Charge to the FIFRA SAP for December 4-7, 2018 Meeting

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 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 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)[1]. In these studies, several groups of experimental animals are exposed daily for a defined period to graduated concentrations of test substance as a gas, volatile substance, or aerosol/particulate. 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.

The anatomy and physiology of human and animal 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. Furthermore, traditional *in vivo* toxicity tests used to extrapolate to humans are resource intensive in terms of animal use, expense, and time. As a result, efforts to develop alternative methods and strategies for hazard identification and characterization have been supported by the Agency.

In 2007, the National Research Council (NRC) presented a long-range vision and strategy to advance 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 [2]. Since then, innovation and progress in the development of new approach methodologies (NAMs) has been rapidly occurring. 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. The development of novel NAMs for hazard identification and characterization is an integral part of addressing knowledge gaps and target the replacement of studies most frequently requested by the EPA.

Recently, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) released a strategic roadmap to provide a comprehensive U.S. national strategy to help with the accomplishment of the NRC's vision[3]. The ICCVAM is comprised of 16 federal

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1 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

2 https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a

3 https://ntp.niehs.nih.gov/iccvam/docs/roadmap/iccvam_strategicroadmap_january2018_document_508.pdf

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 NAMs that provide more human relevant information than typical *in vivo* animals tests and fit the needs of end-users. Consistent with the roadmap, EPA's Office of Pesticide Programs (OPP)[4,5] and Office of Pollution Prevention and Toxics (OPPT)[6] are 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. EPA's OPP and OPPT are currently working together to identify and develop NAMs that may be used to replace *in vivo* inhalation toxicity studies.

An example of a NAM for refining inhalation risk assessment has been submitted to the Agency for the pesticide chlorothalonil. Chlorothalonil is a contact irritant that has been found to be toxic via the inhalation route. Due to the irritant nature of chlorothalonil and animal welfare concerns, the registrant (Syngenta Crop Protection) indicated that a 90-day inhalation toxicity study was not feasible to fulfill the regulatory requirement of a subchronic inhalation study. Subsequently, Syngenta proposed an alternative approach using an *in vitro* assay (MucilAir™ using human nasal tissue) to characterize the hazard of chlorothalonil and derive a point of departure (POD) for use in human health risk assessment. In order to calculate human equivalent concentrations for the purposes of human health risk assessment, an *in vitro* POD has been proposed in conjunction with surface concentrations of deposited chlorothalonil particles from a computational fluid dynamic (CFD) model for the upper airway of a human. As a proof of concept, Syngenta also used the calculated human equivalent concentrations for pesticide operators/applicators to provide potential risk estimates they believe are supported by this proposed approach.

The Agency is soliciting advice from the FIFRA Scientific Advisory Panel (SAP) on the derivation of the POD from the *in vitro* assay and the integration of the *in vitro* POD for calculation of human equivalent concentrations for the inhalation risk assessment. Chlorothalonil will be presented as a case study to solicit advice on the proposed overall approach expected to be applied to other pesticides or industrial chemicals in the future.

DRAFT CHARGE QUESTIONS:

1. As part of its submission (MRID 50610402 and summarized in Section 2.2.4 of the Agency's issue paper), Syngenta has provided a biological understanding of the irritation resulting from chlorothalonil exposure. This includes an adverse outcome pathway where epithelial cell damage occurs from initial respiratory exposure to chlorothalonil

4 https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science

5 https://www.regulations.gov/docketBrowser?rpp=25&so=DESC&sb=commentDueDate&po=0&dct=SR&D=EPA-HQ-OPP-2016-0093

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

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. Please comment on the biological understanding of the irritation caused by exposure to contact irritants, such as chlorothalonil, via the inhalation route and how this understanding informs the applicability of the *in vitro* testing.

2. Changes in transepithelial electrical resistance (TEER), lactate dehydrogenase (LDH) release, and resazurin metabolism were measured in an *in vitro* test system (MucilAir™ using human nasal tissue) as described in MRID 50317702 and summarized in Section 2.2.4 of the Agency's issue paper. Please comment on the strengths and limitations of using the *in vitro* test system to evaluate a variety of membrane and cell damage endpoints as markers of cellular response. Please include in your comments a consideration of the study design and methods, appropriateness of the selected measures, robustness of the data, and sufficiency of reporting.
3. A CFD model for the upper airway of a human was used in the proposed approach to determine surface deposition of discrete particle sizes (monodisperse) in regions of the respiratory tract and adjusted for amount of active ingredient as described in MRID 50610403 and summarized in Section 2.2.3 of the Agency's issue paper. Since operators are exposed to distributions of particle sizes (polydisperse), percent contributions of each discrete particle size were calculated based on a particle size distribution derived for operators applying liquid formulations and used to determine cumulative deposition in each region of the respiratory tract as described in MRID 50610402 and summarized in Section 2.2.5 of the Agency's issue paper. Please comment on the strengths and limitations of using the CFD model results to calculate cumulative deposition, including the assumptions and calculations made to account for polydisperse particle sizes.
4. Human equivalent concentrations were calculated for operators applying liquid formulations in the proposed approach using the benchmark dose level from the *in vitro* measurements and the cumulative deposition as described in MRID 50610402 and summarized in Section 2.2.5 of the Agency's issue paper. Please comment on the calculation of the human equivalent concentrations.
5. The proposed approach to refine inhalation risk assessments for contact irritants has been presented with chlorothalonil as a proof of concept. Please comment on the strengths and limitations of using this proposed approach for other contact irritants, as well as its potential to be used for other chemicals that cause portal of entry effects in the respiratory tract.