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Strategies To Address Mutagenic Impurities Derived from Degradation in Drug Substances and Drug Products

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ABSTRACT: This paper outlines strategies in alignment with ICH M7 for systematically assessing the potential risk posed by mutagenic degradants in active pharmaceutical ingredients (API) and formulated products. A mutagen risk assessment (MRA) process that involves degradation should include results from focused drug substance and drug product stress testing experiments (e.g., at elevated temperature, a wide pH range in solution, oxidative, and photolytic stress) as well as accelerated and long-term stability studies in the solid-state. While the MRA may include hypothetical (theoretically predicted) degradation products from *computer based* and/or *knowledge-based* approaches, investigations for numerous hypothetical degradation products whose significance have not been verified experimentally should not be initiated based on these results alone. Drug substance and drug product stress (forced degradation) studies should be designed to generate a comprehensive range of potential degradants that encompass all degradation products likely to form under typical ICH storage conditions. As a result of the absence of definitive regulatory guidance covering stress testing (including strategies for impurity identification/elucidation) there are different approaches used within the industry. Three general strategies for triggering structure elucidation of degradants (and hence inclusion in a MRA) are outlined, all of which are consistent with the approaches outlined in ICH Q1A, Q3A/B, and M7. The first approach for triggering structure elucidation is centered around long-term and accelerated stability and ICH Q3A/B thresholds; the second approach focuses on the "major" degradation products and pathways observed during stress testing using an algorithm for defining the threshold for "major" degradation products; the third approach focuses on those degradation products observed during stress testing that meet criteria derived from thresholds that have been scaled from ICH Q3A/B identification thresholds. Regardless of the chosen strategy, it is proposed that only those major degradation products observed at significant levels in stress testing, ICH accelerated, or long-term stability studies be included in the MRA process as this reflects the degradants most likely to be seen in marketed products. Such an approach is consistent with ICH M7. The overall strategy should be based on a risk assessment, where potential degradation products are determined to be either relevant and addressed or irrelevant and excluded from further consideration. The approaches described herein provide an appropriate framework to assess the risk posed by mutagenic impurities (MIs) arising as a result of either drug substance and/or drug product degradation.

1. INTRODUCTION

1.1. Regulatory Perspective. The safety of impurities present in drug substances (DSs) and drug products (DPs) is a major concern within the pharmaceutical industry and for regulatory agencies. The control of impurities (i.e., organic, inorganic impurities, and residual solvents) is generally addressed by ICH guidelines covering both DS and DP.[1−4] However, within the range of impurities that need to be assessed with regard to safety, a specific area of concern are mutagenic impurities (MIs) as well as potentially mutagenic impurities (PMIs; defined as containing a structure that alerts for mutagenicity, but where an Ames test has not been performed). In 2007 the EMA introduced a specific guideline addressing genotoxic impurities (supplemented by an associated question and answer document).[5−7] This was followed in December 2008 by the issuance of a draft FDA guideline.[8] The FDA guidance was not finalised, due to MIs being adopted as a formal ICH topic in 2011.[9] The scope of the guidance for MIs includes synthesis related impurities and those species resulting from degradation of the DS or DP. In 2014, the International Conference on Harmonisation (ICH) published the Step 4 ICH M7 document on "DNA reactive (mutagenic) impurities", a subset of genotoxic impurities.[9] As a consequence throughout the remainder of this article such impurities will be referred to as mutagenic impurities.

1.2. Challenges Associated with the Assessment of the Risk Posed by (Potentially) Mutagenic Degradants.

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Strategies for the assessment of risk derived from MI and PMIs arising from synthetic routes have been described previously. Much has been written about the use of science-based mutagen risk assessments (MRAs) to evaluate the risk to drug substance quality.[10−21] In these assessments, the workflow includes evaluation of the starting materials and intermediates in tandem with the process chemistry to arrive at a holistic view of potential process impurities and their possible purgeability in subsequent steps. Once this is completed, as outlined by ICH M7, two or more (Q)SAR tools such as DEREK for Windows (Lhasa Ltd.) or Leadscope (Leadscope Inc.), SciQSAR (Scimatics, Inc.), or CASEUltra (MultiCASE Inc.) should be used to assess whether these impurities (or related compounds) have any structural alerts for mutagenicity. It should be noted that the two (Q)SAR tools should be complementary (expert and statistical based systems). The selection of chemical structures for (Q)SAR screening arising from the DS synthesis should be focused on the starting materials, intermediates, and reagents. Additionally, major byproducts and significant process related impurities are usually determined during the development of the synthetic route and process as well as included in the (Q)SAR screening. The difficulty that arises for inclusion of degradation-related impurities in (Q)SAR screening is that the evaluation process must encompass a projection about which degradation products will form over the product's shelf life, and such a projection is not a trivial exercise.

Active pharmaceutical ingredients are typically small organic molecules that possess a variety of functional groups, resulting in a wide array of potential decomposition pathways. Accurate prediction of potential degradation pathways is further complicated because of the relatively small amount of published information available regarding the degradation of pharmaceuticals, the length of time required to assess molecular stability, and the significant effects of physical form on molecular stability. Consequently, degradation processes sometimes yield chemical structures that are not initially expected, predicted or even seen under ICH storage conditions. Furthermore, understanding of the degradation pathways associated with a compound often evolves during the development process. These pathways are often not fully elucidated before the final formulation is established and are generally unknown prior to the start of clinical studies.

ICH M7 discusses "potential" degradants as those that may be "reasonably expected to form during long term storage conditions". The relevant (i.e., "actual") degradants include those degradants that form above the ICH Q3A/B reporting thresholds during storage of the drug substance and drug product in the proposed long-term storage conditions and primary and secondary packaging. These "actual" degradants should be included in the MRA process. To determine the relevancy of potential degradants, a robust science-based risk assessment should be conducted, which may include:

- Further examination through well-designed stress testing studies;[22−24]
- Accelerated stability testing (also known as "exaggerated storage conditions");[23] or well-designed kinetically equivalent shorter term stability studies (cf. section 8.4 of ICH M7) and;
- Confirmation through long-term ICH stability studies of both the drug substance and formulated drug product.

It is worth noting that while prediction of potential degradation pathways via in silico tools or knowledge-based approaches can help in the development of a mechanistic understanding of the possible degradation pathways, the overall strategy should be based on risk assessment. Potential degradation products should be determined to be either *relevant* and addressed via risk mitigation, or *nonrelevant* and excluded from further consideration.

Currently, there is no consensus approach within the industry to assess the risk of potential degradation products that may be mutagenic and to which a patient could be reasonably exposed (i.e., over the shelf life).[18] Therefore, the purpose of this paper is to provide systematic strategies for evaluating the risk of mutagenic impurities formation arising through DS/DP degradation. The article does not deliberately seek to specify the exact experimental details of individual studies as the nature of the studies will depend on a number of factors that need to be evaluated on a case-by-case basis.

2. RISK ASSESSMENT PROCESS FOR THE EVALUATION OF MUTAGENIC DEGRADANTS

2.1. Stability-Related MRA Process Overview. ICH M7 states that "actual and potential degradation products likely to be present in the final drug substance or drug products and where the structure is known should be evaluated for mutagenic potential...". Thus, a critical aspect of a mutagen risk assessment (MRA) of drug substance or drug product degradation is the determination of degradation pathways and associated degradation products that are relevant to the manufacturing processes and/or proposed packaging and storage conditions.

Stress Studies. Well-designed stress testing studies, as recommended by ICH Q1A and discussed in more detail by others,[22,24,25] can yield a set of potential degradation products, whose chemical structures can be included in the risk assessment. Such products are termed "potential" since they may or may not form during ICH long-term and accelerated stability studies (see ICH Q1A(R2) for a definition of stress testing and ICH M7 section 5.2[9]).

Accelerated Stability Studies. Evaluation of ICH accelerated stability testing results (typically 40 °C/75%RH for 6 months for solid oral dosage forms, as per ICH Q1A) may also reveal degradation products to be included in the assessment. Such studies may encompass a range of stability conditions ranging from open storage to studies performed in the final packed product. Those degradants formed under these conditions are also included in the set of "potential degradation products".

Long-Term ICH Stability Studies. Degradation products formed on the proposed long-term ICH stability storage conditions in the proposed primary and secondary packaging (as per ICH Q1A and M7) should also be included in the MRA and are included in the set of "actual degradation products".

A crucial part of any evaluation of degradation products is that the risk assessment is commensurate with requirements of the relevant guidelines.[1−3,6,8,9] These guidelines advocate that any such assessment should focus on probable/likely impurities, and hence, any risk assessment of degradants should similarly focus on primary degradation pathways and their associated major degradants. Hypothetical degradants not observed experimentally would not need to be included for assessment in the MRA.

Once the structures of the degradation products are elucidated, the structures are screened in (Q)SAR models. From ICH M7 (Section 6), a positive (Q)SAR result indicates that the degradant is a class 1 or 2 (known mutagenic carcinogen or known mutagen) or 3 (an alerting structure

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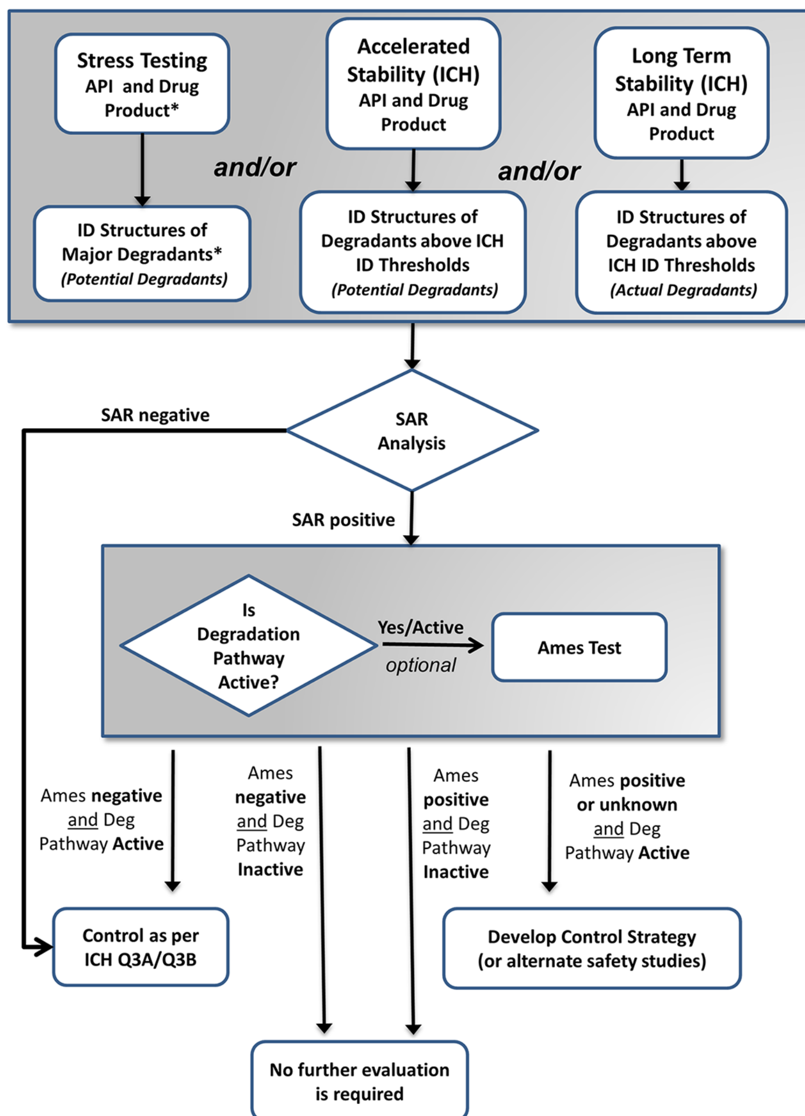
Scheme 1. Proposed Process Flow for Assessing Degradants in Drug Substance and Drug Product



unrelated to the structure of the active pharmaceutical ingredient). If the (Q)SAR evaluation is positive, in vitro tests such as Ames testing may be undertaken in order to assess the mutagenicity. The Ames test allows further classification of a Potential Mutagenic Impurity (PMI) as either a MI (Ames positive) or a nonmutagenic "standard" Q3A/Q3B[1,2] degradant (Ames negative). If the degradant in question is shown to be Ames positive, then further targeted studies should be performed to determine "relevancy" (i.e., is the pathway active in the DS or DP), and how much of the degradant will actually form over the shelf life in the proposed primary and secondary packaging. If needed, appropriate mitigation and control strategies may then be devised and implemented.

Alternatively, accelerated or long-term stability studies may be performed *instead* of an Ames test in order to evaluate whether or not the potential degradant in question is formed at levels of concern, based on permissible safety limits. If the potential degradant does not form at levels (defined by ICH M7) approaching the permissible limits over the shelf life, then the potential degradant does not need to be further assessed in the MRA because it has been shown to be irrelevant. See Scheme 1 for the proposed process flow.

2.2. In Silico Tools for Prediction of Potential Degradation Products. Prior to conducting stress or other stability studies, in silico predictions are useful to help consider potential degradation pathways and potentially guide the strategy for analytical method development. Currently, there are relatively few commercial software programs designed specifically for predicting degradation pathways. Historically, several companies developed their own predictive software applications, such as Pfizer's Delphi.[26] In addition, in partnership with Pfizer and Eli Lilly, CambridgeSoft also developed a free, online database, the Pharma Drug Degradation Database ("Pharma D3").[27] Pharma D3 does not have predictive capabilities but serves as an excellent repository of known drugs and their associated degradation products. The most recent commercially available predictive degradation software is Zeneth (Lhasa Ltd.).[28] Zeneth is a chemical degradation prediction application that utilizes a rules-based software platform with a growing number of chemical transformations in its knowledge base to predict possible

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degradation products from a given molecular structure. Using a software package such as Zeneth, in concert with in cerebro knowledge gleaned from chemical principles and expertise, a more comprehensive approach can be taken regarding the possible degradation pathways available to the DS/DP (such as solution pH, environmental conditions (acid, base, oxidative—radical initiators, hydrogen peroxide, metals, or light) as well as excipients and impurities). The objective is to provide an extensive list of degradation products that may be formed via degradation of a particular drug structure.

Generally, in silico predictive tools have a tendency to "over-predict", i.e., prediction of more degradation products than actually occur.[29] Consequently, while hypothetical assessment of degradation products and pathways may provide potentially useful starting points for deciding which degradation products need to be considered in the MRA process, such assessment tools are not a substitute for stress testing studies. It is proposed that in silico prediction tools should not be used to initiate investigations (e.g., "fishing" or "hunting" exercises that evaluate numerous hypothetical structures (predicted via in silico or other means) whose significance has not been verified experimentally via appropriate stability studies). This proposal is consistent with the ICH M7 guidance that evaluations should focus on "reasonably expected" impurities.

2.3. Using Stress Testing (Forced Degradation) to Select Degradation Products for Identification. Stress testing, also known as forced degradation or purposeful degradation, is utilized in the pharmaceutical industry to learn more about DS and DP stability, discern the degradation pathways, inform formulation strategies, and develop/validate stability indicating methods.[30,31] The authors view stress testing studies as a critical tool for defining the potential degradants to be included in the MRA process as well as a filter for any hypothetically predicted degradation products.

Due to the lack of detail in the ICH guidance documents in relation to stress testing, the exact conditions of the stress tests are interpreted differently by individual companies leading to differences in approaches.[17,25,31,32] However, there is general industry consensus that only the major degradation products arising from these studies should be evaluated.[32,33] In line with ICH Q1A,[23] stress testing of the drug substance consists of exposure to conditions including acid/base in solution, oxidation, and solid state exposure to light and thermal/humidity stress (temperatures that are at least 10 °C above accelerated stability). Stress testing of the drug product typically involves exposure to elevated temperature and humidity, along with photostressing as per ICH Q1B. These conditions provide thorough coverage of the environmental conditions that the DS or DP may reasonably be exposed to and, hence, can be expected to address all relevant degradation pathways.

Due to the disparate strategies in the area of forced degradation, we suggest that any of the three following strategies can be considered. The choice of strategy can be dependent on a number of criteria, which may include the phase of development, disease indication, and familiarity with the primary degradation pathways of the molecular scaffold (chemical series).

2.3.1. Approach 1: Structure Identification after Observation in Accelerated or Long-Term ICH Stability Studies. The first strategy is the simplest to implement and uses forced degradation (at appropriate levels) to help guide the accelerated and long terms stability studies. The samples generated for the forced degradation studies are used for analytical method development of the stability indicating method. Forced degradation results in the generation of larger quantities of degradation products (both in the number of degradants and the total amount), making the degradants easier to detect via the available analytical methods. Degradation product structures are not necessarily elucidated unless they are also observed in accelerated or long-term ICH stability studies as described in ICH M7.

2.3.2. Approach 2: Structure Identification through Use of an Algorithm in Forced Degradation Studies. The second approach utilizes forced degradation studies to delineate potential degradation products and pathways, with structure elucidation focused on "major" degradation products; which are defined by the algorithm below. The systematic approach is based on work by Alsante et al.[33] The algorithm involves defining a major degradation product as a degradant at levels greater than 10% of the total degradation *and* also >25% of the largest individual degradant (see Figures 1 and 2 for an illustration). This strategy has been successfully applied[18] and has been shown to reliably focus on the major degradation pathways, creating a reasonable number of "potential" degradants while comprehensively including "actual" degradants (i.e., "actual" degradants are a subset of "potential"). Table 1 adds further details regarding the comparison to the two criteria.

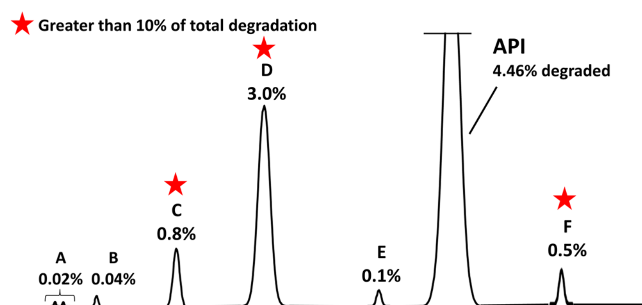

Figure 1. Criterion no. 1: Identify largest impurity only if it comprises at least 10% of the total degradation for appropriately degraded samples. Total degradation can be determined from either assay or peak versus total measurement but should not be more than 20%. A sample chromatogram is illustrated.

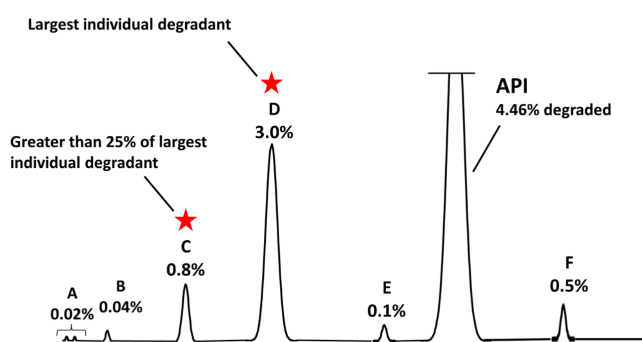

Figure 2. Criterion no. 2: Identify additional peaks only if they are greater than 25% of the largest impurity. A sample chromatogram is illustrated.

2.3.3. Approach 3: Structure Identification through Use of Kinetic Equivalence and Scaled ICH Q3B Thresholds. A third approach has also been developed based on the concept of

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Table 1. Peaks Selected for Identification Based on Meeting Criteria 1 (Identify Largest Impurity Only if It Comprises at Least 10% of the Total Degradation for Appropriately Degraded Samples) and Criteria 2 (Identify Additional Peaks Only if They Are Greater than 25% of the Largest Impurity)[a]

peak	% area or % weight	Criteria 1 assessment (rationale)	Criteria 2 assessment (rationale)	selected for identification (meets Criteria 1 and Criteria 2)
A	0.02	no (too small)	no (too small)	no
B	0.04	no (too small)	no (too small)	no
C	0.8	yes (meets criteria; 0.8%/4.46%)	yes (0.8%/3.0%)	yes
D	3.0	yes (largest impurity 3.0%/4.46%)	yes (largest impurity)	yes
E	0.1	no (too small)	no (too small)	no
API	95.54	API (4.46% degraded)	API	API
F	0.5	no (too small; 0.5%/4.46%)	no (too small)	no

[a]In this case, only two degradation products are selected for identification from the forced degradation study.

kinetic equivalence (described below) and ICH Q3B thresholds. This strategy is more complex than the other two approaches. Even though it is based on advanced kinetics concepts and prorated (scaled) ICH Q3B thresholds, at this time the benefit of this approach to mitigate the risk of the formation or observation of PMIs over the latter two strategies has yet to be fully established.

2.3.3.1. Defining a Relevant Duration for Storage in Forced Degradation Experiments: Kinetic Equivalence. Generally, solid state stress testing is aimed at achieving a "kinetic equivalence" to at least 6 months storage at 40 °C/75% RH, and at least 2× ICH Q1B light exposure, up to 10% loss, whichever comes first.[24,32−34] It should be noted that the relative humidity of the system also plays a key role in the rate of degradation and thus design of short-term studies to create a "kinetic equivalence" should take both temperature and humidity into account.[35,36] Two main options are available: (1) building the sensitivity to relative humidity into the Arrhenius relationship (e.g., using the Accelerated Stability Assessment Program (ASAP) protocol[35−39]) or (2) maintaining the same relative humidity at the higher stress temperatures. While both options are feasible, the latter approach offers a simpler conceptual perspective and is the focus of the discussion here for the third approach.

Accurate Arrhenius-based rate predictions require knowledge of the energy of activation (E_a) of degradation for the drug substance or drug product being studied. If the E_a is not known, an E_a of 19.87 kcal/mol (83.14 kJ/mol) as per United States Pharmacopeia guidance[40] is recommended here as a reasonably conservative approach for calculating the mean kinetic temperature. The E_a has a significant effect on the rate of degradation as a function of temperature, and this is illustrated in Table 2.

The different E_a's shown in the table, ranging from a very low 12 to 29.8 kcal/mol were chosen for specific reasons. An E_a of 12 kcal/mol corresponds to the low end of documented E_a's for drug-like molecules and reflects a lower thermodynamic hurdle to degradation relative to higher E_a processes.[43] An E_a of 17 kcal/mol corresponds to the assumption that 6 months storage at 40 °C/75% RH is equivalent to 2 years at 25 °C/75% RH. An E_a of 19.87 kcal/mol corresponds to the recommendation from the USP regarding the estimated mean kinetic temperature.[40] This value is similar to one based on activation energies that were determined by studies of drug degradation in solution (as opposed to the solid state).[44] An E_a of 25.8 kcal/mol corresponds to the "Joel Davis rule",[42] where 3 months storage at 40 °C/75% RH was assumed to be equivalent to 2 years at 25 °C/60% RH. Lastly, an E_a of ~29.8 kcal/mol corresponds to an experimentally determined average E_a from more than 100 solid state drug degradation studies (50 compounds).[39] Additional evidence for the validity of this surprisingly high average E_a for solid state drug degradation has been independently obtained during ASAP studies within Lilly (unpublished data).[32]

Table 3 offers an illustrative example of the significant effect of E_a on the rate of degradation at different temperatures by calculation of the number of days it would take to achieve a kinetic equivalence to 6 months at 40 °C/75% RH. Considering a

Table 3. Number of Days Calculated to Achieve a 6 Months Storage Condition at 40 °C for Reactions with Different E_a Assuming Arrhenius Kinetics[a]

temp. (°C)	number of days (E_a = 12 kcal/mol)	number of days (E_a = 15 kcal/mol)	number of days (E_a = 19.87 kcal/mol)	number of days (E_a = 25.8 kcal/mol)	number of days (E_a = 29.8 kcal/mol)
25	482	615	912	1470	2040
30	345	405	524	718	888
40	182.5	182.5	182.5	182.5	182.5
50	100	86.5	67.8	50.5	41.4
60	57.2	42.8	26.8	15.1	10.3
70	33.7	22.1	11.1	4.8	2.8
80	20.5	11.8	4.9	1.7	0.8

[a]Note that 182.5 days is being used to equate 6 months (one half of one year).

Table 2. Rate of Degradation (Relative to 25 °C) Assuming an Arrhenius Kinetic Relationship

temp. (°C)	relative rate[a] (E_a = 12 kcal/mol)[24,41]	relative rate[a] (E_a = 17 kcal/mol)	relative rate[a] (E_a = 19.87 kcal/mol)[40]	relative rate[a] (E_a = 25.8 kcal/mol)[42]	relative rate[a] (E_a = 29.8 kcal/mol)[39]
25	1	1	1	1	1
30	1.4	1.6	1.7	2.1	2.3
40	2.6	4.0	5.0	8.1	11.2
50	4.8	9.2	13.5	29.2	49.3
60	8.4	20.4	34.1	97.7	198.9
70	14.3	43.2	81.9	304.8	739.8
80	23.6	86.6	187	891.2	2554.7

[a]The relative rate is meaningful only within individual columns. Relative rates across rows should not be inferred.

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Table 4. Proposed ID Thresholds[a] for the Kinetic Equivalence for Degradation Products Derived from Drug Substance and Drug Product

maximum daily dose (mg)	ID threshold from ICH Q3B[2] (%)	ID threshold derived from ICH Q3B for stressed samples degraded 1−5% (%)	ID threshold derived from ICH Q3B for stressed samples degraded >5−10% (%)	ID threshold derived from ICH Q3B for stressed samples degraded >10−15% (%)	ID threshold derived from ICH Q3B for stressed samples degraded >15−20% (%)
>2000	0.10	0.25	0.5	0.75	1.0
>10−2000	0.2	0.5	1.0	1.5	2.0
>1−10	0.5	1.25	2.5	3.75	5.0
<1	1.0	2.5	5.0	7.5	10.0

[a]ICH Q3B provides threshold for identification based on relative amount of the impurity in the drug product and based on the total daily intake (μg). The ID threshold that yields the lower total daily intake should be used for the assessment in stress studies. The amount of degradation corresponds to the percentage of the peak area.

degradation reaction with an E_a of 19.87 kcal/mol (USP mean kinetic temperature energy of activation), a stress testing study at 70 °C would yield the kinetic equivalence at the temperature of an accelerated stability study (40 °C, 6 months) in approximately 11 days. This assumes that the degradation pathways at 70 °C are the same as those at lower temperatures. As discussed earlier, humidity may play a role in the reaction rate, so it is advisible to maintain the same relative humidity (e.g., 75% RH) in the stress study and in the accelerated stability study unless the sensitivity to relative humidity has been determined (e.g., using the ASAP protocol). It is worth noting that a degradation reaction with an E_a of 29.8 kcal/mol can reach kinetic equivalence to 6 months storage at 40 °C in less than 3 days.

Hydrolysis and oxidation are the two principal degradation pathways for most drugs.[25,33] The drug substance acid/base and oxidation solution-based stress studies ensure that any potential hydrolytic or oxidative reactions will be triggered, even if they do not readily occur in the solid state. The acid/base conditions are designed to accelerate hydrolytic reactions via lowering of the E_a through acid or base catalysis. The oxidative conditions (e.g., via peroxides or radical initiated reactions) are designed to significantly accelerate normal oxidative kinetics. Such studies enhance mechanistic understanding and provide an assessment of a wide range of potential degradation pathways. Therefore, inclusion of potential degradation products from these studies in the MRA process represents a conservative and sufficiently thorough view of risk.

2.3.3.2. Identification of Potential Degradants Formed under Stressed Conditions. With the concept of kinetic equivalence, it is helpful to consider the decision process for the identification (ID) of the structures of individual degradants that are formed in stress studies as this forms the basis of the third approach. In alignment with ICH Q1A and with Q4 in the EMA Q&A on mutagenic impurities[7] (for Phase III and beyond), structure elucidation of degradants that form on long-term stability studies is not required when the levels are below ICH identification thresholds. Thus, using ICH Q3B ID thresholds[2] for drug product (column 1, Table 4) as a starting point, for the third strategy we propose thresholds for identification of degradants typically observed in stress testing.

It is important to understand that these identification thresholds reflect the typically larger degradation levels and higher number of degradation products formed under stress conditions. Such studies are designed to identify primary degradation pathways and their associated major degradants. If one conservatively assumes that an acceptable potency loss for a drug product over the shelf life may be 2%, a relationship to stress testing can be established. Degradation of 5−10% in a stress testing experiment is 2.5−5 times higher than the 2% degradation that would be acceptable at the end of the shelf life. Thus, in this third strategy based on kinetic equivalence, an ID threshold for degradants arising during stress testing should be 2.5 to 5-fold higher than the corresponding thresholds for ID in the drug product under normal storage conditions.

Table 4 provides ID thresholds for degradants formed during stress testing scaled from the ICH impurity ID thresholds as a recommended lower limit in the kinetic equivalence strategy. By including major degradants observed in the forced degradation studies together with any degradants formed under accelerated or long-term stability studies, the significant effort required to identify numerous low level degradants that might be present in a stressed sample, but which have no

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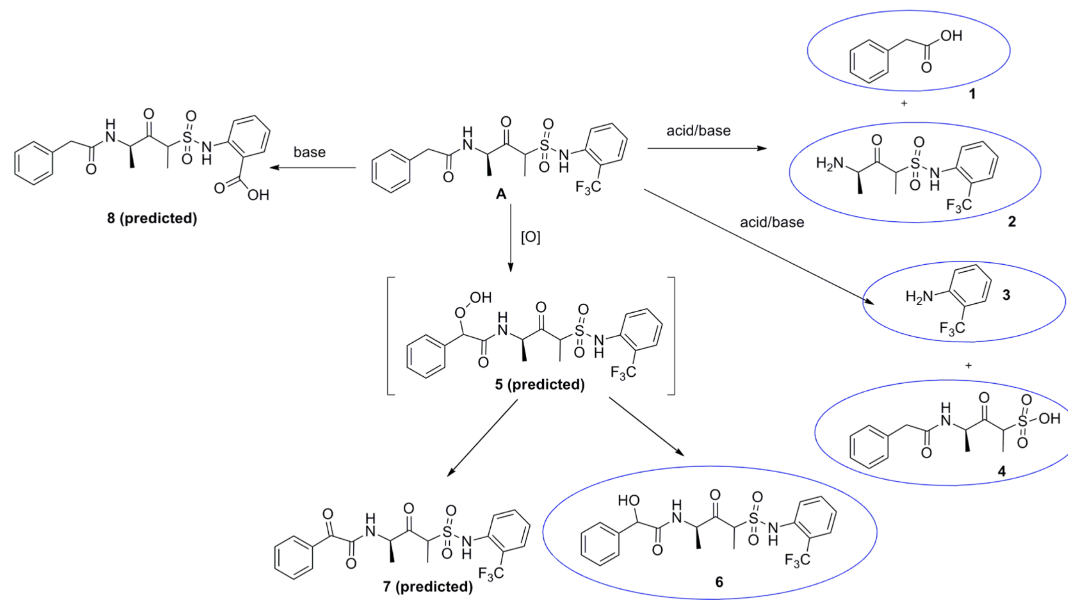
Scheme 2. Degradation Scheme for Molecule A (Solid Oral Dosage Form Product)[a]



[a]The molecule does not have a chromophore with absorbance >290 nm,[45] so no light-induced reactions were predicted or observed. Circled structures are potential or actual degradation products. Other structures have been predicted based on computer and knowledge based assessments.

relevance to the product when stored under ICH conditions over its shelf life, may be avoided.

2.4. Overall Risk Assessment Process: Case Study Example. The following case study serves to provide an example of how the individual aspects of a risk assessment are combined. Scheme 2 consists of both hypothetical degradants (i.e., speculated based on chemistry principles) and potential degradants (i.e., observed during stress testing). Circled structures have been observed in stress testing, accelerated, or formal ICH stability studies above specified thresholds (see Table 4 or ICH Q3A/B) and thus were identified.

2.4.1. Structures To Be Included in the MRA. Using the rationale described above, degradants **1**−**4** and **6** should be included in the MRA since they were observed during stress testing and/or stability studies. Degradants **7** and **8** are hypothetical degradation products that were not observed during stress testing and, therefore, do not need to be included in the MRA. Degradant **5** is a proposed intermediate in the degradation pathway to **6** that was not observed during stress testing or other stability studies above the recommended thresholds in Table 3; therefore, **5** may not need to be included in the MRA. However, since **5** is a potential intermediate in the formation of **6** (which was observed), further consideration may be warranted since the degradation pathway to **6** is known to be active. This example demonstrates that it is important to consider the thresholds as described in the proposed approaches in concert with sound scientific considerations.

2.4.2. (Q)SAR of the Structures in the MRA. In the example above, the structures for degradants **1**−**6** were evaluated using (Q)SAR tools, and two degradants were listed as having a mutagenic concern (aniline **3** and hydroperoxide **5**). These degradants were categorized as ICH M7 Class 3,[46] having a different (Q)SAR alert from the parent (which did not itself flag for mutagenicity). As a consequence, it would be recommended that the risk of formation (in the DS and DP over the shelf life) should be evaluated in the MRA. Note that of the two flagged structures, only aniline **3** was observed under stressed conditions, whereas the hydroperoxide **5** was implicated by the formation of degradant **6**. Please see the following section for additional considerations for the risk assessment of the hydroperoxide intermediate **5**.

2.4.3. Bringing the MRA Together: Assessing the Identified Structures. In the preceding section, two structures were flagged for further scrutiny (aniline **3**, and the hydroperoxide, **5**) from the (Q)SAR evaluation. These are the structures of interest for risk assessment. The hydroperoxide **5** was inferred as a result of the formation of **6** and not observed under any conditions (including appropriate oxidizing stress conditions). The formation of hydroperoxides resulting from autoxidation processes is a common degradation pathway for drug products because many excipients contain oxidizing impurities or are susceptible to autoxidation themselves.[47] Detection of hydroperoxides relies on the relative stability of the specific hydroperoxide. These species are often formed as unstable intermediates (i.e., reactive intermediates) in a degradation pathway and may not be subsequently observed in the final product. This is exemplified by the oxidative degradation of RG12915 where the authors followed the time course of the oxidation reaction by observing the formation of degradation products in aqueous solution in the absence of an additional catalyst or reagent. The authors showed that the hydroperoxide was observed at measurable levels after 200 h but was barely detectable after 400 h.[48] The reduction in the hydroperoxide was matched by a commensurate increase in the secondary oxidation products, which helped demonstrate the transient nature of the hydroperoxides leading to more stable products.

In the example presented here, the hydroperoxide **5** was predicted, but not observed above the thresholds outlined in Table 4 during stress testing. Structure **5** may simply be a transient intermediate with a short lifetime, or it may be so unstable that it does not survive the aqueous analytical workup and analysis. In either case, the patient would not be expected to be physiologically exposed to hydroperoxide **5**. Therefore, in this case, hydroperoxide **5** should be included in the risk

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assessment with the conclusion that no further evaluation of **5** is required. There are cases where a hydroperoxide is observed in stress studies.[49−51] In these instances, the analytical results have confirmed stability of the PMI and its ability to accumulate and survive the analytical procedure. In such cases, consideration should be given to potential patient exposure to the PMI and risk mitigation activities should be planned and documented within the risk assessment. A fundamental prerequisite for the drug product MRA is that the formulated drug product needs to be explicitly considered. The API (or degradant) may react faster or slower in the presence of excipients or in a drug product dosage form.

If aniline **3** was observed as a degradant under acid and base hydrolysis stress testing at levels above that proposed in the selected approach, further consideration would be appropriate. In the MRA, an important question to be addressed is whether aniline **3**, formed from hydrolysis of the API, can be controlled to safe levels.

2.5. Additional Considerations for Mutagen Risk Assessments for Drug Product. Scheme 1 outlines a comprehensive general strategy for assessing the risk of formation of mutagenic degradation products in both drug substance and drug product. Risk assessment of both API and the final drug product are warranted due to the introduction of excipients and packaging materials, even though these are generally perceived to be inert and unreactive. These components should still be considered as part of a comprehensive mutagen risk assessment (e.g., via direct reaction with the drug substance or through leaching into the delivered dosage form). Brusick[52] reviewed pharmaceutical excipients from a MRA perspective and concluded that the inherent risk was low. Existing excipients are viewed as safe for human use in medicinal products based on decades of long-term usage without any significant issues. This view has been recently endorsed by ICH M7, where excipients and their associated degradation products/impurities are considered to be out of scope of the proposed guidance.[9]

2.5.1. Excipients and Packaging. While excipients themselves are out of scope, it is important to understand the role of excipients in stabilizing, destabilizing, or reacting with the active ingredient in DPs. A combination of stress studies/real time stability storage can help elucidate the most likely degradation pathways. This process typically begins with excipient compatibility testing to assess which excipients or combinations of excipients result in a stable formulation (and which lead to instability). Once this is understood it becomes possible to delineate formulation and packaging strategies to minimize product instability and be assessed in long-term stability studies or the kinetic equivalent of 6 months storage at 40 °C/75%RH in the final market drug product presentation. Mutagenic degradants can then be controlled in an analogous fashion to any other degradant.

Extractables and leachables are related drug product issues that may require assessment. Extractables are chemical compounds that are forcibly removed from drug product container closure systems, packaging, or from devices under rigorous laboratory conditions. Leachables are chemical entities, either organic or inorganic, that can migrate under normal use/conditions into a drug product type from the container closure system and packaging components in direct or indirect contact with a formulation. While considered out of scope of ICH M7, readers are directed to published FDA guidance documents[53−55] and to published work regarding the leachable safety concern thresholds proposed by the Product Quality Research Institute (PQRI).[56,57]

2.6. Photostability. As mentioned in Section 2.3, solid state stress testing is carried out under conditions that are harsher than the long-term and accelerated stability studies, and when appropriately designed, retains the kinetic information to allow correlation to accelerated or long-term stability studies. Photostability considerations require a different approach. Drug substances and products can be protected from light since primary or secondary packaging configurations can be easily designed to block all light transmission, whereas packaging alone cannot protect from temperature/humidity excursions during shipping, distribution and storage. In addition, in-use photostability studies for *iv* or topical dosage forms may be critical since these formulations may be exposed to a significant amount of light during administration to the patient. A combination of in-use photoexposure tests[58,59] and standard ICH Q1B[60] confirmatory photostability tests can provide good understanding of the photostability concerns and required packaging. The ICH Q1B confirmatory photostability test specifies a minimum exposure of 1.2 million lux·h for visible light and 200 $W{\cdot}h/m^2$ for UVA light; these exposures were intended to correspond to approximately three months of continuous exposure to ultraviolet (UV) and visible light without protective packaging in the pharmacy, warehouse, or at home.[61] Typical photostress testing conditions usually apply 2−5 times the specified ICH Q1B UV and visible dose, with 2-fold being the minimum recommended light exposure for stress studies.[62] If no degradation products are observed in the DS or DP after photostressing with or without packaging above the specified thresholds, then it is proposed that no further work is required. However, should a photodegradant be observed above the levels described in the three approaches previously described, then it is suggested that identification of the photodegradants be undertaken.

Unlike kinetics of thermal reactions in solution that typically follow the exponential temperature-dependent kinetics outlined by the Arrhenius relationship, the second law of photochemistry states that (under typical (pharmaceutically relevant) lighting conditions where two photon events are statistically negligible) photolytic reactions follow a linear relationship with light dose, i.e., there is a one-to-one relationship between the number of absorbed photons and the number of excited species.[63] Furthermore, there is a direct correlation between the degradation products that are formed and the number of excited species. If a photodegradation product is observed at a certain level after 2× ICH Q1B light exposure, then it is likely to be observed at approximately half that level when exposed to the light exposure dictated by ICH Q1B (1× ICH). If the photodegradation product turns out to be mutagenic, then control via light-protective packaging becomes a viable control strategy to ensure product quality. Based on ICH Q1B, photostability testing can be carried out on the unprotected DS or DP and then with subsequent protection (primary and then secondary packaging, as required). Assuming that protection from light may be achieved by packaging, appropriate packaging remains a viable control option for MIs/PMIs generated by photodegradation. Another option is to test the light that is transmitted through protective packaging by quantitative light transmission and to calculate the expected amount of any photodegradant. This constitutes a sound scientific way to assess whether the level of the degradant would be expected to

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approach or exceed the TTC or staged TTC thresholds. Further action may then be taken in line with ICH M7 (see sections 5 and 8.4).

2.7. Analytical Considerations. Due to the potential need for highly sensitive MI/PMI analyses (e.g., in the ppm to subppm range), having an appropriate analytical method is necessary, but in some cases difficult to achieve. This is especially true for reactive intermediates, unstable degradation products and for drug products where matrix effects provide an even greater challenge toward achieving appropriate method sensitivity. Further discussion and a decision tree to help choose an analytical technique and method can be found elsewhere.[64−67]

3. CONCLUSION

The recent publication of ICH M7 guidance on mutagenic impurities has been widely embraced by Industry and its application to drug substance synthesis is now well understood. Many organizations now have clearly defined operating procedures to ensure that the risk posed by mutagenic impurities arising through drug substance manufacture is appropriately evaluated and effectively controlled. One area that is not as developed and provides opportunity for further discussion is that of mutagenic impurities that are formed during storage of drug substance, or during manufacture and/or storage of drug product, i.e., mutagenic degradation products. This manuscript outlines three possible systematic approaches for evaluating the risk arising through drug substance/drug product degradation for the formation of mutagenic impurities (or potentially mutagenic impurities).

The initiation of a MRA process that involves degradation can start with hypothetical (predicted) mutagenic degradation products that may arise in the drug substance or product using a combination of in silico and/or expert evaluation. However, it is recommended that in silico prediction tools alone should not be used to initiate extensive investigations (e.g., "fishing" or "hunting" expeditions) for hypothetical degradation products whose significance has not been verified experimentally. Three strategies are described for building the set of degradation products to be structurally identified and included in the MRA. The first approach for triggering structure elucidation is centered around long-term and accelerated stability studies and ICH Q3A/B thresholds; the second approach focuses on the "major" degradation products and pathways observed during stress testing using an algorithm for defining "major; and the third approach focuses on those degradation products observed during stress testing that meet criteria derived from thresholds that have been scaled from ICH Q3B identification thresholds.

The use of a diverse set of solution stress conditions represents a good way to accelerate the degradation kinetics and yield a set of potential degradation products that may be considered for inclusion in the MRA along with actual and potential degradation products generated by accelerated and long-term stability studies.

A decision tree has been developed to illustrate the possible options based on the observations from stability experiments and safety studies. Following this process, the actual degradants from long-term ICH stability studies and potential degradation products from stress testing and ICH accelerated stability studies are evaluated via (Q)SAR tools and Ames testing in combination with consideration of the activity of the degradation pathway. This allows for subdividing degradants into (1) those that may be controlled by ICH Q3A/ICH Q3B approaches ((Q)SAR negative and/or (Q)SAR alerting but negative in the Ames test), or (2) those where no additional work is required (where the degradation pathway is not active regardless of Ames result) and (3) those that require comprehensive and appropriate mitigation strategies (active degradation pathway and a (Q)SAR alerting structure and/or positive Ames test).

It is hoped that the approaches described herein will provide an appropriate, scientifically sound, and practical framework to assess and address the risk posed by mutagenic impurities arising as a result of either drug substance and/or drug product degradation. Ultimately, the goal is to provide assurance of safety to the patient over the shelf life of the drug product.

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Notes

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ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ASAP	Accelerated Stability Assessment Program
DS	Drug Substance
DP	Drug Product
E_a	activation energy
MRA	Mutagen Risk Assessment
GRAS	Generally Recognized as Safe
MI	Mutagenic Impurity
ID	Identification
PMI	Potentially Mutagenic Impurity
(Q)SAR	(Quantitative) Structure Activity Relationship
TTC	Threshold of Toxicological Concern

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