Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal products

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1. Introduction

The synthesis of drug substances involves the use of reactive chemicals, reagents, solvents, catalysts, and other processing aids. As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated veterinary medicinal products (VMPs). While VICH GL10: Impurities in New Veterinary Drug Substances (Ref. 1) and VICH GL11 (Ref. 2): Impurities in New Veterinary Medicinal Products provide guidance for qualification and control for the majority of the impurities, limited guidance is provided for those impurities that are DNA reactive. The purpose of this guideline is to provide a practical framework that is applicable to the identification, categorisation, qualification, and control of these mutagenic impurities, to limit potential carcinogenic risk associated with the exposure to potentially mutagenic impurities. This guideline is intended to complement VICH GL10 and VICH GL11.

This guideline considers both safety and quality risk management in establishing levels of mutagenic impurities that are expected to pose negligible carcinogenic risk. It outlines recommendations for assessment and control of mutagenic impurities that remain or are reasonably expected to remain in the final drug substance or VMP.

The approach of this guideline is based on that of ICH guideline M7 (Ref. 3) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk which was used as a template, with amendments introduced in order to cover the issues specific to VMPs.

2. Scope of guideline

This document is intended to provide guidance for new veterinary drug substances and new VMPs, including cases where drug substances can be used in both human and veterinary drug products.

Post-approval submissions of authorised products (i.e., variations), or applications for new marketing authorisations for VMPs that include drug substances that have previously been present in authorised VMPs are also in the scope of this guideline, but only in cases where:

- Changes to the synthesis of the drug substance result in new impurities or increased acceptance criteria for specified impurities;

- Changes in the formulation, composition or manufacturing process result in new degradation products or increased acceptance criteria for specified degradation products;

- Changes in indication, dosing regimen or target species are made which significantly affect the acceptable exposure level.

Assessment of the mutagenic potential of impurities as described in this guideline is not intended for the following types of drug substances and drug products: biologicals/biotechnologicals, peptides, oligonucleotides, radiopharmaceuticals, fermentation products, herbal products, and crude products of animal or plant origin. This guideline does not apply to drug substances and drug products intended for advanced cancer indications or cases where a drug substance intended for other indications is itself genotoxic at therapeutic concentrations and may be expected to be associated with an increased cancer risk. Exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the drug substance. Assessment of the mutagenic potential of impurities as described in this guideline is not intended for excipients used in existing authorised products, flavouring agents, colourants, or perfumes. Application of this guideline to leachables associated with VMP packaging is not intended, but the safety risk assessment principles outlined in this guideline for limiting potential carcinogenic risk can be used if warranted.

The guideline aims to describe a framework for setting acceptable limits for genotoxic impurities with, in some cases, different considerations for companion and/or food-producing animals. It focuses on risk (management) for the target animal, which is expected to receive a health benefit from exposure to the VMP, and for the human consumer, who may be exposed to residues via food of animal origin and who does not receive a direct health benefit. It is therefore important that the consumer's exposure is below the 'virtually safe dose'.

DNA reactive impurities should also be considered as part of the user risk assessment (URA) in which the applicant needs to assure that the 'virtually safe dose' will not be exceeded.

3. Legal basis

Directive 2009/9/EC specifies that, in relation to the drug substance, information on the levels, nature and safety of predictable impurities shall be provided. In relation to the finished product, the Directive specifies that maximum levels of individual and total degradation products should be specified. The guidelines named below address these requirements more specifically and this document should be read in conjunction with these.

VICH GL10: Guideline on impurities in new veterinary drug substances (EMEA/CVMP/VICH/837/99-Rev.1) (Ref. 1)

VICH GL11: Guideline on impurities in new veterinary medicinal products (EMEA/CVMP/VICH/838/99-Rev.1) (Ref. 2)

VICH GL18(R): Impurities: Residual solvents in new veterinary medicinal products, actives substances and excipients (Revision) (EMA/CVMP/VICH/502/99-Rev.1) (Ref. 4)

In addition, VICH GL23: Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing (EMA/CVMP/VICH/526/2000), Ref. 5 provides useful background in relation to the evaluation of genotoxic impurities.

4. General principles

This Guideline provides an approach for assessing actual and potential impurities that have a potential to directly cause DNA damage and that are likely to arise during the synthesis and storage of a new drug substance, and during manufacturing and storage of a new VMP. It should ensure that such impurities are controlled to safe levels even when present at levels below the VICH GL10/11 qualification threshold. Impurities present above the qualification threshold should additionally be qualified regarding other toxicity endpoints according to VICH GL 10/11. Other types of genotoxins that are non-mutagenic typically have threshold mechanisms and usually do not pose carcinogenic risk at the levels ordinarily present as impurities.

From a target animal safety perspective, acceptable limits for mutagenic impurities in VMPs corresponding to an intake with a theoretical 1 in 100,000 excess lifetime risk of cancer can be justified, in analogy to the approach for human patients. This represents a small theoretical increase in risk when compared to overall lifetime incidence of developing any type of cancer, but is acceptable as the animal is expected to receive a health benefit from the VMP. Acceptable intakes can be derived using substance specific carcinogenicity data for the concerned impurity, or be based on the threshold of toxicological concern (TTC) for genotoxic carcinogens, resulting in an acceptable intake of 0.025 µg/kg bw/day. For target animals, the approaches to derive acceptable intakes are specified in section 8.

It is noted that established cancer risk assessments are based on lifetime exposures. Estimation of risk based on Less-Than-Lifetime (LTL) exposures can result in higher acceptable intakes of impurities and still maintain comparable risk levels. The calculation of LTL acceptable intakes for mutagenic impurities is described in ICH M7 (Ref. 3). In order to apply this concept for VMPs, the expected lifetime of the animal species in years, as well as the total number of treatment days, should be taken into consideration. For companion animals, additional potential justifications for exceeding the (default) TTC-based acceptable intake of 0.025 µg/kg bw/day other than less-than-lifetime exposure, may apply, including: treatment of a life-threatening condition, limited therapeutic alternatives, or where the impurity is a known substance and exposure will be much greater from other sources (e.g. food or endogenous metabolism).

For food-producing animals the TTC-based acceptable intake of 0.025 µg/kg bw/day (or the substance–specific acceptable intake) should not be exceeded, since consumers exposed to residues via food of animal origin are not expected to receive a health benefit. It can be pragmatically assumed that the amount of impurity ingested by the consumer will be lower than the 'virtually safe dose', if the amount of the impurity to which the target animal is exposed is below 0.025 µg/kg/day. A higher dose of the impurity applied to the target animal (as described for companion animals) may be justified in exceptional cases. The applicant needs to ensure that consumer exposure is below the 'virtually safe dose' and that consumer safety is not affected. Any deviation from this guidance should be supported with suitable data.

Appendix 1 contains an example showing how the acceptable intake for target animals (e.g. TTC-based) is converted to a specific concentration limit for a drug substance.

The presence of DNA reactive impurities to which the user may be exposed as a result of treating companion or food-producing animals should be addressed as part of the user safety assessment, i.e., the applicant needs to ensure that the user's exposure is below the 'virtually safe dose'.

Where a potential risk has been identified for an impurity, an appropriate control strategy taking into account understanding of manufacturing processes and/or analytical controls should be developed to ensure that the mutagenic impurity is at or below the acceptable level.

There may be cases when an impurity is also a metabolite of the drug substance. In such cases the risk assessment that addresses mutagenicity of the metabolite can qualify the impurity.

5. Considerations for authorised products

This guideline is not intended to be applied retrospectively (i.e., to VMPs marketed prior to adoption of this guideline). However, some types of post-approval changes warrant a reassessment of safety in relation to mutagenic impurities. This section applies to these post-approval changes for VMPs marketed prior to, or after, the adoption of this guideline. Section 9.5 (Lifecycle Management) contains additional recommendations for VMPs marketed after adoption of this guideline.

5.1. Post approval changes to the drug substance chemistry, manufacturing, and controls

Post-approval submissions (variations) involving the chemistry, manufacturing, and controls on the drug substance should include an evaluation of the potential risk associated with mutagenic impurities from changes to: the route of synthesis, reagents, solvents, or process conditions after the starting material. Specifically, changes should be evaluated to determine if they result in any new mutagenic impurities, or higher acceptance criteria for existing mutagenic impurities. Re-evaluation of impurities not affected by such changes is not required. For example, when only one aspect of the manufacturing

process is changed, the assessment of risk from mutagenic impurities should be limited to whether any new mutagenic impurities result from the change, whether any mutagenic impurities formed during the affected step are increased, and whether any known mutagenic impurities from up-stream steps are increased. Regulatory submissions associated with such changes should describe the assessment as outlined in Section 10. Changing the site of manufacture of drug substance, intermediates, or starting materials, or changing raw materials supplier will not require a reassessment of mutagenic impurity risk.

When a new manufacturer of drug substance, intermediate or starting material is proposed, evidence that the substance or material produced by this manufacturer is produced using the same route of synthesis for the substance already used in an existing VMP marketed in the EU, is considered to be sufficient evidence of acceptable risk regarding mutagenic impurities and an assessment in accordance with this guideline is not required. If this is not the case, then an assessment in accordance with this guideline is expected.

5.2. Post approval changes to the drug product chemistry, manufacturing, and controls

Post-approval submissions involving the VMP (e.g., change in composition, manufacturing process, dosage form) should include an evaluation of the potential risk associated with any new mutagenic degradation products or any proposal to increase the acceptance criteria for existing mutagenic degradation products. If appropriate, the regulatory submission should include an updated control strategy. Re-evaluation of the drug substance(s) associated with VMPs is not recommended or expected, provided there are no changes to the drug substance(s). Changing the site of manufacture of a VMP will not require a reassessment of mutagenic impurity risk.

5.3. Changes to the clinical use of authorised products

Changes to the clinical use of authorised VMPs that can warrant a re-evaluation of the mutagenic impurity limits include a significant increase in approved dose or an increase in duration of use. Re-evaluation may also be warranted in case of a change in, or addition of, an indication, such as from a serious or life-threatening condition, where higher acceptable intakes were justified, to an indication for a less serious condition, where the existing impurity acceptable intakes may no longer be appropriate.

5.4. Other considerations for authorised products

Application of this guideline to authorised VMPs is warranted if there is specific cause for concern. The existence of structural alerts in an impurity alone is considered insufficient to trigger follow-up measures, unless it is a structure in the cohort of concern (i.e., high potency mutagenic carcinogens for which the TTC is not sufficiently protective, such as aflatoxin-like-, N-nitroso-, and alkyl-azoxy compounds).

A specific cause for concern would be new relevant hazard data on the impurity (classified as Class 1 or 2, i.e., known mutagenic carcinogens and known mutagens with unknown carcinogenic potential, see Table 1), generated after the overall control strategy and specifications for authorisation were established. These new relevant hazard data should be derived from high-quality scientific studies consistent with relevant regulatory testing guidelines, with data records or reports readily available. Similarly, a newly discovered impurity that is a known Class 1 or Class 2 mutagen that is present in an authorised VMP could also be a cause for concern. In both of these cases, when the applicant becomes aware of this new information, an evaluation in accordance with this guideline should be conducted.

6. Drug substance and veterinary medicinal product impurity assessment

Actual and potential impurities that are likely to arise during the synthesis and storage of a new drug substance, and during manufacturing and storage of a new VMP should be assessed.

The impurity assessment is a two-stage process:

- Actual impurities that have been identified should be considered for their mutagenic potential.

- An assessment of potential impurities likely to be present in the final drug substance is carried out to determine if further evaluation of their mutagenic potential is required.

The steps as applied to synthetic impurities and degradation products are described in Sections 6.1 and 6.2, respectively.

6.1. Synthetic impurities

Actual impurities include those observed in the drug substance above the VICH GL10 reporting thresholds. Identification of actual impurities is expected when the levels exceed the identification thresholds outlined by VICH GL10. However, it is acknowledged that some impurities below the VICH GL10 identification threshold may also have been identified.

Impurities found above the VICH GL10 reporting threshold, but below the VICH GL 10 identification threshold should be considered in the mutagenicity evaluation as potential impurities.

Potential impurities in the drug substance can include starting materials, reagents, by-products and intermediates in the route of synthesis, from the starting material to the drug substance.

The risk of carryover into the drug substance should be assessed for identified impurities that are present in starting materials and intermediates, and impurities that are reasonably expected by-products in the route of synthesis from the starting material to the drug substance. As the risk of carryover may be negligible for some impurities (e.g., those impurities in early synthetic steps of long routes of synthesis), a risk-based justification could be provided for the point in the synthesis after which these types of impurities should be evaluated for mutagenic potential.

For starting materials that are introduced late in the synthesis of the drug substance (and where the synthetic route of the starting material is known) the final steps of the starting material synthesis should be evaluated for potential mutagenic impurities.

Actual impurities where the structures are known, and potential impurities, as defined above, should be evaluated for mutagenic potential as described in Section 7.

6.2. Degradation products

Actual drug substance degradation products include those observed above the VICH GL10 reporting threshold during storage of the drug substance, in the proposed long-term storage conditions and primary and secondary packaging.

Actual degradation products in the VMP include those observed above the VICH GL11 reporting threshold during storage of the VMP in the proposed long-term storage conditions and primary and secondary packaging, and also include those impurities that arise during the manufacture of the VMP. Identification of actual degradation products is expected when the levels exceed the identification thresholds outlined by VICH GL 10/11. However, it is acknowledged that some degradation products below the identification threshold may also have been identified. Degradation products found above the

VICH GL10/11 reporting threshold, but below the VICH GL 10/11 identification threshold should be considered in the mutagenicity evaluation as potential degradation products.

Potential degradation products in the drug substance and VMP are those that may be reasonably expected to form during long term storage conditions. Potential degradation products include those that form above the VICH GL 10/11 identification threshold during accelerated stability studies (e.g., 40°C/75% relative humidity for 6 months), but are yet to be confirmed in the drug substance or VMP under long-term storage conditions in the primary packaging.

Knowledge of relevant degradation pathways can be used to help guide decisions on the selection of potential degradation products to be evaluated for mutagenicity, e.g., from degradation chemistry principles, relevant stress testing studies, and development stability studies.

Actual and potential degradation products likely to be present in the final drug substance or VMP, and where the structure is known, should be evaluated for mutagenic potential as described in Section 7.

7. Hazard assessment elements

Hazard assessment involves an initial analysis of actual and potential impurities by conducting database and literature searches for carcinogenicity and bacterial mutagenicity data in order to classify them as Class 1, 2 or 5 according to Table 1. If data for such a classification are not available, an assessment of Structure-Activity Relationships (SARs) that focuses on bacterial mutagenicity predictions should be performed. This could lead to classification as Class 3, 4 or 5.

Table 1. Impurities Classification with Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions, see also Appendix 2 (Decision tree of impurity classification)

Class	Definition	Proposed action for control (details in Section 8 and 9)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)	Control at or below acceptable limits (TTC-based acceptable intake)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (TTC-based acceptable intake) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

*Or other relevant positive mutagenicity data indicative of DNA-reactivity related induction of gene mutations (e.g., positive findings in *in vivo* gene mutation studies)

A computational toxicology assessment should be performed using (Q)SAR methodologies that predict the outcome of a bacterial mutagenicity assay. Two (Q)SAR prediction methodologies that complement each other should be applied. One methodology should be expert rule-based and the second methodology should be statistical-based. (Q)SAR models utilizing these prediction methodologies should follow the general validation principles set by the Organisation for Economic Co-operation and Development (OECD).

The absence of structural alerts from the two complementary (Q)SAR methodologies is sufficient to conclude that the impurity is of no mutagenic concern, and no further testing is recommended (Class 5 in Table 1).

If warranted, the outcome of any computer system-based analysis can be reviewed with the use of expert knowledge in order to provide additional supportive evidence on relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.

To follow up on a relevant structural alert (Class 3 in Table 1), either adequate control measures could be applied or a bacterial mutagenicity assay with the impurity alone can be conducted. An appropriately conducted negative bacterial mutagenicity assay (see Note 1) would overrule any structure-based concern, and no further genotoxicity assessments would be recommended. These impurities should be considered non-mutagenic (Class 5 in Table 1). A positive bacterial mutagenicity result would warrant further hazard assessment and/or control measures (Class 2 in Table 1). For instance, when levels of the impurity cannot be controlled at an appropriate acceptable limit, it is recommended that the impurity be tested in an *in vivo* gene mutation assay (e.g., transgenic rodent gene mutation assay, OECD TG 488) in order to understand the relevance of the bacterial mutagenicity assay result under *in vivo* conditions. The selection of other *in vivo* genotoxicity assays should be scientifically justified, based on knowledge of the mechanism of action of the impurity and expected target tissue exposure. *In vivo* studies should be designed taking into consideration existing VICH genotoxicity guidelines.

An impurity with a structural alert that is shared (e.g., same structural alert in the same position and chemical environment) with the drug substance or related compounds can be considered as non-mutagenic (Class 4 in Table 1) if the testing of such material (drug substance or related compounds) in the bacterial mutagenicity assay was negative.

8. Risk characterisation

As a result of the hazard assessment described in Section 7, each impurity will be assigned to one of the five classes in Table 1. For impurities belonging in Classes 1, 2, and 3, the principles of risk characterization used to derive acceptable intakes are described in this section.

8.1. Threshold of Toxicological Concern (TTC) based acceptable intakes

From the point of view of target animal safety, a TTC-based acceptable intake of a mutagenic impurity of 0.025 µg/kg bw per day is considered to be associated with a small theoretical increase in risk and would usually be used for mutagenic impurities present in VMPs intended for long-term treatment and where no carcinogenicity data are available (Classes 2 and 3).

8.2. Acceptable intakes based on compound-specific risk assessments

8.2.1. Mutagenic impurities with positive carcinogenicity data (Class 1)

Compound-specific risk assessments to derive acceptable intakes should be applied instead of the TTC-based acceptable intakes where sufficient carcinogenicity data exist. For a known mutagenic carcinogen, a compound-specific acceptable intake can be calculated based on carcinogenic potency and linear extrapolation as a default approach (see also Addendum to ICH M7, Ref. 3). Alternatively, other established risk assessment practices, such as those used by international regulatory bodies, may be applied either to calculate acceptable intakes or to use already existing values published by regulatory authorities.

Compound-specific calculations for acceptable intakes can be applied case-by-case for impurities which are chemically similar to a known carcinogen compound class (class-specific acceptable intakes), provided that a rationale for chemical similarity and supporting data can be demonstrated.

8.2.2. Mutagenic impurities with evidence for a practical threshold

The existence of mechanisms leading to a dose response that is non-linear or has a practical threshold is increasingly recognised, not only for compounds that interact with non-DNA targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage. The regulatory approach to such compounds is based on calculation of a permitted daily exposure.

The permitted daily exposure (PDE) is preferably derived from the No Observed (Adverse) Effect Level (NO(A)EL) in the most relevant animal study. The modifying (uncertainty) factors comprise factors to account for, e.g., extrapolation between species, variability between individuals, and/or short-term toxicological studies (as described in VICH GL18, Appendix 3).

8.3. Acceptable intakes in relation to less-than-lifetime (LTL) exposure for companion animals

Standard risk assessments of known carcinogens assume that cancer risk increases as a function of cumulative dose. Thus, the cancer risk of a continuous low dose over a lifetime would be equivalent to the cancer risk associated with an identical cumulative exposure, averaged over a shorter duration.

The TTC-based acceptable intake of 0.025 µg/kg bw/day is considered to be protective for a lifetime of daily exposure. To address LTL exposures to mutagenic impurities in pharmaceuticals, an approach is applied in which the acceptable cumulative lifetime dose is uniformly distributed over the total number of exposure days during LTL exposure. This would allow higher daily intake of mutagenic impurities than would be the case for lifetime exposure and still maintain comparable risk levels for daily and non-daily treatment regimens.

The LTL concept can only be applied for companion animals; however, the approach described in the ICH M7 (Ref. 3) guideline uses an estimated human lifespan of 70 years. A parallel approach cannot be directly applied to companion animals, due to the large variety of their expected lifespans. If the applicant proposes increased acceptable intakes of mutagenic impurities for limited treatment periods, then a scientifically justified description of how the LTL concept is used will be required. The proposed increased acceptable intake depends on factors such as breed-specific mean life span, duration of treatment, number of treatment days, severity of indication, limited therapeutic alternatives, etc.

In analogy with ICH M7 (Fig. 1), a linear relationship between the daily intake of a mutagenic impurity and the number of treatment days should be provided by the applicant, although the individual

example depends on the animal species. The calculation should be based on the TTC-based acceptable intake (i.e. 0.025µg/kg bw/day) using the formula:

0.025 µg/kg bw x (365 days x Expected years lifetime) divided by total number of treatment days

Estimation of risk based on the LTL approach is not accepted for substances administered to food-producing animals as consideration needs to be given to potential consumer exposure to residues, which could be chronic (potential lifetime exposure is assumed) even if target animal exposure is for only a short duration.

8.4. Acceptable intakes for multiple mutagenic impurities

The TTC-based acceptable intakes should be applied to each individual impurity. Higher values of the total mutagenic impurities need to be justified by the applicant.

8.5. Exceptions and flexibility in approaches

For impurities present in VMPs for food producing animals, as a matter of principle, since consumers exposed to residues via food of animal origin have no health benefit, the TTC-based acceptable intake of 0.025 µg/kg bw/day may not be exceeded. Potential exceptions require full justification by the applicant.

For impurities present in VMPs for use in companion animals, possible reasons for departing from the standard approach might include:

- Higher acceptable intakes may be justified when exposure to the impurity will be much greater from other sources e.g., food, or endogenous metabolism (e.g., formaldehyde).

- Case-by-case exceptions to the use of the appropriate acceptable intake may be justified in cases of severe disease, reduced life expectancy or where there are limited therapeutic alternatives.

- Compounds from some structural classes of mutagens can display extremely high carcinogenic potency (cohort of concern), i.e., aflatoxin-like-, N-nitroso-, and alkyl-azoxy structures. Intakes even below the TTC are theoretically associated with a potential for a significant carcinogenic risk and a case-by-case approach using e.g., carcinogenicity data from closely related structures, if available, should be developed to justify acceptable intakes for authorised VMPs. Principally, these substances should not occur as an impurity of a drug substance or a VMP, due to their extremely high carcinogenic potency.

- Where available data were generated using a route of administration other than that by which the VMP will be administered, consideration will need to be given to the validity of any conclusions (e.g., data generated by the dermal or subcutaneous route may not be relevant for oral exposure).

9. Control[1]

A control strategy is a planned set of controls, derived from current product and process understanding that assures process performance and product quality. A control strategy can include, but is not limited to, the following:

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[1] Several references to ICH documents are included in the guideline. Whilst veterinary products are outside the scope of these ICH documents there are no corresponding VICH documents and the principles outlined in these ICH documents may also be relevant to veterinary products. By inclusion of these references it is not the intention to introduce any additional requirements for veterinary products, on the contrary they are included in order to facilitate flexibility and to allow the applicant the option of using different approaches.

- Controls on material attributes (including raw materials, starting materials, intermediates, reagents, solvents, primary packaging materials);

- Facility and equipment operating conditions;

- Controls implicit in the design of the manufacturing process;

- In-process controls (including in-process tests and process parameters);

- Controls on drug substance and VMP (e.g., release testing).

When an impurity has been characterised as Classes 1, 2, or 3 in Table 1, it is important to develop a control strategy that assures that the level of this impurity in the drug substance and VMP is below the acceptable limit. A thorough knowledge of the chemistry associated with the drug substance manufacturing process, and of the VMP manufacturing process, along with an understanding of the overall stability of the drug substance and VMP is fundamental to developing the appropriate controls. Developing a strategy to control mutagenic impurities in the VMP is consistent with risk management processes principles identified in ICH Q9 (Ref. 6). A control strategy that is based on product and process understanding and utilisation of risk management principles will lead to a combination of process design and control and appropriate analytical testing, which can also provide an opportunity to shift controls upstream and minimise the need for end-product testing.

9.1. Control of process related impurities

There are 4 potential approaches for the development of a control strategy for a drug substance:

Option 1

Include a test for the impurity in the drug substance specification with an acceptance criterion at or below the acceptable limit using an appropriate analytical procedure.

For an Option 1 control approach, it is possible to apply periodic verification testing. Periodic verification is justified when it can be shown that levels of the mutagenic impurity in the drug substance are less than the acceptable limit for at least 6 consecutive pilot scale or 3 consecutive production scale batches. If this condition is not fulfilled, a routine test in the drug substance specification is required. See Section 9.3 for additional considerations.

Option 2

Include a test for the impurity in the specification for a raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion at or below the acceptable limit using an appropriate analytical procedure.

Option 3

Include a test for the impurity in the specification for a raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion above the acceptable limit of the impurity in the drug substance, using an appropriate analytical procedure coupled with demonstrated understanding of fate and purge and associated process controls that assure the level in the drug substance is below the acceptable limit without the need for any additional testing later in the process.

This option can be justified when the level of the impurity in the drug substance will be less than the acceptable limit by review of data from laboratory scale experiments (spiking experiments are encouraged) and where necessary supported by data from pilot scale or commercial scale batches. See Case Examples 1 and 2 in appendix 4. Alternative approaches can be used to justify Option 3.

Option 4

Understand process parameters and impact on residual impurity levels (including fate and purge knowledge) with sufficient confidence that the level of the impurity in the drug substance will be below the acceptable limit, such that no analytical testing is recommended for this impurity (i.e., the impurity does not need to be listed on any specification).

A control strategy that relies on process controls in lieu of analytical testing can be appropriate if the process chemistry and process parameters that impact levels of mutagenic impurities are understood, and the risk of an impurity residing in the final drug substance above the acceptable limit is determined to be negligible. In many cases, justification of this control approach based on scientific principles alone is sufficient. Elements of a scientific risk assessment can be used to justify an option 4 approach. The risk assessment can be based on physicochemical properties and process factors that influence the fate and purge of an impurity, including chemical reactivity, solubility, volatility, ionizability and any physical process steps designed to remove impurities. The result of this risk assessment might be shown as an estimated purge factor for clearance of the impurity by the process (Ref. 7).

Option 4 is especially useful for those impurities that are inherently unstable (e.g., thionyl chloride that reacts rapidly and completely with water), or for those impurities that are introduced early in the synthesis and are effectively purged.

In some cases an Option 4 approach can be appropriate when the impurity is known to form, or is introduced late in the synthesis; however, process-specific data should then be provided to justify this approach.

9.2. Considerations for control approaches

For Option 4 approaches, where justification based on scientific principles alone is not considered sufficient, as well as for Option 3 approaches, analytical data to support the control approach is expected. These could include, as appropriate, information on the structural changes to the impurity caused by downstream chemistry (fate), analytical data on pilot scale batches, and in some cases, laboratory scale studies with intentional addition of the impurity (spiking studies). In these cases, it is important to demonstrate that the fate/purge argument for the impurity is robust and will consistently assure a negligible probability of an impurity residing in the final drug substance above the acceptable limit. Where the purge factor is based on developmental data, it is important to address the expected scale-dependence or independence. In the case that the small-scale model used in the development stage is considered to not represent the commercial scale, confirmation of suitable control in pilot scale and/or initial commercial batches is generally appropriate. The need for data from pilot/commercial batches is influenced by the magnitude of the purge factor calculated from laboratory or pilot scale data, point of entry of the impurity, and knowledge of downstream process purge points.

If Options 3 and 4 cannot be justified, then a test for the impurity on the specification for a raw material, starting material or intermediate, or as an in-process control (Option 2) or drug substance (Option 1) at the acceptable limit should be included. For impurities introduced in the last synthetic step, an Option 1 control approach would be expected unless otherwise justified.

The application of 'As Low As Reasonably Practicable' (ALARP) is not necessary if the level of the mutagenic impurity is below acceptable limits. Similarly, it is not necessary to demonstrate that alternate routes of synthesis have been explored.

In cases where control efforts cannot reduce the level of the mutagenic impurity to below the acceptable limit and levels are as low as reasonably practical, a higher limit may be justified based on a benefit/risk analysis.

9.3. Considerations for periodic testing

The above options include situations where a test is recommended to be included in the specification, but where routine measurement for release of every batch may not be necessary. This approach, referred to as periodic or skip testing in VICH GL39 (Ref. 8) could also be called 'Periodic Verification Testing'. This approach may be appropriate when it can be demonstrated that processing subsequent to impurity formation/introduction clears the impurity. It should be noted that allowance of Periodic Verification Testing is contingent upon use of a process that is under a state of control (i.e., produces a quality product that consistently meets specifications and conforms to an appropriately established facility, equipment, processing, and operational control regimen). If upon testing, the level of the mutagenic impurity fails to meet the acceptance criteria established for the periodic test, the drug producer should immediately commence full testing (i.e., testing of every batch for the attribute specified) until the cause of the failure has been conclusively determined, corrective action has been implemented, and the process is again documented to be in a state of control. As noted in VICH GL39 (Ref. 8), regulatory authorities should be notified of a periodic verification test failure to evaluate the benefit/risk of previously released batches that were not tested.

9.4. Control of degradation products

For a potential degradation product that has been characterised as mutagenic, it is important to understand if the degradation pathway is relevant to the drug substance and VMP manufacturing processes and/or their proposed packaging and storage conditions. A well-designed accelerated stability study (e.g., 40 °C/75% relative humidity, 6 months) in the proposed packaging, with appropriate analytical procedures, is recommended to determine the relevance of the potential degradation product. Alternatively, well designed kinetically equivalent shorter term stability studies at higher temperatures in the proposed commercial package may be used to determine the relevance of the degradation pathway prior to initiating longer term stability studies. This type of study would be especially useful to understand the relevance of those potential degradation products that are based on knowledge of potential degradation pathways but not yet observed in the VMP.

Based on the result of these accelerated studies, if it is anticipated that the degradation product will form at levels approaching the acceptable limit under the proposed packaging and storage conditions, then efforts to control formation of the degradation product are expected. In these cases, monitoring for the drug substance or VMP degradation product in long term primary stability studies at the proposed storage conditions (in the proposed commercial pack) is expected, unless otherwise justified. Whether or not a specification limit for the mutagenic degradation product is appropriate will generally depend on the results from these stability studies.

If it is anticipated that formulation development and packaging design options are unable to control mutagenic degradation product levels to less than the acceptable limit and levels are as low as reasonably practicable, a higher limit may be justified based on a risk/benefit analysis.

9.5. Lifecycle management

This section is intended to apply to those products approved after the publication of this guideline.

Quality system elements and management responsibilities, such as those described in ICH Q10 (Ref. 9) are intended to encourage the use of science-based and risk-based approaches at each lifecycle

stage, thereby promoting continual improvement across the entire product lifecycle. Product and process knowledge should be managed from development through the commercial life of the VMP up to and including product discontinuation.

The development and improvement of a drug substance or VMP manufacturing process usually continues over its lifecycle. Manufacturing process performance, including the effectiveness of the control strategy, should be periodically evaluated. Knowledge gained from commercial manufacturing can be used to further improve process understanding and process performance and to adjust the control strategy.

Any proposed change to the manufacturing process should be evaluated for the impact on the quality of drug substance and VMP. This evaluation should be based on understanding of the manufacturing process and should determine if appropriate testing to analyse the impact of the proposed changes is required. Additionally, improvements in analytical procedures may lead to structural identification of an impurity. In those cases the new structure would be assessed for mutagenicity, as described in this guideline.

Throughout the lifecycle of the VMP, it will be important to reassess if testing is recommended when intended or unintended changes occur in the process. This applies when there is no routine monitoring at the acceptable limit (Option 3 or Option 4 control approaches), or when applying periodic rather than batch-by-batch testing. This testing should be performed at an appropriate point in the manufacturing process.

In some cases, the use of statistical process control and trending of process measurements can be useful for continued suitability and capability of processes to provide adequate control on the impurity. Statistical process control can be based on process parameters that influence impurity formation or clearance, even when that impurity is not routinely monitored (e.g., Option 4).

All changes should be subject to internal change management processes as part of the quality system. Changes to information filed and approved in a dossier should be reported to regulatory authorities in accordance with regulations and guidelines.

10. Documentation

Information relevant to the application of this guideline should be provided. For actual and potential process related impurities and degradation products, where assessments according to this guideline are conducted, the mutagenic impurity classification and rationale for this classification should be provided:

- This would include the results and description of *in silico* (Q)SAR systems used, and as appropriate, supporting information to arrive at the overall conclusion for Class 4 and 5 impurities;

- When bacterial mutagenicity assays are performed on impurities, study reports should be provided.

Justification for the proposed specification and the approach to control should be provided. For example, this information could include the acceptable intake, the location and sensitivity of relevant routine monitoring. For Option 3 and Option 4 control approaches, a summary of knowledge of the purge factor, and identification of factors providing control (e.g., process steps, solubility in wash solutions, etc.) is important.

Notes

Note 1 To assess the mutagenic potential of impurities, a single bacterial mutagenicity assay can be carried out with a fully adequate protocol according to VICH GL23(R) (Ref. 5) and OECD 471 (Ref. 10). The assays are expected to be performed in compliance with Good Laboratory Practices (GLP) regulations. Any deviations should be described in the study report (for example, if the test article is not prepared or analysed in compliance with GLP regulations). In some cases, the selection of bacterial tester strains may be limited to those proven to be sensitive to the identified alert. For impurities that are not feasible to isolate or synthesise, or when compound quantity is limited, it may not be possible to achieve the highest test concentrations recommended for a VICH-compliant bacterial mutagenicity assay, according to the current testing guidelines. In this case, bacterial mutagenicity testing could be carried out using a miniaturised assay format with proven high concordance to the VICH-compliant assay to enable testing at higher concentrations, with justification.

Definitions

Acceptable intake: In the context of this guideline, an intake level for mutagenic impurities in target animals which is associated with a small theoretical increase in cancer risk, or for serious/life-threatening indications where risk and benefit are appropriately balanced.

Acceptable limit: Maximum acceptable concentration of an impurity in a drug substance or VMP derived from the acceptable intake and the daily dose of the drug substance.

Acceptance criterion: Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.

Alerting structure: Molecular substructures or reactive groups that are related to the carcinogenic and mutagenic properties of chemicals.

Control strategy: A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and VMP materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.

Cumulative intake: The total intake of a substance that an animal is exposed to over time.

Degradation Product: A molecule resulting from a chemical change in the drug substance brought about over time and/or by the action of light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system.

DNA-reactive: The potential to induce direct DNA damage through chemical reaction with DNA.

Expert knowledge: In the context of this guideline, expert knowledge can be defined as a review of pre-existing data and the use of any other relevant information to evaluate the *accuracy of an in silico model* prediction for mutagenicity.

Genotoxicity: A broad term that refers to any deleterious change in the genetic material, regardless of the mechanism by which the change is induced.

Impurity: Any component of the drug substance or VMP that is not the drug substance or an excipient.

Mutagenic impurity: An impurity that has been demonstrated to be mutagenic in an appropriate mutagenicity test model, e.g., a bacterial mutagenicity assay.

New Drug Substance: The designated therapeutic moiety, in the EU legislation referred to as active substance, that has not been previously registered in a region or member state in a veterinary medicinal product (also referred to as a new molecular entity or new chemical entity). It can be a complex, simple ester, or salt of a previously approved drug substance.

New veterinary medicinal product: A veterinary medicinal product produced from chemically synthesised drug substances not previously registered in a region or member state.

(Q)SAR and SAR: In the context of this guideline, refers to the relationship between the molecular (sub) structure of a compound and its mutagenic activity using (Quantitative) Structure-Activity Relationships derived from experimental data.

Purge factor: Purge reflects the ability of a process to reduce the level of an impurity, and the purge factor is defined as the level of an impurity at an upstream point in a process divided by the level of an impurity at a downstream point in a process. Purge factors may be measured or predicted.

Structural alert: In the context of this guideline, a chemical grouping or molecular (sub) structure which is associated with mutagenicity.

Threshold of Toxicological Concern: The TTC concept was developed to define acceptable exposure levels for unstudied chemicals that may pose a risk of carcinogenicity or other toxic effects. The methods upon which the TTC is based are generally considered to be very conservative, since they involve a simple linear extrapolation from the dose giving a 50% tumour incidence (TD_{50}) to a 1 in 10^6 incidence, using TD_{50} data for the most sensitive species and most sensitive site of tumour induction. The assessment of acceptable limits of mutagenic impurities in drug substances and VMPs may be based on the TTC and results in a human intake (or exposure) value of 1.5 µg/day, corresponding to a theoretical 10^{-5} increased lifetime risk of cancer.

Virtually safe dose: In the context of this guideline the 'virtually safe dose' is a level of (human) exposure to a genotoxic carcinogen associated with a tumour incidence of ≤ 1 in 10^6. A virtually safe dose can be calculated based on substance-specific carcinogenicity data by linear extrapolation from a TD_{50}. In the absence of substance-specific data, the TTC approach delivers a generic dose of 0.0025 µg/kg bw per day or 0.15 µg/person per day.

References

1. VICH GL10: Impurities in new veterinary drug substances (EMEA/CVMP/VICH/837/99-Rev.1)

2. VICH GL11: Impurities in new veterinary medicinal products (EMEA/CVMP/VICH/838/99-Rev.1)

3. ICH guideline M7(R1): Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (EMA/CHMP/ICH/83812/2013)

4. VICH GL18(R): Impurities: Residual solvents in new veterinary medicinal products, active substances and excipients (Revision) (EMA/CVMP/VICH/502/99-Rev.1)

5. VICH GL 23(R): Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing (EMA/CVMP/VICH/526/2000)

6. ICH guideline Q9: Quality risk management (EMA/CHMP/ICH/24235/2006)

7. Teasdale A., Elder D., Chang S-J, Wang S, Thompson R, Benz N, Sanchez Flores I (2013). Risk assessment of genotoxic impurities in new chemical entities: strategies to demonstrate control. Org Process Res Dev 17:221-230.

8. VICH GL39: Test procedures and acceptance criteria for new veterinary drug substances and new medicinal products: chemical substances (EMEA/CVMP/VICH/810/04)

9. ICH guideline Q10: Pharmaceutical quality system (EMA/CHMP/ICH/214732/2007)

10. OECD Guideline for Testing of Chemicals; Section 4; Test No 471: Bacterial Reverse Mutation Test; 1997

Appendix 1

Conversion of the TTC-based acceptable intake to a specific concentration limit for a veterinary drug substance (example)

A chemist/engineer needs to calculate the concentration in ppm for a mutagenic impurity X present in drug substance Y, which is administered chronically to dogs with a therapeutic dose of 25 mg/kg bw/day.

The TTC-based acceptable intake (AI) for companion animals is 0.025 µg/kg bw/day and the intake of mutagenic impurity X by the animals should not exceed this limit.

TTC-based AI is expressed in µg/kg bw/day

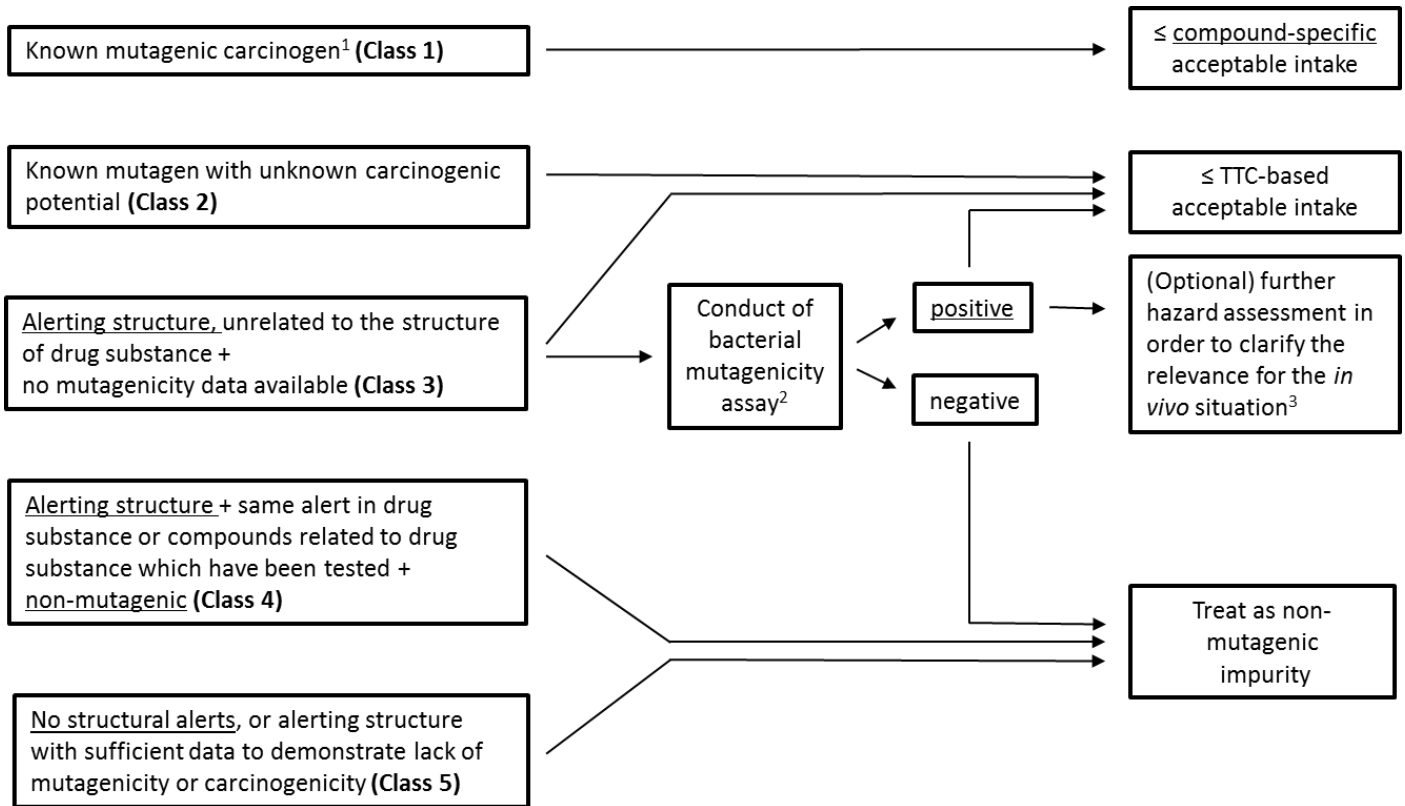Dose of the drug substance is expressed in g/kg bw/day

The ppm limit = TTC-based AI in µg divided by the daily dose in grams

In the specific example the dose is 0.025 g/kg bw/day and the maximum allowable concentration of mutagenic impurity X in veterinary drug substance Y is

0.025 µg divided by 0.025 g = 1 ppm

Appendix 2

Decision tree of impurity classification

Known mutagenic carcinogen[1] **(Class 1)**	→	≤ compound-specific acceptable intake

Known mutagen with unknown carcinogenic potential **(Class 2)**	→	≤ TTC-based acceptable intake

Alerting structure, unrelated to the structure of drug substance +
no mutagenicity data available **(Class 3)** → Conduct of bacterial mutagenicity assay[2] →

- positive → (Optional) further hazard assessment in order to clarify the relevance for the *in vivo* situation[3]
- negative → ≤ TTC-based acceptable intake

Alerting structure + same alert in drug substance or compounds related to drug substance which have been tested +
non-mutagenic **(Class 4)** → Treat as non-mutagenic impurity

No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity **(Class 5)** → Treat as non-mutagenic impurity

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[1] For class I, a compound-specific acceptable intake can be calculated based on carcinogenic potency and linear extrapolation as a default approach, see chapter 8.2.1.

[2] bacterial mutagenicity assay or (Q)SAR methodologies that predict the outcome of a bacterial mutagenicity assay

[3] For instance, when levels of the impurity cannot be controlled at an appropriate acceptable limit, it is recommended that the impurity be tested in an in vivo gene mutation assay or other appropriately justified in vivo genotoxicity assay in order to understand the relevance of the bacterial mutagenicity assay result under in vivo conditions.

Appendix 3

Scope scenarios for application of the guideline

Scenario	Applies to Drug Substance	Applies to VMP	Comments
Applications for new marketing authorisations			
Registration of new VMP that includes new drug substances	Yes	Yes	Primary intent of the Guideline
Registration of new VMP that includes existing drug substance(s), but this drug substance is used for the first time by the applicant, or represents a new source for the applicant.	yes	yes	Primary intent of the Guideline
Registration of new VMP that includes an existing drug substance(s), which is from the same source as used in an existing VMP already authorised by the applicant. Synthesis of the drug substance is consistent with previously approved processes.	No	Yes/No	Drug substance: As long as the synthesis of the drug substance is consistent with previously approved methods, and mutagenic impurity risk has previously been assessed, then re-evaluation of mutagenic impurity risk is not necessary. The applicant would need to demonstrate that no changes have been made to a previously approved process. VMP: In scope if there are differences in the indication, dosing regimen or target species compared with already authorised VMPs. Also in scope if there are differences in the VMP formulation, composition or manufacturing process which result in new degradation products or increased acceptance criteria for existing degradation products (compared with already authorised VMPs)
Registration of new VMP that includes an existing drug substance, which is from the same source as used in an existing VMP already authorised by the applicant. Synthesis of the drug substance is **different** to	Yes	Yes/No	Drug substance: If the synthesis of the drug substance is different to that previously approved, then re-evaluation of mutagenic impurity risk is necessary. VMP In scope if there are differences in the indication, dosing regimen or target species compared with already authorised VMPs.

that previously approved.			Also in scope if there are differences in the VMP formulation, composition or manufacturing process which result in new degradation products or increased acceptance criteria for existing degradation products (compared with already authorised VMPs)
Post-approval submissions of authorised products			
A new formulation of an approved VMP is filed	No	Yes	See Section 5.2
A new manufacturer of the drug substance is registered. Synthesis of the drug substance is consistent with previously approved processes.	No	No	As long as the synthesis of the drug substance is consistent with previously approved methods, then re-evaluation of mutagenic impurity risk is not necessary, provided mutagenic impurity risk has previously been assessed. The applicant would need to demonstrate that no changes have been made to a previously approved process/product. Refer to Section 5.1.
A new manufacturer of the drug substance is registered. Synthesis of the drug substance is different to that previously approved.	Yes	No	If the synthesis of the drug substance is different to that previously approved, then re-evaluation of mutagenic impurity risk is necessary.

Appendix 4

Case examples to illustrate potential control approaches

Case 1: Example of an option 3 control strategy

An intermediate X is formed two steps away from the drug substance and impurity A is routinely detected in intermediate X. The impurity A is a stable compound and carries over to the drug substance. A spike study of the impurity A at different concentration levels in intermediate X was performed at laboratory scale. As a result of these studies, impurity A was consistently removed to less than the TTC-based limit in the drug substance even when impurity A was present at 1% in intermediate X. Since this intermediate X is formed only two steps away from the drug substance and the impurity A level in the intermediate X is relatively high, the purging ability of the process has additionally been confirmed by determination of impurity A in the drug substance in multiple pilot-scale batches and results were below the TTC-based limit. Therefore, control of the impurity A in the intermediate X with an acceptance limit of 1.0% is justified and no test is warranted for this impurity in the drug substance specification.

Case 2: Example of an option 3 control strategy: based on predicted purge from a spiking study using standard analytical methods

A starting material Y is introduced in step 3 of a 5-step synthesis and an impurity B is routinely detected in the starting material Y at less than 0.1%, using standard analytical methods. In order to determine if the 0.1% specification in the starting material is acceptable, a purge study was conducted at laboratory scale where impurity B was spiked into starting material Y with different concentration levels up to 10% and a purge factor of > 500 fold was determined across the final three processing steps. This purge factor applied to a 0.1% specification in starting material Y would result in a predicted level of impurity B in the drug substance of less than 2 ppm. As this is below the TTC-based limit of 50 ppm for this impurity in the drug substance, the 0.1% specification of impurity B in starting material Y is justified without the need for providing drug substance batch data on pilot scale or commercial scale batches.

Case 3: Example of an option 2 and 4 control strategy: control of structurally similar mutagenic impurities

The Step 1 intermediate of a 5-step synthesis is a nitro-aromatic compound that may contain low levels of impurity C, a positional isomer of the step 1 intermediate and also a nitro-aromatic compound. The amount of impurity C in the step 1 intermediate has not been detected by ordinary analytical methods, but it may be present at lower levels. The step 1 intermediate is positive in the bacterial mutagenicity assay. The step 2 hydrogenation reaction results in a 99% conversion of the step 1 intermediate to the corresponding aromatic amine. This is confirmed via in-process testing. An assessment of purge of the remaining step 1 nitro-aromatic intermediate was conducted and a high purge factor was predicted based on purge points in the subsequent step 3 and 4 processing steps. Purge across the step 5 processing step is not expected and a specification for the step 1 intermediate at the TTC-based limit was established at the step 4 intermediate (Option 2 control approach). The positional isomer impurity C would be expected to purge via the same purge points as the step 1 intermediate and therefore will always be much lower than the step 1 intermediate itself and therefore no testing is required and an Option 4 control strategy for impurity C can be supported without the need for any additional laboratory or pilot scale data.

Case 4: Example of an option 4 control strategy: highly reactive impurity

Thionyl chloride is a highly reactive compound that is mutagenic. This reagent is introduced in step 1 of a 5 step synthesis. At multiple points in the synthesis, significant amounts of water are used. Since thionyl chloride reacts instantaneously with water, there is no chance of any residual thionyl chloride to be present in the drug substance. An Option 4 control approach is suitable without the need for any laboratory or pilot scale data.