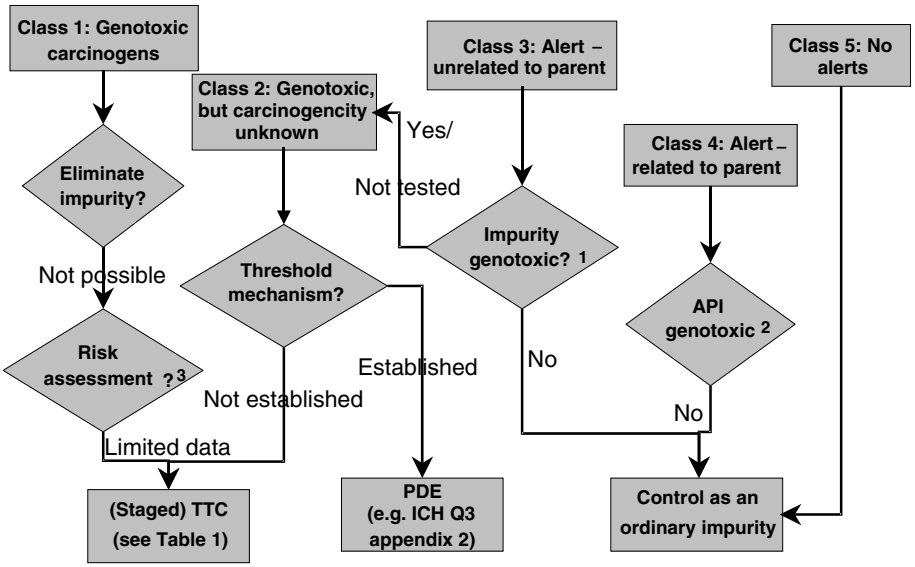

Fig. 2. Categorization, qualification, and risk assessment of Impurities (Note: Impurity levels for Classes 1, 2, and 3 may always be controlled to the staged TTC levels in Table 1). [1]Either tested neat or spiked into the API and tested up to ⩽250 μg/plate. [2]If the API is positive, then a risk-benefit analysis is required. [3]Quantitative risk assessment to determine ADI.

3.2.2.1. Class 2(a) genotoxic impurities with sufficient evidence for a threshold-related mechanism. For impurities with sufficient evidence of threshold-based genotoxicity (i.e., compounds that do not react with DNA, such as spindle poisons, topoisomerase inhibitors, and DNA synthesis inhibitors), it is possible to determine exposure levels with little risk of genotoxicity. One approach is described in the ICH Q3C Note for Guidance on Impurities: Residual Solvents for calculating "Permitted Daily Exposures" (PDE) for class 2 solvents. These exposure levels are derived from no observed effect levels or lowest-observed effect levels in the most relevant study.

3.2.2.2. Class 2(b) genotoxic impurities without sufficient evidence for a threshold-related mechanism. Compounds in this group include compounds for which there is evidence of direct DNA reactivity, e.g., demonstrated DNA binding or DNA adduct formation. Such compounds are usually bacterial mutagens and unequivocal in vitro clastogens. Very frequently these compounds are also positive in in vivo genotoxicity assays. For these genotoxic impurities for which there is insufficient evidence of a threshold-related mechanism, levels should be controlled using the staged TTC approach (Table 1).

Example: an aryl epoxide is found in the API intended for chronic use. It is identified as a mutagen in the Ames assay but rodent carcinogenicity data are lacking. Alternative synthetic routes and purification schemes have been considered and it has not been reasonably possible to avoid traces of this impurity in the drug substance. If discovered at the time of registration, a TTC approach using lifetime exposure estimates would be employed with a 1.5 μg/day limit. If discovered earlier in the drug development process, a staged TTC approach using limited duration of exposure would be used and a suitable specification determined (see Table 1). As an example, this compound could be specified at no greater than 240 ppm for clinical trials of up to four weeks in duration at a maximum dosage of the API of 500 mg/day (Table 2).

3.2.3. Class 3—Alerting structure, unique, and of unknown genotoxic (mutagenic) potential

When an impurity contains a structural alert but does not have experimental data, the qualification strategy may vary depending on expected toxicological risk and process and analytical understanding. Without experimental data, an impurity may be controlled to the staged TTC levels specified in Table 1. It is understood that this approach reflects a balance between chemical and analytical efforts in relation to more detailed toxicological hazard identification testing while assuring clinical safety. Alternatively, testing of the impurity in a genetic toxicology assay (typically a bacterial reverse mutation assay) may be conducted to gain toxicological understanding of structural predictions.

If experimental data are to be generated, the API may be spiked with the impurity as described below or the isolated/synthesized impurity may be tested as such. The level of spiking needed to provide a sufficient sensitivity of detection is driven by knowledge about the potency of mutagenic chemicals with this alerting structure in the standard bacterial reverse mutation assay strains. Based on data collected for over 250 bacterial mutagens, a mutagenic effect is normally detectable at levels at or below 250 μg/plate (~10 μg/ml) in a standard bacterial reverse mutation assay (Cyr et al., 2005; Ku et al., 2001). Therefore, the impurity may be evaluated as part of the API as long as the impurity is present at a minimum concentration of 250 μg/plate.

Table 2
Relationship between acceptable daily intake (ADI) levels and daily dose of a pharmaceutical (active pharmaceutical ingredient, API) as derived from Table 1 for daily doses between 0.1 and 3000 mg (the dark shaded areas in the upper parts of the tables represent levels of impurities below 100 ppm; the lighter shaded areas in the lower parts of the tables represent acceptable daily intake levels of a genotoxic impurity in the API of higher than 0.5%, which would be "capped" at 0.5%)

Daily dose of API (mg)	Concentration of impurity (%)					Concentration of impurity (ppm)				
	Acceptable (maximum) daily intake (ADI) and duration of exposure					Acceptable (maximum) daily intake (ADI) and duration of exposure				
	≤4 weeks ADI = 120 μg	1–3 months ADI = 40 μg	3–6 months ADI = 20 μg	6–12 months ADI = 10 μg	>12 months ADI = 1.5 μg	≤4 weeks ADI = 120 μg	1–3 months ADI = 40 μg	3–6 months ADI = 20 μg	6–12 months ADI = 10 μg	>12 months ADI = 1.5 μg
3000.0	0.004	0.001	0.0007	0.0003	0.00005	40	13	7	3	0.5
2000.0	0.006	0.002	0.0010	0.0005	0.00008	60	20	10	5	0.8
1500.0	0.008	0.003	0.0013	0.0007	0.00010	80	27	13	7	1.0
1200.0	0.010	0.003	0.0017	0.0008	0.00013	100	33	17	8	1.3
1000.0	0.012	0.004	0.0020	0.0010	0.00015	120	40	20	10	1.5
900.0	0.013	0.004	0.0022	0.0011	0.00017	133	44	22	11	1.7
800.0	0.015	0.005	0.0025	0.0013	0.00019	150	50	25	13	1.9
700.0	0.017	0.006	0.0029	0.0014	0.00021	171	57	29	14	2.1
600.0	0.020	0.007	0.0033	0.0017	0.00025	200	67	33	17	2.5
500.0	0.024	0.008	0.0040	0.0020	0.00030	240	80	40	20	3.0
400.0	0.030	0.010	0.0050	0.0025	0.00038	300	100	50	25	3.8
300.0	0.040	0.013	0.0067	0.0033	0.00050	400	133	67	33	5.0
200.0	0.060	0.020	0.0100	0.0050	0.00075	600	200	100	50	7.5
100.0	0.120	0.040	0.020	0.0100	0.00150	1,200	400	200	100	15
90.0	0.133	0.044	0.022	0.011	0.00167	1,333	444	222	111	17
80.0	0.150	0.050	0.025	0.013	0.00188	1,500	500	250	125	19
70.0	0.171	0.057	0.029	0.014	0.00214	1,714	571	286	143	21
60.0	0.200	0.067	0.033	0.017	0.00250	2,000	667	333	167	25
50.0	0.240	0.080	0.040	0.020	0.00300	2,400	800	400	200	30
40.0	0.300	0.100	0.050	0.025	0.00375	3,000	1,000	500	250	38
30.0	0.400	0.133	0.067	0.033	0.00500	4,000	1,333	667	333	50
25.0	0.480	0.160	0.080	0.040	0.00600	4,800	1,600	800	400	60
20.0	0.600	0.200	0.100	0.050	0.00750	6,000	2,000	1,000	500	75
10.0	1.20	0.400	0.200	0.100	0.015	12,000	4,000	2,000	1,000	150
9.0	1.33	0.444	0.222	0.111	0.017	13,333	4,444	2,222	1,111	167
8.0	1.50	0.500	0.250	0.125	0.019	15,000	5,000	2,500	1,250	188
7.0	1.71	0.571	0.286	0.143	0.021	17,143	5,714	2,857	1,429	214
6.0	2.00	0.667	0.333	0.167	0.025	20,000	6,667	3,333	1,667	250
5.0	2.40	0.800	0.400	0.200	0.030	24,000	8,000	4,000	2,000	300
4.0	3.00	1.00	0.500	0.250	0.038	30,000	10,000	5,000	2,500	375
3.0	4.00	1.33	0.667	0.333	0.050	40,000	13,333	6,667	3,333	500
2.0	6.00	2.00	1.00	0.500	0.075	60,000	20,000	10,000	5,000	750
1.0	12.00	4.00	2.00	1.00	0.150	12,0000	40,000	20,000	10,000	1,500
0.9	13.33	4.44	2.22	1.11	0.167	133,333	44,444	22,222	11,111	1,667
0.8	15.00	5.00	2.50	1.25	0.188	150,000	50,000	25,000	12,500	1,875
0.7	17.14	5.71	2.86	1.43	0.214	171,429	57,143	28,571	14,286	2,143
0.6	20.00	6.67	3.33	1.67	0.250	200,000	66,667	33,333	16,667	2,500
0.5	24.00	8.00	4.00	2.00	0.300	240,000	80,000	40,000	20,000	3,000
0.4	30.00	10.00	5.00	2.50	0.375	300,000	100,000	50,000	25,000	3,750
0.3	40.00	13.33	6.67	3.33	0.500	400,000	133,333	66,667	33,333	5,000
0.2	60.00	20.00	10.00	5.00	0.750	600,000	200,000	100,000	50,000	7,500
0.1	>100%	40.00	20.00	10.00	1.50	>1 million	40,0000	200,000	100,000	15,000

While this is a general guidance, some classes of bacterial mutagens (e.g., many sulfonic acid esters) may be missed by this approach since they require higher concentrations to be detected in the bacterial reverse mutation test (Glowienke et al., 2005) and they are considered to be trans-species rodent carcinogens (Ashby and Paton, 1993; Matthews et al., 2005). Hence, an evaluation of the available literature data on compounds with similar structures must be made when judging the adequacy of evaluating impurities as part of the API.

Another option is to isolate or synthesize the impurity and to test it, typically as previously outlined in a bacterial reverse mutation assay. If determined to be a bacterial genotoxin, the compound will be evaluated as a Class 2 impurity using the TTC. If it is not mutagenic, this experimental data outweighs the structural alert and the impurity will be controlled as a typical impurity per ICH Quality Guidelines Q3A(R), Q3B(R), and Q3C.

3.2.4. Class 4—Alerting structure; related to the API

Many generic rule-based structural alerts are quite unspecific and must be assessed in light of chemical constraints and existing experimental testing data. In cases where an impurity has a structural alert that is shared with the parent molecule, thorough genotoxicity testing of the parent is usually sufficient to qualify the structurally similar impurity. When genotoxicity tests on the parent are negative, limits for the impurity will be set in accordance with procedures used during development to identify, qualify, and set limits for "ordinary impurities."

3.2.5. Class 5—No alerting structure or sufficient evidence for absence genotoxicity

Limits for the impurity will be set in accordance with procedures used during development to identify, qualify, and set limits for "ordinary impurities."

The usefulness of this procedure in practice has been evaluated by Dobo et al. (2005) for a range of structurally alerting impurities that commonly occur in syntheses of active pharmaceutical ingredients. In their experience when chemicals were classified as structurally alerting based on published data including those in a computer structure–activity system, 21 of 27 (about 78%) were confirmed as mutagenic in the Ames test, while of those considered non-alerting only 10 of 178 were mutagenic in the Ames test. Thus, there was a high degree of concordance (92%) when compared with the results of Ames mutagenicity testing.

3.3. Step 3: risk assessment methods and determination of allowable daily intake (ADI) for mutagenic/carcinogenic compounds

Following the classification of the impurity, the determination of acceptable concentrations in drug substance and drug product is based on the qualification and risk assessment strategies outlined below and summarized in Fig. 2.

If sufficient 2-year rodent data are available to determine the carcinogenic potency, compound-specific calculation of risk should be conducted. Different methods are available to achieve this, including the use of cancer slope factors, TD_{50}/TD_{25} values, or the use of MTD information.

3.3.1. Carcinogen with a published or calculated slope factor

Certain databases such as the US EPA Integrated Risk Information System (IRIS—http://www.epa.gov/IRIS/index.html) contain calculated cancer potency values for specific compounds based upon the highly conservative linearized model. Using the reported slope factor value, the daily dose associated with the specific risk (10^{-6} or 10^{-5}) can be calculated.[5] Alternatively, data can be analyzed with available software, calculating the benchmark dose and performing the recommended linear extrapolation. If the dataset is not available from which a slope factor can be calculated, one can be estimated based on the TD_{50} (see following section). Using summary data from 191 carcinogens, a slope factor of $0.87/TD_{50}$ was determined (Gaylor and Gold, 1995).

3.3.2. Carcinogen with published or calculated TD_{50}

The TD_{50} is defined as the average daily dose estimated to halve the probability of remaining tumor-free at a specified tissue site throughout a 2-year study (Gold et al., 1984). A compilation of bioassays with a TD_{50} analysis has been published (Gold and Zeiger, 1997). Alternatively, if a complete bioassay dataset is available for a given chemical, the TD_{50} can be calculated (Peto et al., 1984). The TD_{50} may be used to calculate the daily dose associated with the specific risk (10^{-6} or 10^{-5}). As an alternative to using the TD_{50}, a simplified approach using the TD_{25} has been proposed (Dybing et al., 1997; Sanner et al., 1997, 2001). This simplified approach yields similar results.

3.3.3. Carcinogen with incomplete data (Use MTD to estimate slope)

For situations where evidence for carcinogenicity exists, but there are incomplete data to calculate a slope factor or a TD_{50}, a slope factor can be estimated from a maximum tolerated dose (MTD) based on the results of a 90-day study. The correlation is due in part to the convention of running bioassays at dose levels equal to the MTD and 1/2 MTD. Gaylor and Gold (1995) reported that the virtual safe dose (VSD; 10^{-6} risk) can be estimated by the relationship VSD = MTD/740,000. The result of this equation is estimated to be within a factor of 10 of the VSD that would be obtained from a rodent carcinogen based on a 2-year NCI/NTP chronic bioassay.

[5] A 1 in 10^6 (or 10^{-6}) risk is defined as the calculated dose giving rise to 1 additional case of cancer in a population of 1 million people over the course of 70 years exposure.

3.3.4. Presumed genotoxic carcinogen without animal cancer data: use of the animal cancer potency curve and the TTC

If available information is not sufficient to calculate a compound-specific risk, data derived from several hundred compounds tested in 2-year bioassays has been used to develop a TTC concept and can be used to determine allowable daily intakes. Thus for Class 1, 2, and 3 impurities it is possible to derive an allowable daily intake from a probabilized estimate of risk using information from animal cancer potency data. Determination of allowable daily intake of potentially genotoxic impurities in drug substance or drug product is based on an understanding of dose–response relationships for carcinogenicity observed for several hundred compounds positive in chronic rodent bioassays. There was remarkable consistency among several efforts that defined cancer potency curves for rodent carcinogenicity studies (Ashby and Tennant, 1988; Fiori and Meyerhoff, 2002; Gold et al., 1984, 1989; Munro et al., 1999; Rulis, 1986).

The TTC was developed by the FDA as a 'threshold of regulation' for food contact materials (Rulis, 1986) by the Center for Food Safety and Nutrition. It was derived by simple linear extrapolation from the carcinogenic potencies, measured as the dose giving a 50% tumor incidence (TD_{50}) of more than 700 carcinogens. The methods and assumptions used to derive the TTC were conservative, using TD_{50} data from the most sensitive species and sites. A TTC figure of 1.5 μg/day was associated with a 1 in 10^6 lifetime cancer risk and was termed a virtually safe dose (VSD) for any chemical, even if it should later prove to be a carcinogen (Rulis; Federal Register 1993). In the recent evaluation by Kroes et al. (2004) for mutagens/alerting structures and their tumorigenicity, a TTC of 0.15 μg/day was associated with a 1 in 10^6 lifetime excess cancer risk. For pharmaceuticals, a higher limit of ~1.5 μg/day giving a 1 in 10^5 lifetime risk is considered acceptable, as there is a benefit associated with their intake, exposure is intentional, and use is infrequently for a lifetime. This remains a highly conservative number given the high lifetime risk of cancer in the population at large. Because the thresholds were derived using highly conservative estimates, they should be regarded as a lower limit for the control of most genotoxic impurities. The cancer potency data indicate that some structural groups of compounds, the "cohort of concern" (COC), would require control to levels lower than the TTC due to their high carcinogenic potency; examples include aflatoxin-like, *N*-nitroso-, and azoxy-compounds (genotoxic COC compounds; Kroes et al., 2004) for which compound-specific risk assessments would be required. These classes are unlikely to occur in drugs. The Draft CHMP Guideline indicates that a level of a potential genotoxic impurity above 1.5 μg/day may be acceptable in some circumstances, e.g., if treating a life-threatening condition where no safer alternatives are available, if life expectancy is less than 5 years, or if there is significant exposure to the impurity from other sources such as food. In addition, higher levels may be acceptable for short-term exposure (Bos et al., 2004) and are outlined in this paper.

Defining a level of acceptable risk is an approach consistent with the drinking water standards in the World Health Organization (1996) and United States Environmental Protection Agency (1991). The WHO considers 10^{-5} and the USEPA considers 10^{-6} to 10^{-4} as acceptable cancer risks for drinking water (USEPA, 1991). A negligible cancer risk of 10^{-6} has been used to develop the TTC, which is an acceptable intake for unknown compounds. The TTC is used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for flavoring substances and the U.S. Food and Drug Administration (FDA) for indirect food additives (JECFA, 1996; USFDA, 1995). The draft CHMP Guideline uses the TTC approach and a risk level of 10^{-5} to establish 1.5 μg/day as a virtually safe dose for most classes of genotoxic impurities (CHMP, 2004).

Based on risk/benefit considerations, the authors believe that risk levels of 10^{-6} to 10^{-5} are justified for pharmaceuticals and their use in clinical trials and the general population. The allowable daily intake (ADI) of genotoxic impurities for short-term exposures presented in Table 1 are based on a 10^{-6} risk because of the common inclusion in clinical studies of healthy volunteers, for whom there is normally no pharmacological benefit. In principle, a linear extrapolation from lifetime daily exposures to short-term daily exposures is defensible (Bos et al., 2004) and basing ADIs on a conservative 10^{-6} risk level is a mechanism to ensure safety for these short duration studies. The ADI for exposures greater than 12 months in duration is based on a 10^{-5} risk, because individuals in these longer-term clinical studies have the target indication and are intended to benefit from treatment.

3.3.5. Rationale for modifications of the lifetime TTC based on duration of exposure

Doses determined from 2-year rodent bioassay cancer curves have been used to define allowable daily doses for a human lifetime, or 70 years. Linear back extrapolation from rodent carcinogenicity data are performed in order to calculate the TTC limits, a procedure which very likely overestimates the risk but for which currently no defendable alternative exists, specifically for genotoxic carcinogens (Kroes et al., 2004). The theoretical foundation for a linear/non-thresholded dose–response relationship lies ultimately in the stochastic mode of action of genotoxins. Inherent to this concept is that an exposure to a defined cumulative dose (D) of a carcinogen will lead to a similar additional tumor incidence (I_c), irrespective of the combination of dose rate (i.e., daily dose, d), and duration of exposure (i.e., number of treatment days, n).

$$I_c \leftrightarrow \mathrm{D} = \mathrm{d} \times n (= \mathrm{d} \times 25,570 \text{ for lifelong [70 year] exposure}).$$

Following an evaluation of both multistage models and initiation–promotion models, Bos et al. (2004) concluded that it is possible, with an appropriate dose-rate correction factor, to safely administer the equivalent of an acceptable

lifetime cumulative dose in an acute manner (even within a single day), except perhaps when considering particularly sensitive subpopulations. For application on genotoxic impurities in the pharmaceutical area, Table 1 identifies ⩽1 month as the minimum, >12 months as the maximum exposure periods and >1–3 months, >3–6 months, >6–12 months as intermediate scenarios. The upper level of >12 months is based on the conservative assumption that any pharmaceutical that is taken for more than 12 months is likely to be taken for such long periods that there would be no reliable basis to assume a less than lifetime risk scenario. These exposures define a practical range that will allow the safe development of most pharmaceuticals. However, the algorithm used in Table 1 is dependent on the product of daily dose and exposure period such that doubling the duration, halves the daily allowable dose. Therefore, other durations could be calculated if needed. It is recognized that allowable daily doses of genotoxic impurities may exceed qualification thresholds for drug substance and drug product as defined in ICH Q3A(R) and ICH Q3B(R). In these cases, appropriate testing to qualify these impurities is warranted; however, the risk assessments defined in this document apply.

The proposal to distribute the lifetime dose over a shorter exposure period is straightforward. Potential effects of altering cellular repair processes, saturation of biological processes, or organotropism at doses in excess of the derived 10^{-5} risk level have already been controlled in the animals studies used to back extrapolate to the 10^{-6} risk level (Bos et al., 2004). It is recognized that there may be some uncertainty on the extrapolation of long-term exposures to short-term exposures. An evaluation of the available animal carcinogenicity data for short versus lifelong exposure experiments yields evidence that the back calculation of short-term risk from lifetime data can in some cases underestimate the short-term risk (Halmes et al., 2000). The more stringent standard of 10^{-6} cancer risk adequately covers the uncertainty of shorter exposure durations. Since the levels in Table 1 have been derived using highly conservative assumptions, they should comply with the general multifactorial exposure background of human volunteers and patients including polypharmacy as they do for indirect food additives. It is proposed that the conservatism built into the TTC level would be compatible with allowing the TTC value of 1.5 μg/day/lifetime for each of at least three impurities (independent of whether their genotoxicity is related to the same structural moiety or different ones), since the excess cancer risk would remain close to 1 in 100,000.

3.3.6. Considerations given for pediatric and other sensitive subpopulation exposures

Carcinogenic risks to populations vary depending on stage of maturation or unique susceptibilities. Few data exist to characterize variable risks to different populations; however, there is support for young individuals being more susceptible to carcinogenicity than older individuals (Drew et al., 1983; Kari et al., 1993; Land et al., 2003; USEPA, 2005). It has been proposed to set allowable levels of genotoxic impurities for short-term exposures in pediatric or young adult patients at one-tenth those of the general adult population unless justified otherwise. If other sensitive subpopulations of individuals are identified, these predispositions can be factored into the allowable daily intake. However, given the generally highly conservative nature of the proposed values in this paper, it is doubtful if there is a need for further adjustments for sensitive populations.

4. Balancing toxicologically driven genotoxic impurity limits with generally acknowledged and achievable quality goals

One of the critical quality-related attributes of the drug substance and drug product is the impurity profile, which needs to be controlled according to certain specified *concentrations*. The presence of a small amount of an impurity will result in a low concentration when dispersed in a large amount of drug substance, thereby posing significant testing and manufacturing challenges. Conversely, unacceptably high concentrations of the impurity may result if the same amount of impurity is dispersed in a small amount of drug substance. For example, 20 μg of an impurity translates to a concentration of 10 ppm if dispersed in a dose of 2 g and a concentration of 20,000 ppm if dispersed in a dose of 1 mg.

Toxicological concerns are appropriately addressed by controlling the daily intake (i.e., the *amount*) of a potentially toxic impurity. Table 2 shows the relationship between the concentration of an impurity, the daily dose of the drug, its period of intake, and the ADI of the impurity. This analysis demonstrates that the staged TTC approach derived using fundamental, risk-based principles of toxicology allows for reasonable upper limits of potentially genotoxic impurities for situations when the daily dose is high and the amount of impurity present is low.

Having addressed the combination of low levels of impurity and a high daily dose of the drug, it is also important to consider the other extreme where a high ADI of an impurity is dispersed in a relative low daily dose of the drug. For example, if the daily clinical dose were 10 mg for up to 4 weeks, the staged TTC would permit the presence 1.2% of a genotoxic impurity in this investigational product (Table 2). While this may be toxicologically justifiable, it may be necessary to limit the levels of the impurities to ensure the overall quality of the drug substance and the drug product. Since the ICH Quality Guidelines governing impurities (Q3A (R), Q3B (R), and Q3C) do not apply to the clinical phase of development, it is suggested here that an upper limit of 0.5% of potential genotoxic impurities be applied to low-dose drugs in clinical trials not exceeding 12 months in duration (Table 1). Nevertheless, different upper limits may be appropriate and could be considered on a case-by-case basis, e.g., for so-called "microdose" or PET imaging approaches where the very

low dose drug product may preclude analytical resolution at this level of sensitivity.

The above proposed steps must also take into account that an in-depth understanding of the chemical process as well as the development of very sensitive methods for the detection of impurities is acquired through laboratory and manufacturing experience. This level of understanding generally grows in parallel with the execution of the non-clinical safety assessment. The level of understanding may be poor or incomplete in the initial clinical phases, when the populations exposed are small and the duration of exposure is short and when changes in manufacture of the drug substance and drug product occur frequently. Consequently, in the early phases of development, identification, characterization, and control of potential genotoxic impurities to levels at the TTC, which can be in the low ppm range, are especially difficult. This goal has to be balanced against potential clinical study delays and thus ultimate delay in progressing important medicines to people in need. As compounds progress through clinical development to registration, synthetic processes, control of impurities, and analytical methods to detect and quantitate impurities evolve and improve. These improvements result in modified synthetic routes and, as a consequence, associated impurity profiles of the investigational product. It is therefore critical that a pragmatic approach to application of limits to potential genotoxic impurities, which nevertheless takes adequate account of the associated risks, is adopted in the initial phases of clinical development.

5. Conclusions

The control of impurities bearing a genotoxic potential in pharmaceutical products has received more and more attention over the past years. The inherent difficulties of true or hypothesized linear dose effect relationships have led to diverse strategies and risk calculations to achieve a rational level of control. Hence, a unified approach for pharmaceutical product development and marketing would be useful. The ultimate risk concern for genotoxicants is carcinogenicity but carcinogenicity data are not available in most cases. Hence, a risk assessment based on surrogate data such as structure–activity relationships and limited genotoxicity testing in bacterial reverse mutation tests, knowledge about the relationship between genotoxicity and carcinogenicity, and a generic determination of virtually safe exposure levels for the world of genotoxic carcinogens is proposed. The applicability of this concept in practice is clearly demonstrated by Dobo et al. (2005) for a range of structurally alerting compounds that are used as starting materials or are present as intermediates in the synthetic process of pharmaceuticals. The risk assessment is based on a generic calculation of a Threshold of Toxicological Concern (TTC) for carcinogens based on lifetime exposure. With few exceptions of very highly potent carcinogens, this level can be set at $\sim$1.5 μg/day and reflects an appropriate maximum risk level of 1/100,000 excess cancer case, which is in compliance with expectations of the society at large and extremely conservative given the lifetime cancer risk in the population of over 1 in 4. For shorter than lifetime exposures such as those occurring in clinical trials of pharmaceutical candidate compounds, multiples of this level can be accepted, albeit with an increased risk calculation stringency of 1 in a million to take into account (a) the treatment of volunteers and (b) limited knowledge about the benefit of the drug. This approach, together with a proactive process analysis of the chemistry behind the synthesis of the pharmaceutical product and matching analytical capabilities, ensures patient and volunteer safety and may not hinder inappropriately the fastest possible development of new medicines to improve patient health.

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