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RESEARCH ARTICLE

Setting occupational exposure limits for unstudied pharmaceutical intermediates using an in vitro parallelogram approach

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Abstract

Occupational exposure limits for unstudied pharmaceutical synthetic intermediates are often established under the assumption that penultimate and near-ultimate intermediates have the same structure-activity and dose-response as the ultimate active pharmaceutical ingredient (API). This is seldom the case because moieties that render biological activity to the API are often protected or modified for synthetic purposes. Incorrectly assuming that intermediates have biological activity similar to the API may lead to excessive exposure controls that in turn impose unnecessary ergonomic hazards on workers and greatly reduces the scale and efficiency of production. Instead of assuming intermediates have the same toxicity profile as the API, it is feasible to use a parallelogram approach to establish exposure limits for synthetic intermediates using low-cost in vitro data. By comparing in vitro responses of intermediates to structurally similar data-rich molecules such as the API, occupational exposure categories can be established for unstudied intermediates. In this contribution (1) methods for setting occupational exposure limits for data-poor compounds are reviewed; (2) applications and limitations of in vitro assays are discussed; (3) two exposure categorization examples are presented that rely on an in vitro parallelogram approach; and (4) inherent safeguards for uncertainties in pharmaceutical risk assessment are identified. In vitro hazard and dose-response information for unstudied intermediates that are structurally similar to well-studied APIs can greatly enhance the basis for setting occupational exposure limits for unstudied synthetic intermediates.

Keywords: *API; containment; control; ergonomics; exposure; hazard; occupational; OEL; OEB; parallelogram; pharmaceutical; potent compound; risk assessment*

Introduction

Occupational exposure bands

Chemical process development of active pharmaceutical ingredients (APIs) is a dynamic process that involves handling of many unstudied starting compounds and synthetic intermediates. These molecules typically have little or no hazard information, much less the dose-response data needed for setting occupational exposure limits (OELs). In order to choose an appropriate strategy to protect workers from chemical hazards when toxicity data are insufficient to quantify an OEL, it is common practice in the pharmaceutical industry to develop control- or performance-based in-house exposure limit ranges, often called occupational exposure bands (OEBs). OEBs are typically an order-of-magnitude estimate of airborne concentrations (in units of micrograms per cubic meter of air, $\mu g/m^3$) that should not be exceeded and are established using default 'safety' factors that conservatively adjust the exposure limit downward for uncertainties in the dataset. Figure 1 depicts a decision process for developing occupational exposure criteria for intermediates depending on the availability of toxicity data.

The performance-based OEB, like the better-refined OEL each map to a set of engineering and administrative controls designed to prevent worker exposure to airborne substances above the exposure limit (Naumann et al. 1996). These sets of controls are often called *exposure* control categories or bands. As control levels increase to afford more exposure protection to workers, costs increase dramatically, worker efficiency goes down and most important from a health and safety perspective, the level of ergonomic hazard to employees can increase

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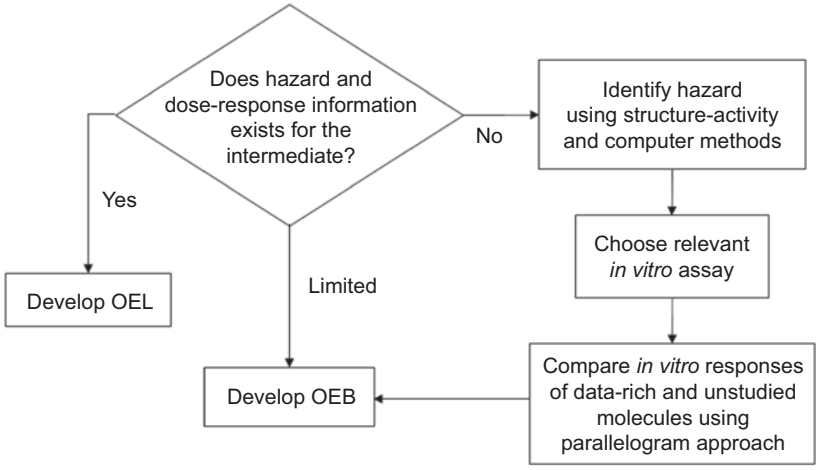

Figure 1. Process for establishing occupational exposure criteria depending on the availability of toxicity data. This algorithm integrates an in vitro parallelogram approach for evaluating synthetic intermediates that lack toxicity data. OEL = occupational exposure limit; OEB = occupational exposure band.

substantially. Unless robotic equipment is available, at the highest level of exposure control, work must be performed in complex glove box isolators that significantly reduce production scale and worker mobility. Additionally, the increased grip-strength required to use heavy isolator gloves substantially reduces worker endurance (Fleming et al. 1997). While it is essential that OEB determinations be protective from an exposure standpoint, it is crucial that they are not set at unnecessarily conservative levels from an ergonomic hazard perspective. In vitro data in a parallelogram approach shown in Figure 2 can provide the hazard and dose-response information needed to establish OEBs for unstudied penultimate and near-ultimate intermediates.

When in vitro data are absent or when comparative approaches may be useful, both the threshold of toxicological concern (TTC) and the uncertainty factor (UF) approach can be used to assign exposure limits to data-poor synthetic intermediates. The commonly used TTC and UF approaches will be discussed briefly followed by a discussion of how in vitro data can be used to establish OEBs.

Threshold of toxicological concern approach

The TTC approach to setting exposure limits is a straightforward method that is well-suited for APIs or other compounds when at least some structure-activity information is available. The TTC concept has a long regulatory history, particularly with regard to food additives, and is based on the idea that low-level exposures with negligible risk can be established for chemicals with unknown toxicity using empirical structure-activity relationships (Kroes et al. 2004). The TTC approach has also been used to establish exposure and clean-out limits in pharmaceutical manufacturing (Dolan et al. 2005). In the TTC approach, drug compounds are assigned to one of three order-of-magnitude acceptable daily intake (ADI) categories of 1, 10, or 100 μg/day, depending on likelihood of carcinogenicity, or potency and toxicity. Assuming 100% absorption by inhalation, the ADI can be related to the OEL by the amount

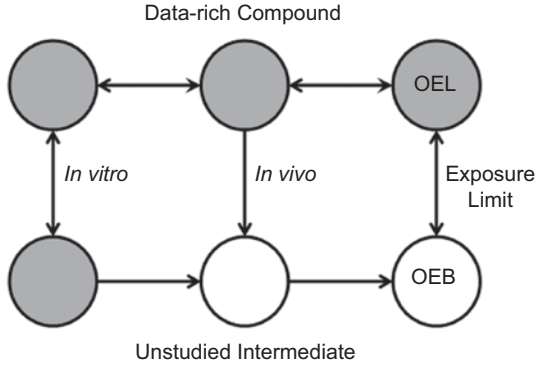

Figure 2. The notion behind this parallelogram is that an occupational exposure band (OEB) can be estimated for unstudied pharmaceutical intermediates that have structure-activity similarity to a data-rich compound such as the API. By comparing the in vitro dose-response of the unstudied intermediate to results for the data-rich positive control (e.g. the API) for the same assay in the context of what is known about the data-rich compound, an OEB can be estimated for the unstudied intermediate. Shaded circles represent data that are routinely available or can be readily obtained. Unfilled circles represent data that are not routinely available or can not be easily obtained. Double-headed arrows indicate relationships that can be compared. Single-headed arrows indicate estimates. OEL = occupational exposure limit. The difference between the OEL and OEB is discussed in the Introduction.

of air inhaled in an 8-h work day, usually 10 m^3 of air by default.

$$\text{ADI}(\mu\text{g/day}) = \text{OEL}(\mu\text{g/m}^3) \times 10\,\text{m}^3/\text{day} \quad (1)$$

Although the validity of the TTC approach has been demonstrated for many chemicals, several chemical structure classes have been explicitly excluded from the TTC approach due to unusually high carcinogenic potency. These include aflatoxin-like compounds, N-nitroso-compounds and azoxy-compounds. In addition, the databases used to derive and verify TTC values did not include proteins, organometallics and nanomaterials (Kroes et al. 2004). There are also several examples of especially bioactive or potent APIs with

exposure limits below those predicted by the TTC approach. As shown in Table 1, OELs and OEBs have been established for hormones, nanomaterials, opioids, proteins, and proteasome pathway inhibitors below the TTC lower limit of 1 μg/day. For these pharmaceuticals and especially their synthetic intermediates, the in vitro parallelogram approach discussed later can provide a data-driven alternative to the TTC approach.

Uncertainty factor approach

When limited dose-response data are available for non-carcinogenic compounds, or threshold data are available for non-mutagenic carcinogens, by far the most common approach for setting occupational exposure limits is to divide an experimentally measured low- or no-effect dose (i.e. the point-of-departure) by uncertainty factors (Dourson and Stara 1983). This 'safety' or uncertainty factor (UF) approach has been used for regulatory purposes for more than 50 years and usually involves a default divisor of 10 for each category of uncertainty in the database (Lehman and Fitzhugh 1954). Uncertainty categories may include (1) human-to-human variability, (2) experimental animal-to-human variability, (3) low-effect dose to estimate a no-effect dose or exposure level, (4) acute effect data to estimate a chronic effect level, (5) use of a lowest therapeutic dose (LTD) to estimate a no-effect level, (6) route-to-route extrapolation (e.g. inhalation to oral), and (7) pharmacokinetic variability. For perspective, the US EPA has held that 3000 should be the maximum combined uncertainty factor for four categories of uncertainty, and if additional uncertainties are present, the database is considered inadequate for risk assessment (USEPA 2002).

For unstudied synthetic intermediates lacking toxicity data, there could easily be many more categories of uncertainty than those mentioned above. Additionally, there are a large number of studies reviewed by Naumann in which 10-fold factors were demonstrated to be unnecessarily conservative (Naumann and Weideman 1995). Without additional dose-response information to reduce uncertainty, default factors can easily overwhelm what is actually known and play a greater role in the occupational risk assessment than scientific knowledge. Most importantly, exposure limits determined using well-intended but unnecessarily conservative default assumptions will trigger exposure controls that impose needless ergonomic hazards on pharmaceutical workers and unnecessarily increase production costs.

Parallelogram approach

Given the unsuitability of the TTC approach for certain classes of compounds and the possibility of excessively conservative exposure limits using the UF approach, the in vitro parallelogram approach shown in Figure 2 can provide a data-driven alternative. A large battery of cell and tissue culture methods have become available, driven by the ethical need to reduce animal testing and by the need for regulators to evaluate a backlog of tens-of-thousands of chemicals in commerce. Unlike the challenges faced in regulatory risk assessment where there is little or no toxicity data for entire classes of chemicals, most near-ultimate and penultimate API synthetic intermediates have structure-activities that correspond closely with a well-studied API. When this is the case, the activity of the API can inform selection and interpretation of low-cost in vitro screening assays, and the API can serve as a positive-control for testing. The parallelogram approach is a systematic comparison of pre-clinical and clinical data that are available for the structurally similar API, coupled with new in vitro dose-response information for unstudied intermediates and the API. Taken together this information can be used to infer the potential for unstudied intermediates to produce an adverse effect in vivo, and this information can provide dose-response information for the critical adverse effect.

In vitro toxicity assays for occupational risk assessment

At the outset, it is important to note that occupational exposure limits, and especially OEBs, are not fine lines between safe and dangerous. Rather, the goal of an occupational exposure limit in pharmaceutical development and manufacturing is to rank the hazards of a compound so appropriate containment and other exposure controls can be implemented. Because exposure control and containment is categorical, it is necessary only to establish an occupational exposure band or range, rather than develop a highly refined risk-based single-value exposure limit.

For occupational assessments, often the first question asked is whether an unstudied drug compound has potential to cause direct-acting respiratory, dermal, or ocular toxicity, or sensitization. There are numerous low-cost in vitro assays that rely on reconstructed epithelium that can be used to

Table 1. Examples of compounds with OELs/OEBs* less than the lower TTC-ADI limit of 1 μg/m³.

Class	Compound	Critical effect	ADI (μg/day)	OEL or OEB (μg/m³)	Method
Hormone	Estradiol	Endocrine activity	0.4	0.04	UF
Nanomaterial	Dendrimer	Lung cell cytotoxicity	0.1–1	0.01–0.1	In vitro
Opioid	Carfentanil	Somnolence	0.4	0.04	PSM
	Sufentanil	Somnolence	0.5	0.05	PSM
Protein	Interferon	Immunomodulation	0.1–1	0.01–0.1	UF
Ubiquitin-proteasome pathway inhibitor	Proprietary	Neurotoxicity	0.1–1	0.01–0.1	In vitro

* Currently there is no uniformly accepted methodology for setting pharmaceutical OEL/OEBs. Values provided are based on the methodology used by Cambrex Corp. ADI = acceptable daily intake; OEL = occupational exposure limit; OEB = occupational exposure band; UF= uncertainty factor approach; PSM = potency scaling to morphine; In vitro = In vitro parallelogram approach.

test for direct-acting toxicity. Fluorescent antibodies against immune mediators may be used to test for sensitization potential using the same in vitro systems. The second question for the occupational risk assessment has to do with the potential for toxic effects at remote target organs following systemic absorption. For this type of assessment, the question is not whether an unstudied penultimate or near-ultimate molecule is toxic per se; rather the question is whether the molecule is more or less toxic to the same target organ tissue compared to a structurally-similar data-rich comparison API.

Even with recent advances in the application of in vitro systems for risk assessment, it is important to recognize that there remain two fundamental difficulties that hamper use of in vitro assays for hazard and risk assessment that can be collectively called the part-to-whole problem (Hansson 1998). First, it is uncertain how to apply in vitro results to whole-organism toxicodynamic outcomes such as impaired fertility or cancer development. Secondly, in vivo pharmacokinetic absorption, distribution, metabolism, and elimination (ADME) parameters do not fully apply to in vitro systems, making it generally unfeasible to directly compare nominal media concentrations to in vivo target organ doses. Additionally, it should be noted that in vitro screening results in isolation are usually 'bottom-up' or inductive, meaning that such results are hypothesis-generating rather than hypothesis-confirming or deductive. For this reason, hazard and dose-response information for unstudied molecules derived from in vitro data should be used only in a parallelogram comparison to the results of the data-rich positive control API for the same assay.

Selecting in vitro toxicity screening assay

Despite the limitations of in vitro data, the toxicodynamic problem for direct-acting toxicants can be largely overcome using engineered tissue systems such as reconstructed human epidermis, cornea, and human airway epithelium (e.g. Epithelix Sàrl, Geneva, Switzerland, and MatTek Corporation, Ashland, MA). Because it is clear that different tissues respond uniquely and unexpectedly to the same compound at the gene-expression and molecular level, it is important to select a tissue-type that is representative of the target organ for the critical or adverse effect (Maier et al. 2007). When guided by foreknowledge of the target organ for a related data-rich API, the critical toxicity endpoints, the dose-response, and pharmacokinetics, it is possible to choose relevant cell lines for in vitro testing (e.g. American Type Culture Collection, Manassas, VA, USA; and European Collection of Cell Culture, Wiltshire, UK). In this way, in vitro exposure of a relevant cell type to both the API and unstudied compound can provide a good picture of potential toxicity to remote target tissues following systemic absorption. When viewed in parallel to what is already known about the API and in view of the in vitro data for the API from the same assay in a parallelogram approach (Figure 2), information from in vitro systems can provide substantial information about the likely in vivo bioactivity of the unstudied intermediate.

It is not feasible to provide an up-to-date summary of all available in vitro techniques because of the large number of assays and the pace at which new approaches are being developed. In addition to the body of peer-reviewed scientific literature on the subject, overviews of recent development of in vitro assays can be found in a series of workshop reports produced by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods (NICEATM-ICCVAM) and the European Centre for Validation of Alternative Methods (ECVAM).

Both ECVAM and NICEATM-ICCVAM have focused their efforts on the reduction and replacement of animal testing primarily to address toxic substance regulation. As such, only a sub-set of validated methods is applicable to occupational risk assessment. For example, screening tests for lethality by oral exposure are not useful because oral exposure and lethality are not usually relevant to occupational scenarios. Similarly, direct eye irritation or skin corrosivity may not be useful because highly effective personal protective equipment (PPE) is routinely employed in pharmaceutical manufacturing to protect against such exposures. Additionally, the Local Lymph Node Assay (LNNA) for dermal sensitization is certainly relevant to occupational settings, but it requires the use of animals and is relatively expensive. Fluorescent antibodies can be used to illuminate immune mediator release in reconstructed epithelium systems, and thus it is possible to assess potential for sensitization without the use of animals.

An additional source of information for availability and suitability of in vitro toxicity assessment assays and methods is available through collaborative work underway by the National Institutes of Health (NIH) and the US Environmental Protection Agency (EPA). This project utilizes the NIH Chemical Genomics Center's (NCGC) high-speed, automated screening robots to conduct high throughput toxicity profiling using cells and isolated molecular targets. Summaries of this work are available (Collins et al. 2008; Kavlock et al. 2009). Detection of cytotoxicity using *trans*-epithelial electrical resistance (TEER) is used in this high throughput screening project and was also used to detect cytotoxicity in the reconstituted airway epithelium system in the second case example presented below.

Case example 1: Myelosuppressive oncology drug

Oncology drugs and synthetic intermediates that may target rapidly dividing cells are often encountered in chemical development and manufacturing. Typically, these compounds are not pharmacologically potent per se because plausible acute exposures are unlikely to produce adverse effects. Repeat dosing of these drugs in patients, however, often produces dose-limiting myelosuppression, in particular neutropenia. For this reason, neutropenia is often the critical effect for occupational risk assessment of oncology APIs. Because numerous synthetic intermediates of these drugs are

structurally similar to the API, it is necessary in occupational hazard assessment to determine if unstudied isolated intermediates, to which workers may be exposed, have myelosuppressive activity or are cytotoxic.

Consider the example of oncology drugs based on taxane chemistries such as paclitaxel or the synthetic analog docetaxel. Paclitaxel is a mitosis-inhibiting taxane that occurs naturally and was originally isolated from the bark of the pacific yew tree (Wani et al. 1971). Unfortunately, paclitaxel occurs naturally at extremely low concentrations. This necessitates the use of semi-synthetic pathways to make paclitaxel (or docetaxel) from more abundant naturally occurring low-activity taxanes. Semi-synthetic processes to make taxane APIs typically involve several synthetic steps whereby side-chains are attached to the taxane substructure to make chemically protected intermediates.

Because extensive toxicity and dose-response data are available for docetaxel, it is possible to establish an OEL of 5 μg/m^3. The question that remains to be answered for the occupational risk assessment is what bioactivity and dose-response characteristics do isolated taxane synthetic intermediates have relative to docetaxel. Once determined, OEBs for intermediates can be appropriately scaled by comparison to the dose-response of docetaxel.

Materials and methods

Toxicity of protected taxane intermediates was evaluated using the validated GLP-compliant human cord blood myeloid progenitor *colony forming unit granulocyte-macrophage* (CFU-GM) and *erythroid* (CFU-E) assays (Pessina and Bonomi 2007). Colony-forming units are multi-potential myeloid stem cells that give rise to progenitors committed to differentiate into different lineages of mature blood cells. The resulting cell-type depends on which cytokines and growth factors are added to the culture medium. The technique is quantified from the number of surviving progenitors as a function of exposure-level under maximally stimulating cytokine concentrations that are able to support the proliferation and differentiation of the progenitors to be detected.

Relevant in vitro test concentrations were selected by scaling against established molar inhibitory concentration 50% (IC_{50}) values for the API (docetaxel). In the present case, the lowest nominal in vitro concentration was chosen to be one order-of-magnitude lower than the IC_{50} for docetaxel (10^{-9} M). In order to compare dose-responses, additional nominal test concentrations were spaced by one order-of-magnitude from 10^{-5} M down to 10^{-10} M. Clonogenic progenitor lineages were established in methylcellulose-based media. The mean ± 1 SD was calculated for triplicate cultures. The laboratory (ReachBio LLC, Seattle, Washington) was blinded to the identify of the test compounds and docetaxel served as the positive control, with blank and vehicle as negative controls. IC_{50} values were determined from a dose-response curve generated by plotting the compound concentration vs the percentage of control colony growth using Origin®8. A sigmoidal curve was generated and the inhibitory concentration (μM) was calculated using the Boltzman equation and the slope of the curve at midpoint was determined by Origin®8.

Results

CFU-GM/E results for docetaxel and intermediates are presented in Table 2. In vitro results confirmed that the protected docetaxel intermediates did not exhibit myelosuppressive activity at the highest concentration tested, and CFU formation for intermediates was comparable to negative controls (Table 3). Blinded results for docetaxel were consistent with published IC_{50} values of ~ 10^{-9} M (Pessina et al. 2003).

Interpretation

The parallelogram for evaluating the docetaxel intermediates is shown in Figure 3. Because no suppression of CFU formation was observed for the intermediates and because structure-activity supported this conclusion, the OEL for the protected intermediates was set to an OEB that brackets a default nuisance dust criterion of 500 μg/m^3 (i.e. OEB = 100–1000 μg/m^3). This enabled manufacturing operations to occur in low containment prior to the last synthetic step in which docetaxel is made.

When using in vitro methods for hazard evaluation, it is necessary to assess the potential for other adverse effects and in vivo bioactivation of otherwise non-toxic intermediates. For example, it is noteworthy that many terpenes such as taxanes have the potential to cause hypersensitivity reactions in some individuals. Also noteworthy for synthetic intermediates in general is the likelihood that reactive moieties of intermediates can not be biotransformed by phase I enzymes because these moieties are chemically protected for synthetic purposes.

For these docetaxel intermediates, activity analysis predicted no bioactivation would occur in vivo and thus a bioactivation step (e.g. microsomal incubation) did not precede the CFU assay. Finally, it is our experience that a nuisance dust criterion of 500 μg/m^3 is sufficient to prevent hypersensitivities to most large molecule sensitizers such as taxane derivatives.

Case example 2: Direct-acting respiratory toxicant

There are numerous reports that show occupational respiratory irritation and asthma are major categories of work-related health problems and many of the reactive compounds described to be asthmagens are either APIs or chemicals used in their production (UKHSE 2001). Especially notable among asthmagenic APIs are antibiotics such as penicillin, cephalosporin and spiramycin, and opiate concentrates and derivatives. Acidic anhydrides and many amines are asthmagens commonly used in pharmaceutical synthesis (Jarvis et al. 2005). In addition, modern pharmaceutical manufacturing can expose

Table 2. Human myeloid and erythroid progenitor proliferation results for docetaxel, and near- and penultimate intermediates. Similar to negative controls, no inhibition of colony forming units was observed for docetaxel intermediates.

Test material	CFU-E	CFU-E	Erythroid (total)	CFU-GM	CFU-GEMM	Total CFU
Standard	8±2	15±5	23±5	49±10	1±1	72±14
Solvent control	6±1	16±2	22±3	52 +/1 10	ND	74±7
Int 1 10^{-5} M	5±2	15±2	20±1	47±10	1±1	68±11
Int 1 10^{-6} M	4±1	11±4	14±4	55±5	ND	69±9
Int 1 10^{-7} M	4±1	15±4	19±4	53±5	ND	72±3
Int 1 10^{-8} M	5±1	13±1	18±2	48±1	1±1	67±13
Int 1 10^{-9} M	5±1	13±1	18±2	51±5	ND	69±3
Int 1 10^{-10} M	6±1	15±4	21±4	46±6	ND	67±10
Int 2 10^{-5} M	4±1	16±3	20±3	50±8	ND	70±7
Int 2 10^{-6} M	3±2	14±2	18±2	51±11	ND	69±13
Int 2 10^{-7} M	4±1	13±3	17±3	43±5	ND	60±8
Int 2 10^{-8} M	3±1	18±2	22±2	53±9	ND	74±11
Int 2 10^{-9} M	3±1	18±1	21±2	55±6	ND	76±7
Int 2 10^{-10} M	4±2	16±2	20±2	50±13	ND	70±14
Dctxl 10^{-5} M	ND[1]	ND[1]	ND[1]	ND[1]	ND	ND[2]
Dctxl 10^{-6} M	ND[1]	ND[1]	ND[1]	ND[1]	ND	ND[2]
Dctxl 10^{-7} M	ND[1]	ND[1]	ND[1]	ND[1]	ND	ND[2]
Dctxl 10^{-8} M	2±2	3±2[1]	4±2[1]	ND[1]	ND	4±2[2]
Dctxl 10^{-9} M	4±1	15±3	19±3	51±12	ND	69±13
Dctxl 10^{-10} M	6±2	18±3	24±4	55±4	ND	79±1

[1] $p \leq 0.001$; [2] $p \leq 0.0001$.
ND = none detected; Int = intermediate; Dctxl = docetaxel; CFU = colony forming unit; CFU- E = erythroid, CFU-GM = granulocyte monocyte/macrophage, CFU-GEMM = multipotential progenitor cells.

Table 3. IC_{50} values for intermediates and docetaxel on human myeloid and erythroid progenitor proliferation.

Test Material	IC_{50} myeloid progenitors	IC_{50} erythroid progenitors
Int 1	no inhibition	no inhibition
Int 2	no inhibition	no inhibition
Docetaxel	3×10^{-9} M	6×10^{-9} M

Int = intermediate.
'no inhibition' at maximum concentration tested (10^{-5} M).

workers to proteins, fermentation bacteria, organometallic catalysts, and nanomaterials, all of which have little toxicity information and may produce respiratory effects. Because inhalation exposure is usually the most relevant route of exposure in occupational settings, it is especially useful to have human airway toxicity information.

To demonstrate how an in vitro reconstructed human airway epithelium system can be used to assess direct-acting airway toxicity, consider the example of a novel cyclodextrin nano-delivery entity for pulmonary drug delivery. Synthesis of this delivery entity begins with beta-cyclodextrin (β-CD) followed by the addition of a series of alkylating moieties to the β-CD framework that ultimately yield the delivery entity (the latter absent an alkylating moiety). It is known a priori that the delivery entity does not exhibit pulmonary toxicity at relevant exposure concentrations and there is no evidence to suggest β-CD is particularly toxic to pulmonary cells (Matilainen et al. 2008). For the occupational risk assessment, a question to be answered is whether any of the isolated intermediates substituted with alkylating moieties have greater cytotoxicity than β-CD.

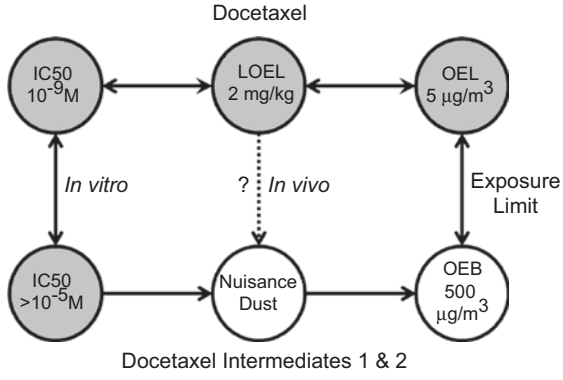

Figure 3. Parallelogram approach to hazard and dose-response assessment of docetaxel near-ultimate and penultimate intermediates. In vitro data demonstrate that unlike docetaxel, Intermediates 1 and 2 did not inhibit formation of colony forming units. This suggests the critical effect of docetaxel (neutropenia) does not apply to the intermediates and that an appropriate occupational exposure band (OEB) would be a default nuisance dust criterion of 500 µg/m^3. IC50 = inhibitory concentration, 50%; OEL = occupational exposure limit; LOEL = lowest observed effect level.

Materials and methods

Toxicological evaluation of synthetic cyclodextrin intermediates was done using the MucilAir™ in vitro reconstructed normal human tracheal-bronchial epithelial system (Constant et al. 2008). Histologically, this reconstructed human tissue is a highly differentiated pseudo-stratified mucocilliary phenotype that secretes mucin and exhibits microvilli having a ciliated apical surface. Among the advantages of this system compared to most mono-cultures is that the culture system is serum-free and can simulate the in vivo

condition for deposition and cilliary clearance of insoluble particulates and thus avoids confounding by surfactants or solvents used for solubilization. Although numerous endpoints can be evaluated with this tissue system, perhaps the most relevant for occupational risk assessment are effects on mucocilliary clearance (cilia function), tissue morphology, tissue integrity, and cell viability. Effects on cilia function and tissue morphology were evaluated by visual comparison to controls using microscopy. Tissue integrity and cell metabolic viability measurements were done using transepithelial electrical resistance (TEER) and Alamar Blue fluorometry, respectively, as described below.

Tissue integrity by TEER

In vitro electrophysiological techniques have been used for many decades and have been adapted to sterile measurement of cultured epithelial cells (Steele et al. 1992). When tissue cultures become confluent there is a sharp increase in TEER and when cells begin to die and confluence is lost, TEER diminishes. In this way, TEER was used to evaluate the integrity of the reconstructed airway epithelium following exposure to β-CD and derivatives.

Cell metabolic viability by Alamar Blue fluorometry

Alamar Blue is a proprietary mixture that contains non-toxic resazurin dye optimized for mitochondrial reduction and inhibition of non-specific reduction of resazurin (Lancaster and Fields 1996). Because Alamar Blue substitutes for molecular oxygen as an electron acceptor in the final step of the cytochrome respiratory chain, it does not interfere with respiration and is non-toxic. As such, it is a good short-term indicator of cellular aerobic metabolism. Resazurin reduction leads to loss of oxygen and gain of hydrogen to form the reduced product resorufin, a highly fluorescent molecule that was detected fluorometrically and optimized (excitation 530 nm; emission 590 nm) as described previously (Page et al. 1993).

Selecting nominal test concentrations

Nominal in vitro test concentrations were established based on the maximum concentration that could be reached in the human lung airway surface liquid volume (ASL) in an 8-h work day, assuming 100% deposition, no clearance at a given occupational exposure limit (OEL), and a human in vivo ASL of 47.3 ml as was used in an airway model (Hasan and Lange 2007). Spiked media were sonicated by the lab (Epithelix Sàrl, Geneva, Switzerland) prior to treatment in order to simulate an inhalable particle size for any insoluble test material. The following relationship was used to calculate a convenient range of nominal micromolar media concentrations (NCs) that correspond to OELs in the range of 0.01–1000 μg/m^3 under the stated conservative assumptions:

$$NC = \frac{OEL \times BR \times ET}{MW \times ASL \times 1000} \quad (2)$$

where NC is the nominal media concentration (mM), OEL is a selected occupational exposure limit to be tested (μg/m^3), BR is a default adult breathing rate (25 L/min), ET is a default work day exposure time (480 min), MW is the molecular weight of the test material (mg/mmol), ASL is the airway surface liquid volume (47.3 ml), and 1000 is a unitless conversion factor.

Results

Each compound was evaluated in the reconstructed airway epithelium system for tissue integrity by TEER shown in Figure 4, cell viability by Alamar Blue shown in Figure 5, and cilia function and cell morphology by microscopy. A summary of all measures for cytotoxicity are presented in Table 4. The halogenated Intermediate 3 consistently produced cytotoxicity for all measures at a 10-fold lower concentration than β-CD or intermediates 1 or 2.

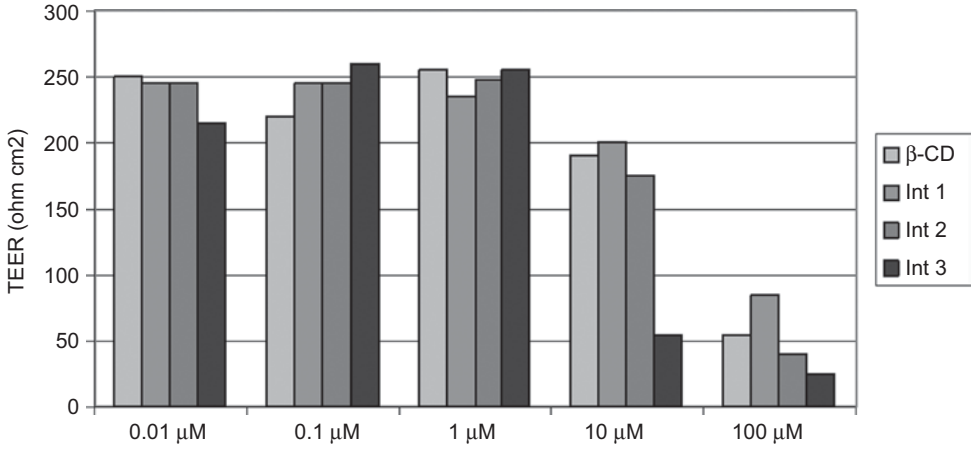

Figure 4. Constructed airway epithelium tissue integrity measurements using trans-epithelial electrical resistance (TEER). Intermediate (Int) 3 caused substantially greater loss of confluence (cytotoxicity) at a concentration 10 μM lower than β-cyclodextrin (β-CD) or the other intermediates. All compounds caused cytotoxicity at 100 μM.

Interpretation

The parallelogram for evaluating the more cytotoxic halogenated cyclodextrin (intermediate 3) is shown in Figure 6. Using this model, in vitro cytotoxicity data suggest that a protective OEB should be an order-of-magnitude lower than for β-CD or intermediates 1 and 2. Because β-CD would be regarded as a nuisance dust and because intermediates 1 and 2 responded in a manner similar to β-CD, the OEB for these compounds was set to an OEB that brackets a default nuisance dust criterion of 500 μg/m^3 (i.e. OEB = 100–1000 μg/m^3). Because cytotoxicity occurred at a 10-fold lower concentration, the OEB for the more cytotoxic halogenated intermediate 3 was set an order-of-magnitude lower than the default nuisance dust criterion (i.e. 10–100 μg/m^3).

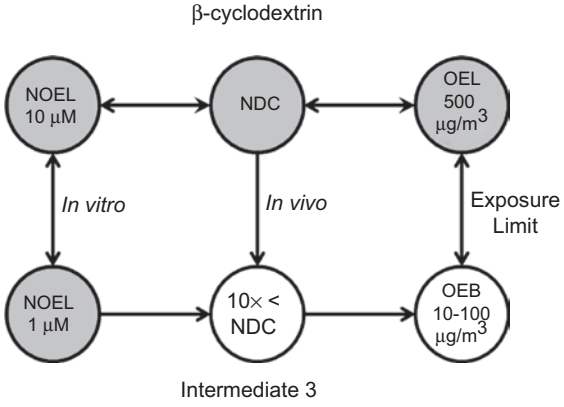

Figure 5. Parallelogram approach to hazard and dose-response assessment of β-cyclodextrin penultimate Intermediate (Int) 3. In vitro data demonstrate that unlike β-cyclodextrin and Int 1 and Int 2, Int 3 exhibited lung cytotoxicity at a 10-fold lower concentration than the default nuisance dust criterion of 500 μg/m^3. NDC = nuisance dust criterion; NOEL = no observed effect level.

Table 4. In vitro data summary for β-cyclodextrin and intermediates. Intermediate 3 produced toxicity at a concentration 10-fold lower than β-cyclodextrin and intermediates 1 and 2.

Test material	Estimated EC_{50} (viability) (μM)	Altered tissue integrity (ATI) (μM)	Altered tissue morphology (μM)	Altered cilia function (μM)
β-CD	> 100	10–100	10	100
Int 1	100	10–100	10	100
Int 2	≈ 100	10–100	10	100
Int 3	10–100	1–10	1	10

ATI = Altered Tissue Integrity; Int = intermediate; β-CD = beta-cyclodextrin; EC_{50} = effective concentration, 50% = molar concentration that produces 50% of the maximum response.

Backstops for uncertainty

As demonstrated, in vitro hazard and dose-response information for unstudied synthetic intermediates that are structurally similar to well-studied compounds can greatly enhance the underpinnings of risk assessment and thus improve the basis for making risk management decisions. Nonetheless, occupational risk assessment of unstudied API synthetic intermediates will remain uncertain so long as in vivo data are lacking. Despite this uncertainty, when making risk management decisions, it is essential to recognize and consider safeguards that can balance the conflicting goals of mitigating exposure risk and minimizing the ergonomic hazards imposed by unnecessarily conservative exposure controls. These safeguards include integration of API-specific toxicodynamic factors into risk management decisions, appropriate use of PPE, and containment verification with good hygiene practices.

Toxicodynamic factors

Acute-acting potent APIs with large therapeutic indices

Fortunately, most modern potent pharmaceuticals have a large therapeutic index (TI) whereby the acute dose-response

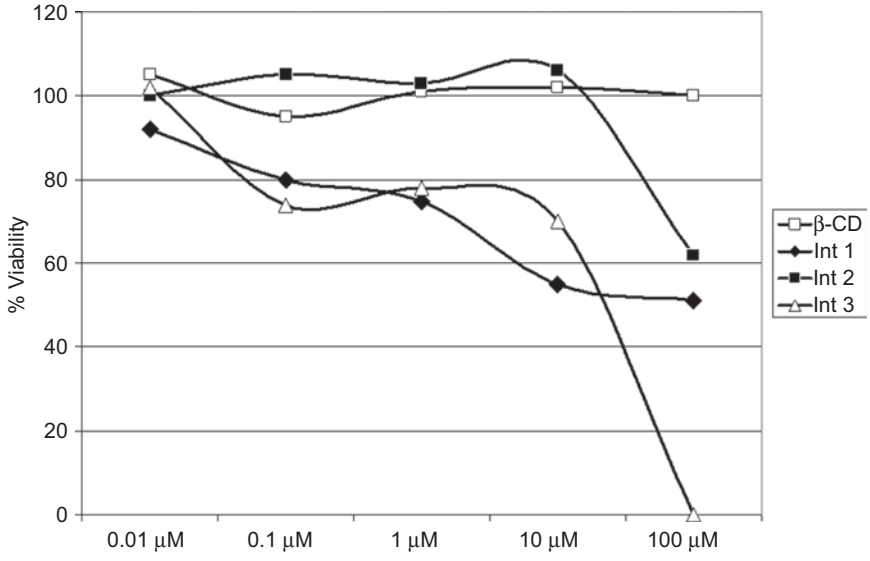

Figure 6. Constructed airway epithelium cell viability measurements using Alamar Blue redox dye fluorometry. Percentage viability normalized to controls. β-cyclodextrin (β-CD) did not alter cell viability relative to controls. Intermediate (Int) 3 caused 100% cell death at 100 μM. Int 2 and Int 3 produced moderate reductions in cell viability at concentrations greater than 1 μM and 10 μM, respectively.

for the desired pharmacological effect is orders-of-magnitude smaller than the acute dose-response for the adverse critical effect (i.e. toxicity). Although it would be unethical to intentionally do so, technically it would be safe for workers to be exposed to APIs at normal dosing levels if the API does not pose a health or performance hazard (e.g. analgesics and statins). For such APIs and related intermediates, if containment is designed (as it should be) to prevent exposure at pharmacological levels and the TI is large, then there is an intrinsic safety margin in the unlikely event of containment failure. This condition affords a strong rationale for less stringent containment or redundancy, especially if doing so is balanced by decreased ergonomic hazard.

Chronic-acting APIs with cytotoxic or cytostatic activity

In contrast to acute-acting potent pharmaceuticals, cytotoxic or cytostatic oncology drugs often have remarkably low acute toxicity, but may cause adverse health effects if exposure is chronic. Although by definition, occupational exposure limits are calculated so as to be protective of chronic occupational inhalation exposure, in chemical development and batch manufacturing, pharmaceutical workers are potentially exposed to airborne particulates only for short periods up to several hours over a few days. Under this scenario, if containment is designed to protect down to chronic exposure levels, additional protection is afforded when potential exposures would be only of short duration. While it is not recommended that occupational exposure limits be adjusted for less than chronic exposures, in cases where ergonomic hazards can be reduced, it may be justified to use less rigorous containment and redundancy for chronic toxicants if accidental exposure would be of short duration.

Personal protective equipment and hygiene monitoring

An additional way to protect against uncertainty in the risk assessment when relying on in vitro methods is to employ PPE. The US OSHA recommends that PPE devices alone should not be relied on to provide protection, but should be used in conjunction with other safeguards (OSHA 2005). In many instances, the hazard and dose-response information gained from in vitro testing data can provide the additional safeguard needed to justify the use of PPE in lieu of backup containment. In vitro hazard data from screening tests can also be used to assess exposure routes and pathways that should be protected with PPE. Regardless of the approaches used to address uncertainty in the hazard and toxicity assessment, it is essential to conduct exposure assessment and hygiene monitoring to verify that containment and handling practices are working as designed.

Discussion

Protecting the health and safety of pharmaceutical workers is a multi-faceted, multi-disciplined endeavor that requires careful consideration of chemical exposure, physical-chemical hazards and ergonomic risk factors. Ergonomic factors are often overlooked and can be a significant contributor to workplace injury; and, paradoxically, increased exposure protection increases ergonomic risk, to say nothing of greatly decreasing worker endurance, efficiency and production scale. Although no workplace statistics are available on musculoskeletal injuries attributable explicitly to containment equipment, work-related upper limb disorders (known to be associated with work in isolators) were the third leading cause of occupational injury in the pharmaceutical industry between 2001 and 2004 (UKHSE 2009).

Given the ergonomic hazards associated with increased containment, the practice of highly conservative risk assessment based on default uncertainty factors should be exchanged in favor of risk assessment that employs in vitro assay data to reduce biological uncertainty. It is also recommended that risk assessment of unstudied compounds using in vitro data should not be done in isolation, but rather should rely heavily on comparisons to the positive control data-rich API using a parallelogram approach.

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Declaration of Interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of this paper.

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