Organic Process Research & Development **2010,** *14,* 986–992

Risk Assessment of Genotoxic Impurities in Marketed Compounds Administered over a Short-Term Duration: Applications to Oncology Products and Implications for Impurity Control Limits

Courtney M. Callis,* Joel P. Bercu, Keith M. DeVries, Linda K. Dow, David K. Robbins, and David L. Varie

Health, Safety, & Environmental, Analytical Sciences Research & Development, and Chemical Product Research & Development, Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Indiana 46285, U.S.A.

Abstract:

Controlling impurities during drug development improves product quality and minimizes safety risks to the patient. Recent regulatory guidance on genotoxic impurities (GTIs) state that identified GTIs are unusually toxic and require lower reporting, identification, and qualification limits than outlined in the International Conference on Harmonization (ICH) guideline "Impurities in New Drug Substances Q3A(R2)." [*ICH Harmonized Tripartite Guideline: Impurities in New Drug Substances (Q3A)*, (R2); International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2006.] Patient safety is always the underlying focus, but the overall impurity control strategy is also driven by appropriate "as low as reasonably practicable" (ALARP)[2] procedures that include assessment of process capability and associated analytical techniques. In combination with ALARP, safe and appropriate GTI levels are currently identified using chronic toxicology-based limits calculated under the standard assumption of 70-years for exposure duration. This paper proposes a risk assessment approach for developing GTI limits based on shorter-term exposure durations by highlighting marketed anticancer compounds with limited dosing schedules (e.g., 2 years). These limits are generally higher than the defaulted threshold of toxicological concern (TTC of 1.5 μg/day) and can result in more easily developed and less complex analytical methods. The described approach does not compromise safety and can potentially speed life-saving medicines to patients.

Introduction

Control of impurities in active pharmaceutical ingredients (API) is an important aspect of drug development, ensuring product quality and minimizing safety risks. Different impurities may be observed throughout the development lifecycle of an API as the chemistry and process understanding evolve. Impurities that are potentially genotoxic and/or carcinogenic are of particular concern because they have been considered "unusually toxic", and therefore, are not adequately tested in the preclinical safety assessment studies typically used to support qualification of impurities according to the International Conference on Harmonization (ICH) Q3A(R2) guideline.[1] Potentially genotoxic impurities (PGIs) contain structural alerts for genotoxic effects, while genotoxic impurities (GTIs) test positive in validated genotoxicity assays (e.g., Ames). Carcinogenic impurities produce positive results for oncogenicity in bioassays conducted in two rodent species over standard lifespans. Acceptable levels of genotoxic and carcinogenic impurities in the API change as a function of adjustments in dose and duration of the administered pharmaceutical.[3] A control strategy for GTIs is based on the same types of knowledge required to design a control strategy for nongenotoxic impurities (non-GTIs), i.e., understanding the source and entry point of the impurity, the fate of the impurity, the ability of purification and processing operations to remove the impurity, and the acceptable level of the impurity in the API. Consideration of the ALARP principle (as low as reasonably practicable)[2,4] for impurities may achieve tighter control than toxicology-based limits require. GTI control typically must be demonstrated at very low (ppm) levels in the API or synthetic intermediates. Such limits have significant implications for process design and the analytical methods for detection and monitoring needed to ensure acceptable control of a GTI.[2,5] Overall impact can include increased resource costs and time delays with no additional patient benefit. The risk assessment approach described herein mitigates the impact for anticancer compounds with limited dosing schedules (e.g., ≤2 years) by providing higher GTI limits on the basis of shorter-term exposure durations.

* Author to whom correspondence may be sent. Telephone: (317) 277-5067. Fax: (406) 586-0917. E-mail: callis_courtney_m@lilly.com.

(1) *ICH Harmonized Tripartite Guideline: Impurities in New Drug Substances (Q3A)*, (R2); International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), **2006**.
(2) Pierson, D. A.; Olsen, B. A.; Robbins, D. K.; DeVries, K. M.; Varie, D. L. *Org. Process Res. Dev.* **2009**, *13*, 285–291.
(3) Müller, L.; Mauthe, R. J.; Riley, C. M.; Andino, M. M.; Antonis, D. D.; Beels, C.; DeGeorge, J.; De Knaep, A. G.; Ellison, D.; Fagerland, J. A.; Frank, R.; Fritschel, B.; Galloway, S.; Harpur, E.; Humfrey, C. D.; Jacks, A. S.; Jagota, N.; Mackinnon, J.; Mohan, G.; Ness, D. K.; O'Donovan, M. R.; Smith, M. D.; Vudathala, G.; Yotti, L. *Regul. Toxicol. Pharmacol.* **2006**, *44*, 198–211.
(4) *Guideline on the Limits of Genotoxic Impurities*, CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, 28 June 2006
(5) Liu, D. Q.; Chen, T. K.; McGuire, M. A.; Kord, A. S. *J. Pharm. Biomed. Anal.* **2009**, *50*, 144–150.

Published on Web 06/07/2010
10.1021/op1000226

Proposed Modified Threshold of Toxicological Concern (TTC)

Both the United States Food and Drug Administration (USFDA) (draft)[6] and European Medicines Agency (EMEA)[4,7] guidance documents clarify certain aspects of applying a threshold of toxicological concern (TTC) concept as a default approach to determine the upper limit for GTIs in clinical trial and marketed material. The described application applies to GTIs without adequate carcinogenicity information or evidence of a threshold, and extends the EMEA and FDA paradigms to include a longer duration of ≤2 years. In most cases, regulators consider a TTC value of 1.5 μg/day over a chronic duration of 70 years to be acceptable for exposure to a GTI. The TTC was developed from a database of known rodent carcinogens.[8] Linear extrapolations from the daily chronic dose that induced tumors in half the test animals over background at the end of their lifespan (TD_{50} values in mg/kg/day) were plotted for each carcinogen in the database, and the distribution was used to identify an appropriate exposure level below which there would be no significant additional risk.[8] The 1.5 μg/day TTC is generally considered to be conservative and commensurate with a 1 in 100,000 excess cancer risk, or the risk of one excess cancer over background in a population of 100,000 individuals exposed daily for a 70-year lifetime. Both agencies state that higher limits might also be acceptable for GTIs in certain marketed compounds, but no publication provides clear guidance. The draft FDA guidance on limits for GTIs states, "... a TTC value higher than 1.5 μg per day may be acceptable...in situations where the anticipated human exposure will be short-term, for the treatment of life- threatening conditions, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources."[6] The ICH S9 guideline further addresses flexibility for genotoxic impurities in anticancer compounds: "For genotoxic impurities, several approaches have been used to set limits based on increase in lifetime risk of cancer. Such limits are not appropriate for pharmaceuticals intended to treat patients with advanced cancer, and justifications described above [disease being treated and the patient population, the nature of the parent pharmaceutical (pharmacologic properties, genotoxicity, and carcinogenic potential, etc.), duration of treatment, and the impact of impurity reduction on manufacturing] should be considered to set higher limits."[9]

On the basis of the principles behind dose-effect and time relationships for DNA-reactive carcinogens, this paper proposes an approach that modifies the adopted "staged TTC"[3] methodology to address short-term duration of exposure to genotoxic and carcinogenic impurities in marketed anticancer compounds. The proposed modified approach targets an acceptable excess cancer risk of 1 in 100,000 to align with regulatory precedence for chronically administered drug product. This modified approach also takes into account the duration of delivery, which is typically not more than two years for oncolytics used to treat late-stage, recurring, and/or aggressive disease. Figure 1 compares the current approach based on regulatory guidance with the proposed strategy for marketed anticancer compounds. Since the lifetime cumulative dose associated with the 1.5 μg/day TTC is approximately 38 mg, the lifetime average daily dose (LADD)[10] over two years would be approximately 50 μg/day [i.e., 38 mg/(365 days × 2 years)]. For weekly exposures over two years, the acceptable impurity level would be approximately 360 μg/week [i.e., 38 mg/(52 weeks × 2 years)]. The underlying assumption is that risk is not reduced via exposure of a GTI over an intermittent duration (e.g., once weekly). This is a conservative approach considering a recovery period may allow for repair and ultimate reduction of the patient's susceptibility to cancer. A comprehensive assessment should also address threshold effects such as potential target-organ toxicity based on structure,[8] and should consider the ICH Q3A thresholds[1] since qualification studies (i.e., toxicology studies) of the API with the impurity present are intended to detect threshold effects. Since the TTC is an accepted cutoff for genotoxic compounds when carcinogenicity information is not available,[3,4,6] assessment of known carcinogenic impurities should follow a similar approach, but incorporate calculation of cancer risk from the actual cancer data, typically measured in rodents.

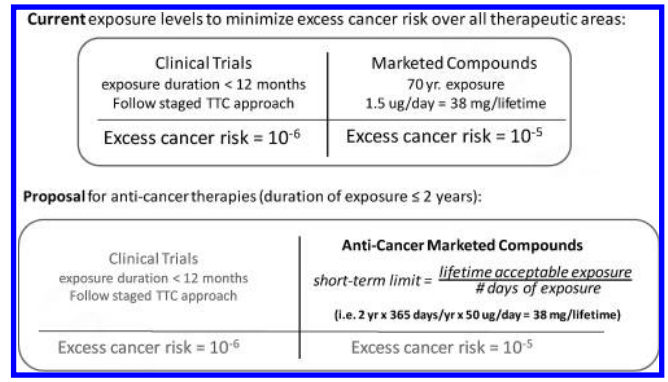


Figure 1. **Current regulatory versus proposed risk assessment paradigms including duration of administration and targeted excess cancer risk.**

Table 1 highlights some example oncolytics and whether it would be appropriate to apply the modified staged TTC approach, and Table 2 describes toxicology limits for specific genotoxic or carcinogenic impurities. In all cases, an acceptable toxicology limit based on a two-year dosing duration, an API dose of 1000 mg, and the risk assessment methodology described below are substantially higher than limits determined using current risk assessment parameters. For acetamide, a compound with an established carcinogenicity potency, the acceptable limit increases from 10 to 2450 ppm. This acceptable

(6) Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches. Draft Guidance; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Silver Spring, MD, U.S.A., December 2008.

(7) *Question & Answers on the CHMP Guideline on the Limits of Genotoxic Impurities*, EMEA/CHMP/SWP/431994/2007; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, June 2008.

(8) Kroes, R.; Renwick, A. G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma, A.; Schilter, B.; Schlatter, J.; van Schothorst, S. F.; Vos, J. G.; Wurtzen, G. *Food Chem. Toxicol.* **2004**, *42*, 65–83.

(9) *Nonclinical Evaluation for Anticancer Pharmaceuticals S9*; International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), October **2009**.

(10) Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001B; United States Environmental Protection Agency: Washington, D.C., March 2005.

Table 1. **Examples of oncolytics with durations of use appropriate for application of the modified TTC to address GTI**

treatment	FDA labeled indication	duration of use	modified TTC appropriate?[a]
Imatinib	chronic myeloid leukemia[b]	chronic[b]	no
Tamoxifen	prophylaxis of breast cancer in high risk women[c]	daily for 5 years[c]	no
Carboplatin	advanced ovarian carcinoma (palliative treatment of recurrent disease)[d]	likely <2 years[d]	yes

[a] The modified TTC is appropriately applied for oncolytics that are likely to have a limited duration of use (i.e., ≤2 years) based on approved indications to treat late-stage, recurring, and/or aggressive disease. The modified TTC is not appropriately applied for oncolytics used in prophylaxis, supportive care, or for indications where duration of use could exceed 2 years. [b] Imatinib. Drugdex Drug Evaluations. Corporate Solutions from Thomson Micromedex [Online], last modified July 6, 2009, Healthcare Series, Vol. 143. (accessed January 25, 2010). [c] Tamoxifen. Drugdex Drug Evaluations. Corporate Solutions from Thomson Micromedex [Online], last modified September 18, 2009, Healthcare Series, Vol. 143. (accessed January 25, 2010). [d] Carboplatin. Drugdex Drug Evaluations. Corporate Solutions from Thomson Micromedex [Online], last modified October 2, 2009, Healthcare Series, Vol. 143. (accessed January 25, 2010).

limit also takes into consideration potential noncarcinogenic effects (e.g., developmental toxicity, repeat-dose toxicity, etc.), but would be lowered to 1000 ppm based on recommendations for impurities from ICH Q3A(R2) (i.e., 1 mg or 0.15% whichever is less).[1] Limits for GTIs without known carcinogenicity potency, such as isopropyl mesylate, increase from 1.5 to 350 ppm. These higher limits may have significant implications for the development of suitable analytical techniques to enable the GTI control strategy. This topic will be discussed in the final section.

Toxicological Rationale for Proposed Modified TTC

Toxicology risk assessment is a pragmatic application of the science of toxicology. Prospective risk assessment approaches are intended to conservatively fill the gaps in scientific knowledge such that potential exposure to a specific chemical is considered to have a negligible health impact. While gaps in scientific knowledge hinder assessment of cancer risk following short-term exposure to genotoxic or carcinogenic compounds, the modified staged TTC approach described herein is a practical application of the current understanding of the science to GTI limits in marketed anticancer API used to treat late-stage or recurring cancer.

The application of both dose and duration in toxicology is not a new concept and was described mathematically in the 1920s as $c \times t = k$ (i.e., Haber's Law),[11] where c is the concentration, t is time, and k is a constant (for a toxicological outcome). Druckery (1967)[12] applied this concept to carcinogens, but modified the equation to $d \times t^n = k$, where d is the daily dose and n is greater than one, depending on the properties of the carcinogen. The implication of Druckery's work is that the daily dose and time relationship may not be linear for some carcinogens. If the total cumulative dose was the same, the risk would be reduced in the shorter duration with higher daily doses as compared with chronic duration exposure at lower doses. However, Rozman and Doull (2001)[11] point out that this relationship is likely due to the biological properties for some of the chemicals in the study (e.g., nitrosamines), which have a short pharmacokinetic (PK) half-life (i.e., ~10 min) and a long pharmacodynamic (PD) half-life of DNA adducts (i.e., weeks to months). This is an important consideration because the $c \times t$ concept has been shown to be influenced both by PK and PD specific to the chemical of interest.

Halmes et al. (2000)[13] explored tumor incidence as a function of dose and time by comparing stop exposure data (exposure time ranging from 13 to 66 weeks) with 2-year exposure data in chronic carcinogenicity studies for 11 chemicals. The stop-exposures were adjusted to average lifetime exposures, and an estimated carcinogenic risk at 1% incidence over background was compared for both the short and chronic duration exposures to test the $c \times t$ concept for a small subset of carcinogens. The overall conclusion was that the $c \times t$ concept held in many cases (less than a 2-fold change in cancer risk), but in some instances there was an increase or decrease in cancer risk when comparing short, high-dose studies with bioassays. This result is not surprising since the evaluated studies compared external doses (e.g., oral) and did not consider the systemic PK/PD of the carcinogens.

For compounds where only the genotoxicity hazard is known (e.g., Ames assay results), the biological complexities of a molecule in the body are not known. In fact, many times PK/PD data are not known for chemicals tested in cancer bioassays. An analysis of the literature by Rozman and Doull[11] indicates that the $c \times t$ concept holds true for most chemicals, but there may be some variability dependent on a number of factors including dynamics and kinetics. An understanding of these limitations is critical for practical application of the science to "real world" situations. Further data such as half-life, types of adducts formed, or short-term versus long-term *in vivo* genotoxicity data could show the relevance of Haber's rule. Such data were generated for ethyl methanesulfonate as follow-up to contamination in Viracept[14] but is rarely generated for a GTI or PGI.

For intermittent or short-term exposure to unavoidable GTIs via pharmaceuticals, the concept of lifetime cumulative dose should be considered on the basis of precedence of available guidances. Standard risk assessments of known carcinogens operate under the assumption that cancer risk increases as a function of cumulative dose. The United States Environmental Protection Agency (USEPA) "*Guidelines for Carcinogen Risk Assessment*"[10] advocate this assumption stating that "... a high dose of a carcinogen received over a short period of time is equivalent to a corresponding low dose spread over a lifetime," so that the cancer risk of a continuous low dose over a lifetime

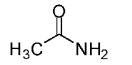
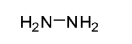
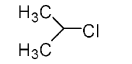
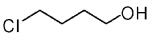
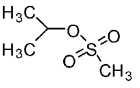
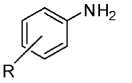
(11) Rozman, K. K.; Doull, J. *J. Pharmacol. Exp. Ther*. **2001**, *296*, 663–668.

(12) Druckery, H. Quantitative Aspects in Chemical Carcinogenesis. In *Potential Carcinogenic Hazards from Drugs*; Truhaut, R., Ed.; UICC Monograph Series, Vol. 7; Springer-Verlag: New York, 1967; pp 60−78.

(13) Halmes, N. C.; Roberts, S. M.; Tolson, J. K.; Portier, C. J. *Toxicol. Sci*. **2000**, *58*, 32–42.

(14) Gocke, E.; Ballantyne, M.; Whitwell, J.; Muller, L. *Toxicol. Lett*. **2009**, *190*, 286–297.

Table 2. **Toxicology limits for genotoxic impurities (GTIs)**

Compound	GTI Category[a]	Carcinogenicity Slope Factor (CSF) [b]	Acceptable Lifetime Cumulative Dose[c] *(daily chronic limit)*[d]	Acceptable 2-year weekly dose *(short-term limit)*[d]	Chosen limit for 2-year weekly dosing[e]
Acetamide 	Category 1 (Animal carcinogen)[f]	0.07/mg/kg/day[g]	255 mg *(10 ppm)*	2450 mcg[h] *(2450 ppm)*	1000 ppm[i]
Hydrazine 	Category 1 (Animal Carcinogen)[j]	3.0/mg/kg/day[j]	5960 mcg *(0.2 ppm)*	50 mcg[k] *(50 ppm)*	50 ppm
Isopropyl Chloride 	Category 2 (+ Ames)	NA[l]	TTC at 38 mg *(1.5 ppm)*	350 mcg[m] *(350 ppm)*	350 ppm
4-Chloro-1-butanol 	Category 2 (+ Ames)	NA[l]	TTC at 38 mg *(1.5 ppm)*	350 mcg[m] *(350 ppm)*	350 ppm
Isopropyl mesylate 	Category 2 (+ Ames)[n]	NA[l]	TTC at 38 mg *(1.5 ppm)*	350 mcg[m] *(350 ppm)*	350 ppm
Aniline Alert 	Category 3 (Structural Alert)	NA[l]	TTC at 38 mg *(1.5 ppm)*	350 mcg[m] *(350 ppm)*	350 ppm

[a] Based on GTI classification scheme as described by Dobo, K. L.; Greene, N.; Cyr, M. O.; Caron, S; Ku, W. W. *Regul. Toxicol. Pharmacol*. **2006**, *44*, 282−293. [b] Derived from positive carcinogenicity bioassay data and defined by US EPA as: "... an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent." See Integrated Risk Information System (IRIS) Glossary. Last updated March 16, 2010. [Online: http://www.epa.gov/ncea/iris/help_gloss.htm (accessed April 27, 2010).] [c] One in 100,000 excess cancer risk; 10^{-5} = CSF [in $(mg/kg/day)^{-1}$] × exposure (mg/kg/day); cumulative dose calculated over 70-year lifetime, assuming exposure for 365 days per year. [d] Limits relative to a daily dose of 1000 mg API. [e] To protect for noncarcinogenic effects, the chosen limit should be the lower of the appropriate GTI and ICH Q3A limits. [f] Classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) based on rodent toxicity data. Conservatively considered genotoxic based on Chieli, E.; Aliboni, F.; Saviozzi, M.; Malvaldi, G. *Mutat. Res.* **1987**, *192*, 141−143. [g] California Environmental Protection Agency. Office of Environmental Health Hazard Assessment. Toxicity Criteria Database: Cancer Potency. [Online: http://www.oehha.org/risk/chemicaldb/cancerpotency.asp?name=acetamide&number=60355 (accessed April 27, 2010).] [h] Limit based on carcinogenicity potency value and protective for threshold effects observed in dietary and reproductive/developmental toxicity studies as reported in: British Industrial Biological Research Association (BIBRA) Toxicology International. *Toxicity Profile. Acetamide*; 2006. [i] Limited by ICH Q3A qualification threshold (i.e., 0.15% or 1 mg, whichever is less). [j] United States Environmental Protection Agency. Integrated Risk Information System (IRIS). Hydrazine/Hydrazine Sulfate (CASRN 302-01-2). Last revised February 1, 1991. [Online: http://www.epa.gov/ncea/iris/subst/0352.htm (accessed April 27, 2010)]. [k] Permissible daily exposure for neurological effects observed in cancer patients using hydrazine as a chemotherapeutic; using the proposed modified TTC approach, the limit based on carcinogenicity for an exposure duration ≤2 years would be 57 μg. [l] Not available. [m] Lifetime Cumulative Dose/Intermittent Duration of Exposure = 38 mg/(52 days/year × 2 years). [n] Glowienke, S.; Frieauff, W.; Allmendinger T.; Martus, H. J.; Suter, W.; Müller, L. *Mutat. Res.* **2005**, *581*, 23−34. Note that structural similarity suggests possible threshold for genotoxicity as described for ethyl methanesulfonate in: Müller, L.; Gocke, E. *Toxicol. Lett*. **2009**, *190*, 330−332.

would be equivalent to the cancer risk associated with an identical cumulative exposure averaged over a shorter period of time (or a lifetime average daily dose - LADD). However, USEPA acknowledges that this approach is not always applicable and might not hold for more intense, less frequent exposures. The USEPA cancer risk assessment guidelines caution: "This approach becomes more problematical as the exposures in question become more intense but less frequent, especially when there is evidence that the agent has shown dose-rate effects." Furthermore, if higher daily exposure occurs over a sensitive life stage, then USEPA recommends additional conservatism such as the application of safety factors depending on the sensitivity of the carcinogen.

Bos et al. (2004)[15] completed an independent analysis of the current state of science for short-term exposure to carcinogens and provided guidance on acceptable exposure. Like USEPA, Bos et al. suggested that certain sensitive subpopulations may require a more conservative assessment. In contrast to USEPA, Bos guidance provides that a single exposure could be equivalent to the total cumulative lifetime dose in a single

(15) Bos, P. M.; Baars, B. J.; van Raaij, M. T. *Toxicol. Lett*. **2004**, *151*, 43–50.

day, or the virtually safe dose (the chronic daily dose associated with a negligible risk value) multiplied by 25,000 (the number of days in a lifetime). In 2006, Pharmaceutical and Research Manufacturers of America (PhRMA)[3] adopted a similar approach to allow higher daily exposures of GTIs with unknown carcinogenic potential in clinical trial material. However, PhRMA applied additional conservatism for phase I clinical trials mainly because subjects (e.g., volunteers) lacked benefit from the drug substance. Therefore, the acceptable risk target was lowered from 10^{-5} to 10^{-6}. PhRMA coined this approach the "staged TTC."[3] EMEA CHMP[7] and the USFDA[6] agreed with the fundamental concepts of the staged TTC, but divided the acceptable daily doses for each phase of clinical trials by a factor of 2 to account for uncertainties that may exist from linearly extrapolating a cumulative lifetime dose over a short-term duration. Regardless, the underlying principles of the EMEA CHMP and USFDA strategies align with Haber's rule (i.e., effect is a function of concentration and time, or $c \times t = k$). Thus, based on the scientific and regulatory precedence, taking into account duration of exposure for marketed anticancer compounds would allow control of potential exposure to a negligible excess cancer risk (1 in 100,000) with a higher daily allowance during shorter or intermittent durations.

Effect of Modified TTC on Chemistry Control

Regardless of the GTI limit deemed acceptable, a control strategy must be established for each genotoxic impurity introduced or formed in the API process.[16] A GTI control strategy is based on the same types of process knowledge needed to design a control strategy for non-GTI. This includes defining the point of entry and source (e.g., starting material, solvent, processing chemistry) of the impurity and the ability of purification and processing operations to remove the impurity. GTI control must also be considered in concert with an overall API control strategy. An API process design that eliminates the introduction or production of GTIs is, in principle, ideal. This may not be possible (or desired) when all aspects of the control strategy and patient requirements are considered. Robust control strategies for non-GTIs, residual solvents, and the API physical characteristics (e.g., polymorph, surface area, particle size) are also essential. For example, use of acetonitrile as a solvent may introduce acetamide into a process and thus may not be a preferred choice for a solvent, especially in the final step. However, if acetonitrile provides uniquely robust impurity removal or enables the crystallization of a more stable or more bioavailable polymorph of the API, the use of acetonitrile provides direct benefit to the patient if a suitable acetamide control strategy can be implemented.

GTI control strategy design requires analytical methods with appropriate sensitivity to quantitate the GTI level in the starting materials/reagents, reaction mixtures, isolated intermediates, and if necessary, the API. This mapping work is necessary, irrespective of acceptable GTI limit. However, the magnitude of the GTI limit may have greater impact on the effort and technology required to develop analytical methodology and necessary purification operations to remove the GTI. We have qualitatively evaluated the impact of GTI control on the development efforts required for API processes requiring control of two common GTIs, acetamide and 4-chloro-1-butanol, over a recent two year period. Acetamide was evaluated due to its origin from acetonitrile, as described above. The 4-chloro-1-butanol impurity is often formed in reaction mixtures containing tetrahydrofuran (THF) and HCl. In each project evaluated, standard processing operations (e.g., extraction, crystallization, chemical/processing degradation, distillation) were sufficient to reduce or control the levels of these two GTIs to levels below acceptable limits. Except in cases where the GTI entered the process in the final processing step, control of the GTI to acceptable levels was achieved by purification of an intermediate.

Each API process and the impurities of concern will present unique GTI control challenges. In the above assessment of acetamide and 4-chloro-1-butanol, we found that the magnitude of the GTI limit had a greater impact on the effort required for analytical method development than on the development of chemistry and processing operations.

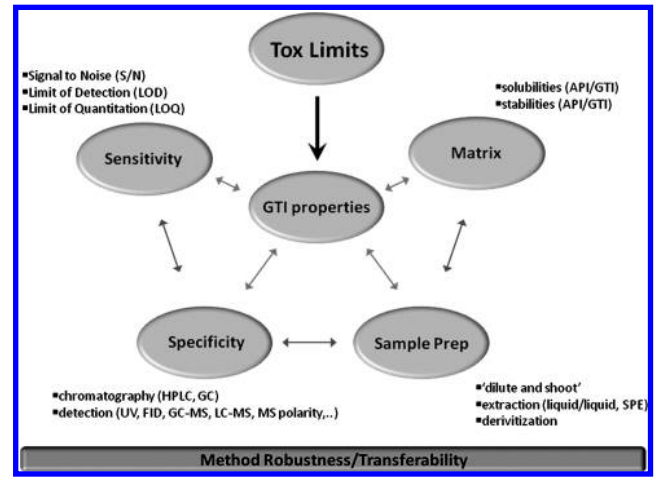


Figure 2. **Lower toxicology limits can increase the difficulty of developing robust and transferable analytical methods due to factors associated with characteristics of the analyses and due to properties of the GTIs.**

Effects of Modified TTC on Analytical Method Development

Establishing a suitable assay to quantitate GTIs requires an assessment of the interactions between key parameters.[5] Specificity, sensitivity, sample preparation, and matrix contributions, along with the properties of the GTI itself, must all be evaluated (Figure 2). When considering potential risk to the patient, genotoxic impurities must often be controlled to much lower levels than required by the ICH Q3A(R2) guideline for non-GTIs. Thus, as the toxicological limit decreases, the challenges associated with these parameters typically increase, and specialized analytical methods must be developed that are capable of quantifying analytes at parts per million (ppm) or even parts per billion (ppb) levels.

The given toxicological limit dictates the required limit of detection (LOD) and limit of quantitation (LOQ) for the analytical method. Methods must be adequately sensitive,

(16) For a recent example of the design and development of control strategies for sulfonate ester GTIs in an API see: Cimarosti, Z.; Bravo, F.; Stonestreet, P.; Tinazzi, F.; Vecchi, O.; Camurri, G. *Org. Process Res. Dev.* **2010**, *14*, DOI: 10.1021/op900242x(Web release date: 6 Nov 2009).

capable of detecting and quantifying the GTI across a wide concentration range that brackets the given limit. We recommend that a method should have an LOQ of at least 2−10 times below the toxicology limit to allow acquisition of trending data, facilitate route development, and guide the control strategy. The ability to detect and quantitate a GTI, however, can be impacted by a number of factors, including complications from the matrix. Poor sample solubility, poor recovery of the analyte from the sample matrix, interferences from the main component or other impurities, or degradation products in the main component itself can compromise both selectivity and sensitivity. Sample preparation is also critical to achieving the appropriate LOD and LOQ. A variety of approaches are available for preparing samples for analysis with the goal of introducing as much analyte into the detector as possible while minimizing matrix contributions. These range from the simplest 'dilute and shoot' approach to more complicated extractions and derivatizations.

The specificity of a method is its ability to distinguish a signal response related to an analyte from signal contributions due to other sources, such as matrix and background. Obtaining adequate specificity is critical to the development of trace level methods for GTIs due to the direct impact on signal-to-noise (S/N), and is achieved through the combination of effects from the sample preparation, chromatography, and detection technique. While common UV and FID detectors are very sensitive, they often lack the specificity required to differentiate an analyte from matrix artifacts and other low level impurities at trace levels. Alternatively, mass spectrometry (MS) is an ideal technique for GTI analysis. When operated as a mass filter, specificity and S/N are maximized by detecting only selected species related to the analyte, based on their unique mass-to-charge ratio (*m*/*z*), while ignoring all other species present. Instruments capable of detection via accurate mass, such as time-of-flight (TOF) MS, may be used in certain applications with even greater improvements in specificity and S/N. One caveat with MS is that the analyte must be ionized (either positively or negatively) for detection, and due to the nature of the various ionization techniques currently available, none are considered universal.

The amount of information needed to characterize any analytical method increases as the drug candidate progresses along the development pathway. Although not typically required for early phase drug development activities, a thorough evaluation of the parameters outlined above can provide a high level of confidence in the data. An assessment of method specificity and sensitivity ensures the GTI can be detected well below the toxicology limit without concern for interferences from the matrix or background. A response curve bracketing the toxicology limit allows measurement of the rejection efficiency across a range of values for the chemistry being developed. An evaluation of in-matrix standard spike recovery demonstrates method efficiency. We suggest a reasonable spike recovery range for ppm or ppb levels of between 70 and 130%. The stability of the analyte, or its derivative, is understood to the extent that it fits within the scope of use of the method.

Early on, these data support process development efforts and provide confidence in the GTI control strategy of the given synthetic route. As the molecule progresses through clinical development, the GTI method becomes critical to monitoring API manufacture and ensuring patient safety. As dose and duration of the compound evolve, the methods are revisited and requalified as necessary. If required in a quality control setting, the challenge becomes the development of a robust method that performs consistently in a broad spectrum of laboratories by a variety of analysts. The knowledge gained from early development using advanced instrumentation by experienced personnel may be applied to simplify and standardize a method as much as is reasonably practical, without compromising the quality of the results.

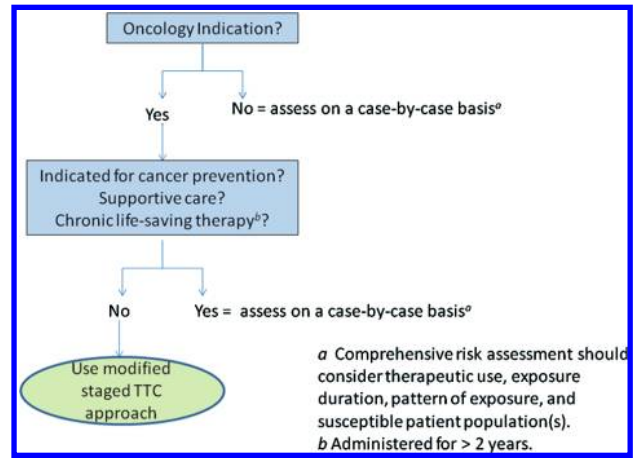


Figure 3. **Decision tree to guide application of the proposed strategy for oncolytics and the possible extension of the proposed strategy to drug substances with short-duration, non-lifesaving indications.**

Adopting higher acceptable GTI limits for marketed compounds targeted for short-term exposure does not necessarily impact analytical methodology used during the early phases of drug development since appropriate methods are required to assess process capabilities and demonstrate control at low levels. Higher limits do, however, typically result in methods that are quicker to develop and require less sophisticated expertise and analytical capabilities since standard sample preparation techniques and instrumentation can be used. In a situation where the limit for acetamide was 130 ppm, for example, we developed a method quickly using a dilute and shoot sample preparation approach with GC/MS detection. In a second case, the solubility of the sample matrix, combined with a toxicological limit of 0.3 ppm, necessitated an organic/aqueous extraction in order to appropriately prepare the sample. Although these assays were not required in a quality control setting, the former case required only days to develop while the latter required weeks. The former is also more robust and amenable to lab transfers. Thus, the limits adjusted by duration of exposure can potentially speed medicines to the market, while assuring patient safety.

Conclusion

The scientific basis supporting higher limits for intermittent or short-term exposure to unavoidable GTIs is rooted in the application of both dose and duration in toxicology (i.e., Haber's Law). Precedence established by available regulatory guidelines indicates that cancer risk increases as a function of cumulative dose. These guidance documents support limits that protect for

the lifetime cumulative dose over a shorter-term duration. The strategy proposed here supports GTI limits in oncolytics administered daily over two years that are commensurate with an exposure of approximately 50 μg /day and a 1 in 100,000 excess cancer risk. Therefore, the proposed limit is 30-fold higher than the currently outlined staged approach for clinical trial material administered over more than 12 months (i.e., 1.5 μg/day TTC associated with a 1 in 100,000 excess cancer risk over a chronic duration of 70 years). This proposal maintains that the higher GTI limits for intermittent or short-term exposure via pharmaceuticals are associated with negligible patient safety risk in comparison with chronic exposure at the TTC. In application, additional considerations might include PK/PD effects as well as mechanism.

This modified staged TTC approach did not consider the life-saving benefit of anticancer compounds or that the therapeutic dose of many anticancer drugs has inherent risks, including genotoxicity. The current guidance documents from EMEA, USFDA, and ICH allow for higher limits with life-saving medications, especially for medications indicated for advanced cancer, but the principles applied in this manuscript restrict higher limits to a negligible cancer risk of 10^{-5} despite the benefit of the medication. Therefore, one could apply the same principles for nonlife-saving medications provided that differences in exposure duration, pattern of exposure, and susceptible patient populations are considered (Figure 3). In many cases, the modified staged TTC limit will be below the ICH Q3A limit, but above the chronic TTC. If the modified staged TTC exceeds the ICH Q3A limit, Q3A thresholds should guide the final recommended limit to protect for noncarcinogenic effects. The extension of the risk assessment strategy discussed above to short-duration non-lifesaving indications warrants further regulatory and scientific discussion.

Received for review January 28, 2010.

OP1000226