Issue Paper

Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)

EPA's Office of Chemical Safety and Pollution Prevention

August 30, 2018

Table of Contents

List of Acronyms

ACGIH: American Conference of Governmental Industrial Hygienists

AIC: Akaike Information Criterion

BMD: Benchmark Dose

BMD_{sd}: BMD for One Standard Deviation Change

$BMDL_{sd}$: Lower Bound of the 95% Confidence Interval on the BMD_{sd}

BMR: Benchmark Response

CEN: European Committee for Standardization

CF: Composite Factor

CFD: Computational Fluid Dynamic

DDEF: Data-Derived Extrapolation Factor

EF_{AD}: Extrapolation Factor for Interspecies Toxicodynamics

$EF_{AK:}$ Extrapolation Factor for Interspecies Toxicokinetics

EF_{HD}: Extrapolation Factor for Intraspecies Toxicodynamics

EF_{HK}: Extrapolation Factor for Intraspecies Toxicokinetics

EPA: Environmental Protection Agency

EURL ECVAM: European Union Reference Laboratory for Alternatives to Animal Testing

FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act

GD: Guidance Document

GSD: Geometric Standard Deviation

HEC: Human Equivalent Concentrations

ICATM: International Cooperation on Alternative Test Methods

ICCVAM: Interagency Coordinating Committee on the Validation of Alternative Methods

ISO: International Organization for Standardization

LC_{50}: Median Lethal Concentration

LDH: Lactate Dehydrogenase

LOAEC: Lowest Observed Adverse Effect Concentration

LOAEL: Lowest Observed Adverse Effect Level

LOC: Level of Concern

MMAD: Mass Median Aerodynamic Diameter

MOE: Margin of Exposure

MPPD: Multiple Path Particle Deposition

MSDS: Material Safety Data Sheets

NAM: New Approach Methodology

NICEATM: National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NOAEC: No Observed Adverse Effect Concentration

NOAEL: No Observed Adverse Effect Level

NRC: National Research Council

OCSPP: Office of Chemical Safety and Pollution Prevention

OECD: Organisation for Economic Co-operation and Development

OPP: Office of Pesticide Programs

OPPT: Office of Pollution Prevention and Toxics

OSHA: Occupational Safety and Health Administration

OVS: OSHA Versatile Samplers

POD: Point of Departure

PSD: Particle Size Distribution

RfC: Reference Concentration

SAP: Scientific Advisory Panel

SC: Suspension Concentrates

SDS: Sodium Dodecyl Sulphate

SE: Suspo-Emulsions

TD: Toxicodynamic

TEER: Transepithelial Electrical Resistance

TK: Toxicokinetic

TSCA: Toxic Substances Control Act

UF_A: Interspecies Factor for Animal-to-Human Extrapolation

UF_H: Intraspecies Factor for Differences in Sensitivity Among Humans

WDG: Water Dispersible Granule

WOE: Weight of Evidence

List of Figures

List of Tables

1.0 Introduction

1.1 Background

The mission of the Environmental Protection Agency's (EPA) Office of Chemical Safety and Pollution Prevention (OCSPP) is to protect humans and the environment from potential risks associated from exposure to pesticides and toxic chemicals. In order to achieve this, two offices within OCSPP are responsible for evaluating these potential risks. The Office of Pesticide Programs (OPP) regulates the use of all pesticide chemicals, while the Office of Pollution Prevention and Toxics (OPPT) evaluates new and existing chemical substances (excluding, among others, pesticides, tobacco and tobacco products, food, food additives, drugs, and cosmetics).

EPA regulates chemicals under authority granted by statutes, such as the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA), which allow the Agency to require or request a suite of data and relevant information from pesticide registrants and chemical manufacturers to support scientifically-based risk assessment. To assess the potential hazard of a chemical for human health risk assessment, toxicological studies in laboratory animals are used to provide information on a wide range of adverse health outcomes, routes of exposure, exposure durations, species, and lifestages. EPA's test guidelines for pesticides and toxic substances are harmonized with those established by the Organisation for Economic Co-operation and Development (OECD) and specify EPA-recommended methods to generate data that is submitted to EPA[1]; however, it should be noted that the statutory requirements differ between FIFRA and TSCA for data requirements (e.g., breadth and issues which trigger data requirements).

Under 40 CFR Part 158[2], the OPP requires toxicology data to support registration of food and non-food use pesticides. The regulations give EPA substantial discretion to make registration decisions based on what the Agency deems are the most relevant and important data for each action. The actual data and studies required may be modified on an individual basis to fully characterize the use and properties of specific pesticide products under review (40 CFR §158.30). Also, the data requirements may not always be considered appropriate. For instance, the properties of a chemical or an atypical use pattern could make it impossible to generate the required data or the data would not be considered useful in the Agency's evaluation. Therefore, the Agency may waive data requirements, but must ensure that sufficient data are available to make the determinations required by the applicable statutory standards (40 CFR §158.45). The 40 CFR also provides EPA with broad flexibility under 158.75 to request additional data beyond the Part 158 data requirements that may be important to the risk management decision. Alternative methods and approaches can be considered and accepted for these additional data, when appropriate.

[1] https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances
[2] https://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title40/40cfr158_main_02.tpl

For OPPT, there are various sections in the TSCA that include animal testing-related provisions[3], the most prominent including Sections 4, 5, 6, and 8. Section 4 of TSCA, entitled *Testing of Chemical Substances and Mixtures*, refers to EPA's authority to require health and environmental effects testing be conducted in most cases relevant to a determination of an unreasonable risk of injury to health or the environment (Section 4(a)). When such testing is required, TSCA further requires EPA to "reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures…." (Section 4(h)(1). Sections 5 and 6 of TSCA pertain to new chemicals and existing chemicals, respectively. Section 8, entitled *Reporting and Retention of Information*, has a subsection (e) which requires the Administrator to be notified of any substantial risk information.

Recently, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) released a strategic roadmap[4] to provide a comprehensive U.S. national strategy to help with the accomplishment of the National Research Council's (NRC) vision of toxicity testing in the 21st century, which promotes studying the potential hazards of a chemical at a cellular or tissue level rather than using whole animal testing.[5] The ICCVAM is comprised of 16 federal regulatory and research agencies, including EPA, that require and/or utilize toxicological and safety testing information. The strategic roadmap is reliant on interagency collaboration and public-private partnerships to develop new approach methodologies (NAMs) that provide relevant information and fit the needs of end-users. Consistent with the roadmap, EPA's OPP[6,7] and OPPT[8] have been committed to supporting NAM development and implementation by generating a process for evaluating alternative approaches to traditional *in vivo* acute toxicity studies to meet regulatory requirements.

1.2 Inhalation Risk Assessment: Typical Methods Using *In Vivo* Laboratory Animal Data

The Agency conducts human health risk assessments to evaluate the potential health effects of pesticides and toxic chemicals in residential and occupational settings based on the use pattern or conditions of use. For pesticides, anticipated exposures are based on legally enforceable pesticide labels that provide critical information about how to safely and legally handle and use pesticide products. Exposures from multiple routes are often assessed by OPP and OPPT as appropriate, including oral (dietary and incidental), dermal, and inhalation.

[3] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/frank-r-lautenberg-chemical-safety-21st-century-act
[4] A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. https://ntp.niehs.nih.gov/pubhealth/evalatm/natl-strategy/index.html
[5] https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a
[6] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science
[7] https://www.regulations.gov/docketBrowser?rpp=25&so=DESC&sb=commentDueDate&po=0&dct=SR&D=EPA-HQ-OPP-2016-0093
[8] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

For evaluating effects via the inhalation route, registrants and manufacturers conduct subchronic inhalation toxicity studies according to test guideline requirements (OPPTS 870.3465, 40 CFR Part 798, OECD TG 412 and 413)[9]. In these studies, several groups of experimental animals (rat is the preferred species) are exposed daily for a defined period of time to graduated concentrations of test substance (one concentration per group) as a gas, volatile substance, or aerosol/particulate. During the period of administration, the animals are observed daily to detect clinical signs of toxicity. At the end of the study, animals are sacrificed and necropsied, and appropriate histopathological examinations carried out. These studies are used to determine the lowest concentration where adverse effects are observed following repeated inhalation exposure, which is referred to as the lowest observed adverse effect concentration (LOAEC). The highest concentration tested at which no adverse effects were observed would be used to establish a no observed adverse effect concentration (NOAEC) for the study.

When selecting endpoints for human health risk assessment, the Agency reviews all toxicological data available to identify toxicity endpoints (effects observed in toxicity studies that are considered treatment related/adverse), as well as the dose levels needed to elicit these effects following chemical exposure. These dose levels are then used to identify a point of departure (POD). The POD is typically a dose where no adverse effects have been observed, and is used as a quantitative starting point for risk assessment for the route (oral, dermal, or inhalation) and duration (single day to chronic) of exposure under evaluation. When considering toxicological endpoints, anticipated routes of exposure are preferably matched with appropriate toxicity studies performed via the same route. Therefore, route-specific inhalation studies are optimal for evaluating risk via the inhalation route; however, these studies are not always available or cannot be used due to hazard concerns identified in the toxicological database (e.g., concern for developmental effects not evaluated in the route-specific study). When route-specific data are not available or used, route-to-route extrapolation may be necessary.

If a route-specific inhalation study has been selected to evaluate inhalation exposures from a chemical, exposure concentrations in the animal study are converted to human equivalent concentrations (HECs) and doses according to the Agency's Reference Concentration (RfC) Methodology (U.S. EPA 1994), when appropriate. This conversion allows for exposure duration adjustments (daily and weekly) to account for differences between the animal toxicity study and expected human exposures. The conversion also allows for application of a dosimetry adjustment factor that accounts for the physical nature of the inhaled material (i.e., gas, volatile substance, or aerosol/particulate), and species differences in ventilation rate and respiratory tract architecture that contribute to the pharmacokinetic differences between the test species and humans.

To provide appropriate safety margins for assessing human health risks, uncertainty factors are applied. Typically, this includes a 10X interspecies factor for animal-to-human extrapolation (UF_A) and a 10X intraspecies factor for differences in sensitivity among humans (UF_H). If the

[9] 40 CFR Part 798 Health Effects Testing Guidelines: https://www.ecfr.gov/cgi-bin/text-idx?SID=974304441e2db6c31db7a6b6a37f5572&mc=true&node=pt40.32.798&rgn=div5;
Series 870 Health Effects Test Guidelines: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines
OECD test guidelines: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

RfC Methodology has been applied, the interspecies extrapolation factor may be reduced from 10X to 3X due to the calculation of HECs that account for pharmacokinetic (not pharmacodynamic) interspecies differences. Additional factors may also be applied to account for deficiencies or uncertainties in the toxicology database (e.g., extrapolation from a lowest observed adverse effect level (LOAEL) to a no observed adverse effect level (NOAEL), uncertainty from a data gap, extrapolation to longer durations).

1.3 Comparison of Rat & Human Respiratory Tract

The anatomy and physiology of human and rodent respiratory tracts differ in several ways that can impact changes in airflow and deposition of inhaled substances and, therefore, influence the animal to human dose response extrapolation. For example, airway size (length and diameter), cell types and distribution, and composition of secretory products vary across species (Clippinger et al. 2018, Schlesinger 1984; U.S. EPA 1994). Additionally, branching patterns differ across species. Human airways have a more symmetrical dichotomous pattern than rodents. The more symmetrical dichotomous pattern is prone to deposition at branching points leading to higher concentrations at these points compared to rodents (Clippinger et al. 2018; Schlesinger 1984).

The structures that provide an initial barrier to inhaled air and particles are the nasal cavity and larynx, which have notable differences between rats and humans. The nasal cavity consists of nasal turbinates where particles deposit primarily through inertial impaction. Humans have three turbinates that are relatively simple in shape, while the architecture of the nasal turbinate systems in rats is more convoluted than humans with complex folding and branching patterns (Harkema et al., 2006). In conjunction with the obligate nasal breathing of rodents, this results in greater deposition in rats as compared to humans.

There is also significant interspecies variability in overall surface area and cellular composition/distribution of the nasal surface epithelium. On average, the surface area of the human extrathoracic, tracheobronchial, and pulmonary regions are 200 cm^2, 3200 cm^2, and 54 $m^{2,}$ respectively. In contrast, the average surface area in the rat in those regions are 15 cm^2, 22.5 cm^2, and 0.34 m^2, respectively (U.S. EPA 1994). In most animal species, there are four types of nasal epithelium: 1) squamous epithelium, 2) non-ciliated cuboid or columnar transitional epithelium, 3) ciliated pseudostratified cuboid or columnar respiratory epithelium, and 4) olfactory epithelium. However, the distribution of these epithelial populations and nasal cell types within these populations will differ across species (Harkema et al., 2006). Furthermore, rats have a higher percent coverage of the nasal cavity in olfactory epithelium that leads to a more heightened sense of smell as compared to humans.

In addition, there is an anatomical difference between rats and humans in the larynx. The larynx is involved in sound production and protects against food aspiration. In rats, cartilage associated with the ventral pouch is U-shaped and the larynx and trachea in rats form a relatively straight line from the nasal turbinates, which enhances the deposition of aerosols in the rat larynx (Kaufmann et al., 2009). As a result, the larynx can be a common site of injury in laboratory inhalation toxicity studies with rats. In contrast, in humans the U-shaped pouch is absent and the larynx is more sharply angled to the oro-nasal cavity (Kaufmann et al., 2009). As a result, when considering risk assessment for humans, determining the relevance of laryngeal lesions seen in rat *in vivo* studies is complicated by these anatomical differences.

Due to critical differences between rat and human respiratory tracts, the ability of *in vivo* testing to correctly predict effects in humans can be affected. As a result, NAMs that take into consideration the differences may serve as a refinement for human health risk assessment.

1.4 Using New Approach Methodologies (NAMs) to Refine Inhalation Risk Assessment

1.4.1 Overview: Alternative Test Methods

Traditional *in vivo* toxicity tests used to extrapolate to humans are resource intensive in terms of animal use, expense, and time. Typically, uncertainty factors must also be applied to account for differences between the species tested and humans. As a result, efforts to develop alternative methods and strategies for hazard identification and characterization have been supported by the Agency. These efforts are consistent with the recommendations presented in the NRC's vision of toxicity testing in the 21st century, as well as the National Academy of Science's report on how to integrate and use data from emerging techniques to improve risk-related evaluations[10].

Alternative test methods and strategies have a common goal which are historically defined by the 3Rs: reduction (promoting use of fewer experimental animals), refinement (procedures to minimize animal pain and distress), and replacement (test systems that use phylogenetically lower species or avoids animal use). Strategies include using more than just toxicity test methods to characterize hazard (e.g., use of analog/read across techniques and tiered testing approaches to characterize a given human health or environmental endpoint). Collectively, alternative test methods and strategies can be referred to as NAMs, a term intended as a broadly descriptive reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment.

Innovation and progress in the development of NAMs is rapidly occurring. EPA is working with multiple national/international organizations to identify NAMs for hazard identification and characterization, including individual government organizations (e.g., Health Canada), the ICCVAM, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), the International Cooperation on Alternative Test Methods (ICATM), and the OECD[11]. The advancement of biological knowledge aids in the development of novel *in vitro* assays and better computational models that integrate *in vitro*, *in vivo*, and *in silico* data. The

[10] http://dels.nas.edu/Report/Using-21st-Century-Science-Improve/24635

[11] List of alternative methods accepted by US agencies through ICCVAM - https://ntp.niehs.nih.gov/pubhealth/evalatm/accept-methods/index.html and list of ICCVAM Guidance Documents: https://ntp.niehs.nih.gov/pubhealth/evalatm/accept-methods/guidance/index-2.html; List of alternative methods listed as "regulatory acceptance/standards" completed according to the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM) through its Tracking System for Alternative Methods towards Regulatory Acceptance (TSAR) - http://tsar.jrc.ec.europa.eu/; and List of alternative methods/strategies presented by health endpoints in the OECD - http://www.oecd.org/chemicalsafety/testing/oecdguidelineapproachbyendpoints.htm; and others such as Alttox.org – table of validated and accepted alternative methods: http://alttox.org/mapp/table-of-validated-and-accepted-alternative-methods/

development of novel NAMs for hazard identification and characterization is integral to address the knowledge gaps and target the replacement of studies most frequently requested by the EPA.

1.4.2 *In Vitro* Test Systems for Inhalation Toxicity

There are several *in vitro* tools available to evaluate inhalation toxicity that have been summarized in Clippinger *et al.* (2018). These include lung-on-a-chip models, *ex vivo* lung slices, and *in vitro* cell cultures. The lung-on-a-chip model is a microphysiological system that replicates the microarchitecture of the tracheobronchial airways and alveoli in order to provide predictions of physiological responses in human lung tissue. Although this model is promising and may advance rapidly, it does not appear to be a feasible option for regulatory applications at this time due to issues with transferability, lack of throughput, and lack of commercial availability with lung-on-a-chip models. *Ex vivo* precision-cut lung slices reflect the natural microanatomy of the respiratory tract, as well as its functional response to an inhaled chemical. Although *ex vivo* lung slices collected from human donor lungs can be maintained for weeks, thickness of tissue slices vary due to lack of a standardized method and this variation can impact comparative functionality (Clippinger et al., 2018). Consequently, *ex vivo* lung slices are also not ready for regulatory applications, but may be an option in the future as the science advances.

In vitro cell cultures range in complexity from simple submerged culture systems to three-dimensional models. Due to the medium that covers simple cell cultures, the assays do not allow for direct exposure at the air-liquid interface and are less human relevant for evaluation of respiratory chemicals. On the other hand, three-dimensional models cultured from airway epithelial cells at the air-liquid interface can mimic particular regions of the human respiratory tract, including barrier function, mucous production, and cilia function. Three-dimensional models have been used successfully to study infection and toxicity in the respiratory system (Mathis et al. 2013, Neilson et al. 2015, Essaidi-Laziosi et al. 2017) and are the focus of the case study described below.

An understanding of *in vitro* and *in vivo* dosimetry is essential when using any of the *in vitro* systems. Although NAMs are often validated by comparing to *in vivo* tests, there are inherent differences between animals and humans as discussed in Section 1.3 that make this comparison challenging. Therefore, integrating human relevant exposure information into the evaluation of *in vitro* results is crucial.

Selection of an appropriate and relevant NAM should be determined in a fit-for-purpose context. As noted above, there are advantages and limitations associated with available *in vitro* systems. The Agency recognizes that the science will continue to evolve as methods continue to advance and additional tools become available; however, in order to address current science questions, the best tool currently available based on the state of the science should be employed. At this time, EPA considers *in vitro* models that allow direct exposure at the air-liquid interface, such as the three-dimensional models, to be the best available tools to evaluate human respiratory tract toxicity.

2.0 Case Study Using a Respiratory Irritant: Chlorothalonil

The Agency has received an example of an alternative approach to refine inhalation risk assessment for the pesticide chlorothalonil. Syngenta Crop Protection (hereafter referred to as Syngenta), one of the registrants of chlorothalonil products, submitted a proposed approach using a POD derived from an *in vitro* assay (MucilAir™). In order to calculate HECs for the purposes of human health risk assessment, the *in vitro* POD was used in conjunction with surface concentrations of deposited chlorothalonil particles derived from a computational fluid dynamic (CFD) model. As a proof of concept, Syngenta used the calculated HECs to provide potential risk estimates for chlorothalonil.

Syngenta initially presented a proposal for refining the inhalation risk assessment for chlorothalonil using the MucilAir™ assay in 2014. The Agency recognized the value of this proposal for chlorothalonil, as well as other contact irritants, and supported the movement to a NAM in lieu of *in vivo* laboratory animal testing. Early in the process EPA reached out to the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to collaborate on the review of Syngenta's proposed approach. EPA encouraged further development and determined that external peer view and public dialogue would be needed prior to applying the proposed approach to human health risk assessments for contact irritants, such as chlorothalonil. As a result, the EPA has brought the proposed approach to refine the inhalation risk assessment for chlorothalonil to the FIFRA Scientific Advisory Panel (SAP) for review. The approach is being considered by OPP and OPPT for applicable pesticides and industrial chemicals.

The Agency is specifically soliciting advice from the SAP on the methods used to derive the POD from the *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. The SAP evaluation is not a review of the CFD model *per se*, but rather a review of how the CFD model was applied for the refinement of the inhalation risk assessment. Chlorothalonil is being presented as a case study to solicit advice on the proposed methodological approach, which is expected to be applied, when applicable, to other pesticides and industrial chemicals in the future. This case study is not intended to represent the final conclusions for the human health risk assessment for the case study chemical, chlorothalonil. As noted later in this document, consultations with Syngenta on some aspects of this approach (e.g., exposure assumptions for different occupational handler activities) are still on-going; however, this should not impede the ability for the SAP to review the overall proposed approach.

The remainder of this document briefly summarizes the proposed approach and includes relevant information from EPA regarding regulatory application. It provides key information and values for the approach. As a result, not all details and values provided by Syngenta will be discussed. The following sections are organized by the following:

- *Section 2.1 Inhalation Risk Assessment for Chlorothalonil* provides a discussion of the inhalation toxicology data available for chlorothalonil and a summary of previously conducted inhalation risk assessments.

- *Section 2.2 Source to Outcome Approach* provides a list of documents submitted by Syngenta and is comprised of 6 subsections:
    - *Section 2.2.1 Source* summarizes the chlorothalonil products currently registered by Syngenta where the proposed approach could be applicable and presents the percent of chlorothalonil expected in diluted products during application according to label directions.
    - *Section 2.2.2 Exposure* discusses information available to derive a human relevant particle size distribution (PSD).
    - *Section 2.2.3 Dosimetry* provides information on the CFD simulations and the resulting deposition calculated for the maximum percent of chlorothalonil in a diluted product.
    - *Section 2.2.4 Outcome* describes the data obtained from the *in vitro* assay (MucilAir™) and derivation of the POD.
    - *Section 2.2.5 Chlorothalonil Inhalation Risk Assessment Utilizing Refined Approach* provides step-wise calculations for site-specific HECs and presents risk estimates using the most health protective HEC.

2.1 Inhalation Risk Assessment for Chlorothalonil

Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile) is a broad-spectrum, non-systemic protectant pesticide mainly used as a fungicide to control fungal foliar diseases of vegetable, field, and ornamental crops. It is also used as a wood protectant, antimold and antimildew agent, bactericide, microbiocide, algaecide, insecticide, and acaricide. Residential/non-agricultural uses include use on golf courses, on home gardens, as a wood preservative, and in paint formulations.

Chlorothalonil is a contact irritant that has been found to be toxic via the inhalation route. It is classified as a Toxicity Category I for acute inhalation (median lethal concentration (LC_{50}) ≤ 0.05 mg/L). Non-lethal effects observed in acute inhalation studies included clinical signs indicative of respiratory tract effects, such as nasal discharge, difficulty breathing, decreased activity/lethargy, respiratory rales, ptosis, and piloerection. Consistent with its effects as a respiratory irritant, chlorothalonil also causes severe eye irritation (Toxicity Category I) in acute studies.

In the most recent risk assessment (G. Kramer; 23-DEC-2010; D370486), a repeat dose inhalation study was not available for chlorothalonil and there were concerns that using an oral endpoint may underestimate risk via the inhalation route due to the high lethality and clinical signs consistent with respiratory-tract irritation observed in acute inhalation toxicity studies. Furthermore, a NOAEC was not established in several acute inhalation toxicity studies carried out with technical-grade chlorothalonil or end-use product formulations. As a result, the lowest concentration tested in the critical acute inhalation toxicity study (MRID 43678403) of 0.002 mg/L was used as the POD to assess inhalation risks from use of chlorothalonil. For acute exposures, a total uncertainty factor of 100 was applied which incorporated a 10X UF_H, a 3X UF_A since the RfC Methodology was applied to calculate HECs, and a 3X uncertainty factor for extrapolation of a lowest observed adverse effect concentration (LOAEC) to a NOAEC. For short- and intermediate-term exposures, the same uncertainty factors were applied and an additional 10X was applied to account for extrapolating from an acute study to longer durations

resulting in a total uncertainty factor of 1,000. The 2010 risk assessment identified inhalation risk concerns for short- and/or intermediate-term exposure for residential handlers using paint, post-application exposure from inhaling vapors from treated paint, bystander volatilization exposure, and occupational handler exposure. As part of this action, the Agency requested a 90-day inhalation study.

In response, the chlorothalonil registrants submitted four inhalation studies – a range finding acute inhalation toxicity study (MRID 49184807), an acute inhalation toxicity/tolerability study (MRID 49184808), an acute pilot toxicokinetic study (MRID 49184809), and a 2-week inhalation toxicity study (MRID 49184810). Table 2.1.1 summarizes the study design for these four studies. All of the studies, except the toxicokinetic study, were conducted with a formulation (Bravo Weather Stik 720 SC) containing approximately 54% chlorothalonil. A NOAEC was not established in any of the inhalation toxicity studies. Clinical signs related to respiration (e.g., labored/rapid breathing, gasping, wheezing, rales) were noted following acute and repeat dosing. Epithelial degeneration and/or necrosis (with and without ulceration) in the nasal cavity, larynx, lung, and trachea were the primary histopathological findings observed in the respiratory tract across the toxicity studies. In the 2-week toxicity study, squamous cell metaplasia in the larynx was observed for all concentrations tested and squamous cell hyperplasia in the nasal cavity was seen at the highest dose tested. Following 14 days of recovery in the 2-week toxicity study, the effects were either absent or reduced in incidence and/or severity.

Although these studies provided further information on chlorothalonil toxicity via the inhalation route, the Agency did not consider these studies sufficient to fulfill the requirement of a 90-day inhalation toxicity study given the high toxicity demonstrated. Syngenta, however, indicated that a 90-day inhalation toxicity study was not feasible due to the irritant nature of chlorothalonil and animal welfare concerns. Subsequently, Syngenta proposed an alternative approach using an *in vitro* assay to characterize the hazard of chlorothalonil and derive a POD for establishing HECs by integrating relevant particle size distributions (PSDs) for expected human exposures.

Table 2.1.1. Study Design of Inhalation Studies Submitted to the Agency Following Registration Review DCI

Study	Test substance	Number of animals	Chlorothalonil concentrations (mg/L)	Exposure Duration
Range-finding acute inhalation study (MRID 49184807)	Bravo Weather Stik 720 SC (53.7% chlorothalonil)	5 Sprague-Dawley rats/sex/concentration/ exposure duration	0 (air control), 0 (vehicle control), 0.004, 0.015 or 0.030	2, 4, or 6 hours
Acute inhalation toxicity/tolerability study (MRID 49184808)	Bravo Weather Stik 720 SC (53.7% chlorothalonil)	4 Sprague-Dawley rats/sex/concentration	0 (air control), 0 (vehicle control), 0.031, 0.077, or 0.107	6 hours
Acute pilot toxicokinetic study (MRID 49184809)	^{14}C-chlorothalonil (98.1% radiochemical purity)	7 Sprague-Dawley male rats/concentration	0.0029 or 0.026	5 hours
2-week inhalation toxicity study (MRID 49184810)	Bravo Weather Stik 720 SC (53.7% chlorothalonil)	25 Sprague-Dawley male rats/concentration (10 evaluated at end of exposure; 5 each at recovery times of 48 hours, 7 days, or 14 days)	0 (air control), 0 (vehicle control), 0.0011, 0.0029, 0.0096, or 0.0143	6 hours/day, 5 days/week

2.2 Source to Outcome Approach

Syngenta has utilized a source to outcome approach as a framework for integrating human exposure and hazard characterization for a refined inhalation risk assessment. This approach is comprised of 4 components – source, exposure, dosimetry, and outcome – that are used to refine the inhalation risk assessment for chlorothalonil. The following are the primary documents submitted by Syngenta to describe the information utilized for each component and the overall proposed approach:

- MRID 50610404 – particle size characterization of agricultural sprays collected on personal air monitoring devices (Flack and Ledson, 2018)
- MRID 50610403 – computational modeling of aerosol dosimetry in the respiratory tracts of the rat and human (Corley et al, 2018)
- MRID 50317702 – *in vitro* measurement of the airway irritation potential of Bravo 720 SC formulation using MucilAir™ tissues from five difference donors (Vinall, 2017)
- MRID 50610401 – benchmark dose (BMD) analysis of MucilAir™ data to establish a toxicological POD for use in human risk assessment (Li et al., 2018)
- MRID 50610402 – a source to outcome approach for inhalation risk assessment (Flack et al., 2018)

2.2.1 Source

The proposed approach has been developed to evaluate exposure and risk from applying chlorothalonil liquid formulations or solids that are diluted in water and applied as a liquid (summarized in Table 1 of MRID 50610402). The approach could be applied in a similar fashion for mixer/loader exposure scenarios with liquid formulations. Liquid formulations of chlorothalonil can be applied using hand-held, groundboom, chemigation, airblast, and aerial equipment. End use products currently registered by Syngenta that are formulated as liquids include suspension concentrates (SC) and suspo-emulsions (SE). Following dilution of SC and SE products with water according to label instructions, spray applications contain 0.3%-4.5% (w/v) chlorothalonil. Additionally, Syngenta has registered water dispersible granule (WDG) formulations of chlorothalonil, which are solids that are diluted in water and applied as a liquid. For the WDG formulations, the spray applications contain 0.2%-4.9% (w/v) chlorothalonil. Therefore, the maximum percent of chlorothalonil in a diluted spray that a handler is expected to apply is 4.9%.

2.2.2 Exposure

Spray nozzles are used for occupational applications to maximize deposition on a target, while also minimizing drift. Nozzles break liquids into droplets, create spray patterns, and propel droplets in designated directions. Syngenta has investigated the effect of nozzle type or spray quality (e.g., fine, medium, coarse) on PSD of sprays containing chlorothalonil and found the percentage of spray volume in the inhalable range (<100 µm) increases as the spray quality becomes more fine (MRID 50610404). Since different activities utilize different spray qualities,

the PSD of aerosols is dependent on the type of activity performed during the period of exposure to a pesticide. Additionally, Syngenta conducted a study to compare air sampling from Occupational Safety and Health Administration (OSHA) versatile samplers (referred to as OVS tubes hereafter) and Respicon particle samplers using various nozzle spray qualities with a chlorothalonil dilution (5% v/v Bravo Weather Stik®). OVS tubes are frequently used in occupational settings to collect aerosols by placing the device in the breathing zone of a worker. The Respicon particle sampler is a cascade impactor consisting of multiple stages that separate airborne particles into three size fractions (inhalable, thoracic, and respirable) that have defined mathematical descriptions that are internationally recognized[12]. Syngenta concluded from this comparative study that OVS tubes capture the inhalable fraction (< 100 µm) and proportions of size fractions were similar across spray qualities. Additional details regarding the methods used and data obtained from these analyses can be found in MRID 50610404 and MRID 50610402.

For the purposes of presenting the proposed approach to the SAP, Syngenta has mathematically derived a human relevant PSD for inhalable particles for spray applicators. As mentioned above, mathematically defined distributions of the inhalable, thoracic, and respirable size fractions have been established and are used as the basis of the PSD for the spray applicators in this assessment. The thoracic portion of the inhalable fraction is defined as a cumulative density function of a lognormal distribution with a mass median aerodynamic diameter (MMAD) of 11.64 µm and a geometric standard deviation (GSD) of 1.5. The respirable portion of the inhalable fraction is defined as a cumulative density function of a lognormal distribution with a MMAD of 4.25 µm and a GSD of 1.5. To establish a human relevant PSD for spray applicator exposures, a maximum cut-off of 100 µm for particles that are inhalable was incorporated into the derivation of a cumulative density function (or an "adjusted" inhalable fraction) resulting in a PSD with a median geometric diameter of 35 µm and GSD of 1.5. Since inhalation dosimetry models require particle sizes to be characterized by aerodynamic diameter (e.g., MMAD), the geometric diameter must be converted using the density of the particle. Chlorothalonil formulations use water as the carrier; therefore, applying the density of water (1 g/cm^3) will yield equivalent geometric and aerodynamic cumulative density functions (i.e., MMAD = 35 µm and GSD = 1.5). Additional details regarding the derivation of the PSD for the "adjusted" inhalable fraction for applicators can be found in Section 4.2 of MRID 50610404.

Table 2.2.2.1. Summary of Particle Size Distribution (PSD) for Proposed Approach

Exposure Scenario	Mass Median Aerodynamic Diameter (MMAD) (µm)	Geometric Standard Deviation (GSD)
Spray Application of Liquids	35	1.5

2.2.3 Dosimetry

Dosimetry models are used to determine internal doses of a chemical and provide information that aids in the understanding of the relationship between an external exposure and a biological

[12] Definitions of particle size fractions based on agreed upon criteria set forth by the International Organization for Standardization (ISO), American Conference of Governmental Industrial Hygienists (ACGIH), and European Committee for Standardization (CEN).

response. For the proposed approach, Syngenta predicted deposition of chlorothalonil in site-specific regions of the human upper respiratory tract (i.e., vestibule, respiratory, olfactory, pharynx, larynx, and trachea) using a CFD model similar to previously published models (Corley et al. 2012, Corley et al. 2015, Kabilan et al. 2016). CFD has been used in many scientific fields to analyze fluid flows and there is a multitude of literature available on CFD theory and application. CFD models for the upper respiratory tract have been developed for several species, including rats (e.g., Kimbell et al. 1993, Kimbell et al. 1997), monkeys (e.g., Kepler et al. 1998), and humans (e.g., Subramaniam et al. 1998). For these models, a computational mesh based on species-specific anatomical data are used to develop airflow patterns that are used in conjunction with boundary conditions, chemical-specific diffusivity, and mass transfer coefficients to predict localized deposition of inhaled material in units of mass per unit area (e.g., mg/cm^2/breath).

Simulations were performed for monodisperse, spherical particles sizes of 1, 3, 5, 10, 15, 20, and 30 µm. Each simulation assumed a 1 mg/L aerosol concentration and resting nasal breathing; however, the model may be reconfigured for oral breathing and multiple breathing patterns in the future, if needed. Since total particle deposition was approximately 99% at 30 µm, simulations for particles greater than 30 µm were not included in the proposed approach since negligible penetration of the larger particles was predicted. Since the CFD model is essentially generating results for a generic water droplet (i.e., non-chemical specific), the results need to be adjusted for the amount of chlorothalonil in the diluted product. The deposition of chlorothalonil is proportional to the amount of active ingredient being applied; therefore, the CFD results were multiplied by the maximum percent of chlorothalonil in a diluted product (4.9%) (Table 2.2.3.1). Deposition at the 75th percentile was selected because it is the highest concentration area that is not affected by stochastic variations in the modelling.

Additional information regarding the CFD model can be found in MRID 50610403 and summarized in Section 6.0 of MRID 50610402.

Table 2.2.3.1. Human CFD simulation results for 1 mg/L aerosol, assuming 4.9% (w/w) chlorothalonil formulation for aerosol sizes ranging from 1 to 30 µm MMAD.

Aerosol diameter (µm)	Deposition at 75th percentile (mg chlorothalonil/cm^2/breath) adjusted for 4.9% (w/w) chlorothalonil					
	Vestibule	Respiratory	Olfactory	Pharynx	Larynx	Trachea
1	5.15E-05	3.66E-05	6.27E-05	2.05E-05	2.59E-05	8.82E-06
3	4.07E-05	2.92E-05	5.44E-05	1.51E-05	2.98E-05	9.26E-06
5	6.86E-05	3.43E-05	1.51E-04	1.78E-05	3.70E-05	7.64E-06
10	1.95E-03	5.39E-05	2.12E-05	6.47E-05	1.68E-04	1.56E-05
15	3.49E-03	3.48E-05	1.17E-05	4.19E-05	1.01E-04	1.68E-05
20	3.31E-03	2.73E-05	7.79E-06	2.22E-05	3.21E-05	6.71E-06
30	1.81E-03	2.27E-05	0.00E+00	6.76E-06	1.23E-05	2.56E-06

Table taken from page 19 of MRID 50610402.

2.2.4 Outcome

As described in Section 2.1, there have been challenges with fulfilling the requirement of a 90-day inhalation toxicity study due to the contact irritation caused by chlorothalonil. Syngenta has provided a biological understanding of the irritation resulting from chlorothalonil exposure

(Section 7.0 of MRID 50610402). This includes an adverse outcome pathway[13] where epithelial cell damage occurs from initial inhalation exposure to chlorothalonil and causes cell death. Following repeated exposure, the repeated cell death results in a metaplastic response and transformation of respiratory epithelium into stratified squamous epithelium (Figure 1). As such, Syngenta considered available *in vitro* models for assessing damage to respiratory epithelial cells and identified MucilAir™ as the optimal model for the proposed approach. A sufficient amount of chlorothalonil is needed at the cell surface to result in cell death in this pathway. Therefore, the *in vitro* test system is mimicking the *in vivo* exposure for the initial interaction of chlorothalonil with respiratory cells. Furthermore, by protecting for the initial cell damage caused by chlorothalonil exposure, effects that would be caused from repeated exposure would also be prevented.

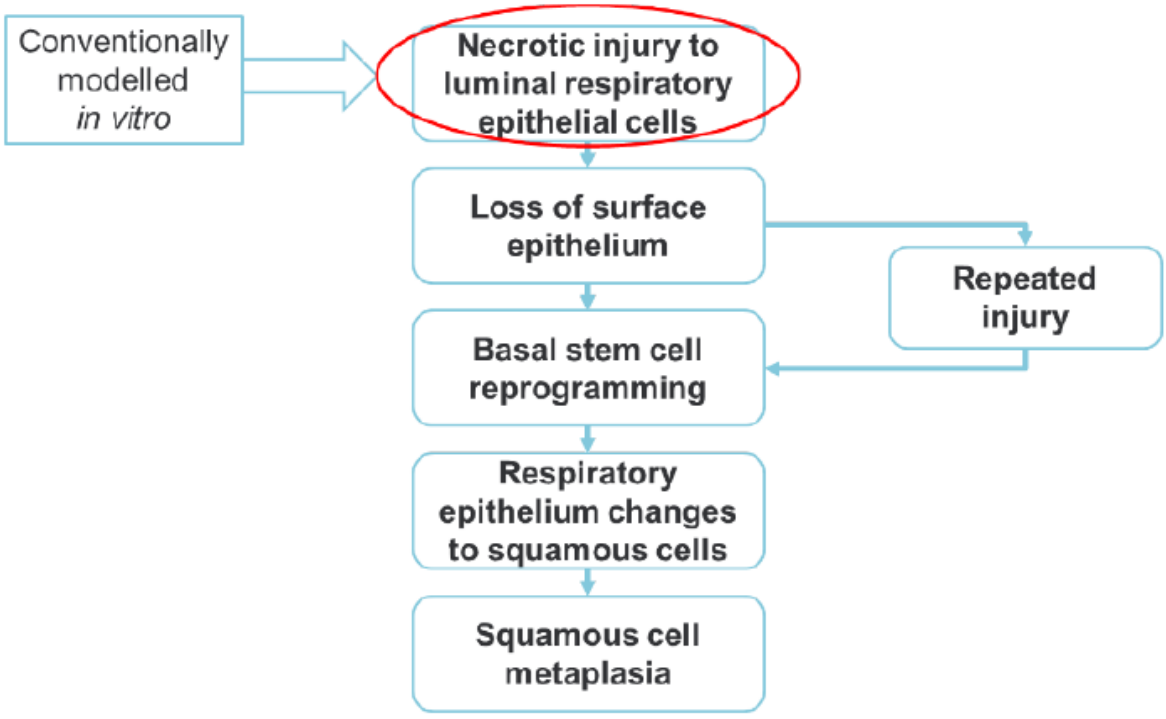

Figure 1. Adverse outcome pathway for irritant induced laryngeal squamous metaplasia adapted from Renne et al. 2009 (figure taken from MRID 50610402).

MucilAir™ is a three-dimensional *in vitro* test system derived from human epithelial cells from the nasal, tracheal, or bronchial tissues of donors. The condition of the tissues was determined by viewing a subset of tissues (n = 4 per plate) by microscopy to verify form and correct cilia function. For the proposed approach, the cells were collected from nasal tissue of 5 separate healthy donors; however, it is noted that the cellular composition of the nasal, tracheal, and bronchial epithelia is the same and consists of basal, ciliated, and goblet cells. Therefore, similar responses are expected across tissue types for the evaluation of cell damage from irritation.

[13] An adverse outcome pathway links a molecular initiating event to progressive levels of biological organization at the individual or population level. As such, although the terminology is different, the concepts of adverse outcome pathway and mode of action are similar – an adverse outcome pathway is conceptually similar to establishing key events in a mode of action.

Furthermore, it should be noted that the nasal tissue model was the only model available from Epithelix at the time of the studies. Certificates of analysis including donor information (e.g., age, sex, smoker), cell information (e.g., cell type, date of seeding), and quality control results (e.g., sterility, tissue integrity, etc.) were provided for each donor (Appendix 1 of MRID 50317702).

Cell viability can be determined using numerous parameters, but it is typically defined by the integrity of the outer cell membrane. If the cell membrane is damaged, substances that are typically prohibited from traversing the cell membrane are able to cross it. As a result, measurements may evaluate membrane integrity directly or by using dyes that indicate substances have moved across the membrane due to cell damage. For the proposed approach, cell damage was evaluated using measurements of transepithelial electrical resistance (TEER), resazurin metabolism, and lactate dehydrogenase (LDH). As such, these measurements are being used to determine if cell damage and/or death has occurred from the initial respiratory exposure to chlorothalonil described in the adverse outcome pathway above.

TEER is a commonly used measurement of the integrity of tight junctions between cells in the membrane by an electrical resistance meter. Decreases in this measurement would indicate loss of barrier integrity. Resazurin is a dye that can be reduced by viable cells with active metabolism, resulting in a product that is fluorescent. As a result, the measured fluorescence is proportional to the number of viable cells and reduced fluorescence indicates low cell viability. LDH is an enzyme released when cells suffer sufficient membrane damage indicative of cytotoxicity that leads to cell death. The released LDH can convert resazurin into its fluorescent product. Therefore, similar to resazurin metabolism, the measured fluorescence is proportional to the number of viable cells; however, in this case, an increase in fluorescence indicates low cell viability. Evaluation of these *in vitro* endpoints using MucilAir™ has been shown to predict *in vivo* respiratory toxicity (Sivars et al., 2018). In particular, TEER and resazurin measurements had 88% sensitivity and 100% specificity.

Chlorothalonil was applied to MucilAir™ at 10 dose levels ranging from 2 to 200 mg/L (6 replicates/dose/donor)[14] using dilutions of a formulation containing 54.7% chlorothalonil (Bravo 720). Tissues were exposed for 24 hours and evaluation of irritation potential was assessed by TEER measurements, LDH release, and resazurin metabolism (MRID 50317702). For each endpoint, BMD modeling was used to determine a BMD for one standard deviation change (BMD_{sd}) and the lower bound of the 95% confidence interval on the BMD_{sd} ($BMDL_{sd}$) (MRID 50610401). Use of the BMD_{sd} is consistent with the EPA's Benchmark Dose Technical Guidance[15]. Benchmark response (BMR) selections are made on a case-by-case basis and take into account statistical and biological information. In the absence of information to determine the level of response to consider adverse, a change equal to one control standard deviation from the control mean is used.

[14] Note: higher dose levels (up to ~5000 mg/L) were tested with sample sizes varying across doses and studies. These higher doses were used for a preliminary assessment of the data with BMD modeling. The analyses can be found in Appendix VII of MRID 50610401.

[15] https://www.epa.gov/risk/benchmark-dose-technical-guidance

For Syngenta's BMD analyses, doses were log transformed and fit with a modified Hill model. It was noted that the transformation assumed negative controls to be 1, rather than zero; however, this does not have an impact on the modeling in this case due to the flat response at the low doses. The Agency performed its own BMD analyses on the untransformed data for comparison and found the Hill model to best fit the data (Appendix A). Both models (Hill for untransformed and modified Hill for transformed) were found to fit the data well visually. Akaike information criterion (AIC) values indicate the relative fit of a model for a dataset (i.e., a lower AIC value indicates that a model fits the data better than a model with a higher AIC). Overall, the untransformed data provided similar or lower AIC values than the transformed data; however, the BMD and BMDL values obtained using the transformed data were lower and, therefore, considered protective.

The three endpoints provided similar BMD_{sd} results (Table 2.2.4.1). An overall mean $BMDL_{sd}$ value from all three endpoints was 80.79 mg/L. Conversion of this value to a tissue concentration using the internal diameter of the MucilAir™ well inserts (33.18 mm^2) results in a $BMDL_{sd}$ value of 0.0073 mg/cm^2 (Equation 1; Section 7.2 of MRID 50610402).

$$BMDL_{sd}\ (mg/cm^2) = BMDL_{sd}\ (mg/L) \times \frac{30\ \mu L \times 1 \times 10^{-6}\ L/\mu L}{31.88\ mm^2 \times 0.01\ cm^2/mm^2}$$

Equation 1

Additional information regarding the conduct and results of the *in vitro* measurements can be found in MRID 50317702 and summarized in Section 7.0 of MRID 50610402.

Table 2.2.4.1. Chlorothalonil $BMDL_{sd}$ values in mg/cm^2 calculated from MucilAir™ data

$BMDL_{sd}$ (mg/cm^2)			
TEER	LDH	Resazurin	Geometric Mean
0.00724	0.00794	0.00678	0.0073

Values taken from page 25 of MRID 50610402.

2.2.5 Chlorothalonil Inhalation Risk Assessment Utilizing Refined Approach

2.2.5.1 Calculation of Human Equivalent Concentrations (HECs)

In order to calculate HECs, Syngenta has described steps that integrate the information described thus far, including the PSD for the "adjusted" inhalable fraction for applicators, the CFD model results assuming the maximum percent of chlorothalonil in a diluted product of 4.9%, and the $BMDL_{sd}$ based on the *in vitro* results (Section 8.0 of MRID 50610402). These steps include:

1. Calculation of cumulative site-specific deposition of polydisperse particles in each region of the upper respiratory tract.
2. Calculation of site-specific total deposition for the relevant exposure duration.
3. Calculation of the site-specific HEC using the total deposition and $BMDL_{sd}$.

Step 1: Calculation of cumulative site-specific deposition

The CFD model (as described above in Section 2.2.3 and MRID 50610403) provided results for discrete particles sizes (i.e., monodisperse) ranging from 1 to 30 µm in a single breath; however, spray applicators will be exposed to distributions of these particle sizes (i.e., polydisperse). The percent contribution of each discrete particle size was determined mathematically using the PSD for the "adjusted" inhalable fraction for applicators described in Section 2.2.2 (MMAD = 35 µm, GSD = 1.5) and are presented in Table 2.2.5.1.1.

Table 2.2.5.1.1. Percent Contribution of Discrete Particles to the Relevant Particle Size Distributions (MMAD = 35 µm, GSD = 1.5)

Aerosol Diameter (µm)	Percent Contribution
1	$3.43x10^{-14}$%
3	$6.06x10^{-6}$%
5	0.0034%
10	1.44%
15	12.80%
20	32.89%
30	52.87%

Taken from page 28 of MRID 50610402.

For each region of the upper respiratory tract, the deposition of each particle size was calculated by multiplying the percent contribution of a particle size by the predicted deposition from the CFD model (assuming the maximum percent of chlorothalonil in a diluted product of 4.9% as described above in Section 2.2.3). For example, the deposition in the larynx of a 10 µm particle would be calculated by multiplying $1.68x10^{-4}$ mg/cm^2/breath (from Table 2.2.3.1) by 1.44% (Table 2.2.5.1.1). After calculating the deposition of each particle size for a given region in this manner, the cumulative site-specific deposition per breath was then calculated as the sum of depositions across particle sizes (Table 2.2.5.1.2). Additional details regarding the calculation of cumulative site-specific deposition for each region of the upper respiratory tract can be found in Section 8.1.1 of MRID 50610402.

Table 2.2.5.1.2. Cumulative particle deposition for 1 mg/L aerosol in site-specific regions of the respiratory tract for each exposure scenario assuming 4.9% (w/w) chlorothalonil formulation.

Exposure Scenario	Cumulative deposition amount (mg chlorothalonil/cm^2/breath)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	2.62E-05	4.37E-06	1.72E-05	3.25E-05	5.93E-06

Taken from page 30 of MRID 50610402.

Step 2: Calculation of site-specific total deposition

Before calculating HECs, relevant breathing rates and exposure duration must be incorporated to determine the total daily deposition of chlorothalonil for each region of the upper respiratory tract since the site-specific deposition estimates in Table 2.2.5.1.2 were calculated per breath. The total deposition for each region of the respiratory tract was calculated by multiplying the cumulative site-specific deposition (from Table 2.2.5.1.2) by a breathing rate of 12.7

breaths/min, an exposure duration of 8 hours, and a conversion factor of 60 min/hr. The breathing rate was derived from the minute ventilation of 8.3 L/min and the exposure duration is the default used by EPA to evaluate occupational handler activities. Site-specific total deposition values are presented in Table 2.2.5.1.3. For additional details regarding the calculation of site-specific total deposition, see Section 8.1.2 of MRID 50610402.

Table 2.2.5.1.3. Total deposition of chlorothalonil in site-specific regions of the respiratory tract for each exposure scenario assuming 4.9% (w/w) chlorothalonil formulation

Exposure Scenario	Total deposition amount (mg chlorothalonil/cm^2)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	0.16	0.027	0.10	0.20	0.036

Taken from page 30 of MRID 50610402.

Step 3: Calculation of site-specific HECs

The final step is the calculation of the HECs. Since each CFD simulation assumed a 1 mg/L aerosol concentration, site-specific HECs can be calculated by simply dividing the geometric mean $BMDL_{sd}$ of 0.00730 mg chlorothalonil/cm^2 by the total deposition calculated for each region of the upper respiratory tract in Table 2.2.5.1.3. Site-specific HECs are presented in Table 2.2.5.1.4. The lowest HEC was obtained for the larynx (0.037 mg/L); therefore, it was selected to calculate subsequent risk estimates since it is protective of the other regions of the upper respiratory tract. Additional details regarding the calculation of HECs can be found in Section 8.1.3 of MRID 50610402.

Table 2.2.5.1.4. HEC values assuming 4.9% (w/w) chlorothalonil formulation

Exposure Scenario	HEC (mg/L)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	0.046	0.27	0.070	0.037	0.20

Taken from page 31 of MRID 50610402.

2.2.5.2 Calculation of Spray Applicator Risk Estimates

For risk assessment, default uncertainty factors are commonly applied to extrapolate toxicity data derived from animal models to humans (interspecies or UF_A) and to account for human variability (intraspecies or UF_H). The Agency has provided guidance on the process for identifying reliable data that are useful for quantifying interspecies and intraspecies differences to serve as the basis for empirically deriving data-derived extrapolation factors (DDEFs)[16]. When using DDEFs, interspecies and intraspecies extrapolation factors are divided into two components representing toxicokinetic (TK) variability and toxicodynamic (TD) variability.

[16] https://www.epa.gov/risk/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and

Therefore, four DDEFs can be calculated given sufficient information. Two extrapolation factors for interspecies extrapolation are: 1) extrapolation factor covering interspecies toxicokinetics (EF_{AK}) to account for TK variability and 2) extrapolation factor for interspecies toxicodynamics (EF_{AD}) to account for TD variability. Similarly, the two extrapolation factors for intraspecies extrapolation are: 1) extrapolation factor covering intraspecies toxicokinetics (EF_{HK}) to account for TK variability and 2) extrapolation factor for intraspecies toxicodynamics (EF_{HD}) to account for TD variability. The composite factor (CF) is calculated after the appropriate DDEF values for interspecies and intraspecies differences in TK and TD have been derived as shown in Equation 2.

$$CF = EF_{AK} \times EF_{AD} \times EF_{HK} \times EF_{HD} \qquad \text{Equation 2}$$

The CF calculation is analogous to calculating composite uncertainty factors when using the 10X defaults for UF_A and UF_H. If data are only available to develop a DDEF for one component of extrapolation or another, the remaining extrapolation is done by an appropriate default procedure.

For the proposed approach with chlorothalonil, there are data to inform both of the interspecies factors (EF_{AK} and EF_{AD}). Since the CFD model directly predicts the deposition of chlorothalonil in the human upper respiratory tract, animal-to-human extrapolation is not needed and the EF_{AK} can be reduced to 1X. The TD response was directly measured for humans since the POD is a BMDL based on measured endpoints in a human derived system; therefore, EF_{AD} can also be reduced to 1X. At this time, there is not sufficient information to inform the intraspecies factors. As a result, a default 10X UF_H should be retained.

As discussed in Section 2.1, additional uncertainty factors were previously applied for inhalation risk assessment of chlorothalonil exposures to account for lack of a NOAEC and extrapolation to longer durations. The proposed approach has negated the need for these additional uncertainty factors. The total uncertainty factor is 10 (1X EF_{AK}, 1X EF_{AD}, and 10X UF_H); therefore, the level of concern (LOC) for inhalation risk assessment is a margin of exposure (MOE) < 10.

Using the most health protective HEC value of 0.037 mg/L calculated for the larynx, risk estimates were calculated for representative spray applicator scenarios, including:

- aerial application to soybeans, cranberries, and pistachio
- airblast application to stone fruit
- groundboom application to golf courses and sod farms

Assumptions for area treated and inhalation unit exposures are consistent with EPA assumptions for evaluating these occupational scenarios for pesticide application. Inhalation exposures (expressed as air concentrations in mg/L) were calculated for comparison with the HEC assuming the maximum application rate on the product label and a breathing rate of 8.3 L/min. Margins of exposure (MOEs) are calculated by dividing the human equivalent concentration by

the inhalation exposure. All MOEs were greater than the LOC and ranged from 170 to 17,000 without additional respiratory protective equipment (Table 13 of MRID 50610402).

3.0 Conclusions

For evaluating effects via the inhalation route, registrants and manufacturers typically conduct *in vivo* subchronic inhalation toxicity studies in rats; however, traditional *in vivo* studies are resource intensive in terms of animal use, expense, and time. Additionally, NAMs that take into consideration critical anatomical and physiological differences between rats and humans may serve as a refinement for human health risk assessment. Consequently, NAMs are being investigated that may predict a human response using human relevant methods that incorporate an underlying mechanistic understanding of effects resulting from exposure to inhaled chemicals. These efforts to develop and implement NAMs are supported by EPA's OPP and OPPT and are consistent with the recommendations presented by the NRC's vision of toxicity testing in the 21st century and the recent strategic roadmap released by ICCVAM.

An alternative approach to refine inhalation risk assessment for spray applicators has been presented in a source to outcome framework for contact irritants using the pesticide chlorothalonil as an example. Total deposition for each region of the respiratory tract was calculated using CFD models results assuming the maximum percent of chlorothalonil in a diluted product and a mathematically derived PSD for the "adjusted" inhalable fraction (i.e., MMAD = 35 µm, GSD = 1.5). Using the total deposition and $BMDL_{sd}$ of 0.00730 mg/cm^2 identified from the *in vitro* assay (MucilAir™), the most protective HEC of 0.037 mg/L was calculated for the larynx that can be used to generate risk estimates for inhalation risk assessment. The overall approach being presented to the SAP is expected to be applied, when applicable, to other pesticides and industrial chemicals in the future.

Application of this approach for chlorothalonil would be the first time a POD is derived using an *in vitro* assay for an EPA pesticide risk assessment. The reliability and relevance of the MucilAir™ assay and its use in the proposed approach was evaluated using criteria outlined in the recently released strategic plan for TSCA[17]. The Agency has concluded that each criterion has been met for the use of MucilAir™ with contact irritants (Appendix B).

The POD was obtained from measured endpoints using an *in vitro* assay derived from human tissues and the CFD model directly predicts the deposition of chlorothalonil in the human respiratory tract. Therefore, the proposed approach allows for the refinement of inhalation risk assessment by incorporating direct measurement of a response in human tissues and human relevant exposure information. Consequently, animal-to-human extrapolation is not needed and challenges with interpretation of effects observed in rat *in vivo* studies are avoided.

[17] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

Following the SAP meeting, the Agency will consider the recommendations from the panel and incorporate the appropriate information and changes to the methodological approach presented. The Agency will continue to work with Syngenta to identify appropriate exposure assumptions (i.e., PSDs for mixer/loader and applicator scenarios). Additionally, since the underlying data for the CFD model utilizes data from a study conducted with human subjects, the Agency will review all relevant human data and studies in accordance with the Human Studies Rule, which could include presenting research to the Human Studies Review Board prior to utilizing the proposed approach prior to relying on this CFD model for chlorothalonil or any other chemical if the approach is received favorably by the panel.

4.0 References

Anjilvel, S and B Asgharian, 1995. A multiple-path model of particle deposition in the rat lung. Fund. Appl. Toxicol. 28: 41-50.

Asgharian, B, W Hofmann, R Bergmann2001. Particle deposition in a multiple-path model of the human lung. Aerosol. Sci. Technol. 34: 332-339.

Casati, S, K Aschberger, J Barroso, W Casey, I Delgado, TS Kim, N Kleinstreuer, H Kojima, JK Lee, A Lowit, HK Park, MJ Regimbald-Krnel, J Strickland, M Whelan, Y Yang, V Zuang. 2018. Standardisation of defined approaches for skin sensitization testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods. Arch. Toxicol. 92: 611-617.

Clippinger, AJ, D Allen, AM Jarabek, M Corvaro, M Gaca, S Gehen, JA Hotchkiss, G Patlewicz, J Melbourne, P Hinderliter, M Yoon, D Huh, A Lowit, B Buckley, M Bartels, K Berube, DM Wilson, I Indans, M Vinken. 2018. Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: an international workshop report. Tox In Vitro. 48: 53-70.

Corley, RA, S Kabilan, AP Kuprat, JP Carson, KR Minard, RE Jacob, C Timchalk, R Glenny, S Pipavath, T Cox, CD Wallis, RF Larson, MV Fanucchi, EM Postlethwait, DR Einstein. 2012. Comparative computational modeling of airflows and vapor dosimetry in the respiratory tracts of rat, monkey, and human. Toxicol. Sci. 128(2): 500-516.

Corley, R.A., S Kabilan, AP Kuprat, JP Carson, RE Jacob, KR Minard, JG Teeguarden, C Timchalk, S Pipavath, R Glenny, DR Einstein. 2015. Comparative risks of aldehyde constituents in cigarette smoke using transient computational fluid dynamics/physiologically based pharmacokinetic models of the rat and human respiratory tracts. Toxicol. Sci. 146, 65-88.

Corley et al. 2018. Computational modeling of aerosol dosimetry in the respiratory tracts of the rat and human. MRID 50610403.

Essaidi-Laziosi, M, F Brito, S Benaoudia, L Royston, V Cagno, M Fernandes-Rocha, I Piuz, E Zdobnov, S Huang, S Constant, M Boldi, L Kaiser, C Tapparel. 2017. Propagation of respiratory viruses in human airway epithelia reveals persistent virus-specific signatures. J Allergy Clin Immunol. 141(6): 2074-2084.

Flack and Ledson. 2018. Particle size characterization of agricultural sprays collected on personal air monitoring devices. MRID 50610404.

Flack et al. 2018. A source to outcome approach for inhalation risk assessment. MRID 50610402.

Harkema, JR, SA Carey, JG Wagner. 2006. The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol. Pathol. 34: 252-269.

Hoffmann, W, J Gradinaru, L Farcal, M Caul-Futy, S Huang, L Wiszniewski, N Parissis, S Morath, S Fortaner, T Cole, E Reginato, P Carrupt, S Constant, S Coecke. 2018. Establishment of a human 3D tissue-based assay for upper respiratory tract absorption. Appl. In Vitro Toxicol. 4(2).

Kabilan, S, SR Suffield, KP Recknagle, RE Jacob, DR Einstein, AP Kuprat, JP Carson, SM Colby, JH Saunders, SA Hines, JG Teeguarden, TM Straub, M Moe, SC Taft, RA Corley. 2016. Computational fluid dynamics modeling of Bacillus anthracis spore deposition in rabbit and human respiratory airways. J. Aero. Sci. 99: 64-77.

Kaufmann, W, R Bader, H Ernst, T Harada, J Hardisty, B Kittel, A Kolling, M Pino, R Renne, S Rittinghausen, A Schulte, T Whormann, M Rosenbruch. 2009. 1st International ESTP expert workshop: “larynx squamous metaplasia”. A reconsideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment.

Keeler, JA, A Patki, CR Woodard, DO Fank-Ito. 2015. A computational study of nasal spray deposition pattern in four ethnic groups. J. Aerosol Med. Pulm. Drug Deliv. 28:1-14.

Kelly et al., 2005. Inertial particle deposition In a monkey nasal mold compared with that in human replicas. Inhal. Toxicol. 17: 823-830.

Lei et al. 2018. Benchmark dose (BMD) analysis of Mucilair data to establish a toxicological POD for use in human risk assessment. MRID 50610401.

Mathis, C., Poussin, C., et al., 2013. Human bronchial epithelial cells exposed in vitro to cigarette smoke at the air-liquid interface resemble bronchial epithelium from human smokers. Am. J. Phys. Lung Cell. Mol. Phys. 304 (7), L489–503.

Neilson, L., Mankus, C., et al., 2015. Development of an in vitro cytotoxicity model for aerosol exposure using 3d reconstructed human airway tissue; application for assessment of e-cigarette aerosol. Toxicol. in Vitro 29 (7), 1952–1962.

Renne et al. 2009. Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol. Pathol. 37:5D-73S.

Shanley, KT, P Zamankhan, G Ahmadi, PK Hopke, YS Cheng. 2008. Numerical simulations investigating the regional and overall deposition efficiency of the human nasal cavity. Inhal. Toxicol. 20: 1093-1100.

Sivars, KB, U Sivars, E Hornberg, H Zhang, L. Branden, R Bonfante, S Huang, S Constant, I Robinson, CJ Betts, PM Aberg. 2018. A 3D human airway model enables prediction of respiratory toxicity of inhaled drugs in vitro. Toxicol. Sci. 162(1): 301-308.

U.S. EPA. 1994. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development. EPA/600/8-90/066F.

Vinall, 2017. *In vitro* measurement of the airway irritation potential of Bravo 720 SC formulation using MucilAir™ tissues from five difference donors. MRID 50317702.

Appendix A. Comparison of untransformed and log transformed BMD analysis results.

Donor	Endpoint	Untransformed Data				Log transformed dose			
		P value	AIC	BMD_{sd}	$BMDL_{sd}$	P value	AIC	BMD_{sd}	$BMDL_{sd}$
1	TEER	0.1512	770.1	63.4	54.0	0.104	770.6	58.23	53.32
	LDH	0.99	351.9	108.4	84.8	0.015	369.9	80.86	77.71
	Resazurin	<0.0001	489.9	92.5	61.2	<.0001	490.5	61.73	49.02
2	TEER	0.0101	719.9	75.0	46.8	0.007	721.1	73.10	46.85
	LDH	0.99	308.9	144.6	126.3	<.0001	360.5	110.40	106.50
	Resazurin	<0.0001	526.9	126.8	72.3	<.0001	528.3	60.98	35.42
3	TEER	0.04	702.1	117.8	94.7	0.018	704.3	111.00	102.80
	LDH	0.99	311.6	163.4	128.7	<.0001	417.0	101.90	97.13
	Resazurin	<0.0001	565.4	120.7	90.5	<.0001	565.5	83.42	56.36
4	TEER	0.004	742.9	133.1	124.6	0.000	751.2	121.10	116.20
	LDH	0.99	265.2	160.5	129.5	<.0001	417.0	114.10	110.40
	Resazurin	<0.0001	557.8	88.9	61.7	<.0001	557.5	24.29	12.14
5	TEER	0.29	810.4	179.1	143.2	0.034	817.3	121.70	113.30
	LDH	1.0	439.7	171.9	128.8	0.001	465.8	103.60	96.82
	Resazurin	<0.0001	571.2	135.9	112.8	<.0001	569.2	116.30	87.45

Appendix B. Evaluation of Reliability and Relevance

As discussed in OCSPP's strategic plan to promote the development and implementation of alternative test methods[18], scientific confidence in NAMs must be established to ensure that various *in vitro*, *in silico*, and *in chemico* methods provide equivalent or better scientific quality and relevance for assessing risks as compared to vertebrate animal testing. As defined by the OECD Guidance Document 34 (GD 34)[19], relevance encompasses the regulatory need, usefulness of the alternative method(s) and associated limitations of the test method. Therefore, relevance incorporates *fit for purpose* and *utilization* as a contextual evaluation and application of the NAM or integrated NAMs, and may include a weight of evidence (WOE) analysis, based on all available evidence, for their use in making qualitative or quantitative predictions. Reliability of *in silico* NAMs is derived from transparency and peer review.

Section 4(h)(2) of TSCA requires EPA to develop criteria "for considering scientific reliability and relevance" of NAMs. In the recently released strategic plan for TSCA, criteria were outlined that may be used to evaluate the reliability and relevance of NAMs based on a framework originally developed in Casati et al. (2018). EPA has used these same criteria to evaluate the proposed approach using a POD derived from *in vitro* MucilAir™ data and found that each criterion has been met for contact irritants.

- *The decision context should be clearly defined.*

  The use of an *in vitro* assay (MucilAir™) for chlorothalonil was based on the need for a POD to evaluate inhalation exposures from a contact irritant chemical for human health risk assessment.

- *Where possible, the NAMs should be mechanistically and/or biologically relevant to the hazard being assessed. The chemical domain of applicability of the NAMs should be defined to determine relevance to the TSCA chemical landscape.*

  MucilAir™ is a three-dimensional *in vitro* assay derived from human epithelial cells from the nasal, tracheal, or bronchial tissues of healthy donors allowing for a direct measurement of the response of human tissue to chlorothalonil exposures. A biological understanding of the irritation resulting from chlorothalonil exposure was presented, which included an adverse outcome pathway where epithelial cell damage occurs from initial respiratory exposure to chlorothalonil and causes cell death. Following repeated exposure, the repeated cell death results in a metaplastic response and transformation of respiratory epithelium into stratified squamous epithelium. For the proposed approach, cell damage was evaluated using measurements of TEER, resazurin metabolism, and lactate dehydrogenase LDH. As such, these measurements are being used to determine if cell damage and/or death has occurred

[18] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

[19] OECD *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment:* http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2005)14&doclanguage=en

from the initial inhalation exposure to chlorothalonil described in the adverse outcome pathway in Section 2.2.4.

- *Criteria for selecting reference or training chemicals should be defined and supporting information should be adequately referenced.*

Sodium dodecyl sulphate (SDS) was selected as a positive control for the *in vitro* measurements. Surfactants, such as SDS, are known to compromise the integrity of cell membranes, which facilitates cell lysis. The irritant effects of SDS and similar anionic surfactants has been demonstrated in numerous *in vitro* and *in vivo* studies. SDS is a known skin irritant and is commonly used as a positive control for irritation in the cosmetic industry[20]. Disruption of cell membrane integrity would be expected to be similar in the respiratory system. According to material safety data sheets (MSDS) for SDS and dilutions of SDS, breathing SDS may cause respiratory tract and mucous membrane irritation[21]. Furthermore, MucilAir™ has displayed dose-dependent responses, including TEER and LDH measurements, when treated with SDS[22].

- *The reliability of the NAM should be considered within the context of intended use and accepted best practices within the given field and the variability of the existing animal model.*

Syngenta concluded that the MucilAir™ was the optimal *in vitro* model for the proposed approach. This included consideration of ease of use and maintenance, ability to model cell-cell interactions in response to toxicants, representation of *in vivo* tissue organization, ability to simulate mechanical action of the respiratory tract, suitability for long-term tests, and applicability of results to *in vivo* inhalation toxicity. As described in Section 1.4.2, three-dimensional *in vitro* assays, such as MucilAir™, are the best available tool to evaluate human respiratory tract toxicity given the current state of the science. Evaluation of the *in vitro* endpoints (TEER, LDH, and resazurin) using MucilAir™ has been shown to predict *in vivo* respiratory toxicity (Sivars et al., 2018). In particular, TEER and resazurin measurements had 88% sensitivity and 100% specificity. Furthermore, unlike other assays that have been shown to have transferability issues, MucilAir™ does not appear to have this limitation since it can remain in a homeostatic state for a long period of time. According to the provider (Epithelix), MucilAir™ "remains fully differentiated and functional for over one year in culture"[23]; however, Syngenta did not provide any information or data for stability testing of the cell cultures. The good transferability and high reproducibility of MucilAir™ within and across laboratories has been documented in a recent study (Hoffmann et al. 2018).

- *The NAMs should be transparently described and information made available to the public (e.g., any datasets are publicly available and its known limitations are clearly*

[20] https://www.pharma-excipients.ch/2015/12/08/background-review-for-sodium-laurilsulfate-used-as-an-excipient-ema-report/
[21] Examples: https://www.nwmissouri.edu/naturalsciences/sds/s/Sodium%20lauryl%20sulfate.pdf; https://www.avantorinc.com/Documents/MSDS/USA/SAP/00027162.PDF; https://www.gbiosciences.com/image/pdfs/msds/DG093_msds.pdf
[22] https://www.criver.com/sites/default/files/resource-files/SP-SOT-18-Toxicity-of-SDS-in-the-MucilAir%E2%84%A2.pdf
[23] www.epithelix.com/products/mucilair

described). Information claimed as CBI restrictions may not allow public accessibility of all information in some cases.

Syngenta has submitted several documents to support the proposed approach. Relevant information has been included in these submissions, including particle size data, *in vitro* measurement results, and CFD model results. This included raw data, data summary tables, method descriptions/protocols, and certificates of analysis for MucilAir™ tissues and chemicals. Additionally, a step-wise description of the proposed approach and applicable calculations were included.

- *Uncertainty should be described to the fullest extent possible; both independently and compared to the existing animal model (if possible).*

The proposed approach is capable of reducing the uncertainty of the inhalation risk assessment for point of contact toxicity by directly measuring the response in human tissues using the *in vitro* assay and predicting deposition in the human upper respiratory system with CFD models. The MucilAir™ model is derived from cells of human donors and simulates the structure and function of the human upper respiratory system with pseudostratified, ciliated epithelium which secrete mucus. As such, the uncertainty that arises from extrapolation from an animal model, particularly given the anatomical and physical differences between animal and human respiratory tracts, can be avoided using the *in vitro* assay. However, some uncertainty may arise from delivering the test material to the tissues by pipetting the liquid rather than aerosolization, which is the expected inhalation exposure for humans.

For the proposed approach, a MucilAir™ model using nasal tissue was used; however, it is noted that the cellular composition of the nasal, tracheal, and bronchial epithelia is the same and consists of basal, ciliated, and goblet cells. Therefore, similar responses are expected across tissue types for the evaluation of cell damage from irritation. Furthermore, it should be noted that the nasal tissue model was the only model available from Epithelix at the time of the studies.

There is some uncertainty that arises due to duration differences between the MucilAir™ and expected handler exposures; however, the MucilAir™ exposures are considered protective since the MucilAir™ tissues were exposed for 24 hours to the chlorothalonil dilutions. This is 3X longer than the expected occupational exposures (i.e., 8 hr) and could have resulted in additional cell damage in the assay that may not occur during typical human exposure durations.

Intraspecies variability is still uncertain using the proposed approach. The MucilAir™ tissues only represented 5 individual healthy donors. Variability across these donors was relatively low; however, the low number of donors would not be considered representative of the human population. As such, the default intraspecies uncertainty factor remained at 10X.

There are limited experimental data available to evaluate the model performance of the CFD model. Comparisons can be made with alternative modeling approaches to supplement the

limited data available (e.g., other CFD model simulations, multiple path particle deposition (MPPD) model). It should be noted that there are several differences between the current CFD approach and experimental/alternative modeling approaches that make direct comparisons difficult; however, the data indicate the current CFD model simulations for a single male were within the range observed for other CFD simulations (Keeler et al., 2015), results using the MPPD model (Anjilvel and Asgharian, 1995; Asgharian et al., 2001), and data using nasal molds (Kelly et al., 2005; Shanley et al., 2008).

- *Access and use by third parties should be possible (i.e., the alternative approach must be readily accessible commercially and/or the relevant protocols should be available).*

The selected *in vitro* assay, MucilAir™, is commercially available and relevant protocols may be obtained from the provider, Epithelix[24].

- *The NAMs should undergo an independent scientific review in order to raise confidence in the approach.*

The use of MucilAir™ has been documented in numerous open literature studies that require independent scientific review prior to publication. The Agency is soliciting advice from the SAP on the derivation of the POD from the *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Chlorothalonil is being presented as a case study to solicit advice on the proposed overall approach.

[24] www.epithelix.com