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Safety evaluation of 8 drug degradants present in over-the-counter cough and cold medications

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ABSTRACT

Although the United States Food & Drug Administration (FDA) has provided guidance on the control of drug degradants for prescription drugs, there is less guidance on how to set degradant specifications for FDA OTC monograph drugs. Given that extensive impurity testing was not part of the safety paradigm in original OTC monographs, a weight of evidence (WOE) approach to qualify OTC degradants is proposed. This approach relies on *in silico* tools and read-across approaches alongside standard toxicity testing to determine safety. Using several drugs marketed under 21 CFR 341 as case studies, this research demonstrates the utility of a WOE approach across data-rich and data-poor degradants. Based on degradant levels ranging from 1 to 4% of the maximum daily doses of each case study drug and 10th percentile body weight data for each patient group, children were recognized as having the highest potential exposure relative to adults per body mass. Depending on data availability and relationship to the parent API, margins of safety (MOS) or exposure margins were calculated for each degradant. The findings supported safe use, and indicated that this contemporary WOE approach could be utilized to assess OTC degradants. This approach is valuable to establish specifications for degradants in OTCs.

1. Introduction

Although general guidance pertaining to the assessment of drug degradants is available for drug substances and drug products, specific guidance on how to set degradant specifications for FDA non-application products, such as monographed over-the-counter (OTC) drugs, is not readily available. Under current FDA guidelines, the safety assessment (i.e. qualification) of pharmaceutical drug degradants requires multiple considerations, such as whether the degradant is structurally or metabolically related to the parent active pharmaceutical ingredient (API), whether the degradant has toxicity data and/or structural alerts associated with potential hazard, such as mutagenicity or carcinogenicity, as well as other toxicity tests and considerations specified in key standards and guidance pertaining to drug impurities (Table S1). While these considerations are generally also applicable to over-the-counter (OTC) drugs, some OTC drugs, such as oral antihistamines, have been on the commercial market since the 1940's, prior to the existence of current toxicity testing methods and ICH/FDA guidelines for impurity assessment.

The ambiguity in degradant specifications for OTC drugs leads to challenges in setting USP product monograph degradant acceptance criteria for OTC Monograph products. Across marketed products, there can be a wide range of degradation product levels observed for a particular API due to differing formulations, different manufacturing processes, and batch-to-batch variability, further compounding difficulties in identifying specification limits for all drug products marketed under a monograph or containing the same API under multiple monographs.

These degradant specifications are not intended to be specific to a single product, but can be applied across numerous products containing the API of interest, assuming oral administration of the product. Furthermore, the degradant specifications should not be set higher than the limits qualified by the toxicological assessment. These specifications can then be leveraged for the development of United States Pharmacopeia (USP) Monographs for each product and facilitate interactions between USP, FDA, and industry stakeholders regarding OTC drug degradant qualification procedures. Additional information pertaining

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Abbreviations

API	Active pharmaceutical ingredient
BMDL	Benchmark Dose
CPM	Chlorpheniramine
CPM-NOx	Chlorpheniramine N-oxide
CHPA	Consumer Healthcare Products Association
CFR	Code of Federal Regulations
DEX	dextromethorphan
DEX-keto	17-methyl, (9a,13a,14a)-morphinan-10-one, 3-methoxy
DEX-NOx	Dextromethorphan N-oxide
4,6-DMQ	4,6-dihydroxy-2-methylisoquinolone
4,8-DMQ	4,8-hihydroxy-2-methylisoquinolone
DOX	Doxylamine
DOX-NOx	Doxylamine N-oxide
ECHA	European Chemicals Agency
EMA	European Medicines Agency
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
GRASE	Generally recognized as safe and effective
GLP	Good Laboratory Practice
HED	Human Equivalent Dose
IARC	International Agency for Research on Cancer
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
LSMA	Leadscope Model Applier
LOAEL	Lowest observed adverse effect level
MDD	Maximum daily dose
MOS	Margin of safety
NHANES	National Health and Nutrition Examination Survey
NTP	National Toxicology Program
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OTC	Over-the-counter
PDE	Permitted daily exposure
PE	Phenylephrine
PE-HCl	Phenylephrine hydrochloride
PE-bitartrate	Phenylephrine bitartrate
POD	Point of departure
QSAR	Quantitative structure activity relationship
TDI	Total daily intake
USP	United States Pharmacopeia
WHO	World Health Organization
WOE	Weight of evidence

to the development of acceptance criteria considered herein is presented in companion commentary article "Industry Addresses Challenges of OTC Drug Modernization Request from FDA and USP."

Considering that formal guidance regarding impurity qualification specific to monographed OTC drugs has not been published by regulatory authorities, herein we describe and implement a weight-of-evidence (WOE) approach utilizing multiple lines of evidence where available to independently assess and support safety of identified data-poor degradants in marketed OTC products. This approach results in an evaluation of degradant safety outside of the context of overall product quality (i.e., without consideration of the total product impurity profile). ICH Q3B and ICH Q3C were used as the primary regulatory guidance to support derivation of daily safe exposure limits to each degradant of interest, in conjunction with quantitative structure activity relationship (QSAR) data to support compounds lacking adequate mutagenicity data per ICH M7. Where available, metabolite data showing a clear metabolic relationship between the API of interest and degradant, as well as read-across approaches, were also considered as lines of supportive evidence for safety. Whenever possible and based upon available data, Permissible Daily Exposure (PDE) values were calculated per ICH Q3C guidance for the purposes of deriving a margin of safety (MOS) based upon expected daily patient exposure. If degradant-specific repeat-dose safety studies were available, an exposure margin approach comparing the dose from an animal study to expected daily patient exposures, was considered. As described in this paper, the WOE approach was implemented to assess the safety of 8 degradants present in 5 APIs commonly used in cough and cold medications.

2. Methods

2.1. OTC degradants of interest

The APIs and associated degradants assessed herein are described in Table 1.

2.2. Exposure evaluation

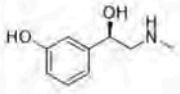
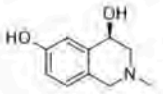
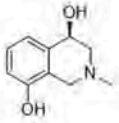
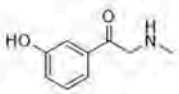
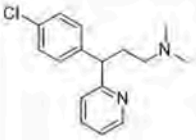
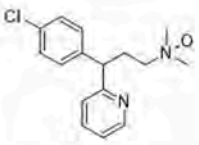
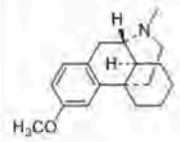
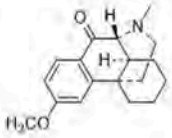
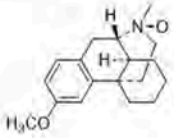
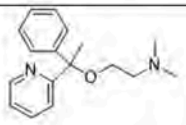
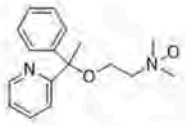
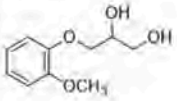
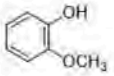
Overall, a total of 8 impurities present in 5 APIs and their salt forms were evaluated. Proposed impurity specification levels were calculated based upon the maximum daily doses (MDD) described in the CFR for each patient population (i.e., 2 year olds, 6–11 year olds, ≥12 year olds) as applicable depending on labeled drug use per 21 CFR 341. For each degradant of interest, the supported specification limit exceeded the ICH Q3B qualification threshold. In order to be as conservative as possible, 10th percentile body weights based upon the 2011–2014 NHANES report (CDC, 2016) were identified for the lowest patient age in each given range per label use (e.g., 2 year old, 6 year old, and 12 year old). Body weights in the NHANES report are intended to reflect those of the United States civilian noninstitutionalized population of all ages from birth to 80 years and over. Male and female weights were identified and the lowest body weight between the sexes was selected. Identified body weights were 11.2 kg (female, 2 year old), 17.8 kg (female, 6 year old), and 36 kg (male, 12 year old). Based upon these pediatric body weights and the standard 60 kg for adults, degradant intakes were estimated on a body weight basis (μg/kg bw/day) (CDC, 2016).

2.3. Development of the weight-of-evidence approach to evaluate safety of drug degradants

Considering the ambiguities in qualification of OTC drug degradants marketed under the existing OTC Monograph system, a WOE approach was considered to assess safety of 8 drug degradants related to common cough/cold APIs including phenylephrine, chlorpheniramine, dextromethorphan, doxylamine, and guaifenesin. This WOE approach is intended to support safety evaluation and regulation of OTC drug degradants while utilizing already existing data primarily held in the public domain. A step-wise framework was developed for assessing each degradant of interest based upon data availability, with multiple routes identified to qualify the proposed safe level of a degradant (see Fig. 1).

- The availability of adequate degradant-specific safety data (especially repeat-dose toxicity studies) in the public domain based upon targeted literature searches OR availability of a study conducted in compliance with Good Laboratory Practice (GLP) addressing preclinical safety of the degradant was the first approach/step.
- Availability of documented *in vivo* metabolism data identifying a metabolic relationship between the API and degradant of interest were considered subsequently.

Table 1
APIs and Associated Degradants of Interest.

API	Degradants	21 CFR Section and Intended Use of API
 **Phenylephrine**[a] CAS# 59-42-7 (free base)	 **4,6-DMQ** 4,6-dihydroxy-2-methylisoquinolone CAS# 23824-24-0 (free base)  **4,8-DMQ** 4,8-dihydroxy-2-methylisoquinolone CAS# 57236-57-4 (R specific); 23824-25-1 (not stereo specific)  **Phenylephrone** CAS# 52093-42-2	21CFR341 (nasal decongestant)
 **Chlorpheniramine** CAS# 132-22-9 (free base)	 **CPM-NOx** Chlorpheniramine N-oxide CAS# 120244-82-8 (free base)	21CFR341 (anti-histamine)
 **Dextromethorphan** CAS# 125-71-3 (free base)	 **DEX-keto** 17-methyl, (9a,13a,14a)-morphinan-10-one, 3-methoxy CAS# 57969-05-8  **DEX-NOx** Dextromethorphan N-oxide CAS# 1177494-18-6	21CFR341 (antitussive)
 **Doxylamine** CAS# 469-21-6 (free base)	 **DOX-NOx** Doxylamine N-oxide CAS# 97143-65-2 (free base)	21CFR341 (anti-histamine)
 **Guaifenesin** CAS# 93-14-1	 **Guaiacol** CAS# 90-05-1	21CFR341 (expectorant)

[a] Includes phenylephrine bitartrate and phenylephrine hydrochloride

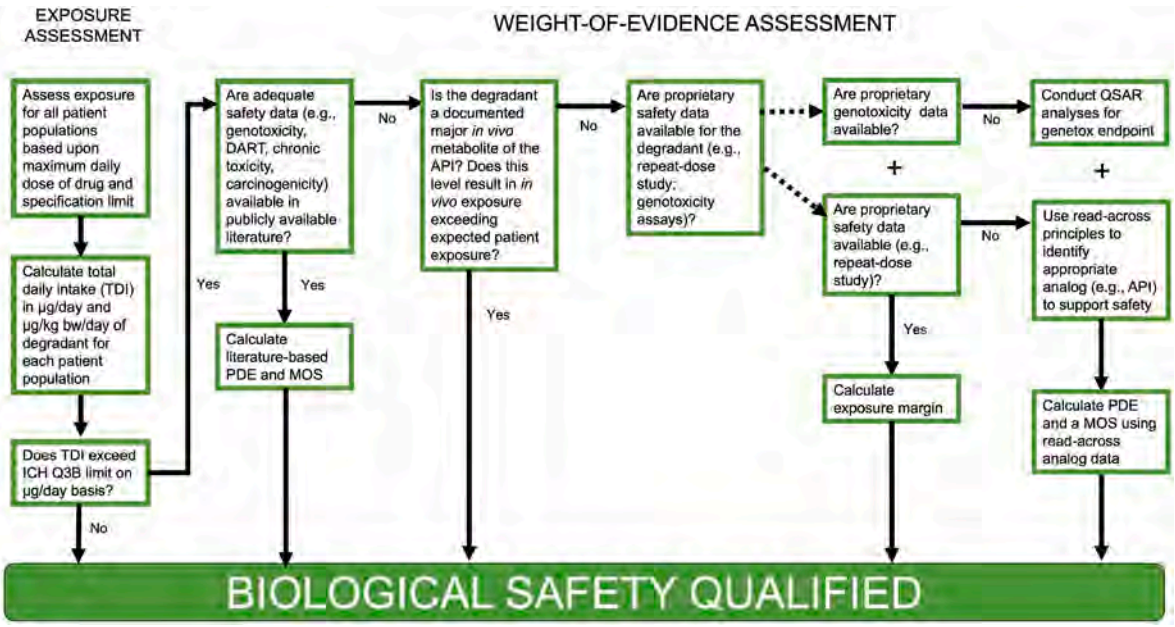


Fig. 1. Decision Flowchart for Assessment of OTC Drug Degradants. A series of steps based upon the standard risk assessment process were considered to conduct WOE assessments of degradants based upon data availability.

- In cases where adequate degradant-specific safety data were not identified, additional approaches were considered:
  - o Generation of predictive QSAR data pertaining to mutagenicity, genotoxicity, and carcinogenicity for degradants with observed data gaps following a literature search.
  - o Application of read-across strategies to identify an analog for use in addressing safety for degradants lacking publicly available repeat-dose safety data and/or lack of GLP-compliant standalone study.

Evaluation of available data assumed oral exposure to identified drug products only, did not consider potential aggregate exposure to the degradants from concomitant use of multiple drug products, and focused on supporting safety of the degradant outside of the context of product quality or overall drug safety. This WOE approach was further bolstered by consideration of the following evidence streams.

1. **Assessment of mutagenic potential per ICH M7 (2023) using two complimentary quantitative structure-activity relationship [QSAR] models.** The expert-rule-based Derek Nexus and statistical-based Leadscope Model Applier (LSMA) QSAR models were deployed to assess compounds for which existing mutagenicity data are inadequate. If structural alerts are not identified by these models, it can generally be concluded that the impurity is not of mutagenic concern. Expert review can also be used to support interpretation of the predicted model outputs, which may result in acceptance or rejection of the (Q)SAR prediction. If there are structural alerts, the exposure to the degradants would need to be controlled below TTC levels, which are intended to be applicable to all patient populations, as described in ICH M7.
2. **Assessment of available metabolism data.** Per ICH Q3B, "degradation products that are also significant/major metabolites [of the API] present in animal and/or human studies are generally considered qualified," (ICH, 2006b). Therefore, in addition to traditional *in vivo* pharmacokinetics data, *in vitro* assays identifying the degradant as a metabolite were also evaluated and used as part of the WOE argument.
3. **Assessment of available repeat-dose toxicity study data.** At minimum, 14- or 28-day studies were available for each degradant of interest or relevant analog and evaluated for use as the point of departure in a PDE calculation. Studies were either described in the public domain or neat degradant qualification studies[1] independently conducted by a Consumer Healthcare Products Association (CHPA) sponsor under GLP conditions. Per FDA guidance regarding study design to support safety of a prescription drug degradant at a specified level, studies ranging from 14 to 90 days are recommended (FDA, 2010a). Doses for studies described in this manuscript were selected by the sponsor in the context of specific product(s) and degradant level(s) identified internally by a CHPA sponsor in order to provide an adequate safety margin considering anticipated exposure to the degradant with administration of the maximum daily dose of the sponsor-specific drug product. Although degradant levels identified by other CHPA member companies may vary, studies were considered applicable to addressing degradant safety in the context of this assessment based upon lack of observable toxicity at all doses assessed. The purpose of the neat degradant qualification studies was not identification of the maximum tolerated dose or dose-response toxicity to support derivation of a PDE; instead, study data were utilized for the purposes of supporting safety of an intended impurity level. In cases where the No Observed Effect Level (NOEL) was the highest dose level tested in the study identified, it is feasible that higher doses are safe.
4. **Consideration of expected patient use and history of use.** Per label instructions for cold, cough, and allergy products (21 CFR 341), extended, chronic use is not expected, although intermittent use is likely over a lifetime. For compounds for which a PDE was derived, this calculation was based upon a chronic point of departure when possible and included safety factors such that the PDE is considered protective of chronic daily exposure.

2.4. Hazard assessment

To assess the potential hazard associated with each degradant, a

[1] A neat degradant qualification study is one where the degradant is assessed as the test article outside of the drug matrix (i.e., not a study where degradant safety is assessed by spiking higher degradant levels in the drug substance or drug product for use as test article).

targeted literature search was conducted to identify applicable systemic toxicity, genotoxicity, pharmacokinetic, and *in vivo* metabolism data, with preference for data for the degradant obtained using standardized or controlled exposures in humans or laboratory animals. A search of the following databases or authoritative sources were included: PubMed, PubChem, Embase, EMA (European Medicines Agency), WHO (World Health Organization), International Agency for Research on Cancer (IARC), US FDA, US NTP (National Toxicology Program), and ECHA (European Chemicals Agency). In instances where the degradant was data-poor but shared structural similarity with the API, publicly available literature was also searched for existing authoritative toxicology reviews and risk assessments for the API. In cases where the API was used as a surrogate structure, an analysis was conducted, consistent with international guidelines and best practices for read-across approaches in hazard and risk assessment from the EMA (2018), ECHA (2008, 2017), and US Environmental Protection Agency (Patlewicz et al., 2017), to determine if the compound exhibited adequate structural similarity, physiochemical properties, and similar anticipated pharmacokinetic, metabolism and toxicity profiles.

For impurities lacking empirical genotoxicity data, an *in silico* approach was considered based upon QSAR modeling using complementary statistical (Leadscope Model Applier, version 3.0.1, Instem) and expert rules-based models (DEREK, version 6.1.0, Lhasa Ltd.), to meet specifications set forth in ICH M7(R2) (ICH, 2023). Derek (v. 6.1.0) and Leadscope Model Applier (v. 3.0.1) (LSMA) were used in the evaluation of drug degradants lacking publicly available genotoxicity data. Endpoints assessed in Derek included carcinogenicity and genotoxicity (including mutagenicity and chromosome damage). In LSMA, endpoints assessed included the statistical models for genotoxicity including clastogenicity *in vitro,* clastogenicity *in vivo,* and gene mutation.

Although these additional endpoints beyond bacterial mutagenicity have not received full regulatory acceptance, they provide valuable data regarding the potential for genotoxicity associated with a compound.

Where available, a combination of these data sources including existing unpublished degradant-specific toxicity and metabolism data, literature findings, and *in silico* approaches, were used to support safety of the impurities.

2.5. Risk assessment

Relevant hazard data were reviewed to identify appropriate point(s)-of-departure (i.e., the most conservative NOEL from repeated, chronic oral exposures, if available). Depending on data availability and relationship to the parent API, an exposure margin or MOS based on PDE derivations, the latter which include uncertainty factors, were calculated for each degradant. An exposure margin was derived for degradants for which the highest dose tested was identified as the study NO(A)EL from an independently-conducted repeat-dose study designed to qualify sponsor-specific degradant levels in the absence of publicly available data in the literature. In cases where adequate repeat-dose studies were available in the public domain for a degradant, the lowest NOEL or non-observed adverse effect level (NOAEL) from these studies were used as the point of departure (POD) in derivation of a PDE.

For compounds with adequate publicly available repeat-dose data for which only a lowest-observed-adverse-effect-level (LOAEL) was identified, dose-response modeling using the US EPA Benchmark Dose Program (Version 3.3.2, March 2023) was used to estimate a benchmark dose ($BMDL_{10}$) per EPA (2012) guidelines. The corresponding BMDL10 is the statistical 95% lower confidence limit of the BMD10. Under risk assessment paradigms used outside of pharmaceutical risk assessment, a BMDL10 is typically preferred over a NOAEL or LOAEL since it considers the dose-response data across the entire range of observation (EPA, 2012). In cases where a BMDL10 could be derived, PDEs using an available LOAEL and the BMDL10 were derived concurrently for comparative purposes.

2.5.1. Derivation of the exposure margin

Derivation of an exposure margin is a standard approach used in risk assessment of product categories such as cosmetics or food impurities, and has also been used in the evaluation of pharmaceuticals. This approach may be utilized to evaluate data derived from neat degradant qualification studies, where study design is not intended to establish a PDE, but instead determine that an intended degradant level present in the drug substance or drug product is safe. This approach traditionally does not explicitly reflect uncertainty in the data as considered in a PDE calculation, and does not include safety factors for interspecies variability, intraspecies variability, study quality, study duration, use of a NOAEL compared to LOAEL, or potential toxicities associated with the selected animal dose (if a LOAEL is used). The exposure margin is calculated as such:

$$\text{Exposure margin} = \frac{\text{Nonclinical NO(A)EL } \left(\frac{\text{mg}}{\text{kg}}\right)}{\text{Maximum human degradant exposure dose } \left(\frac{\text{mg}}{\text{kg}}\right)}$$

For degradants lacking notable toxicity at the highest dose level evaluated as part of a neat degradant qualification study, the ICH Q3C PDE derivation process was not considered as the resulting PDE could be artificially conservative, and reflect an inappropriate extrapolation of the study design (which is intended to support safety of an intended degradant level as opposed to identification of toxicity of the degradant at dose levels anticipated to be significantly higher than exposure from use of a drug product). Therefore, an exposure margin was derived by dividing the nonclinical dose by expected human exposure to the degradant. There is a lack of global harmonization amongst key regulatory authorities and drug sponsors regarding integration of allometric scaling of the nonclinical dose for purposes of identifying an adequate safety margin for degradants (Graham et al., 2021; Mitra et al., 2021; Bercu et al., 2019). In order to further support safety of the degradants evaluated using data derived from a neat degradant qualification study, allometric scaling of the nonclinical dose using interspecies extrapolation factors described in ICH Q3C (2021) to arrive at a Human Equivalent Dose (HED) was also considered (i.e., a factor of 5 for rat studies or 12 for mouse studies) (Graham et al., 2021).

2.5.2. Derivation of the PDE and MOS

The PDE, which is intended to be the maximum acceptable intake of an impurity per day in a pharmaceutical product, was calculated for each degradant, which includes modifying (i.e., safety) factors to account for various uncertainties in the available dataset. The PDE is derived preferably from a NO(A)EL or comparable POD as follows per ICH (2021):

$$\text{PDE} = \frac{[\text{NOEL, NOAEL, LOAEL, etc. }] \times \text{body weight adjustment}}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}}$$

Where.

F1 = A factor to account for extrapolation between species.
F2 = A factor of 10 to account for variability between individuals.
F3 = A factor to account for toxicity studies of short-term exposure
F4 = A factor applied in cases of severe toxicity (e.g., nongenotoxic carcinogenicity, neurotoxicity, reproductive or developmental toxicity).
F5 = A factor applied if the NOEL was not established

The derived PDE values were then compared to the estimated human exposure levels to the impurity to derive a MOS.

$$\text{MOS} = \frac{\text{PDE}}{\text{maximum daily degradant exposure}}$$

A MOS of ≥ 1 indicates that the PDE is higher than the estimated

human exposure and thus is acceptable. A MOS of ≤1 would require additional consideration and may be acceptable under limited conditions depending on additional factors, such as expected consumer use patterns.

3. Results

The WOE framework described was used to address the safety of 8 OTC degradants associated with 5 APIs including 4,6-DMQ, 4,8-DMQ, phenylephrone, CPM-NOx, DEX-keto, DEX-NOx, DOX-NOx, and guaiacol (see Table 1 for additional information). Based upon the findings of literature searches conducted on the degradants and respective APIs in conjunction with existing proprietary data, key lines of evidence were identified for each impurity of interest. Data availability for each degradant of interest is provided in Table 2.

Based upon available data described in Table 2 and the workflow described, there were three primary approaches utilized in determining safety of degradant exposure quantitatively dependent upon the repeat-dose study available as the POD.

1. Compounds with available degradant-specific data in the literature for deriving a PDE and MOS (i.e., guaiacol)
2. Compounds with available degradant-specific data from standalone studies for deriving an exposure margin (i.e., 4,6-DMQ and 4,8-DMQ, CPM-NOx, and DOX-NOx)
3. Compounds requiring implementation of read-across to derive a PDE and MOS (i.e., phenylephrone, DEX-keto, and DEX-NOx)

3.1. Degradant-specific assessments

3.1.1. Degradants with available degradant-specific data in the literature for deriving a PDE

3.1.1.1. Guaiacol. Guaiacol has been reported as a degradant formed by oxidation of parent API guaifenesin, and is not a known *in vivo* metabolite of the API. Guaiacol is listed in 21 CFR 172.515 as a synthetic flavoring substance for direct addition to food for human consumption when used in the minimum quantity required to produce its intended effect, and otherwise in accordance with all the principles of good manufacturing practice. In some markets, guaiacol is used as an expectorant, antiseptic, and local anesthetic, especially for dental purposes (DrugBank, 2023; Hamaguchi and Tsutsui, 2000; Mimura et al., 2005; Reddy et al., 2011).

Based upon a specification limit of 1% for guaiacol and maximum daily doses of 600 mg/day, 1200 mg/day, and 2400 mg/day guaifenesin in patients 2–5 years old, 6–11 years old, and ≥12 years old, respectively, estimated maximum daily exposure to guaiacol was calculated to be 535.7 μg/kg bw/day, 674.2 μg/kg bw/day, and 666.7 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight of 60 kg was calculated to be 400 μg/kg bw/day (see Table S2).

Based upon available data in the public domain, guaiacol was found to be non-genotoxic (Haworth et al., 1983; Pool and Lin, 1982; ECHA 2023; Api et al., 2022; Hikiba et al., 2005; Miyachi and Tsutsui, 2005; Hamaguchi and Tsutsui, 2000). In available oral rodent subchronic and chronic repeat-dose studies, guaiacol exhibited portal of entry effects (e.g., hyperplasia of the forestomach). In rats administered guaiacol (>98% purity) in the diet at 0% or 2% concentrations (equivalent to 1500 mg/kg bw/day) for 28 days, Kawabe et al. (1994) reported significant decreases in body weight, increased relative kidney and liver weights, and increased thickness of the forestomach mucosa, glandular stomach, and esophagus. In a 51-week study conducted by Hirose et al. (1989) assessing the potential chronic toxicity and carcinogenicity of dietary administration of 0% or 1.5% guaiacol in the diet (equivalent to 1000 mg/kg bw/day) in male rats among other catechols, mild- and moderate forestomach focal hyperplasia was observed in 15 of 16 rats receiving guaiacol, but not in control animals. Although hyperplasia was observed, animals administered guaiacol did not develop papillomas, carcinomas *in situ*, and squamous cell carcinomas; however, administration of other catechols including p-t-butylcatechol and p-methylcatechol elicited both hyperplasia and papillomas. Hirose et al. (1989) therefore proposed that the ortho configuration of the alcohol and methoxy substituents, and the methoxy substituent mitigated the hazard potential and potency of guaiacol. Based upon available data, the LOAEL of 1000 mg/kg bw/day identified in the 51-week study was selected for derivation of a PDE (Equation 1).

Equation 1: PDE derivation for guaiacol

$$\mathrm{PDE} = \frac{\mathrm{POD}}{F_1 \times F_2 \times F_3 \times F_4 \times F_5} = \frac{1000\ \mathrm{mg/kg}}{5 \times 10 \times 2 \times 1 \times 3} = 3.333\ \frac{\mathrm{mg}}{\mathrm{kg}}$$
$$= 3333\ \mu\mathrm{g} \Big/ \mathrm{kg}$$

Where.

POD = 1000 mg/kg
F1 = 5, to account for extrapolation from rat to human
F2 = 10, to account for human variation
F3 = 2, due to use of a 51-week study
F4 = 1, due to lack of observed severe toxicities
F5 = 3, due to use of the LOAEL identified from a 51-week bioassay

This PDE (3333 μg/kg) was used for deriving patient population-specific MOS values based upon expected exposure (see Table 4). Considering the patient population of 6–11 year olds exhibited the highest expected exposure concentration (Table 4), the most conservative MOS of 5 was derived (Equation 2).

Equation 2: MOS derivation for Guaiacol

$$\mathrm{MOS} = \frac{\mathrm{PDE}}{\text{maximum daily degradant exposure}} = \frac{3333\ \mu\mathrm{g/kg\ bw/day}}{674.2\ \mu\mathrm{g/kg\ bw/day}} = 5$$

3.1.2. Degradants with available degradant-specific data from standalone studies for deriving an exposure margin

3.1.2.1. 4,6-dihydroxy-2-methylisoquinolone (4,6-DMQ) and 4,8-dihydroxy-2-methylisoquinolone (4,8-DMQ). 4,6-dihydroxy-2-methylisoquinolone (4,6-DMQ) and 4,8-dihydroxy-2-methylisoquinolone (4,8-DMQ) are known data-poor degradants of parent API phenylephrine hydrochloride (PE-HCl) or phenylephrine bitartrate (PE-bitartrate) formed by reaction of PE with trace amounts of formaldehyde. PE-HCl is for use in patients ≥2 years old and PE-bitartrate is only for use in patients ≥6 years old. Considering that 4,6-DMQ and 4,8-DMQ exhibit structural and physicochemical similarities, these compounds were assessed together (see Supplemental Section 1.1; Table S3; Table S4) for additional information pertaining to the read-across).

Based upon a specification limit of 4% each for 4,6-DMQ and 4,8-DMQ and maximum daily doses of 15 mg/day, 30 mg/day, and 60 mg/day PE-HCl in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to either 4,6-DMQ or 4,8-DMQ was calculated to be 53.6 μg/kg bw/day, 67.4 μg/kg bw/day, and 66.7 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight was calculated to be 40 μg/kg bw/day (see Table S2).

At the same 4% specification limit each for 4,6-DMQ and 4,8-DMQ and maximum daily doses of 31.2 mg/day and 62.4 mg/day PE-bitartrate in patients 6–11 years old and ≥12 years old, maximum daily exposure to either 4,6-DMQ or 4,8-DMQ was calculated to be 70.1 μg/kg bw/day and 69.3 μg/kg bw/day, respectively. Exposure in adults using the standard FDA bodyweight was calculated to be 41.6 μg/kg bw/day.

Review of QSAR predictions for potential carcinogenicity,

Table 2
Lines of evidence associated with evaluation of each impurity of interest.

API	Degradant	Lines of evidence					
		QSAR	Empirical genotoxicity data	Metabolite of API	Use of API as surrogate	*In vivo* degradant-specific data in the literature	*In vivo* degradant-specific data from stand-alone study
Phenylephrine	4,6-DMQ	X	–	–	–	N	Y
	4,8-DMQ	X	–	–	–	N	Y[a]
	Phenylephrone	X	–	–	X	N	N
Chlorpheniramine	CPM-NOx	X	–	X	–	N	Y
Dextromethorphan	DEX-keto	X	–	–	X	N	N
	DEX-NOx	X	–	–	X	N	N
Doxylamine	DOX-NOx	X	–	X	–	N	Y
Guaifenesin	Guaiacol	X	–	–	–	Y	N

X = data available; - = no applicable data available; Y = yes; N = no.
[a] Data pertaining to 4,6-DMQ were used to support safety of 4,8-DMQ.

chromosomal damage, genotoxicity, and mutagenicity of 4,6-DMQ and 4,8-DMQ (see Table S5) suggests that the impurities may be considered Class 5 compounds per ICH M7 and treated as non-genotoxic impurities.

No pharmacokinetics, acute toxicity, genotoxicity, carcinogenicity or reproductive/developmental hazard data specific to 4,6-DMQ or 4,8-DMQ were identified in the literature. However, a 14-day GLP-compliant study evaluating the potential toxicity of 4,6-DMQ was available (see Supplemental Section 1.2 for additional details). In this study, male and female Sprague Dawley rats were orally administered 0, 1, 5, or 15 mg/kg bw/day 4,6-DMQ; test article-related effects pertaining to clinical observations, food consumption, body weights, serum chemistry parameters, hematology parameters, urinalysis parameters, or macroscopic/microscopic histopathological changes were not observed. A NOAEL of 15 mg/kg bw/day was concluded.

Use of the ICH Q3C derivation process of 15 mg/kg/day as the POD in a PDE derivation was not considered in the absence of notable toxicity associated with the compound as the resulting PDE could be artificially conservative. Therefore, an exposure margin was derived by dividing the nonclinical dose by expected human exposure. Based upon read-across principles, data pertaining to 4,6-DMQ were considered applicable to 4,8-DMQ, such that the exposure margins were considered applicable to both impurities present in PE-HCl and PE-bitartrate. The most conservative exposure margin values of 223 and 214 were derived for patients 6–11 years old for both PE-HCl and PE-bitartrate, respectively, considering this patient population exhibited the highest expected exposure concentration (Equation 3 and Equation 4; also see Table 5).

Equation 3: Exposure margin derivation for 4,6-DMQ or 4,8-DMQ in PE-HCl

$$\text{Exposure margin} = \frac{\text{Nonclinical NO(A)EL}}{\text{maximum daily degradant exposure}} = \frac{15000\ \mu g/kg\ bw/day}{67.4\ \mu g/kg\ bw/day} = 223$$

Equation 4: Exposure margin derivation for 4,6-DMQ or 4,8-DMQ in PE-bitartrate

$$\text{Exposure margin} = \frac{\text{Nonclinical NO(A)EL}}{\text{maximum daily degradant exposure}} = \frac{15000\ \mu g/kg\ bw/day}{70.1\ \mu g/kg\ bw/day} = 214$$

Application of allometric scaling to the nonclinical NOAEL of 15000 μg/kg bw/day (i.e., 15000 μg/kg bw/day divided by a scaling factor of 5 to account for use of rat data) also resulted in acceptable exposure margins of 45 and 43 for PE-HCl and PE-bitartrate, thus supporting safety of the degradant at anticipated exposure levels.

3.1.2.2. Doxylamine N-oxide (DOX-NOx). DOX-NOx has been reported as a degradant formed by oxidation of parent API doxylamine (DOX). Based upon a specification limit of 3% for DOX-NOx and maximum daily doses of 18.75 mg/day, 37.5 mg/day, and 75 mg/day DOX in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to DOX-NOx was calculated to be 50.2 μg/kg bw/day, 63.2 μg/kg bw/day, and 62.5 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight of 60 kg was calculated to be 37.5 μg/kg bw/day (see Table S2).

DOX-NOx has been shown to be a minor urinary metabolite in rats and rhesus monkeys, and was identified at up to 1% of recovered radiolabel in the urine (Ganes et al., 1986; Slikker et al., 1986; Holder et al., 1985). DOX-NOx was also identified as an *in vitro* metabolite of radiolabeled DOX succinate following incubation with rat hepatocytes (Holder et al., 1987). Considering DOX-NOx is not reported as a major *in vivo* metabolite of DOX, these data were generally considered to support, but alone do not adequately justify, safety of DOX-NOx. QSAR evaluation of the compound for potential carcinogenicity, chromosomal damage, genotoxicity, or mutagenicity resulted in a Class 4 designation per ICH M7 based upon presence of an 1-alkoxymethylbenzene feature, which was a major positive contributing structural feature in a LSMA bacterial mutagenicity model shared with the parent API DOX, which has been shown to be non-mutagenic. DOX-NOx also contained an aromatic N-oxide structural feature, which have been evaluated extensively by Amberg et al. (2019), and concluded to be over-predictive for bacterial mutagenicity. The totality of QSAR data suggested that DOX-NOx could be treated as a non-mutagenic impurity (see Table S5).

No pharmacokinetics, acute toxicity, genotoxicity, carcinogenicity or reproductive/developmental hazard data specific to DOX-NOx were identified in the literature. However, a 28-day GLP-compliant study intended to evaluate the potential toxicity of DOX-NOx as a drug impurity was available (see Supplemental Section 1.3 for additional details). In this study, ICR mice were orally administered 0, 0.03226, 0.3226, and 3.226 mg/kg bw/day DOX-NOx. Test article-related mortality, dose-dependent clinical signs, body weight gain, total body weight, clinical chemistry parameters, hematology parameters, organ weight alterations, or macroscopic/microscopic histopathological changes were reported, although high-dose males exhibited significantly increased food consumption. A NOAEL for the study was not identified by study authors due to lack of observed test article-related toxicity, changes in body weight, or microscopic findings for which to base the NOAEL. It is highly plausible that a NOAEL ≥3.226 mg/kg bw/day exists for this compound. Therefore, an exposure margin was derived by dividing the nonclinical dose by expected human exposure. The most conservative exposure margin of 51 was derived for patients 6–11 years old considering this patient population exhibited the highest expected exposure concentration (Equation 5; also see Table 5). Application of allometric scaling to the nonclinical NOAEL of 3226 μg/kg bw/day (i.e., 3226 μg/kg bw/day divided by a scaling factor of 12 to account for use of mouse data) also resulted in an acceptable exposure margin of 4, thus supporting safety of the degradant at the anticipated exposure level.

Equation 5: Exposure margin derivation for DOX-NOx

$$\text{Exposure margin} = \frac{\text{Nonclinical NO(A)EL}}{\text{maximum daily degradant exposure}} = \frac{3226\ \mu g/kg\ bw/day}{63.2\ \mu g/kg\ bw/day} = 51$$

3.1.2.3. Chlorpheniramine N-oxide (CPM-NOx). CPM-NOx has been reported as a degradant formed by oxidation of parent API chlorpheniramine (CPM). Based upon a specification limit of 3% for CPM-NOx and maximum daily doses of 6 mg/day, 12 mg/day, and 24 mg/day CPM in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to CPM-NOx was calculated to be 16.1 μg/kg bw/day, 20.2 μg/kg bw/day, and 20 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight of 60 kg was calculated to be 12 μg/kg bw/day (see Table S2).

No acute toxicity, genotoxicity, carcinogenicity, or reproductive/developmental hazard data specific to CPM-NOx were identified in the literature. However, CPM-NOx has been detected as a minor *in vivo* metabolite of CPM in humans and rats (Fried et al., 2002; Kasuya et al., 1991). A 28-day GLP-compliant study intended to evaluate the potential toxicity of CPM-NOx as a drug impurity was available (see Supplemental Section 1.4 for additional details). In this study, male and female Sprague Dawley rats orally administered 0, 0.238, 0.476, or 0.952 mg/kg bw/day CPM-NOx did not exhibit test article-related mortalities, changes in clinical observations, or effects on body weight, food consumption, clinical pathology endpoints (hematology, coagulation, clinical chemistry, urinalysis), organ weights, or macroscopic/microscopic histopathological changes. A NOEL of 0.952 mg/kg-bw/day CPM-NOx was identified, which was the highest dose tested. It is highly plausible that a higher NOEL is possible for this compound. The most conservative exposure margin of 47 was derived for patients 6–11 years old considering this patient population exhibited the highest expected exposure concentration (Equation 6; also see Table 5). Application of allometric scaling to the nonclinical NOAEL of 952 μg/kg bw/day (i.e., 3226 μg/kg bw/day divided by a scaling factor of 5 to account for use of rat data) also resulted in an acceptable exposure margin of 9, thus supporting safety of the degradant at the anticipated exposure level.

Equation 6: Exposure margin derivation for CPM-NOx

$$\text{Exposure margin} = \frac{\text{Nonclinical NO(A)EL}}{\text{maximum daily degradant exposure}} = \frac{952\ \mu g/kg\ bw/day}{20.2\ \mu g/kg\ bw/day} = 47$$

3.1.3. Degradants requiring implementation of read-across

3.1.3.1. Phenylephrone. Phenylephrone is a known data-poor degradant of parent API phenylephrine hydrochloride (PE-HCl) or phenylephrine bitartrate (PE-bitartrate) formed by oxidation. Both APIs are used as nasal decongestants (Gelotte and Zimmerman, 2015; FDA, 2006); however, PE-HCl is for use in patients ≥2 years old and PE-bitartrate is only for use in patients ≥6 years old. Based upon a specification limit of 2% for phenylephrone and maximum daily doses of 15 mg/day, 30 mg/day, and 60 mg/day PE-HCl in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to phenylephrone was calculated to be 26.8 μg/kg bw/day, 33.7 μg/kg bw/day, and 33.3 μg/kg bw/day, respectively. Exposure in adults was calculated to be 20 μg/kg bw/day (see Table S2). A similar calculation was carried out for PE-bitartrate, assuming a 2% specification limit for phenylephrone and maximum daily doses of 31.2 mg/day and 62.4 mg/day PE-bitartrate in patients 6–11 years old and ≥12 years old, such that a maximum daily exposure to phenylephrone was calculated to be 35.1 μg/kg bw/day and 34.7 μg/kg bw/day, respectively. Exposure in adults was calculated to be 20.8 μg/kg bw/day.

No *in vivo* or *in vitro* hazard data, including metabolism data, specific to phenylephrone were identified in the literature; review of QSAR predictions for potential carcinogenicity, chromosomal damage, genotoxicity, and mutagenicity of phenylephrone (see Table S5) suggested that the impurity may be considered a Class 5 compound per ICH M7 and treated as a non-genotoxic impurity.

Considering lack of suspected mutagenicity and genotoxicity of phenylephrone as well as close structural and physicochemical similarity to parent API phenylephrine, key data for phenylephrine were used to derive a PDE for phenylephrone. Additional physicochemical information supporting the read-across may be found in Supplemental Section 1.5, Table S10, and Table S11.

PE-HCL was generally considered non-genotoxic based upon a battery of studies conducted by NTP (1987). In two-year bioassays also conducted by NTP in F344/N rats and B6C3F1 mice administered 0, 620, or 1250 ppm (equivalent to 0, 24, or 50 mg/kg bw/day) or 0, 1250, or 2500 ppm (equivalent to 0, 133, or 270 mg/kg bw/day) PE-HCl (99% purity) in the diet, respectively, no evidence of carcinogenicity was reported. Chronic focal inflammation of the liver and prostate was observed at increased incidences in dosed rats, although no neoplastic or nonneoplastic lesions were clearly related to dosing with PE HCl. Incidence of focal cellular change in the liver was increased slightly in high dose male mice. NTP noted that the liver and prostate gland lesions in male rats were similar to those in older, untreated rats, but they were subjectively judged to be more severe and more frequent in dosed male rats. Hepatic focal inflammation and prostate inflammation exhibited increased incidence in treated versus control rats (hepatic focal inflammation, males: 2/50, control; 13/50, low dose; 17/50, high dose; hepatic focal inflammation, female: 17/50, control; 28/50, low dose, 35/50, high dose; prostate inflammation: 10/50, control; 24/50, low dose; 24/50, high dose).

A review of the NTP study by the FDA (2012) identified the low dose of 24 mg/kg bw/day PE-HCl (equivalent to 19.7 mg/kg bw/day phenylephrine[2]) as the LOAEL in rats, based on the hepatic inflammation in both sexes and prostate inflammation in males. As such, a NOAEL in rats was not identified. FDA (2012) identified the low dose of 133 mg/kg bw/day in mice as the NOAEL, based on the hepatic focal cellular change in high-dose male mice at the LOAEL of 270 mg/kg bw/day. Due to the lack of a NOAEL in rats, a benchmark dose ($BMDL_{10}$) for hepatic focal inflammation in male rats was estimated using the Benchmark Dose Program (Version 3.3.2, March 2023) according to EPA (2012) guidelines. The $BMDL_{10}$ of 3.79 mg/kg-day PE-HCl (equivalent to 3.11 mg/kg bw/day PE[3]) for hepatic focal inflammation can be used as the POD to characterize potential liver toxicity associated with oral exposure to phenylephrone. For purposes of comparison, comparative PDEs were calculated using the LOAEL from study data used to derive the $BMDL_{10}$ to highlight how the POD can significantly alter PDE derivation (see Table 3).

The PDE based upon the $BMDL_{10}$ (62 μg/kg) was used for deriving patient population-specific MOS values based upon expected exposure (see Table 4). Although use of the LOAEL resulted in a lower PDE, use of a $BMDL_{10}$ considers dose-response data across the entire range of observation and is based upon a defined response level across studies (Haber et al., 2018). The most conservative MOS of 1.8 was derived for patients 6–11 years old for both PE-HCl and PE-bitartrate, considering this patient population exhibited the highest expected exposure

[2] The PE-HCl dose of 24 mg/kg bw/day was converted to 19.7 mg/kg bw/day PE based upon molecular weights of the salt and freebase forms. Thus, 24 mg/kg bw/day PE-HCl was multiplied by the molecular weight of PE (167.20 g/mol) and divided by the molecular weight of PE-HCl (203.66 g/mol) to arrive at 19.7 mg/kg bw/day PE.

[3] The PE-HCl $BMDL_{10}$ of 3.79 mg/kg bw/day was converted to 3.11 mg/kg bw/day PE based upon molecular weights of the salt and freebase forms. Thus, 3.79 mg/kg bw/day PE-HCl was multiplied by the molecular weight of PE (167.20 g/mol) and divided by the molecular weight of PE-HCl (203.66 g/mol) to arrive at 9.03 mg/kg bw/day PE.

Table 3
Comparative PDE calculations for phenylephrone.

Study	Chronic/carcinogenicity study in rats (NTP 1987)	Chronic/carcinogenicity study in rats (NTP 1987)
Dose-limiting Endpoint/ species	Hepatic focal inflammation (both sexes) and prostate inflammation (males); rat[a]	Hepatic focal inflammation (both sexes) and prostate inflammation (males); rat
Point-of-departure (POD)	**3.11 mg/kg-bw/day $BMDL_{10}$**	**19.7 mg/kg-bw/day LOAEL**
F1: Interspecies MF[b]	5	5
F2: Intraspecies MF[c]	10	10
F3: Study duration MF	1	1
F4: Severity MF	1	1
F5 LOAEL to NOAEL MF	1	10
TOTAL	50X	500X
Permissible Daily Exposure (PDE)	**62 μg/kg-bw/day**	**39 μg/kg-bw/day**

MF = modifying factor.
[a] $BMDL_{10}$ based on hepatic inflammation in females since the BMDL derived was most conservative.
[b] The interspecies MF is based on species-specific scaling factors indicated in ICH (2021).
[c] F2 is a default factor of10 based on ICH (2021).

concentration (Equation 7 and Equation 8).

Equation 7: MOS derivation for phenylephrone in PE-HCl

$$\text{MOS} = \frac{\text{PDE}}{\text{maximum daily degradant exposure}} = \frac{62\ \mu g/kg\ bw/day}{33.7\ \mu g/kg\ bw/day} = 1.8$$

Equation 8: MOS derivation for phenylephrone in PE-bitartrate

$$\text{MOS} = \frac{\text{PDE}}{\text{maximum daily degradant exposure}} = \frac{62\ \mu g/kg\ bw/day}{35.1\ \mu g/kg\ bw/day} = 1.8$$

3.1.3.2. DEX-keto. 17-methyl, (9a,13a,14a)-morphinan-10-one, 3-methoxy (DEX-keto) is a data-poor degradant of dextromethorphan (DEX) hydrobromide formed by oxidation. Safety data pertaining to DEX-keto were not readily identified in publicly available literature. The impurity exhibits a high degree of structural similarity with parent compound DEX, aside from the presence of an additional carbonyl group. The presence of a ketone functional group on DEX-keto is generally not associated with functional group-specific reactivity (e.g., carcinogenicity or mutagenicity), and therefore supports use of DEX hydrobromide as the most relevant analog to DEX-keto for purposes of read-across (Environmental Health and Safety, 2023; Benigni and Bossa, 2011; EMA, 2018). Additional physicochemical information supporting the read-across may be found in Supplemental Section 1.6, Table S12, and Table S13.

Based upon a specification limit of 2% for DEX-keto and maximum daily doses of 30 mg/day, 60 mg/day, and 120 mg/day in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to DEX-keto was calculated to be 53.6 μg/kg bw/day, 67.4 μg/kg bw/day, and 66.7 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight of 60 kg was calculated to be 40 μg/kg bw/day (see Table S2).

No *in vivo* or *in vitro* hazard data, including metabolism data, specific to DEX-keto were identified in the literature; review of QSAR predictions for potential carcinogenicity, chromosomal damage, genotoxicity, and mutagenicity of DEX-keto (see Table S5) suggested that the impurity may be considered a Class 5 compound per ICH M7 and treated as a non-genotoxic impurity. Considering suspected lack of mutagenicity and genotoxicity of DEX-keto as well as close structural and physicochemical similarity to parent API DEX, key data for DEX hydrobromide were used to derive a PDE for DEX-keto.

The following data were identified in the publicly available label and summary basis of approval for Nuedexta® (DEX hydrobromide plus quinidine sulfate). DEX hydrobromide was found to be non-genotoxic *in vitro* and *in vivo* (Avanir Pharmaceuticals, 2010; FDA, 2010b). In a two-year carcinogenicity study in rats which assessed DEX hydrobromide and quinidine, alone and in combination, no biologically significant tumor findings were observed compared to control rats at oral doses of 20 or 50 mg/kg bw/day DEX hydrobromide alone. Further, no biologically significant tumor findings were observed when rats received Nuedexta® (DEX hydrobromide/quinidine) at 5/100, 20/100, or 50/100 mg/kg/day compared to control rats. Thus, the highest tested dose of 50 mg/kg bw/day was considered a NOAEL for carcinogenicity of DEX hydrobromide in rats. Additionally, a 26-week carcinogenicity study in the Tg.rasH2 transgenic mouse also found no evidence of carcinogenic potential when DEX hydrobromide and quinidine, alone and in combination, were tested at oral doses up to 100 mg/kg bw/day each. A NOAEL of 100 mg/kg bw/day (equivalent to 77 mg/kg bw/day dextromethorphan freebase[4]) was identified for DEX hydrobromide in mice, and considered the most relevant POD for use in deriving a PDE considering species-specific effects were not observed for the critical endpoint of interest (Avanir Pharmaceuticals, 2010; FDA, 2010b). A PDE was derived as follows (Equation 9):

Equation 9: PDE derivation for DEX-keto

$$\text{PDE} = \frac{\text{POD}}{F_1 \times F_2 \times F_3 \times F_4 \times F_5} = \frac{77\ mg/kg}{12 \times 10 \times 1 \times 1 \times 1} = 0.642\frac{mg}{kg}$$
$$= 642\ \mu g \Big/ kg$$

Where.

POD = 100 mg/kg
F1 = 12, to account for extrapolation from mouse to human
F2 = 10, to account for human variation
F3 = 1, due to use of a two-year study
F4 = 1, due to lack of observed severe toxicities
F5 = 1, due to use of an identified NOAEL from a two-year bioassay

This PDE (642 μg/kg) was used for deriving patient population-specific MOS values based upon expected exposure (see Table 4). The most conservative MOS of 10 was derived for patients 6–11 years old considering this patient population exhibited the highest expected exposure concentration (Equation 10).

Equation 10: MOS derivation for DEX-keto

$$\text{MOS} = \frac{\text{PDE}}{\text{maximum daily degradant exposure}} = \frac{642\ \mu g/kg\ bw/day}{67.4\ \mu g/kg\ bw/day} = 10$$

3.1.3.3. DEX-NOx. Dextromethorphan N-oxide (DEX-NOx) has been reported as a degradant formed by oxidation of parent API DEX hydrobromide. Similar to DEX-keto, DEX-NOx exhibits a high degree of structural similarity with parent compound DEX hydrobromide, aside from the presence of an aromatic N-oxide feature. This alert was also evaluated at length by Amberg et al. (2019) determined that the alert historically lacked substantive evidence in the literature regarding a putative mechanism of action and in the context of DOX-NOx (i.e., based

[4] The dextromethorphan hydrobromide dose of 100 mg/kg bw/day was converted to 77 mg/kg bw/day dextrmethorphan based upon molecular weights of the salt and freebase forms. Thus, 100 mg/kg bw/day dextromethorphan hydrobromide was multiplied by the molecular weight of dextromethorphan (271.404 g/mol) and divided by the molecular weight of dextromethorphan hydrobromide (352.316 g/mol) to arrive at 77 mg/kg bw/day PE.

on presence of a 1-oxide-pyridinium N-oxide moiety), is not expected to result in genotoxic effects. Additional physicochemical information supporting the read-across may be found in Supplemental Section 1.7, Table S14, and Table S15.

Based upon a specification limit of 3% for DEX-NOx and maximum daily doses of 30 mg/day, 60 mg/day, and 120 mg/day DEX-hydrobromide in patients 2–5 years old, 6–11 years old, and ≥12 years old, estimated maximum daily exposure to DEX-NOx was calculated to be 80.4 μg/kg bw/day, 101.1 μg/kg bw/day, and 100 μg/kg bw/day, respectively. Exposure in adults using the FDA standard body weight of 60 kg was calculated to be 60 μg/kg bw/day (see Table S2).

No *in vivo* hazard data specific to DEX-NOx were identified in the literature. QSAR evaluation of the compound for potential carcinogenicity, chromosomal damage, genotoxicity, or mutagenicity supported a Class 5 designation per ICH M7, and suggested that DEX-NOx may be treated as a non-genotoxic impurity (see Table S5).

Similar to DEX-keto, safety data pertaining to parent API DEX hydrobromide were directly applicable to DEX-NOx for the purposes of deriving a PDE. The same POD, a NOAEL of 100 mg/kg bw/day (equivalent to 77 mg/kg bw/day dextromethorphan) derived from a 26-week carcinogenicity study (FDA, 2010b), was used for the purposes of deriving a PDE of 642 μg/kg bw/day (see Equation 9).

This PDE (642 μg/kg) was used for deriving patient population-specific MOS values based upon expected exposure (see Table 4). The most conservative MOS of 6 was derived for patients 6–11 years old considering this patient population exhibited the highest expected exposure concentration (Equation 11).

Equation 11: MOS derivation for DEX-NOx

$$\text{MOS} = \frac{\text{PDE}}{\text{maximum daily degradant exposure}} = \frac{642\ \mu\text{g/kg bw/day}}{101.1\ \mu\text{g/kg bw/day}} = 6$$

3.2. Summary of qualifications

Implementing these approaches therefore resulted in fit-for-purpose safety justifications for each drug degradant of interest in the context of available data and historical use of their respective drug products. Tables 4 and 5 summarize findings of each degradant evaluation including patient exposure, a safe level (i.e., a PDE or study NOEL), and MOS or exposure margin, as applicable, respectively.

4. Discussion

Herein, a WOE approach that utilizes multiple lines of evidence to assess and support safety of data-poor degradants in marketed OTC drug products was developed and implemented. The approach herein utilized existing data to build a safety profile for impurities in OTC drug products with a long history of safe use. The APIs assessed are marketed for various indications (e.g., nasal decongestant, anti-histamine, antitussive) under OTC monograph 21CFR341 (cough, cold, allergy, bronchodilator, and antiasthmatic drug products for over-the-counter human use). Based upon the WOE approach, these degradants were found to have MOS values or exposure margins ≥1, suggesting that while there is a need to meet all applicable good manufacturing practices and existing product quality standards, presence of the degradants at specified levels in this document are not expected to result in degradant-related patient toxicity.

This approach, which is aligned with the 3Rs (i.e., replacement, reduction, refinement in animal research), incorporates new approach methodologies including read-across and *in silico* strategies to fulfill existing data gaps. Approaches intended to reduce the use of animal testing are consistent with priorities of other US and global authoritative bodies such as US EPA, FDA's National Center for Toxicological Research (NCTR), US NTP, ECHA, EFSA, and EMA, and reflective of animal welfare concerns of consumers. As of 2018, EMA outlined a roadmap highlighting use of new approaches to qualify safety of non-genotoxic degradants including reliance of historical data, use of *in silico* modeling, and implementation of read-across (EMA, 2018). Lines of evidence, which were grounded in ICH Q3C among other FDA guidance, are commonly used in evaluation of prescription drug products, included utilization of publicly available literature and privately held repeat-dose toxicity data, genotoxicity data (*in silico, in vitro,* or *in vivo*), and metabolism data to ascertain potential metabolic relationships between the API and degradants of interest. The historical use of the OTC drug product and expected labeled use scenarios in all relevant patient populations were considered in determining expected exposures to each degradant. Based upon the totality of these data and implementation of the WOE methodology developed, an MOS or exposure margin was calculated.

No single line of evidence was considered wholly adequate to evaluate patient safety of the degradants in these assessments, but rather a holistic, WOE approach with inherent flexibility centered around available data was required for each degradant. Where data were amenable and available, ICH Q3C and FDA guidance was followed. If sufficient data were not available, additional analyses employing the principles of read-across and *in silico* evaluation of genotoxicity were employed. For example, in deriving PDE values for the purposes of calculating an MOS for degradants including phenylephrone, DEX-keto, DEX-NOx, and guaiacol, the most conservative POD identified for the most relevant read-across analog (i.e., respective APIs) was selected when possible, and benchmark dose (BMD) modeling was used when data were amenable to this approach. While BMD modeling has not historically been used in evaluation of drug degradants, this approach is

Table 4
Summary of MOS derivations.

API	Degradant (% specification limit)	Daily exposure level in child (μg/kg-bw/day) assuming 10th percentile bw			Daily exposure level in adult assuming standard FDA bw (μg/kg/day)[a]	PDE (μg/kg-bw/day)	MOS[b]			
		2–5 year old	6–11 year old	≥12 year old			2–5 year old	6–11 year old	12 year old	adult
Phenylephrine bitartrate	Phenylephrone (2%)	–	**35.1**	34.7	20.8	**62**	–	1.8	1.8	3
Phenylephrine hydrochloride	Phenylephrone (2%)	26.8	**33.7**	33.3	20.0	**62**	2	1.8	1.9	3
Dextromethorphan	DEX-keto (2%)	53.6	**67.4**	66.7	40	**642**	12	10	10	16
	DEX-NOx (3%)	80.4	**101.1**	100	60	**642**	8	6	6	11
Guaifenesin	Guaiacol (1%)	535.7	**674.2**	666.7	400	**3333**	6	5	5	8

- = no applicable data available.

[a] Daily exposure levels in adults were calculated assuming the FDA standard 60 kg-bw for adults.

[b] MOS were calculated using the standard formula as follows: **MOS** = **PDE** (in μg/kg-bw/day)/**daily exposure level** (in μg/kg-bw/day); daily exposure values in **bold** are those that resulted in highest exposure on a μg/kg/day basis in comparison to derived PDEs for the purpose of deriving MOS values.

Table 5
Summary of exposure margin derivations.

API	Degradant (% specification limit)	Daily exposure level in child (μg/kg/day) assuming 10th percentile bw			Daily exposure level in adult (μg/kg/day)[a]	Study NO(A)EL (μg/kg bw/day)	Exposure margin[b,c]			
		2–5 year old	6–11 year old	≥12 year old			2–5 year old	6–11 year old	12 year old	adult
Phenylephrine bitartrate	4,6-DMQ (4%) 4,8-DMQ (4%)	–	**70.1**	69.3	41.6	**15000**	–	214	216	361
Phenylephrine hydrochloride	4,6-DMQ (4%) 4,8-DMQ (4%)	53.6	**67.4**	66.7	40.0		280	223	225	375
Doxylamine	DOX-NOx (3%)	50.2	**63.2**	62.5	37.5	**3226**	64	51	52	86
Chlorphenira-mine	CPM-NOx (3%)	16.1	**20.2**	20.0	12.0	**952**	59	47	48	79

- = no applicable data available.

[a] Daily exposure levels in adults were calculated assuming the FDA standard 60 kg-bw for adults.

[b] Exposure margins were calculated using the following formula: **Exposure margin** = **NOEL** (in μg/kg-bw/day)/**daily exposure level** (in μg/kg-bw/day); daily exposure values in **bold** are those that resulted in highest exposure on a μg/kg/day basis in comparison to identified nonclinical NO(A)EL values from available studies to derive exposure margins.

[c] Exposure margins were calculated using the following formula: **Exposure margin** = **NOEL** (in μg/kg-bw/day)/**daily exposure level** (in μg/kg-bw/day); daily exposure values in **bold** are those that resulted in highest exposure on a μg/kg/day basis in comparison to identified nonclinical NO(A)EL values from available studies to derive exposure margins.

less dependent on discrete experimental doses administered to animals and subsequent dose-response curve, and addresses variability and uncertainty resulting from study quality, all of which cannot be ascertained from use of a NOAEL or LOAEL value.

For degradants including 4,6-DMQ, 4,8-DMQ, CPM-NOx, and DOX-NOx, where derivation of a PDE was not considered biologically meaningful due to the maximum doses used in toxicological studies, exposure margin derivations based upon NOEL values identified in 14–28 day studies assessing the degradant were considered. However, considering that toxicities were not observed in any of the 14–28 day studies, it is likely that higher dose levels may be safe. It is of note that these studies were not designed to identify potential toxicities associated with the degradant but instead to qualify safety of the degradant at a sponsor-specified level as described by FDA to support safety of degradants in prescription drugs (FDA, 2010a). Therefore, relatively low exposure margins derived using available data were considered a function of said data as opposed to observed biological response and considered acceptable in the WOE approach in supporting the overall assumption of patient safety. Evaluation of the exposure margins with the addition of an allometric scaling factor to the nonclinical NOAEL values identified in the neat degradant qualification studies to derive HEDs were also found acceptable; this approach was implemented as well considering lack of global regulatory consensus on use of the HED in determining degradant safety (Graham et al., 2021; Mitra et al., 2021; Bercu et al., 2019). Per available literature, there appears to be inconsistency in approach in derivation of HED values which may use ICH Q3C scaling factors or those presented in FDA guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" for the purposes of supporting degradant safety (FDA, 2005; Graham et al., 2021; Bercu et al., 2019). It is of note that the available FDA guidance is applicable to derivation of an intended safe starting dose for a novel API, and therefore may be overly conservative if applied to nonclinical degradant doses evaluated as part of neat degradant qualification studies (especially with consideration to additional safety factors beyond allometric scaling described in the FDA guidance) (Bercu et al., 2019; FDA, 2005).

The WOE approach proposed and applied herein contained several aspects of conservatism further supporting the patient safety. When calculating the expected daily patient exposure levels, the most conservative 10th percentile body weight available for each patient population (i.e., 2–5 year olds, 6–11 year olds, and 12 year olds) was utilized. Additionally, chronic daily exposure was assumed as opposed to intermittent exposure over a lifetime, and patient population-specific MOS calculations are not reflective of expected patient body growth over a lifetime. Combined, these three assumptions are inherently conservative under labeled use of cough-and-cold APIs.

The WOE approach described herein provides a flexible workflow method supporting safety of drug degradants by combining available data for both data-rich and data-poor degradants present in OTC drug products historically marketed under the OTC Monograph system. Some assumptions included in this evaluation include (1) presumed oral exposure to identified drug products only, (2) did not consider potential aggregate exposure to the degradants from concomitant use of multiple drug products, (3) considered expected labeled use only, and (4) focused on supporting safety of the degradant outside of the context of product quality or overall drug safety. The objective of this work was to develop independent safety substantiations for degradants of interest utilizing existing data (in the public domain and privately-held) in the context of OTC drug products marketed for decades in the US. Overall, each degradant of interest was qualified as safe based upon implementation of an integrated WOE approach that utilized elements common in evaluation of drug impurities and across sectors.

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CRediT authorship contribution statement

Amy L. Mihalchik: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Neepa Y. Choksi:** Writing – review & editing, Validation, Software, Methodology. **Amy L. Roe:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Conceptualization. **Michael Wisser:** Writing – review & editing, Resources, Data curation, Conceptualization. **Kylen Whitaker:** Writing – review & editing, Data curation, Conceptualization. **Donna Seibert:** Writing – review & editing, Data curation, Conceptualization. **Milind Deore:** Writing – review & editing, Resources, Investigation, Conceptualization. **Larisa Pavlick:**

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

ToxStrategies, LLC received consulting fees from the Consumer Healthcare Products Association (CHPA; Washington, DC) Quality/Manufacturing Committee – Impurities Working Group for conducting and reporting this work. ToxStrategies, LLC is a consulting firm that provides services to private and public organizations on toxicology and risk assessment issues. The work reported in this article was conducted by the authors during the normal course of employment, and no authors received personal fees. DW is an Associate Editor at Regulatory Toxicology and Pharmacology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data used in support of this manuscript were derived from public and proprietary sources. Detailed data summaries of proprietary studies are provided where applicable.

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Appendix A. Supplementary data

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