

Development of Safety Qualification Thresholds and Their Use in Orally Inhaled and Nasal Drug Product Evaluation

Douglas Ball,^{*,1} James Blanchard,[†] David Jacobson-Kram,[‡] Roger O. McClellan,[§] Timothy McGovern,[‡] Daniel L. Norwood,[¶] W. Mark Vogel,^{||} Ron Wolff,^{|||} and Lee Nagao^{|||}

^{*}Safety Sciences, Groton Laboratories, Pfizer Global Research and Development, Groton, Connecticut 06340; [†]Aradigm Corp., Hayward, California 94545; [‡]Office of New Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland 20993; [§]University of New Mexico Health Sciences Center and Toxicology and Risk Analysis, Albuquerque, New Mexico 87111; [¶]Boehringer Ingelheim Pharmaceuticals, Inc, 900 Ridgebury Road, Ridgefield, CT 06877; ^{||}Safety Sciences, Pfizer Global Research and Development, Ann Arbor, Michigan 48105; ^{|||}Nektar Therapeutics, 150 Industrial Road, San Carlos, CA 94070; and ^{|||}Drinker Biddle and Reath, LLP, Washington, District of Columbia, 20005

Received December 5, 2006; accepted March 3, 2007

Safety thresholds for chemical impurities and leachables in consumer products such as foods and drugs have helped to ensure public health while establishing scientifically sound limits for identification and risk assessment of these compounds. The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group, a collaboration of chemists and toxicologists from the U.S. Food and Drug Administration (FDA), industry, and academia, has developed safety thresholds for leachables and extractables in orally inhaled and nasal drug products (OINDP), for application in United States pharmaceutical submissions. The PQRI safety concern threshold (SCT) is 0.15 $\mu\text{g}/\text{day}$, and the qualification threshold is 5 $\mu\text{g}/\text{day}$. OINDP are important in the treatment of lung diseases such as asthma and chronic bronchitis, as well as systemic diseases such as diabetes. Analysis of extractables and minimization of leachables in OINDP are vital to ensuring the quality and safety of the final product. It is expected that the thresholds developed by the PQRI Leachables and Extractables Working Group will be used by both industry and regulators to ensure and assess such quality and safety in OINDP applications. In this article, we describe the importance of the PQRI safety thresholds in the OINDP pharmaceutical development process; the background and context of safety thresholds for consumer products; how these safety thresholds were developed using well-established, robust databases and quantitative risk assessment approaches; and how these thresholds can be applied in a pharmaceutical safety qualification process, including FDA regulatory perspectives on the use of safety thresholds for OINDP.

Key Words: qualification threshold; safety concern threshold; leachables; extractables; inhalation; nasal; PQRI.

Why Are Extractables and Leachables Important in Inhalation Drug Product Pharmaceutical Development?

Extractables, as defined by the U.S. Food and Drug Administration (FDA), are compounds that can be extracted from elastomeric components, plastic components, or coatings of the container and closure system when in the presence of an appropriate solvent. Leachables are compounds that leach from elastomeric or plastic components or coatings of the container and closure system as a result of direct contact with the drug product formulation (FDA, 1998, 2002b). Extractables are therefore potential leachables, and patients could be exposed to leachables. Leachables in orally inhaled and nasal drug products (OINDP) are generally considered by pharmaceutical regulatory agencies to be of significant safety concern (FDA, 1999) because for some OINDP, leachables could be delivered directly to the diseased lung. For OINDP, toxicological and chemical assessments of leachables and extractables are a critical part of the pharmaceutical development process. Further, extractables and leachables can often impact on the marketing approval of a drug product. OINDP include nasal sprays and inhalation sprays, metered-dose inhaler (MDI) solutions and suspensions, inhalation solutions, and dry-powder inhalers (DPIs). These drug products are used to mitigate the effects of indications such as asthma, emphysema, and allergy symptoms and to deliver medication directly to affected areas such as the nasal passages and lungs (local delivery). Newer inhalation products, such as those for the delivery of insulin to treat diabetes, seek to deliver medication throughout the body, via the lungs (systemic delivery).

Each inhalation or nasal drug product includes a container closure system that is a vital part of the drug product as a whole, facilitating drug delivery and protecting the integrity of the formulation. For example, the MDI consists of a solution or suspension formulation (drug substance or active pharmaceutical ingredient; chlorofluorocarbon or hydrofluoroalkane propellants to facilitate aerosol dose delivery; and surfactants,

¹ To whom correspondence should be addressed at Safety Sciences, Groton Laboratories, Pfizer Global Research and Development, Eastern Point Road MS 8274-1215; Groton, CT 06340. Fax: 860-686-6098. E-mail: douglas.j.ball@pfizer.com.

cosolvents, and other excipients to help stabilize the formulation) and a container closure system with various physical components (metal canister to contain the pressurized formulation, a valve to meter the formulation dose to the patient, elastomer components to seal the valve to the can and contain the pressurized formulation, and an actuator/mouthpiece to facilitate patient self-dosing). The formulation and container closure system are closely integrated in the MDI drug product, which is generally true of all OINDP. The container closure systems for OINDP from which leachables may appear can include not only primary packaging components, e.g., canisters for MDIs and blister packages for DPIs, but also secondary packaging, such as overwraps or labels that are not in direct contact with the drug formulation.

OINDP container closure systems components can consist of various elastomeric (rubber) and polymeric (plastic) materials. These components can be seals, parts of MDI valves, mouthpieces, etc. All such elastomeric and polymeric materials contain relatively low-molecular weight soluble organic chemical entities, either purposefully added to the materials during synthesis, compounding, or fabrication (e.g., polymerization agents, fillers, antioxidants, stabilizers, and processing aids) or present in the materials as a by-product of synthesis, compounding, or fabrication (e.g., oligomers, additive contaminants, and reaction products). All these chemical entities have the capacity to leach into the formulation and be delivered to the patient. Leachables and extractables represent a variety of chemical types and classes, and leachables can be present in

inhalation drug products at widely varying concentrations. Extractables and leachables can also include certain structure types with known safety implications, such as N-nitrosamines, polyaromatic hydrocarbons (PAHs), and mercaptobenzothiozoles (FDA, 1998, 2002b).

Why Do We Need Safety Thresholds?

Modern analytical chemistry has enormous capability for analyzing extractables and leachables. For example, gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry are capable of separating, identifying, and quantifying highly complex mixtures of organic chemical entities such as those produced from controlled extraction studies of OINDP container closure system components (see Fig. 1; Norwood *et al.*, 2005). These analyses are routinely accomplished at very high sensitivity, easily detecting organic leachables at low microgram per canister levels in MDIs (Norwood *et al.*, 1995). The available scientific literature would indicate that many hundreds, if not thousands, of individual chemical entities that can appear as extractables/leachables could potentially be detected, identified, and quantified at similar levels.

However, it is well established that there are levels at or below which organic chemical entities in drug product represent no safety concern to patients. Therefore, the establishment of safety thresholds that are protective of patients for OINDP leachables and extractables can be justified and are

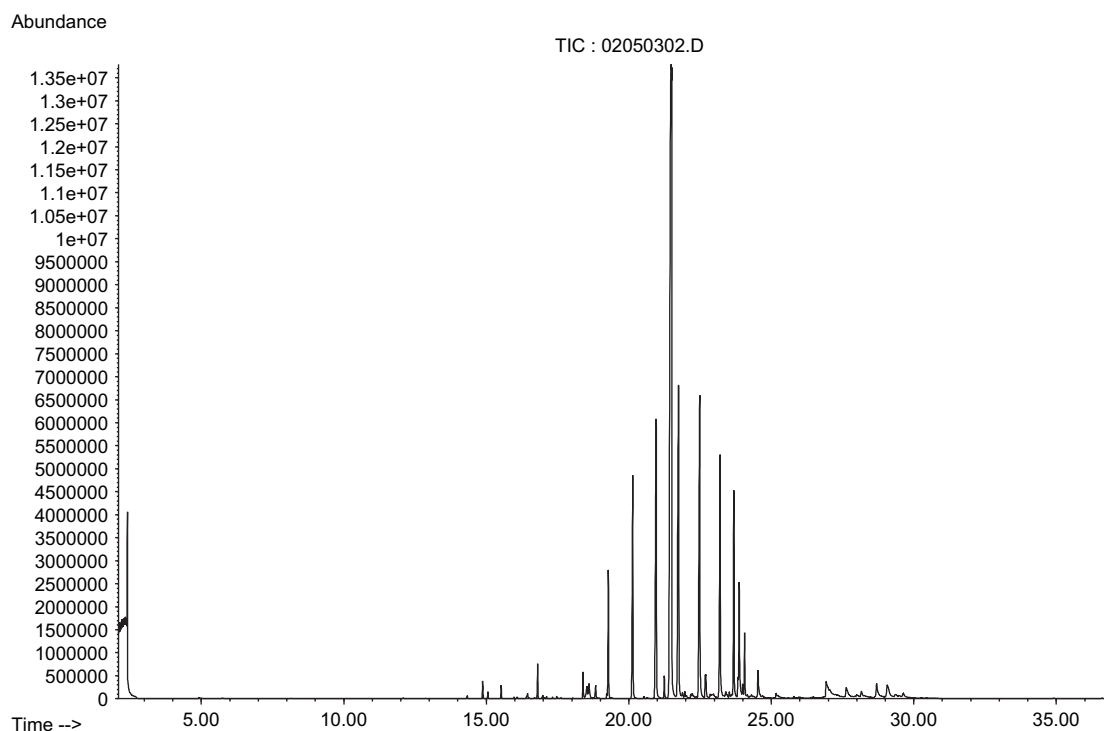


FIG. 1. A gas chromatography/mass spectrometry extractables “profile” of an elastomer (total ion chromatogram of a solvent extract).

believed to be necessary to limit unreasonable and extended evaluations of chemicals present at levels that cannot harm patients. An efficient pharmaceutical development process requires guidance on “how low to go” for the identification and quantification of extractables and leachables.

To ensure quality and safety of OINDP through knowledge of amounts and types of extractables and leachables, safety thresholds must be applied in conjunction with pharmaceutical development “best practices,” which include safety assessment of potential leachables throughout the development program. Such assessment should include at a minimum the following: safety evaluation of supplier information during container closure component selection, safety evaluation of potential leachables during controlled extraction studies on OINDP components, safety evaluation of leachables, and safety input to extractables specifications for routine quality control of extractables.

The PQRI Leachables and Extractables Effort

In 2001, the Product Quality Research Institute (PQRI; <http://www.pqri.org>) initiated a project to develop safety and analytical thresholds for leachables and extractables in OINDP. At the time there was no regulatory guidance available for such thresholds. Furthermore, International Conference on Harmonisation (ICH) thresholds for impurities are not applicable to leachables and extractables in OINDP (ICH, 2003a,b,c).

The PQRI Leachables and Extractables Working Group, consisting of toxicologists and chemists from industry, FDA, and academia, developed a safety concern threshold (SCT) and a qualification threshold (QT) for leachables; an analytical evaluation threshold for extractables and leachables; processes for applying these thresholds; and best practices for selecting OINDP container closure system components and conducting controlled extraction studies, leachables studies, and routine extractables testing.

The PQRI SCT is proposed to be 0.15 µg/day, and the QT is 5 µg/day. The SCT is the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects. The QT is the threshold below which a given noncarcinogenic leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure-activity relationship (SAR) concerns. Below the SCT, identification of leachables generally would not be necessary. Below the QT, leachables without structural alerts for carcinogenicity or irritation would not require compound-specific risk assessment. In 2006, the PQRI recommendations were submitted to the FDA for consideration in the FDA’s development of regulatory recommendations for OINDP (PQRI, 2006).

This article describes, from a U.S. perspective, historical background on the development and application of safety thresholds for consumer products, the development and application of the PQRI safety thresholds for leachables in

OINDP, and FDA regulatory perspectives on the use of these safety thresholds.

CONCEPT AND HISTORY OF SAFETY THRESHOLDS

Over 500 years ago Paracelsus made the astute observation that “All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.” A corollary to this bit of wisdom is that for most or perhaps even all toxicological effects, there exist thresholds: a dose below which an exposure imparts no risk. While most toxicologists would probably agree with this principle, the means for calculating the threshold can sometimes be controversial. In addition, for some adverse health end points, i.e., mutagenesis and carcinogenesis, most regulatory agencies have assumed a lack of a threshold. Practically, this means that any exposure results in some increased risk for mutation and/or cancer. Nevertheless, it is often impossible to reduce human exposures to zero.

Two types of methodologies have evolved in risk assessment: one for calculating safe exposure values for health effects thought to have thresholds and a second for calculating “virtually safe” exposure values for health effects thought to lack thresholds. In both cases, the method involves extrapolation of agent-induced health effects in animals to human risk. For drug development, methodologies for threshold-associated exposures are discussed in *ICH Q3C, Impurities: Guideline for Residual Solvents* (ICH, 2003c). The guideline discusses a method for calculating a PDE, “permissible daily exposure” to a residual solvent. Calculating PDEs for mutagenic and carcinogenic substances is more complex. In this case, a “virtually safe dose” can be established, using animal studies, as a dose that implies a negligible risk when administered over a lifetime. A virtually safe dose has been defined somewhat differently by different regulatory agencies. In general, however, it refers to lifetime exposures that increase the risk of cancer by either one in one million or one in one hundred thousand.

These types of risk assessments are often used to calculate acceptable levels of carcinogens in drinking water, air, and soil at hazardous waste sites. In order to perform such risk assessments, data from rodent lifetime bioassays are required. Data from such studies are often not available for impurities found in drug substances and drug products, and because of the resource intensity and protracted nature of rodent lifetime bioassays, it is generally not practical to test all potentially carcinogenic impurities in such an assay. This conundrum has led toxicologists to devise the concept of the “threshold of toxicological concern” (TTC) (Kroes *et al.*, 2004). These authors define the TTC as “a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health.” The concept of a TTC appears to have originated with FDA’s threshold of regulation for indirect food additives (FDA, 1995).

In general, an impurity exposure level of 1.5 µg per person/day is considered an acceptable threshold below which further qualification for genotoxicity/carcinogenicity concerns would not be required. The QT was originally developed as a “threshold of regulation” by the Center for Food Safety and Applied Nutrition (CFSAN) at the FDA for food contact substances and was further standardized by the CFSAN/FDA in a companion guidance document for food contact substances (FDA, 1995, 2002a). Substances with no known cause for concern that may migrate into food are exempted from regulation as a food additive if present at daily dietary concentrations at or below 0.5 ppb, corresponding to 1.5 µg per person/day based on a total daily consumption of 3 kg of solid and liquid foods. The threshold is an estimate of daily exposure expected to result in an upper bound lifetime risk of cancer of less than 10^{-6} , considered a virtually safe dose. The initial CFSAN/FDA analysis was based on an assessment of 343 carcinogens from a Carcinogenic Potency Database (CPDB) and was derived from the probability distribution of carcinogenic potencies of those compounds (FDA, 1995; Gold *et al.*, 1984; Rulis, 1992). Subsequent analyses of an expanded database of more than 700 carcinogens further confirmed the threshold (Fiori and Meyerhoff, 2002). Additional analysis of subsets of highly potent carcinogens suggested that a threshold of 0.15 µg/day, corresponding to a 10^{-6} lifetime risk of cancer, may be more appropriate for chemicals with structural alerts for potential genotoxicity (Kroes *et al.*, 2004). Some structural groups including aflatoxin-like, N-nitroso, and azoxy-compounds were identified to be of extremely high potency and are excluded from the threshold approach.

U.S. federal regulatory agencies such as the Environmental Protection Agency (EPA) and FDA typically use a 10^{-6} lifetime risk of cancer to determine “acceptable” risk from chemical exposures, although higher risk levels are accepted under certain circumstances, namely, for active pharmaceutical ingredients from which a benefit may be derived. This level of exposure is expected to produce a negligible increase in carcinogenic risk based on the analysis of the CPDB. Additionally, this threshold is considered to be low enough to ensure that the presence of an unstudied compound that is below the threshold will not significantly alter the risk-benefit ratio of a drug product, even if the impurity is later shown to be a carcinogen.

In developing the SCT and QT, the PQRI Working Group carefully considered the assumptions, approaches, and, where possible, exposure data used in developing the threshold of regulation for food additives and the TTC and ICH thresholds for impurities. The Working Group took a relatively conservative approach in the assumptions applied to development of the SCT and QT. These assumptions were in many cases different than those used in development of these other thresholds, taking into account the possibility of mixtures of leachables and a real potential for the presence of carcinogenic leachables. Further, unlike impurities, which are associated with drug

substance or drug product, leachables are not drug related and could possess different toxic characteristics. The approaches to and assumptions made in establishing the SCT and QT are explained below.

SAFETY THRESHOLDS FOR LEACHABLES IN OINDP

Because leachables originate in the container closure system rather than the synthetic pathway, the PQRI SCT and QT are based solely on micrograms per day intake of leachables, unlike ICH thresholds for drug product impurities, which are linked to the dose of the active pharmaceutical ingredient. The PQRI thresholds were defined in relation to estimated safe human inhalation exposures for sets of chemicals assessed for different toxicity end points, an approach similar to that used by others to determine thresholds for orally ingested substances (Blackburn *et al.*, 2005; Cheeseman *et al.*, 1999; Dolan *et al.*, 2005; Fiori and Meyerhoff, 2002; Kroes *et al.*, 2000; Kroes *et al.*, 2004; FDA, 1995; Munro *et al.*, 1996; Rulis, 1992).

Derivation of the SCT

The SCT is based on carcinogenicity end points because carcinogenic effects occur at lower intakes than those associated with noncarcinogenic toxicity. This was previously demonstrated for orally ingested compounds, including those with potent neural, reproductive, or endocrine toxicity (Kroes *et al.*, 2000). Our analysis confirms that genotoxic carcinogenicity is a concern at lower doses of inhaled compounds than acute respiratory irritation or chronic respiratory and systemic toxicity.

The Working Group based the SCT on the potencies of genotoxic carcinogens (i.e., positive for mutations in *Salmonella*, SAL+) in the CPDB, which expresses carcinogenic potency as the TD₅₀, the daily dose inducing a particular tumor type in half the animals that otherwise would not develop the tumor over a lifetime. Human 10^{-6} risk-specific doses were estimated by linear extrapolation from the TD₅₀ (Blackburn *et al.*, 2005; Cheeseman *et al.*, 1999; Fiori *et al.*, 2002; Krewski *et al.*, 1990; Kroes *et al.*, 2004; Rulis, 1992). As expected (Cheesman *et al.*, 1999), SAL+ carcinogens are more potent carcinogens (Fig. 2). The SAL+ compounds are also appropriate for establishing a threshold for structural identification because structural alerts are more predictive for SAL+ than for nongenotoxic carcinogens (Benigni and Zito, 2004). Furthermore, most known human carcinogens are genotoxic (Bartsch and Malaveille, 1989), and the assumption of linear extrapolation of cancer risk is more appropriate for SAL+ compounds than for nongenotoxic compounds, which may exhibit mechanism-based thresholds for tumorigenesis. Too few inhalation studies were represented in the CPDB to establish a threshold based solely on inhalation data. However, the potency of carcinogens tested by inhalation mirrors that of those tested by

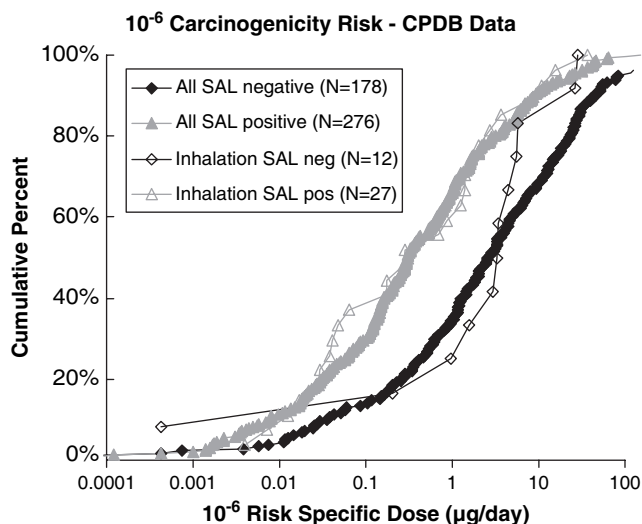


FIG. 2. Carcinogenic potency of genotoxic (SAL+) and nongenotoxic (SAL-) carcinogens from the CPDB from studies conducted in rodents by inhalation or all routes combined.

all routes (Fig. 2); thus, data from all routes should be representative of inhalation carcinogens.

A 10^{-6} risk-specific dose was used as a negligible carcinogenicity risk in our analysis, consistent with the threshold of regulation for indirect food additives (FDA, 1995). The relatively conservative 10^{-6} risk level was considered an appropriate starting point for identification and evaluation of leachable impurities because it is not uncommon for there to be a mixture of multiple leachable impurities with potential genotoxicity issues in an OINDP, and there are examples of potent carcinogens found as leachables in OINDP.

Our estimates of human 10^{-6} risk-specific doses included allometric scaling factors, based on default body weights (70-kg human, 350-g rat, 30-g mouse) to the 0.75 power, used by the U.S. EPA (1992). In U.S. pharmaceutical labeling, dose metrics from carcinogenicity assays are typically scaled to body surface area on a milligram per square meter basis (default body weight to the $2/3$ power). These two specific dose-scaling approaches result in relatively small (< twofold) differences in human dose estimates (FDA, 2005), considering that carcinogen potencies range over several orders of magnitude. Because applying multiple conservative assumptions can unrealistically overestimate carcinogenic risk (Gaylor *et al.*, 1993), the Working Group used a central estimate of risk rather than an upper bound 95% risk estimate and used the geometric mean of potencies from rats and mice rather than using the most sensitive species. Based on these assumptions, the median human equivalent 10^{-6} risk-specific dose for SAL+ carcinogens in the CPDB is 0.36 $\mu\text{g}/\text{day}$, and the median excess cancer risk at the SCT of 0.15 $\mu\text{g}/\text{day}$ is 0.41×10^{-6} . If < 20% of random chemicals are genotoxic carcinogens (Fung *et al.*, 1995; Sawatari *et al.*, 2001), < 7% of all compounds would exceed 10^{-6} increased cancer risk at lifetime intakes < 0.15 $\mu\text{g}/\text{day}$.

day. Thus, a leachable below the SCT is unlikely to have a lifetime excess cancer risk $> 10^{-6}$, and identification of leachables below this threshold generally would not be necessary.

Derivation of the QT

The QT is based primarily on regulatory agency “reference doses” (RfDs) derived by applying safety factors to no-observed-adverse-effect levels (NOAELs) for noncarcinogenic toxicity. The Working Group analyzed 150 inhaled compounds with “Chronic Reference Doses” established by the U.S. EPA, “Minimum Risk Levels” established by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), or “Reference Exposure Levels” established by the California EPA (CAL EPA). The median reference values were 120 $\mu\text{g}/\text{day}$ (10th percentile 1.5 $\mu\text{g}/\text{day}$) for chemicals with reference values based on respiratory toxicity and 1940 $\mu\text{g}/\text{day}$ (10th percentile 5.0 $\mu\text{g}/\text{day}$) for chemicals with systemic toxicity end points. Based on the large safety margins (~ 100 -fold) incorporated in RfDs, leachables at intakes $< 5 \mu\text{g}/\text{day}$ should pose negligible health risks. Compounds with respiratory toxicity and inhalation reference values less than 5 $\mu\text{g}/\text{day}$ are dominated by metals and metal salts and by reactive compounds with readily identifiable structural alerts for irritant potential, such as aldehydes and isocyanates.

In developing the QT, the Working Group focused on the total inhaled dose, which assumes 100% deposition, rather than on the actual dose deposited in the lungs. The 5- $\mu\text{g}/\text{day}$ QT therefore represents a small percentage—between only 1 and 6%—of the quantity of particulate that individuals are normally inhaling. Note that these percentages would be even smaller if comparisons were made to, for instance, air concentrations equal to the National Ambient Air Quality Standard for the respirable fraction (PM_{10}), which is considered protective of public health including sensitive subpopulations.

The Working Group also considered acute respiratory irritation in relation to the QT, since potential airway irritation and bronchoconstriction are concerns for impurities in OINDP (Shaheen *et al.*, 1994). A useful metric of sensory irritation is the RD_{50} , the concentration of an irritant that decreases respiratory frequency by 50% in mice (Alarie *et al.*, 1980). A good correlation was reported between occupational threshold limit values and the value of $0.03 \times \text{RD}_{50}$ (Schaper, 1993). To estimate safe doses for respiratory irritants, the Working Group calculated the microgram dose at the RD_{50} concentration inhaled for 10 min divided by 1000 for a large set of chemicals (Schaper, 1993). The additional 30-fold safety margin for this metric, compared to the value of $0.03 \times \text{RD}_{50}$, should be sufficient to account for sensitive populations such as asthmatics as illustrated in Table 1. The distribution of $\text{RD}_{50}/1000$ doses was quite similar to the distribution of NOAELs for chronic respiratory toxicity. As with the chronic respiratory toxicants, compounds with $\text{RD}_{50}/1000$ values

TABLE 1
Bronchoconstrictor Concentrations in Asthmatics Relative to Occupational Short-Term Exposure Limits and RD₅₀ Values

Compound	STEL (mg/m ³)	RD ₅₀ /1000 (µg/m ³)	Bronchoconstriction in asthmatics	Reference
Nitrogen dioxide	9.4	655	None at 753 µg/m ³	Tunnicliffe <i>et al.</i> (1994)
Sulfur dioxide	13	523	Range = 666–10,500 µg/m ³ (20- to 1.2-fold below STEL)	Rubinstein <i>et al.</i> (1990)
Sulfuric acid	3.0	NA	None at 46 µg/m ³ (65-fold below STEL) Some at 130 µg/m ³ (23-fold below STEL)	Avol <i>et al.</i> (1990)
Formaldehyde	2.45	39	None at 3700 µg/m ³ for 3 h (1.5 × STEL)	Sauder <i>et al.</i> (1987)
Toluene diisocyanate	0.14	4.8	Most at > 14 µg/m ³ (10-fold below STEL) A few at ≤ 7 µg/m ³ (≤ 20-fold below STEL)	O'Brian <i>et al.</i> (1979)

STEL, short-term exposure limit; RD₅₀, concentration decreasing respiratory rate by 50% in mice; NA, not available.

below 5 µg/day were predictably irritant compounds such as aldehydes, isocyanates, and nitriles.

Pediatric populations were also considered. Children would be adequately protected by the proposed QT since it is based upon inhalation reference values that are intended to protect essentially all people, including sensitive subpopulations such as children. When establishing RfD and reference concentration (RfC) values, the EPA identifies the NOAEL, lowest-observed-adverse-effect-level, or benchmark dose or concentration and then divides this value by a series of uncertainty factors; one uncertainty factor relevant to children accounts for variability in toxic response among people, including highly sensitive subjects, such as children and elderly. This intraspecies uncertainty factor usually has a value of 10. This factor can be equally divided into a toxicokinetic variability component with a default value of 3.16 (i.e., 10^{1/2}) and a toxicodynamic variability component also with a default value of 3.16, assuming these components act independently.

The intraspecies uncertainty factor of 10 and the associated subfactors of 3.16 have been justified for children based upon multiple studies that have compared the clinical response to pharmaceutical agents in children versus adults as well as the toxic response to chemical agents in younger versus older animals (Burin and Saunders, 1999; Dourson *et al.*, 2002). For example, the National Academy of Sciences Committee on Pesticides in the Diets of Infants and Children reviewed several human and animal studies and concluded that the 10-fold intraspecies uncertainty factor was sufficient to protect infants and children (Bruckner, 2000). Furthermore, comparison of the toxicokinetic data of 60 xenobiotics and the toxicodynamic data of 49 xenobiotics in adults, children, and other groups has shown that the composite 10-fold factor covers the great majority of the population (Renwick and Lazarus, 1998). Further work is needed to determine whether the default uncertainty factors offer adequate protection for children, especially for inhaled exposure to gases and particles, and for children with lung disease, such as asthma or cystic fibrosis.

The preponderance of current data indicates that a leachable below the QT is unlikely to cause acute or chronic non-

carcinogenic toxicity and in the absence of structural alerts for carcinogenicity or irritation should not require compound-specific risk assessment.

APPLICATION OF SAFETY THRESHOLDS IN THE PHARMACEUTICAL DEVELOPMENT PROCESS FOR OINDP

In general, the use of safety thresholds is considered acceptable in cases where adequate supporting data are available; the Division of Pulmonary and Allergy Products (DPAP) in FDA's Center for Drug Evaluation and Research/Office of New Drugs has incorporated the application of safety thresholds in the qualification process conducted for leachables and extractables in OINDP over the last decade. It is recognized that the appropriate application of safety thresholds has advantages, including a reduction in the unnecessary expenditure of animals, time, effort, and money. This reduced expenditure may allow greater resources to be applied to drug development areas that present more significant safety concerns.

Currently, no formal regulatory guidance on the safety evaluation of leachables and extractables is available. In the absence of formal guidance, the DPAP developed an internal practice which includes the following general approach: identification of the compound, determination of the maximum daily human exposure based upon the proposed product specification (e.g., amount of specific leachable in drug product, typically in units of ppm or microgram per canister), conduct of a SAR assessment for genotoxic/carcinogenic potential through use of published lists (Ashby *et al.*, 1989; Tennant and Ashby, 1991) or software programs such as DEREK (Deductive Estimation of Risk from Existing Knowledge, http://www.lhasalimited.org/index.php?cat_2&sub_cat_64) or Multicase (<http://www.multicase.com>), and review of available toxicology/safety databases or conduct of toxicology studies as deemed necessary, e.g., 14–90 day general toxicology, genetic toxicology. The final safety assessment should be based on a consideration of the maximum expected daily human

exposure, the intended patient population, and the anticipated duration of use.

Specific considerations for the safety assessment of leachables and extractables in OINDP include three primary components: systemic toxicity, local toxicity of the respiratory tree, and mutagenic/carcinogenic potential. The DPAP identified a safety threshold for systemic and local toxicity parameters based on an evaluation of the U.S. EPA's Integrated Risk Information System (IRIS) and Health Effects Assessment Summary Tables (HEAST) databases (Risk Assessment Information System, Chemical Specific Toxicity Values). Database contains information taken from the U.S. EPA's IRIS and HEAST (http://rais.ornl.gov/tox/tox_values.shtml) and concluded that there is no significant safety concern for inhaled chemicals with a maximum expected daily dose of 5 µg/day (100 ng/kg) or less. Therefore, no further toxicity data are needed in most cases when the maximum expected daily human exposure is below the stated threshold.

The safety threshold for systemic toxicity was derived from an evaluation of 36 chemicals listed in the EPA database that were administered via the inhalation route and were associated with systemic toxicity. The presumed safe dose derived from the RfCs for these compounds were all ≥ 100 ng/kg with three exceptions; the three exceptions had a "safe" dose of 80 ng/kg. Considering the large safety factors (1000–10,000) that are incorporated into the calculation of RfCs, a safety threshold of 100 ng/kg was considered reasonable. The safety threshold for respiratory toxicity was derived from an evaluation of 20 chemicals with inhalation data that produced respiratory tract toxicity. All but four of these chemicals had presumed safe daily inhalation exposures greater than 100 ng/kg based on the RfCs, even after incorporation of large safety factors (300–1000). Of note, all the compounds with a presumed safe dose less than 100 ng/kg had a structural alert associated with respiratory irritation. These structural alerts include isocyanates, aldehydes, organic acids, strained heterocyclic rings, and halogenated aromatic rings.

As described earlier, subsequent data analyses conducted by the PQRI Working Group expanded the database evaluation to include the ATSDR and CAL EPA databases. The Working Group also concluded that a threshold of 5 µg/day presented a negligible safety concern for noncarcinogenic effects and recommended a QT of 5 µg/day (100 ng/kg/day, 50-kg person). Thus, the threshold recommended by the Working Group is in agreement with current DPAP practice.

There are some exceptions to the use of the above-described threshold approach for leachables and extractables in inhalation products, and these include compounds identified as respiratory irritants and sensitizers and those that present a known or suspected genotoxic or carcinogenic potential. With regard to respiratory irritants and sensitizers, chemicals should be evaluated for structural alerts associated with irritation or sensitization. This determination is especially important when considering the indicated population for

a given product. Most inhalation products approved to date are indicated for treatment of pulmonary diseases such as asthma. These patients are already considered to have a compromised respiratory function and may be more sensitive to the effects of irritants or sensitizers. If a compound is considered to have an irritant or sensitizing potential, patient risk should be assessed on a case-by-case basis after evaluating the available information for the specific compound. Additionally, the clinical experience with the drug product should be evaluated for evidence of any adverse effects. If no concern is identified for irritancy or sensitization, the safety QT for systemic and local toxicity of 5 µg/day is appropriate. For anticipated clinical exposures greater than 5 µg/day, safety qualification should be conducted for systemic and local toxicity, as described later in this section.

The DPAP currently has no formal policy or practice with regard to a safety threshold for leachables or extractables with an identified or suspected genotoxic or carcinogenic potential. As discussed earlier, the PQRI Working Group proposed an SCT of 0.15 µg/day, a threshold derived from calculated risk-specific doses of genotoxic (SAL+) carcinogens from the CPDB and considered to be a dose below which a leachable would present negligible concern for adverse carcinogenic and noncarcinogenic effects. The proposed SCT for negligible carcinogenic effects expands on the DPAP's previous use of thresholds that focused primarily on general toxicological effects. The PQRI proposal is, however, similar to that described previously by FDA's CFSAN for the safety assessment of food contact materials (FDA, 2002a), and a similar proposal has been made to support safety thresholds for genotoxic impurities (Müller *et al.*, 2006). The proposed approach is supported by a large database, and the applied cancer risk of 10^{-6} is considered appropriate due to the nature of the chemicals that are commonly encountered as leachables and to the lack of any benefit derived from their presence. Notably, high-potency carcinogens (e.g., nitrosamines, PAHs) are excluded from this threshold approach. While the PQRI Working Group recommendation has not yet been formally accepted by the FDA, the proposal is considered in DPAP's safety evaluation of suspected or known carcinogenic leachables and extractables.

Leachables and extractables are considered adequately qualified for genotoxic or carcinogenic potential if they are demonstrated to produce negative results in genotoxicity and/or carcinogenicity assays or, in cases where these data are not available, if they lack structural alerts for these end points. When no concern for genotoxic or carcinogenic potential is identified, a QT of 5 µg/day is appropriate in the absence of supporting general toxicology data and an identified potential for respiratory irritation or sensitization. If a leachable or extractable is a known or suspected genotoxin or carcinogen, appropriate tests should be conducted or a rationale provided to alleviate this concern. Alternatively, consideration should be given toward reducing the drug product specification to a level

associated with the PQRI Working Group–recommended SCT of 0.15 µg/day. If the compound is a known carcinogen, the product specification should be set to a level associated with a cancer risk of less than or equal to 10^{-6} .

In general, when adequate safety data are available to support a proposed product specification, conduct of a compound-specific risk assessment rather than a threshold-based evaluation is recommended. It is anticipated that, in most cases, higher product specifications would be supported from a safety standpoint when data are available to support a compound-specific risk assessment than would be through use of a threshold-based approach. In cases where the initial safety evaluation demonstrates a lack of genotoxic/carcinogenic or airway sensitization risk and the proposed specification is below the QT, additional risk assessment is not necessary.

In cases where the maximum expected human exposure to a leachable is expected to exceed DPAP's QT of 5 µg/day, safety qualification for general toxicity concerns may be provided through evaluation of published toxicity data or relevant regulatory exposure limits such as U.S. EPA air quality standards or through the conduct of inhalation toxicology studies of an appropriate duration (e.g., at least 90 days duration for chronic indications). In some cases, data for chemicals with well-characterized toxicity profiles that have a high degree of structural similarity to a leachable/extractable for which limited safety data are available may be considered.

When toxicology data are used to support proposed product specifications, the most relevant data should be considered. For example, if data are available from studies using both oral and inhalation administration, the data derived from the inhalation studies are usually considered to be most relevant. Safety margins for anticipated human exposures are calculated based upon the NOAELs in animal studies. Generally, a 10-fold safety factor is applied for cross-species extrapolation. In cases where safety data are only available from studies using the oral route of administration, an additional 100-fold safety factor is applied, based on an evaluation of a subset of the EPA HEAST database for which data were available following both oral and inhalation administration. For this subset of chemicals, the presumed safe inhalation doses derived from the RfCs were up to 100-fold lower than the identified RfDs after conversion to a milligram per kilogram per day dose. Therefore, a 1000-fold safety factor (10×100) is typically applied when using animal data from oral studies to support human inhalation use.

The PQRI Leachables and Extractables Working Group's proposed process for safety qualification of leachables incorporating use of the safety concern and QTs is shown in a decision tree format in Figure 3. The decision tree supports FDA recommendations for application of safety thresholds. Note that in some cases, decreasing the level of a leachable to not more than the threshold can be simpler than providing safety data. Alternatively, adequate data could be available in the scientific literature to qualify a leachable. If neither is possible, additional safety testing should be considered. The

studies considered appropriate to qualify a leachable will depend on a number of factors, including the patient population, daily dose, and duration of drug administration.

The following case examples are presented to illustrate the safety qualification process.

Case 1. Bis-2-ethyl-hexyl sebacate (CAS RN 122-62-3)

The proposed product specification corresponded to a maximum daily human exposure of 9.1 µg/day (182 ng/kg/day for a 50-kg individual). The most relevant toxicity study identified for this compound was a published chronic dietary study in rats in which a NOAEL of 200 mg/kg was observed. This dose corresponds to an acceptable human inhalation exposure of 0.2 mg/kg/day (200,000 ng/kg/day) after including a 1000-fold safety factor for cross-species extrapolation and for the use of data derived from oral administration to support inhalation use. Even after incorporation of this safety factor, a greater than 1000-fold safety margin for bis-2-ethyl-hexyl sebacate was present when comparing the acceptable human daily exposure to the maximum anticipated human exposure associated with the proposed product specification.

Case 2. 4-Toluenesulfonamide (CAS RN 70-55-3)

The proposed product specification corresponded to a maximum daily human exposure of 60 µg/day (1200 ng/kg/day). In this case, the sponsor provided no supporting rationale for the proposed specification. However, a review of the literature indicated that only acute toxicity data were available. In this case, the DPAP requested that the sponsor lower the product specification to a level that corresponded to the Division's safety QT of 5 µg/day or provide adequate toxicology data, such as a 90-day inhalation toxicity study, to support their proposed product specification.

Case 3. Acenaphthene (CAS RN 83-32-9)

The proposed product specification corresponded to a maximum daily human exposure of 0.067 µg/day (1.33 ng/kg/day). As in the previous case, only acute toxicity data were available. However, a State of Minnesota drinking water standard for acenaphthene is set at 400 µg/l (U.S. EPA Office of Water, Federal-State Toxicology and Risk Analysis Committee [November 1993], *Summary of State and Federal Drinking Water Standards and Guidelines*). This standard corresponds to an acceptable daily inhalation exposure of 160 ng/kg assuming a daily intake of 2 l/day, a 50-kg body weight, and incorporation of a 100-fold safety factor for the use of oral data to support inhalation use. The calculated acceptable inhalation exposure for humans provided a greater than 100-fold safety margin, when compared to the maximum daily human exposure to acenaphthene through use of the drug product at the sponsor's proposed specification. In addition, the anticipated human exposure was well below the DPAP's safety QT of 5 µg/day.

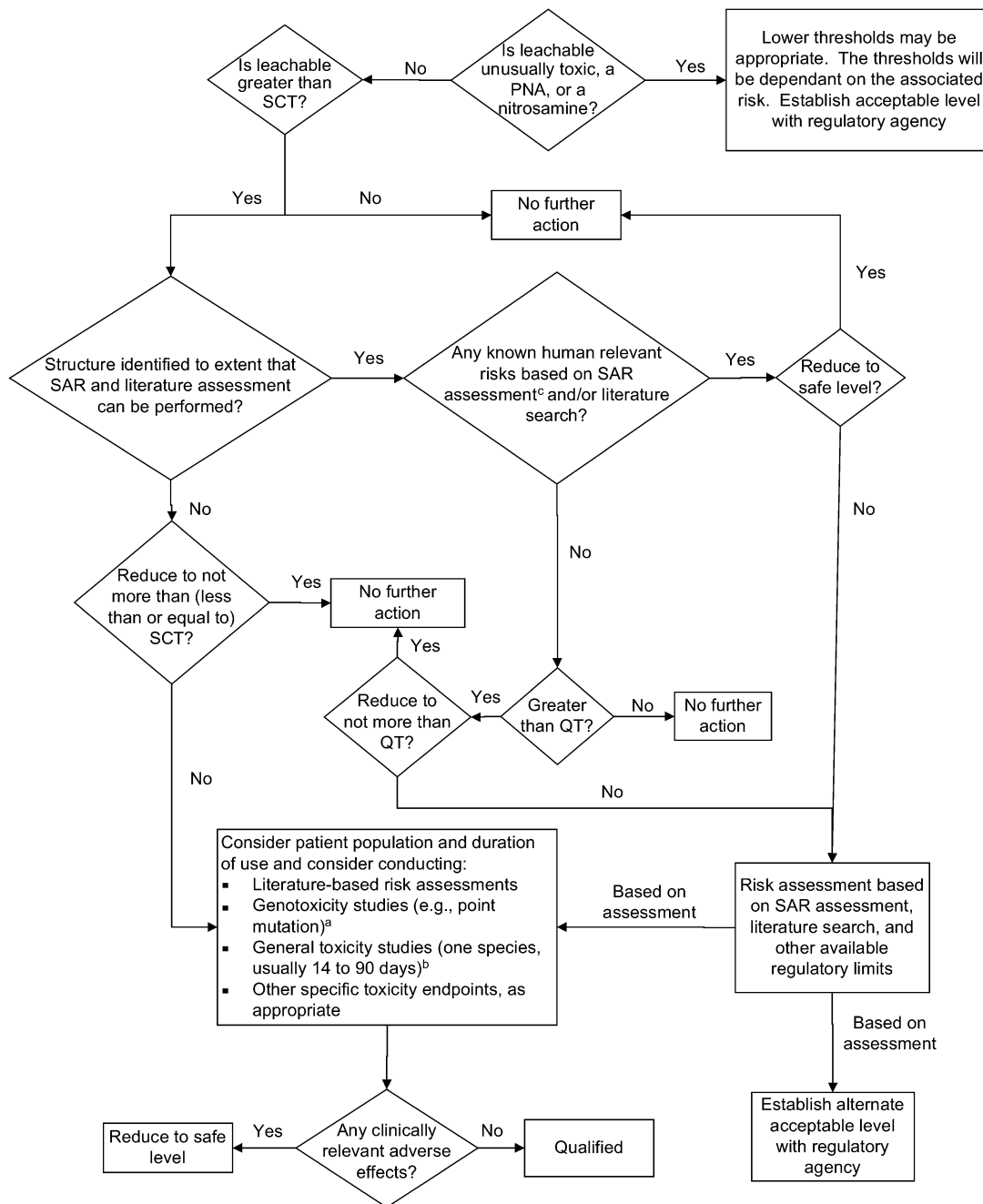


FIG. 3. Decision tree for identification and qualification. ^aIf considered desirable, a minimum screen (e.g., genotoxic potential) should be conducted. A study to detect point mutations, *in vitro*, is considered an appropriate minimum screen. ^bIf general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximize the potential to detect the toxicity of a leachable. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate. ^cFor example, do known safety data for this leachable or its structural class preclude human exposure at the concentration present?

Case 4. Nitrosamines

Nitrosamines can be present in certain rubber components of inhalation devices and are known carcinogens. In one product, six species were identified at various levels. The carcinogenic risk assessment was based on total nitrosamine exposure using

the slope factor calculated for N-nitrosodimethylamine. A maximum human daily exposure of up to 0.04 ng/kg, a level associated with a cancer risk estimate of 10^{-5} , was accepted based on the overall risk-benefit analysis and technological considerations, namely, the inability to manufacture rubber

components that do not potentially leach nitrosamine compounds. Although the Division allowed a level that corresponded to a cancer risk estimate of 10^{-5} , DPAP encourages sponsors to continue to reduce the potential for exposure and to strive to develop methods to eliminate the presence of nitrosamine compounds.

As stated previously, issues arising from the need for adequate safety qualification for leachables and extractables can often delay the approval of drug products. Therefore, some consideration should be given to addressing these issues earlier in product development or providing more substantive qualification information. Some ways in which the safety qualification process can be improved include the selection of materials to limit the number and level of potential leachables, the use of preextraction methods to lower potential exposures, and the submission of a clear rationale to support the safety of proposed product specifications. Often, the DPAP receives submissions containing only the proposed drug product specifications for a given leachable or extractable with no supporting rationale. It must be stressed that submission of an adequate supporting rationale often leads to a more efficient and expeditious review of a submission, with regard to the safety qualification of leachables and extractables.

CONCLUSION

The safety thresholds for leachables in OINDP developed by the PQRI Leachables and Extractables Working Group—an SCT of 0.15 $\mu\text{g}/\text{day}$ and a QT of 5 $\mu\text{g}/\text{day}$ —provide scientifically sound guideposts for industry and regulators to ensure, with a high degree of confidence, the safety of patients using these drug products. These thresholds were developed taking into consideration accepted safety thresholds for indirect food additives and impurities in drug substances, drug products, residual solvents, and genotoxic impurities in drug products. A scientifically sound process using well-established databases, risk assessment approaches, and literature information relevant to inhalation products was used to derive the thresholds. The Working Group also developed a recommended safety qualification process that incorporates application of the SCT and QT. This process supports FDA's recommended processes for application of safety thresholds.

The PQRI thresholds and qualification process were developed with FDA regulatory support and are expected to be used in pharmaceutical development programs and by regulators assessing drug applications. These scientifically justified thresholds will have a significant positive impact on the OINDP development and regulatory review process by removing uncertainty in the development process and directly linking safety with quality of OINDP.

ACKNOWLEDGMENTS

The authors thank the PQRI and the International Pharmaceutical Aerosol Consortium on Regulation and Science and their member organizations for support of this work. Conflicts of interest: None declared.

REFERENCES

- Alarie, Y., Kane, L., and Barrow, C. S. (1980). Sensory irritation: The use of an animal model to establish acceptable exposure to airborne chemical irritants. In *Toxicology: Principles and Practices* (A. L. Reeves, Ed.), pp. 48–92. John Wiley, New York.
- Ashby, J., Tennant, R. W., Zeiger, E., and Stasiewicz, S. (1989). Classification according to chemical structure, mutagenicity to *Salmonella* and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutat. Res.* **223**, 73–103.
- Avol, E. L., Linn, W. S., Shamoo, D. A., Anderson, K. R., Peng, R. C., and Hackney, J. D. (1990). Respiratory responses of young asthmatic volunteers in controlled exposures to sulfuric acid aerosol. *Am. Rev. Respir. Dis.* **142**, 343–348.
- Bartsch, H., and Malaveille, C. (1989). Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series. *Cell Biol. Toxicol.* **5**, 115–127.
- Benigni, R., and Zito, R. (2004). The second National Toxicology Program comparative exercise on the prediction of rodent carcinogenicity: Definitive results. *Mutat. Res.* **566**, 49–63.
- Blackburn, K., Stickney, J., Carlson-Lynch, H. L., McGinnis, P. M., Chappell, L., and Felter, S. P. (2005). Application of the threshold of toxicological concern approach to ingredients in personal and household care products. *Regul. Toxicol. Pharmacol.* **43**, 249–259.
- Burin, G. J., and Saunders, D. R. (1999). Addressing human variability in risk assessment—The robustness of the intraspecies uncertainty factor. *Regul. Toxicol. Pharmacol.* **30**, 209–216.
- Bruckner, J. V. (2000). Differences in sensitivity of children and adults to chemical toxicity: The NAS panel report. *Regul. Toxicol. Pharmacol.* **31**, 280–285.
- Cheeseman, M. A., Machuga, E. J., and Bailey, A. B. (1999). A tiered approach to threshold of regulation. *Food Chem. Toxicol.* **37**, 387–412.
- Dolan, D. G., Naumann, B. D., Sargent, E. V., Maier, A., Dourson, M. (2005). Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. *Regul. Toxicol. Pharmacol.* **43**, 1–9.
- Dourson, M., Charnley, G., and Scheuplein, R. (2002). Differential sensitivity of children and adults to chemical toxicity. II. Risk and regulation. *Regul. Toxicol. Pharmacol.* **35**, 448–467.
- Environmental Protection Agency (EPA) (1992). A cross species scaling factor for carcinogen risk assessment based on equivalence of mg/kg 0.75/day. *57 Fed. Regist.* 24152–24173.
- Environmental Protection Agency (EPA) (1993). Office of Water, Federal-State Toxicology and Risk Analysis Committee. *Summary of State and Federal Drinking Water Standards and Guidelines*.
- Fiori, J. M., and Meyerhoff, R. D. (2002). Extending the threshold of regulation concept: De minimis limits for carcinogens and mutagens. *Regul. Toxicol. Pharmacol.* **35**, 209–216.
- Food and Drug Administration (FDA) (1995). 21 CFR Sec. 170.39—Food additives: Threshold of regulation for substances used in food-contact articles. *60 (136) Fed. Regist.* 36581–36596.
- Food and Drug Administration (FDA), Center for Drug Evaluation and Research (1998). *Draft Guidance for Industry: Metered Dose Inhaler (MDI) and Dry Powder Inhaler Drug Products (DPI); Chemistry, Manufacturing and Controls Documentation*. U.S. Food and Drug Administration.
- Food and Drug Administration (FDA), Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research (1999). *Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics; Chemistry, Manufacturing, and Controls Documentation*. U.S. Food and Drug Administration.

- Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition, Office of Food Additive Safety (2002a). *Final Guidance for Industry: Preparation of Food Contact Notifications for Food Contact Substances: Toxicology Recommendations*. U.S. Food and Drug Administration.
- Food and Drug Administration (FDA), Center for Drug Evaluation and Research (2002b). *Guidance for Industry: Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products; Chemistry, Manufacturing, and Controls Documentation*. U.S. Food and Drug Administration.
- Food and Drug Administration (FDA), Center for Drug Evaluation and Research (2005). *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*. U.S. Food and Drug Administration.
- Fung, V. A., Barrett, J. C., and Huff, J. (1995). The carcinogenesis bioassay in perspective: Application in identifying human cancer hazards. *Environ. Health Perspect.* **103**, 680–683.
- Gaylor, D. W., Chen, J. J., and Sheehan, D. M. (1993). Uncertainty in cancer risk estimates. *Risk Anal.* **13**, 149–154.
- Gold, L. S., Sawyer, C. B., Magaw, R., Backman, G. M., de Veciana, M., Levinson, R., Hooper, N. K., Havender, W. R., Bernstein, L., Peto, R., et al. (1984). A carcinogenicity potency database of the standardized results of animal bioassays. *Environ. Health Perspect.* **58**, 9–319.
- International Council on Harmonization (ICH) (2003a). ICH Q3A(R1): Impurities in new drug substances. *68(68) Fed. Regist.* 6924–6925.
- International Council on Harmonization (ICH) (2003b). ICH Q3B(R2): Impurities in new drug products. *68(220) Fed. Regist.* 64628–64629.
- International Council on Harmonization (ICH) (2003c). ICH Q3C(R3): Impurities: Guideline for residual solvents. *68(219) Fed. Regist.* 64352–64353.
- Krewski, D., Szyszkowicz, M., and Rosenkranz, H. (1990). Quantitative factors in chemical carcinogenesis: Variation in carcinogenic potency. *Regul. Toxicol. Pharmacol.* **12**, 13–29.
- Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L.-A., Walker, R., and Würtzen, G. (2000). Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. *Food Chem. Toxicol.* **38**, 255–312.
- Kroes, R., Renwick, A. G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J. G., et al. (2004). Structure-based threshold of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* **42**, 65–83.
- Müller, L., Mauthe, R. J., Riley, C. M., Andino, M. M., De Antonis, D., Beels, C., DeGeorge, J., De Knaep, A. G. M., Ellison, D., Fagerland, J. A., et al. (2006). A rationale for determining, testing and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul. Toxicol. Pharmacol.* **44**, 198–211.
- Munro, I. C., Ford, R. A., Kennepohl, E., Sprenger, J. G., Maier, A., and Dourson, M. (1996). Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food Chem. Toxicol.* **34**, 829–867.
- Norwood, D. L., Nagao, L., Lyapustina, S., and Munos, M. (2005). Application of Modern analytical technologies to the identification of extractables and leachables. *Am. Pharm. Rev.* **8**(1), 78–87.
- Norwood, D. L., Prime, D., Downey, B. P., Creasey, J., Satinder, S. K., and Haywood, P. (1995). Analysis of polycyclic aromatic hydrocarbons in metered dose inhaler drug formulations by isotope dilution gas chromatography/mass spectrometry. *J. Pharm. Biomed. Anal.* **13**, 293–304.
- O'Brien, I. M., Newman-Taylor, A. J., Burge, P. S., Harries, M. G., Fawcett, I. W., and Pepys, J. (1979). Toluene di-isocyanate-induced asthma. II. Inhalation challenge tests and bronchial reactivity studies. *Clin. Allergy* **9**(1), 7–15.
- PQRI. (2006). *Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products*. Available at: <http://www.pqri.org>. Accessed April 23, 2007.
- Renwick, A. G., and Lazarus, N. R. (1998). Human variability and noncancer risk assessment—An analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol.* **27**, 3–20.
- Rubinstein, I., Bigby, B. G., Reiss, T. F., and Boushey, H. A., Jr. (1990). Short-term exposure to 0.3 ppm nitrogen dioxide does not potentiate airway responsiveness to sulfur dioxide in asthmatic subjects. *Am. Rev. Respir. Dis.* **141**, 381–385.
- Rulis, A. (1992). Threshold of regulation: Options for handling minimal risk situations. In *Food Safety Assessment. American Chemical Society Symposium Series 484* (J. W. Finley, S. F. Robinson, and D. J. Armstrong, Eds.), pp. 132–139. American Chemical Society, Washington, D.C.
- Sauder, L. R., Green, D. J., Chatham, M. D., and Kulle, T. J. (1987). Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. *Toxicol. Ind. Health* **3**, 569–578.
- Sawatari, K., Nakanishi, Y., and Matsushima, T. (2001). Relationships between chemical structures and mutagenicity: A preliminary survey for a database of mutagenicity test results of new workplace chemicals. *Ind. Health* **39**, 341–345.
- Schaper, M. (1993). Development of a database for sensory irritants and its use in establishing occupational exposure limits. *Am. Ind. Hyg. Assoc. J.* **54**, 488–544.
- Shaheen, M. Z., Ayres, J. G., and Benincasa, C. (1994). Incidence of acute decreases in peak expiratory flow following the use of metered-dose inhalers in asthmatic patients. *Eur. Respir. J.* **7**, 2160–2164.
- Tennant, R. W., and Ashby, J. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 209–227.
- Tunncliffe, W. S., Burge, P. S., and Ayres, J. G. (1994). Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* **344**, 1733–1736.