FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

CONFERENCE CENTER LOBBY LEVEL

ONE POTOMAC YARD (SOUTH BUILDING)

2777 SOUTH CRYSTAL DRIVE

ARLINGTON, VIRGINIA 22202

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FIFRA SAP CHAIR & DFO	
ROBERT E. CHAPIN, PhD.	INDEPENDENT CONSULTANT
SHAUNTA HILL-HAMMOND, PhD	US ENVIRONMENTAL PROTECTION AGENCY
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SONYA K. SOBRIAN, PhD	HOWARD UNIVERSITY COLLEGE OF MEDICINE
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KRISTIE SULLIVAN, MPH	PHYSICIANS COMMITTEE FOR RESPONSIBLE MEDICINE
LISA M. SWEENEY, PhD	CHMM, UES, INC.
EPA STAFF	
RICHARD KEIGWIN	US ENVIRONMENTAL PROTECTION AGENCY

STANLEY BARONE, JR. MS, PhD	US ENVIRONMENTAL PROTECTION AGENCY
MONIQUE PERRON, ScD	US ENVIRONMENTAL PROTECTION AGENCY
ANNA LOWIT, PhD	US ENVIRONMENTAL PROTECTION AGENCY
OTHER PARTICIPANTS	
DR. DOUG WOLF	SYNGENTA CROP PROTECTION, LLC
DR. SHEILA FLACK	SYNGENTA CROP PROTECTION, LLC
DR. ALEX CHARLTON	SYNGENTA CROP PROTECTION, LLC
DR. PAUL HINDERLITER	SYNGENTA CROP PROTECTION, LLC
PUBLIC COMMENTERS	
SONG HUANG, PhD	EPITHELIX
AMY CLIPPINGER, PhD	PETA
CLIVE ROPER, PhD	CHARLES RIVER SCIENCES

TABLE OF CONTENT

OPENING/ADMINISTRATIVE PROCEDURES

DR. SHAUNTA HILL-HAMMOND: Good morning everyone. I would like to welcome you all and thank you for participating in today's public meeting. My name is Shaunta Hill, and I'm the Designated Federal Officer, or DFO, for the FIFRA SAP review of EPA's Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM).

At this time I would like to make some opening remarks with regards to this public meeting. As the DFO, I serve as a liaison between the agency and the panel. It is my responsibility to ensure that all provision of the Federal Advisory Committee Act, also known as FACA, are met regarding the creation, operation, and termination of Executive Branch Advisory Committees.

FIFRA SAP meetings are subject to all FACA requirements. These include open meetings, timely public notice of meetings and document availability, which is provided via the

Office of Pesticide Programs public docket, available at www.regulations.gov.

It is also the responsibility of the DFO, in consultation with the appropriate agency officials, to ensure that all appropriate ethics regulations are satisfied. In this capacity, panel members receive training on the provisions of the Federal Conflict of Interest laws. In addition, each participant has filed a standard governmental financial disclosure report, which has been reviewed by appropriate agency staff.

The FIFRA SAP is a federal advisory committee that provides independent scientific peer review and advice, to the agency, on pesticides and pesticide related issues, regarding impacts of proposed regulatory actions on human health and the environment. The FIFRA SAP only provides advice and recommendations to the EPA. Decision making and implementation authority remain with the agency.

The FIFRA SAP consists of several members. The expertise of these members is augmented through the Food Quality Protection Act

Science Review Board. Science review board members serve as ad-hoc temporary participants in FIFRA SAP activities, providing additional scientific expertise to assist in the reviews conducted by the panel.

Please note that the agency does seek and encourage consensus from the panel. Consensus recommendations will be most useful to the agency; therefore, the chair for this panel has been asked to lead the discussions to promote and facilitate the panel members reaching consensus to the greatest extent possible.

However, there may be instances where the panel will be divided and unable to reach consensus on an issue, this is okay and will be captured in the final report and meeting minutes. In these circumstances, where a consensus is not possible, the committee should be clear providing the majority and minority opinions.

Today's public meeting is held for the FIFRA SAP to discuss charge questions and hear public comments. We have a full agenda, and the meeting times on that agenda are approximate;

thus, we may not keep to the exact times noted due to public deliberations and public comments. Please note that we will strive to ensure adequate time for the agency presentations, public comments, and panel deliberations.

For our presenters, panel members and public commenters, I do ask that you identify yourselves and speak into the microphones provided since this meeting is being webcasted, transcribed, and recorded. Copies of all EPA presentation materials, as well as written public comments are available in the public docket at www.regulations.gov. Please note that the docket number and website are noted on the meeting agenda.

Members of the panel are encouraged to fully consider all written and oral comments submitted for this meeting. For any members of the public who have not preregistered to present comments, please notify me, or another member of the FIFRA SAP staff, if you are interested in making a comment. At this time the agenda is full, however, as we move through the

proceedings, if time allows, we might be able to accommodate additional requests.

At the conclusion of this meeting, the FIFRA SAP will prepare a report as a response to the questions posed by the agency, background materials, presentations, and public comments. This final report will also serve as the meeting minutes. We anticipate the final report, and the meeting minutes, will be completed in approximately 60 to 90 days after this meeting.

Again, I would like to thank everyone for their participation this week. I would like to note that the meeting will be held today, Tuesday, and then with continuation on Thursday and Friday. The meeting will be held in recess, on tomorrow, due to the government closure. At this time, I would like to turn the meeting over to our Chair, Dr. Chapin.

INTRODUCTION OF PANEL MEMBERS

DR. ROBERT CHAPIN: Thank you, Dr. Hill. So, next up we're going to go around the table and have the panelist introduce themselves

and their affiliation. I'm Bob Chapin, I'm an independent consultant. We'll go to Sonya.

DR. SONYA SOBRIAN: Good morning, I'm Sonya Sobrian and I'm from the Howard University College of Medicine, and I'm a developmental neurotoxicologist.

DR. GEORGE CORCORAN: Good morning. My name is George Corcoran. I'm from Wayne State University in Detroit. My areas of expertise are liver injury, biotransformation, and nutrition.

MR. ANDY DUPONT: Hi, I'm Andy Dupont (phonetic). I'm your back up DFO and I'm with the SAP staff.

DR. JAMES BLANDO: Hi, I'm Jim Blando. I'm an Associate Professor at Old Dominion University in Norfolk. Virginia.

DR. HOLGER BEHRSING: Hi, I'm Holger Behrsing. I'm a principal scientist and head of the Respiratory Toxicology Program at the Institute for In Vitro Sciences.

DR. JENNIFER CAVALLARI: Hi, my name is Jen Cavallari, and I'm an associate

professor at the University of Connecticut School of Medicine.

DR. MARIE FORTIN: Hi, I'm Marie Fortin. I'm an assistant director of toxicology at Jazz Pharmaceuticals, and also adjunct professor at Rutgers School of Pharmacy.

DR. STEPHEN GRANT: I'm Stephen Grant. Nova Southeastern University. I'm a genetic toxicologist with experience in in vitro and in vivo systems.

DR. JON HOTCHKISS: Hello, I'm Jon Hotchkiss. I'm an Inhalation Toxicologist, and I run the respiratory toxic group for Dow Chemical.

DR. ALLISON JENKINS: I'm Allison Jenkins, a regulatory toxicologist at the Texas Commission on Environmental Quality.

DR. ROBERT MITKUS: Hi, I'm Rob Mitkus, Regulatory Toxicologist at BASF Corporation.

DR. KATHRYN PAGE: Hi, I'm Kathryn Page. I am Product Safety Toxicologist with the Clorox Company, and I am responsible for all of our programs towards animal testing.

DR. EMILY REINKE: I'm Emily Reinke with the US Army Public Health Center. I am in charge of our in vitro screenings and alternative approaches.

DR. NIKAETA SADEKAR: Good morning, I'm Nikaeta Sadekar. I am inhalation toxicologist at Research Institute for Fragrance Materials. I lead the CET assessment program and the research efforts for in vitro models in respiratory testing.

DR. KRISTIE SULLIVAN: Hi, my name is Kristie Sullivan. I'm the Vice President for Research Policy at the Physicians Committee for Responsible Medicine.

DR. LISA SWEENEY: I'm Lisa Sweeney; I am a risk assessment toxicologist for UES, assigned to US Air Force School of Aerospace Medicine.

DR. RAYMOND YANG: I'm Ray Yang. I'm a retired professor from Colorado State University, consultant toxicologist.

DR. CLIFFORD WEISEL: Cliff Weisel, I'm a professor of Environmental and

Occupational Health Sciences Institute at Rutgers University. I work in exposure science.

DR. ROBERT CHAPIN: Dr. Barone, would you like to say a few words?

WELCOME AND OPENING REMARKS

DR. STANLEY BARONE: I would like to say good morning and welcome to the panel and the ad-hocs. Also, welcome to the public who will be participating, listening in by webinar, and the members of the public who will be participating here through the public comment period. I want to also acknowledge that this panel, this FACA committee, and the robust dialogue that takes place this week, is critically important to the EPA's function; and it's very important to our program, the input that we receive from our federal advisory committee for FIFRA.

I also want to actually introduce myself. I'm the acting office director for the Office of Science Coordination Policy, and the deputy ethics official that oversees this

particular FACA committee and the Science Advisory Committee on Chemicals for tox.

DR. RICHARD KEIGWIN: Good morning, my name is Rick Keigwin, I'm the Director of the Office of Pesticide Programs. And I just wanted to also extend my thanks to the panel for all the work that you've done beforehand, your flexibility as we take tomorrow off to observe the leadership and legacy and honor of former President H.W. Bush.

We know that there are going to be a lot of robust discussions over the next couple of days. This SAP meeting is particularly important to us. We've been working with considerable determination to move away from animal testing, or to reduce animals and the toxicology testing that we require as part of pesticide registration decisions; and we think that this is a very important step in that process.

Just within the past couple of years, for example, we have been systematic replacing the skin sensitization, eye irritation, studies with alternative testing. We've even

begun, over the course of the past year, to expand that effort into some of the ecotoxicology testing that we require specifically in regard to avian toxicity testing. I think this is another important step in that process.

We do look forward to your input and advice. I don't want to take any more of your time, because we know you've got lots to cover today. But again, thank you for your time and we look forward to your input.

DR. ROBERT CHAPIN: Thank you. So, what we get is an introduction to the general concept that we're going to be going through by Dr. Lowit; and then Dr. Perron will sort of give us a deeper dive into their proposal. And then we will take a break.

And as you've seen in the slides that were passed around, we got a long and thorough, and quite wonderful, presentation from Syngenta, which will take us through lunch. And then a little bit of something from Epithelix, the provider of the in vitro model, this afternoon. And we hope to be able to get into

discussion of charge questions -- I guess, into and finish with Charge Question 1 this afternoon.

So, that's the shape of our day. Dana Vogel is apparently out sick and, apparently, Dr. Lowit drew the short straw; so she's going to give us the initial introduction.

ANNA LOWIT: A little introduction. My name is Anna Lowit. I'm the science advisor here in the Office of Pesticide Programs and coordinate a lot of our work moving toward alternatives and reducing animal use. I have the honor as being one of the chairs of the Interagency Coordinating Committee for the Validation of Alternative Methods, otherwise known as ICCVAM.

And as you'll hear from Monique, a little tiny bit of detail, we have a lot of history in this program of moving away from the checkbox approach, using animals, and moving towards more hypothesis-based testing and in vitro. And so, this is a step in that direction, although we've been on this road now for a while.

I want to reiterate our thanks to each and every one of you. It's a lot of work to

just read the materials, and be prepared for the day, and spend the week with us. So we want you to know how much we truly appreciate your contribution. It is really vital to our moving the science forward and ensuring that the risk assessments that we put together are protective of human health. Your contribution is very meaningful, and we absolutely appreciate it.

Dana Vogel does send her regards, although I'm not too upset about spending my day next to her. I did not want her germs. So, hopefully we will see her on Thursday, feeling much better. But I will run through sort of her couple of introductory slides that will set the stage for Dr. Perron, and the Syngenta longer, detailed presentation.

So, as the introduction to the white paper notes, that although the presenters today will be from the pesticide program and Syngenta Crop Protection will focus on the case study for pesticide chemical, our hope here is that the work on Chlorothalonil can be expanded; not only beyond Chlorothalonil to other pesticide chemicals, but into the industrial chemical

space. And for that big reason, the work that we're doing here is a joint activity between the pesticide office and the toxic office; and they are here in the room if people have questions for them.

So, under FIFRA, and under federal statutes, we frequently, in the pesticide space, require substantial amount of testing of animals for regulatory testing. In fact, more animals are used in regulatory testing for pesticides than is done for any other sector.

And the main reason for that, is because on the pharma side they go to humans at some point, and in pesticides all testing is done to the animals. So, there is a great deal of opportunity to work towards reducing our animal use and working towards more meaningful human-based evaluations.

So, not long after the NAS report in 2007, the pesticide program responded to the NAS with a relatively short strategic direction that Monique will talk about a little bit. But since the late 2000s, we've been on this journey to do more science-based assessments and move

away from a checkbox. We're firmly committed to doing this, as you'll see as represented here. But we also understand that we can't do this alone. That nearly every project that we have in this space of reducing animal use, and moving towards in vitro and in silico approaches, is a collaborative effort.

So, you'll see today that Syngenta had come to us a couple of years ago, and we saw the promise of the approach and support the furthering of the science. We have many other projects that we're doing in the space; collaborating with other industry partners, states, Canada, animal rights groups, among others, including some academics.

We work very closely with ICCVAM. We have both, in the toxics office and pesticide office, members on nearly every ICCVAM workgroup; and in fact, that we co-chair a few of them.

And if you're not familiar with what ICCVAM is, it's a committee of committed individuals with literally no budget. That's been requested by Congress under the ICCVAM Authorization Act, to work towards the three R's

of animals, Reduce, Replace, Refine, at the federal government level.

I've had the honor the last few years of chairing that group. And it is, by far, the most fun thing that I do in my job. And so, with that I think I'll turn it over to Monique who will get into the deep dive of the science; and we're looking forward to your comments.

EPA INTRODUCTION PRESENTATION

DR. MONIQUE PERRON: In the meantime I'll introduce myself. My name is Monique Perron. I'm a Senior Toxicologist in the Health Effects Division here at the Office of Pesticide Programs. I'm going to be giving you some background information, how we conduct our inhalation risk assessments, currently using in vivo studies. Some information on new approach methodology, and the agency's efforts to develop and implement them. And then I'll lastly give a brief overview, of the approach being proposed for contact irritants and how this approach fits with the agency's practices and policies.

So, we'll start first with inhalation risk assessment using in vivo studies. I'm not sure if Anna already said this or not; but the regulatory statutes allow the agency to require or request data from pesticide registrants and chemical manufacturers. For OPP, this is the Federal Insecticides, Fungicides, and Rodenticide Act. And for OPPT, it's the Toxic Substances Control Act.

For pesticides, the federal regulations outline data requirements. These are dependent on the use pattern. So, whether it's a food or a nonfood use, the expected routes of exposure, the expected durations of exposure. For OPPT, there are various sections of TSCA that include chemical testing authorities. For example, Section 4 refers to EPA's authority to require health and environmental effects testing to be conducted in most cases relevant to a determination of an unreasonable risk of injury.

Toxicological studies can provide the agency with information on the wide range of adverse health outcomes, different routes of exposure. We get studies through the oral route,

dermal, and inhalation. A duration ranging from acute, all the way to chronic durations. We also get information about species differences and life-stage information. And the breadth and issues, which trigger data requirements for each of our programs differ based on their statutory requirements.

EPA's test guidelines are specified, what the agency recommended methods are. And these are harmonized with OECD guidelines, which uses comparison across studies in chemicals. With respect to inhalation studies, our test guidelines requirements are listed under the guidelines that we have here on this slide.

So, in these studies, several groups of experimental animals are exposed to concentrations of a test substance, either as a gas, a vapor or an aerosol. The rat is the preferred species for these studies; and the animals are observed for clinical signs and then sacrificed and necropsied at the end of the study.

Histopathological examinations are performed, which includes the respiratory tract to look for portal of entry effects. A satellite group may also be included, to evaluate the reversibility, persistence, or a delayed occurrence of effects, after the treatment has ended.

Ultimately, based on these results, the lowest observed adverse effects concentration, or LOAEC, is determine, which is the lowest concentration where adverse effects are observed; as well as a corresponding no observed adverse effect concentration, or NOAEC, which is the highest concentration where no adverse effects are observed.

Inhaled doses depend on several factors. These include the volume of air inhaled per minute, which is dependent on breathing frequency and title volume. The breathing frequency can be affected by the nature of the inhaled material, as well as the activity level; so your breathing frequency will increase as you're doing more strenuous activities versus the more sedentary activities.

The duration of the exposure, the respiratory tract architecture, as well the nature of the inhaled material can also have an impact; since volatile chemicals, the deposition, the rate of uptake is determined by their reactivity and solubility. Whereas, the particles, their size, density, and shape can impact their aerodynamic behavior.

So when the agency conducts and inhalation risk assessment, we use all available toxicological information to characterize the potential health effects and identify a point of departure for risk assessment. The point of departure is typically a dose or concentration where no adverse effects have been observed and is used as a quantitative starting point for risk assessment.

Points of departure are selected for each expected route and duration of exposure. So, inhalation will have its own selected point of departure, for each duration, that's expected based on a use pattern.

Inhalation studies are preferable over oral studies, when evaluating inhalation

exposure, since they provide route specific information. However, the studies may not always be available or cannot be used due to other hazard concerns that we've observed in the database.

In 1994, the EPA published its inhalation reference concentration or RFC methodology, which is used to estimate benchmark values for non-cancer toxicity of inhaled chemicals. In this methodology, a dose metric adjustment factor, or DAF, is applied to account for species-specific relationships. And this is largely influenced by the physical chemical properties of the compound and is also dependent on the type of toxicity observed.

Ultimately, the application of the DAF, using the RFC methodology, accounts for pharmacokinetic differences between test species and humans, and allows for the calculation of a human equivalent concentration, or an HEC that may be used for inhalation risk assessment.

And so, just quickly, the duration adjustments are applied to an animal point of departure, often a NOAEC or a LOAEC if the NOAEC

was not established, to get an adjusted inhalation point of departure. We then applied a DAF to get our HEC, and typically that is in the units of milligrams per liter, or milligrams per meter cubed.

To calculate the risk estimates for inhalation risk assessment, using an in vivo inhalation toxicity study, the HEC is then divided by the inhalation exposure to calculate what we call a margin of exposure. However, most exposure databases, and models, are formatted to output exposures with units of milligrams per kilogram per day. So the HEC is often converted to a human-equivalent dose for these calculations.

In order to do that, a conversion factor and expected daily duration are applied. The conversion factor is derived from a default breathing rate for a 70-kilogram person. And then the expected exposure duration will depend on the exposure scenario. So for example, eight hours is assumed for occupational exposure to reflect a typical work day.

Risk estimates are compared to a level of concern that is determined by the uncertainty factors being applied. Typically, a 10x interspecies factor is applied for animal to human extrapolation; and a 10x intraspecies factor is applied to account for variability among humans. And each of these uncertainty factors have toxicokinetic and toxicodynamic components. Since the RFC methodology accounts for toxicokinetic differences, the intraspecies factor may be reduced to 3x when HECs and HEDs are calculated from an in vivo inhalation toxicity study for risk assessment.

After decades of animal testing, we have learned a great deal about the differences between rodent and human respiratory tracts. The anatomy and physiology, of the respiratory tracts, differ in several ways that can impact changes in airflow and deposition of inhaled substances. This includes the airway size and surface area. The complexity of the turbinate system; so in humans we have three nasal turbinate systems that are relatively simple in shape, while the rats have a more

convoluted system with complex folding and branching patterns.

The overall branching pattern, of the respiratory system in humans, is much more symmetrical and dichotomous than the rodents. The cell composition and distribution, and the anatomy of the larynx; wherein in rats the cartridge associated with the ventral pouch is U-shaped. And the larynx and trachea form a relatively straight line from the nasal turbinate. So as a result, the larynx is a common site of injury in inhalation toxicity studies, conducted with rats. In contrast, that U-shaped pouch is absent in humans, and the larynx is more sharply angled to the oral nasal cavity.

So these critical differences can ultimately affect the ability of in vivo testing, in rats, to correctly predict effects in humans. As a result, new approach methodologies, or NAMs, that take into consideration these differences may serve as a refinement for human health risk assessment.

I'm just going to give some information about new approach methodologies, and the agency's efforts to develop and implement them. The NRC provided a vision of toxicity testing in the 21st century -- about a decade ago -- that promotes studying a hazard at a cellular or tissue level rather than utilizing whole animal testing.

Recently, the Interagency Coordinating Committee on Validation of Alternative Methods, or ICCVAM, released a strategic roadmap to provide a comprehensive US national strategy to accomplish the NRC's vision. ICCVAM is comprised of 16 federal regulatory and research agencies, including EPA, that require and/or utilize toxicological and safety testing information. And this roadmap is relying on interagency collaboration, and public/private partnerships, to develop new approach methodologies that provide relevant information, but also fit the needs of the end-users. Consistent with the roadmap, OPP and OPPT have been committed to supporting the development and

implementation of alternative testing methods, and strategies to meet our regulatory needs.

So, alternative test methods and strategies can be referred to as new approach methodologies or you might often hear me say NAMs. NAM is a term intended as a broadly-descriptive reference to any non-animal technology, methodology approach or combination thereof. And the EPA has been working with multiple national, and international organizations, to identify NAMs for hazard characterization and identification. And these efforts are consistent with the NRC's vision, ICCVAM strategic roadmap, as well as the National Academy of Sciences report on how to integrate and use data from emerging techniques to improve risk-related evaluations.

So, there are several drivers for moving away from the whole animal testing. An obvious driver is ethics to remove animal tests. And this has definitely been a driver in European efforts. There are also clear economic advantages. Most alternative testing is cheaper

and faster, and in some cases numerous chemicals may be tested simultaneously.

Then there's also the case that moving away from whole animal testing is a public health issue. After decades of using whole animal tests, we now have a much better understanding of human physiology. And should use this knowledge, along with the other major advances, in science and technology, to move away from animal models in order to better protect public health.

There have been amazing advancements over the past decade, but little has changed in terms of regulatory toxicology. And we're now at the point where requisite animal testing, that remains in place, will limit our ability to take advantage of the knowledge that we've gained. And ultimately the human relevance of new approaches will be limited or masked. Where clear and understandable differences exist, we have an obligation to pursue the approach that is most human relevant and therefore better predicts public health.

And lastly, legislation in other countries is making it increasingly likely that if we don't decide on a path forward, Congress may do that for us. There are also several obstacles in the way of implementing new approaches. One is the institutionalized use of animal data as the gold standard. It's not enough to just say that you have a test that can predict human toxicity. In almost all cases, you have to show that your data with the new test matches the animal results. But how can you ever do better than the animal data if it's always considered the gold standard?

In some cases, the animal test is preventing us from the adoption of better testing, because the new tests predict human toxicity better. But when they're compared to the animal tests, they don't look like they are performing very well.

Institutional resistance: this is ultimately that people don't like to change, for various reasons, whether it's a financial driver, emotional driver. But some of this resistance is justified; we should question things as we're

moving forward. But we do need to understand the intentional blockage of progress, or non-consideration of alternatives. Just drawing a line in the sand and saying, you know, we're not going to accept these alternative testing.

We need to understand what's causing that intentional blockage and figure out a way to work through that. And then also, harmonization, the weakest link in the chain will determine how strong it is. Companies conduct studies for multiple markets. If one market doesn't accept a new test, then there's no motivation for the company to move to alternative testing. If they have to do the animal tests anyways, and it's accepted by everybody, then the lowest common denominator is going to drive that testing.

So, at OPP and OPPT, we've been working diligently to address these challenges, to support the development and implementation of testing and approaches, that move away from whole animal testing. And these efforts are supported, or encouraged, as part of our regulations.

So for OPPT, TSCA was recently amended and updated. This was the first update in 40 years. The agency is required to review and make determinations regarding the unreasonable risks of injury to health or the environment for new and existing chemicals, with clear and enforceable deadlines for existing chemical reviews. There's no consideration of cost or other non-risk factors, and the agency must consider risks to potentially exposed or susceptible populations.

Section four, each one of TSCA requires the agency to reduce and replace the use of vertebrate animals in chemical testing, through prescribed measures when appropriate. Prior to requesting vertebrate tests, this subsection requires the agency to consider existing information, which includes toxicity information, computational toxicology, bioinformatics, and high throughput screening methods.

Amended TSCA also included a new subsection, that requires EPA to develop a strategic plan to promote development and

implementation of alternative test methods and strategies, to reduce, refine, or replace vertebrate animal testing.

OPPT collaborated with other EPA programs, including OPP; and also sought and received input from other federal agencies, and stakeholders, as part of development of this plan. And the final plan was published in June 2018.

Here at OPP, we have a strategic plan for developing and evaluating new technologies to supplement or replace more traditional toxicity testing and risk assessment. This includes a broader suite of computer-aided methods to better predict potential hazards and exposures, while focusing on testing that informs likely risks of concern.

We are also working to implement improved approaches to minimize the number of animals used. It also includes an improved understanding, of toxicity pathways, to allow for the development of non-animal tests that better predicts how exposure relate to adverse effects.

In 2013, OPP came out with a document on guiding principles for data requirements. This document was developed to help OPP staff focus on information that was most relevant to pesticide assessments and reach the overall goal of ensuring their sufficient information to reliably support registration decisions. But also, at the same time, avoiding the generation of data that doesn't influence the scientific certainty of our decisions. So as such, we can avoid unnecessary use of time and resources, data generation costs, and animal testing.

The guiding principles promotes and optimizes full use of existing knowledge, while also providing consistency across the OPP divisions when determining data needs. Ultimately, decisions regarding data needs are on a case by case basis and consider all of the available information that includes physical chemical properties, metabolism data, toxicological profiles, exposure pattern, and any available human information. We also will consider information on structural analogs.

So, the regulations give OPP substantial discretion to make registration decisions, based on what the agency deems are the most relevant and important data for each action. Under Section 158.30, 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.

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 to the agency's evaluation.

So as a result, Section 158.45 permits the agency to waive data requirements. But they must ensure that sufficient data are available to make the determinations required under our statutes. The 40 CFR also prevents 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.

A large focus of this SAP is the proposed use of in vitro data. EPA and the risk assessment community have a long history of using in vitro studies for genotoxic evaluation. Here at OPP, we've also used in vitro data to inform over 50 cancer mode of actions. So this isn't exactly the first time in vitro data is being used for risk assessment. Also, OPPT has a long history of using NAMs in their new chemical program, such as structure activity relationships and read across; those are often utilized in their program.

In addition to that, there's a large effort in OPP to reduce animal use through the Hazard and Science Policy Council. This committee is comprised of senior toxicologist and exposure scientist across our various divisions.

The guiding principles for data requirements are utilized in a weight of evidence approach. So we consider the integration and intersection of hazard and exposure when we make these decisions.

In 2013, OPP published a guidance document on the weight of evidence determination of data needs. This document covers the subchronic inhalations, subchronic dermal neurotoxicity, and immunotoxicity studies required under Part 158. Although not specifically covered by the guidance, we still have flexibility to waive other guideline and non-guideline studies.

We've been fairly successful in this arena. And from December 2011, till August 2018, the HASPOC considered over 1000 data waiver request, and 957 of them were granted. These waivers covered a range of studies, including several sub-chronic studies, as well as larger studies such as the reproduction toxicity study and chronic carcinogenicity studies.

Each year OPP publishes an annual report on HASPOC savings. For instance, in 2017, HASPOC granted 70 study waivers, and this saved approximately 41,000 animals and $10.4 million dollars in generation costs. And similarly, in 2018, 62 waivers were granted, saving about 16,500 animals and approximately $8.9 million

dollars. And here you can find a link to find that information on an annual basis.

Additional efforts in OPP to reduce animal use include the Chemistry and Acute Toxicology Science Advisory Council, which we like to call CATSAC. This council reviews and provides guidance on bridging and waving acute toxicity studies. Also, recently, we had a retrospective analysis conducted by our Environmental Fate and Effects Division, that concluded that a robust, avian, acute risk assessment can be conducted without subacute data. And as a result, OPP is developing guidance on situations where these data are actually necessary; and a manuscript has also been submitted that summarizes the retrospective results.

We also have efforts moving towards in vitro and computational approaches. For example, multiple non-animal testing strategies demonstrate comparable or superior performance, the mouse local lymph node assay for evaluating skin sensitization. OPP and OPPT are now accepting these alternative approaches under

conditions that are described in an interim science policy document from earlier this year.

Similarly, we have a policy in place to accept non-animal test for eye irritation assays. The slides that you would have received, these are accepted for antimicrobial cleaning products, and we are working to extend that to other classes of pesticides.

We're also working with NICEATM, which is NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods, to analyze dermal absorption triple-pack data. Triple-pack data consists of a rat in vivo, rat in vitro, and human in vitro penetration studies, that we use to refine dermal absorption factors for our risk assessments. The current analysis that we're doing, we're compiling data to determine if we could move to just using the human in vitro data alone.

With respect to inhalation, OPP and OPPT have been collaborating to identify and develop NAMs to replace in vivo inhalation toxicity studies, particularly given what we know

about the differences in the rat and the human respiratory tracts.

Furthermore, the traditional in vivo studies are resource intensive in terms of animal use, expense, and time. We also have unique challenges with respiratory contact irritants that can elicit damage at very low concentrations. So often a no observed adverse effect concentration is established for these chemicals, and animal welfare concerns can arise.

So, there are several in vitro tools available to evaluate inhalation toxicity; and these were well-summarized in a publication earlier this year by Clippinger et al. The lung-on-a-chip model replicates the microarchitecture of the tracheobronchial airways, and the alveoli, in order provide predictions of physiological responses in the human lung tissue.

And although this model is promising and may advance rapidly, it doesn't 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.

Another available tool is the ex vivo precision cut lung slices. These reflect the natural microanatomy of the respiratory tract as well as its functional response to an inhaled chemical. The slices are collected from human donor lungs and can be maintained for weeks; however, the thickness of the slices can vary. And without having a standardized method, that variation can have an impact on the comparative functionality. So, at this time, we don't really see the ex vivo lung slices as being quite ready for regulatory applications either.

In terms of in vitro cell cultures, those can range in complexity from simple submerged culture systems to three-dimensional models. The simple subcultures do not allow for direct exposure at the air liquid interface. On the other hand, the three-dimensional models, cultured from airway epithelial cells at the air liquid interface, can mimic particular regions of the respiratory tract.

We're involved in several ongoing research projects with these in vitro models.

Our colleagues at ORD just finished a pilot study using two dimensional models and are now working on a proof of concept study, using commercially available three-dimensional models but will also include a 2D model in there for comparison.

Additionally, there's an NIEHS project validating a human airway model for identifying acute toxicity. We also have quite a few consultations with registrants and non-profit groups on additional studies, that will help further the science and the potential utilization of these in vitro methods.

So ultimately, the selection of an appropriate NAM is fit for purpose. There needs to be some understanding of in vitro and in vivo dosimetry for these systems; and it's important to be able to intergrade human relevant exposure information into that evaluation.

EPA recognizes the science will continue to evolve as methods continue to advance and additional tools become available. However, in order to address the current science questions, the best tool currently available, based on the state of the science, needs to be

employed. At this time, EPA considers the 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.

To wrap up this section, as we discussed, the in vivo studies are resource intensive in terms of animal use and time and money. And the agency is committed to developing and implementing alternatives that are scientifically valid and human relevant. The regulatory statutes provide us with flexibility or require us to consider alternatives. And when we have the knowledge and the technology available, we need to move to more human relevant models. And NAMs that take into consideration, the anatomical and physiological differences, may serve as a refinement for inhalation risk assessment.

The selection of an appropriate NAM is fit for purpose. It's important to be able to integrate the human-relevant exposure information; and currently, EPA considers in

vitro models that allow direct exposure at the air liquid interface to be the best available tools at this time.

The last section that I'm going to go over, I'll provide a brief overview of the proposed approach to refine inhalation risk assessment for respiratory contact irritants. I will not be providing extensive details on the approach, since the registrants themselves that developed this approach will be presenting these to you. And they will be able to answer any detailed questions at that time.

A proposal for refine inhalation risk assessment using an in vitro model was submitted by Syngenta for the pesticide Chlorothalonil. The agency is required a 90-day inhalation study for Chlorothalonil, given the high toxicity demonstrated in acute and short-term inhalation studies. However, Syngenta indicated that the study was not feasible due to the irritant nature of the chemical and animal welfare concerns.

The agency recognized the value of the proposal, not only for Chlorothalonil, but

also other respiratory irritants and encouraged further development. We also reached out to NICEATM, to collaborate with us on the review; and also OPPT was involved in this review since the approach may also be applicable to industrial chemicals.

In the most recent risk assessment for Chlorothalonil, a repeat in dose inhalation study was not available. However, there were concerns that using an oral point of departure would underestimate the risk, via the inhalation route, due to high lethality and clinical science consistent with respiratory tract irritation observed in acute inhalation toxicity studies.

As a result, a point of departure was derived from an acute inhalation toxicity study, and certainty factors applied included a 10x intraspecies factor. The interspecies factor was reduced to 3x with application of the RfC methodology; and an additional 10x was applied for extrapolation from the acute study to longer durations.

The assessment found inhalation risk estimates of concern for several scenarios,

which included residential handler and post application from paint uses, bystander volatilization relativization, and occupational handler scenarios. And also, as part of this action we requested the 90-day inhalation study.

So, in response to that the registrants submitted four inhalation studies; A range-finding acute study, an acute toxicity/tolerability study, acute pilot toxicokinetic study, and a two-week inhalation toxicity study. A NOAEC was not established from these studies. Clinical science related to respirations, such as labored breathing, gasping, and wheezing, were noted following acute and repeated dosing. Epithelial degeneration and/or necrosis in the nasal cavity, larynx, lung and trachea were the primary histopathological findings across the studies. And in the two-week study, squamous cell metaplasia in the larynx was observed for all concentrations tested. And squamous cell hyperplasia, in the nasal cavity, was also seen at the highest dose tested.

Although these studies provided further information on Chlorothalonil toxicity,

via the inhalation route, the agency did not consider these studies sufficient to fulfill the 90-day study requirements. Subsequently, Syngenta proposed an alternative approach, utilizing a source-to-outcome framework for intergrading exposure and hazard characterization.

This proposed approached derives a point of departure for inhalation risk assessment from an in vitro assay, which is used in conjunction with dosimetry model results to calculate human equivalent concentrations for inhalation risk assessment.

There are four components of the approach: source, exposure, dosimetry, and outcome. This case study is presented for applicators of Chlorothalonil liquid formulations or solids that are diluted in water and applied as a liquid. So, at this time, that is the only scenario that we're looking at. The same approach could potentially be applied for mixers and loaders and other exposure scenarios.

So, for the source component at this time, Syngenta has summarized all applicable

formulations they have currently registered with EPA, and the corresponding percentage of Chlorothalonil expected in the spray applications based on the labels. So, the maximum percent of Chlorothalonil based on those labels is 4.9 percent.

For this case study, and the purposes of this SAP meeting, Syngenta has mathematically derived a human-relevant particle size distribution for inhalable particles for the spray applicators. Distributions of inhalable thoracic and respirable size fractions are internationally recognized. But to establish a human-relevant particle sized distribution for this spray applicator, a maximum cut off of 100 microns was incorporated in order to derive adjustable inhalable fraction.

So, this resulted in a particle size distribution with a median geometric diameter of 35 micrometers, and a geometric standard deviation of 1.5. Since Chlorothalonil formulations use water as the primary carrier, application of the density of water, so one, would yield a mass median aerodynamic diameter

equivalent to this. So, you'd have 35 micrometers as your MMAD, and the geometric standard deviation would remain the same.

The approach then utilizes computational fluid dynamic modeling to predict deposition in regions of the upper respiratory tract. CFD is used by many scientific fields to analyze fluid flows, and CFD models for the upper respiratory tract have been developed for several species including rats, monkeys, and humans. And it uses a computational mesh, based on species specific anatomical data, to determine air flow patterns and predict localized deposition of discrete particle sizes within each region of the respiratory tract.

Syngenta conducted simulations for monodispersed spherical particles that ranged from 1 to 30 micrometers. All the simulations assumed one milligram per liter aerosol concentration and resting nasal breathing. Since these results are representative of a generic water droplet, they were adjusted by the maximum percent of Chlorothalonil in a diluted product; so, about 4.9 percent that I mentioned earlier.

Regional and site-specific deposition profiles were generated for each individual particle size.

As part of their submission, Syngenta has provided a biological understanding of the respiratory irritation caused by Chlorothalonil exposure. This includes an adverse outcome pathway beginning with cell death from initial contact, and transformation of epithelial into stratified squamous epithelium following repeated exposures.

This biological understanding guided Syngenta's consideration of the available in vitro models for assessing damage to respiratory epithelial cells; and ultimately, they selected a three-dimensional in vitro model that allows direct exposure at the air/liquid interface, and they measured for several endpoints that are indicative of cell damage or death.

They identified MucilAir as an optimal model at the time when they considered all of the available in vitro tools. MucilAir is a three-dimensional in vitro test system derived from human epithelial cells. For the proposed

approached, the cells were collected from nasal tissue of healthy donors. This was the only available model at the time. However, the cellular composition of nasal, tracheal, and bronchial epithelial are similar, so we believe that similar responses for cell damage from irritation are expected across the tissue types.

Dilutions of Chlorothalonil were applied to MucilAir at dosage ranging from 2 to 200 milligrams per liter for 24 hours. Cell damage and viability was evaluated using three endpoints, transepithelial electrical resistance, resazurin metabolism and lactate dehydrogenase. Benchmark dose modeling was then used to determine a BMD for one standard deviation, and a BMDL which is the lower bound of the 95 percent confidence interval.

BMDs incorporate and convey more information than the traditional NOAEL/LOAEL approach, since NOAELs/LOAELs are highly dependent on dose spacing and sample size. BMDs can also account for variability and uncertainty in results that are due to study design characteristics. The agency follows a BMD

technical guidance when the BMD approach is being used.

The benchmark response selected is determined on a case by case basis and takes into consideration statistical and biological information. In the absence of information to determine a level of response to consider adverse, one standard deviation from the mean is used. Syngenta's use of the one standard deviation BMD for this case study is consistent with our guidance.

For their BMD analyses, Syngenta log transformed the data and fit it with a modified Hill model. The agency also conducted its own BMD analysis on the untransformed data and found the Hill model to be the best fit. Both analyses found the models to fit the data well visually. We got similar or lower AIC (phonetic) values with the untransformed data, but ultimately the BMD and BMDL values obtained by Syngenta, were lower, and therefore would be considered protective. Across the three endpoints investigated, similar BMD results were

obtained, and the geometric mean was calculated across.

The human equivalent concentrations for inhalation risk assessment were then calculated for each region of the respiratory tract, by integrating the dosimetry and in vitro test results. This included calculations to generate polydisperse particle distributions, since the CFD model was generated for discrete particle sizes. And it also allows for incorporation of relevant exposure durations. The lowest HEC was calculated for the larynx, which would be considered the most health protective for risk assessment purposes.

Uncertainty factor determinations; our agency policy decisions are outside the purview of this panel. However, we wanted to note that with the incorporation of human-relevant data, there may also be an opportunity to reduce uncertainty factors for risk assessment by using this refined approached. The agency has guidance on the process for identifying reliable data that are useful for quantifying inter and intraspecies differences to serve as the basis

for empirically-deriving extrapolation factors. And as I discussed earlier, typically 10x interspecies and intraspecies factors are applied, and each of these consist of a toxicokinetic and toxicodynamic component.

Direct predictions of deposition with the CFD model may inform the interspecies toxicokinetic component. And deriving a point of departure for measurements in a human-derived tissue system may inform the interspecies toxicodynamic component.

For the Chlorothalonil case studies, Syngenta calculated risk estimates for representative spray applicator scenarios. There was a typo on the original slides; this should say, aerial application to soybeans and cranberries, airblast application to pistachio and stone fruit, and groundbloom application to golf courses and sod farms. And using the most health protective HEC value calculated for the larynx, MOEs ranged from 170 to 17,000, and that's without any additional respiratory-protective equipment.

So, I've given a quick overview of the proposed approach with Chlorothalonil as a case study, and how it fits into the agency's policies and practices. However, it should be noted that the case study was used to demonstrate this approach and does not represent final conclusions for the human health risk assessment for Chlorothalonil.

As I mentioned earlier, Syngenta will be providing a more detailed presentation of the proposed approach, and you'll have the opportunity to ask their team of experts any questions you have on the details of this approach. The HECs calculated, using this approach, integrate dosimetry and outcome results allowing for the incorporation of human relevant particle sizes, derivation of a point of departure from endpoints measured in a human tissue in vitro system, and the potential to reduce uncertainty associated with interspecies extrapolation. The agency has a long history of using in vitro data; however, this would be the first time a point of departure, for risk

assessment, would be derived using in vitro data for a pesticide.

This proposed approached is in line with the agency's commitment to develop and implement new approach methodologies and move away from requisite toxicity testing with laboratory animals. It represents a natural step forward, utilizing the knowledge that we've gained over years of whole animal toxicity testing, and the advancement in science and technology to develop an approach that's more human relevant and also meets the regulatory needs of our program.

With respect to TSCA, the reliability and relevance of this approach were also evaluated, using the criteria outlined in OPPTs alternative testing strategic plan. And they were all found to be met. And lastly, we expect that this approach will be applied to other contact irritants, and the potential to be applied to other pesticides and industrial chemicals. So, we are asking the panel, as part of charge question number five, to comment on the

strengths and limitations of using this approach beyond the Chlorothalonil case study.

And then just one more thing to note before we answer any questions; I also just wanted to note some of the additional work that is ongoing and related to this project. We are continuing to work with Syngenta, and representatives from Crop Life America, to identify appropriate exposure assumptions related to the particle sized distributions that should be used for different exposure scenarios; so, mixer/loader versus an applicator.

Additionally, any of the relevant human data and studies associated with the CFD model will be reviewed in accordance with the human studies rule. This will include presentation of relevant research to our human studies review board, prior to using the proposed approach for Chlorothalonil or any other chemical, if the panel receives this approach favorably.

So, with that I would be glad -- or any of our team that is here would be glad to answer any of your questions.

DR. ROBERT CHAPIN: Thank you very much. Questions for Dr. Perron?

DR. RAYMOND YANG: Ray Yang, Colorado State University. Dr. Perron, thanks very much for an excellent presentation. And I do have a recommendation at the end of my discussion. But what I want to say, is I would like to compliment EPA and specifically OPP, OPPT; all the colleagues involved in bringing this about, and also Syngenta and their scientists for advancing this initiative. This is very important. If I'm not mistaken, this is the first time that a new approach is brought to the risk assessment and regulatory domain.

And what I am about to say, the reasons for my compliment to you, the information that I'm going to give -- and I will apologize because it's to you. Most of you probably are very familiar with what I'm about to say. But I want to enter into the record to demonstrate how important this particular initiative is. Okay.

NTP was established in 1963. Prior to that is NCIs bioassay program. And in more than 60 years, we have, so far, less than

600 chronic toxicity carcinogenicity studies, technical report, okay. EPA IRIS, a couple of months ago I checked, probably is in the order of 500 some, probably less than 600 chemicals in IRIS database.

Now relatively simpler versions, PPRTV. And for those of you who are not familiar with PPRTV, this is the EPA Superfund program, it represents Provisional Peer-Review Toxicity Value. I was told 10 years ago, back in Cincinnati as a visiting scientist, that the original PPRTV was only a few pages. And at the time I was at Cincinnati, 10 years ago, it's a book. And the situation, I believe, is not getting any better, meaning, it will take an awful lot of time to even produce the PPRTV.

Now, using EPA's own database, your scientist, Rusty Thomas, and his colleagues, at National Center for Computational Toxicology, they set up this database called dashboard, chemistry dashboard, CompTox/Chemistry Dashboard. How many chemicals we are talking about, 760,000 chemicals. Therefore, using the traditional

method of toxicity testing and risk assessment, we will never catch up.

Now this is only a single chemical; we're not talking about mixture yet. Now just for your and my gain, a mixture combination follows the formula of 2 to the N power minus one. If you have a 25-component chemical mixture, you are talking about more than 33 million combinations just for one dose, okay.

And therefore, it is critically important that we use high throughput, use in vitro, use computational methodology, use all of these resources and so on and so forth, to develop new methodology. And that is why I compliment OPP and OPPT, because this represents forward thinking. And I salute you.

Now after this, I want to give you a recommendation. Maybe you are already doing this, or Syngenta already is doing this. You are advancing a new approach. Whenever you're dealing with a new approach, the most critical thing is validation, validation, validation. So, how do you validate? Now, my suggestion to you -- - and you might have better methodology -- is

first you assume IRIS risk assessment is the gold standard. I say this, because I know there are scientists who even question the accuracies and so on of IRIS risk assessment.

You assume that, and you use a testing set of chemicals which have been well studied, such as a respiratory irritant, such as formaldehyde, and you have probably a lot in the inventory. And use this entire suite of methodology testing data, derive your BMDL values and also derive your risk assessment and compare with what's in the IRIS.

The more you have, the more to serve as your defense for the new approach. This is my recommendation to you. And thank you very much, and thanks to each of you for this initiative.

DR. ROBERT CHAPIN: Thank you, Dr. Yang. I was getting ready to ask if you had slides that you needed to present. Let's see, other questions or comments? Yeah?

DR. JAMES BLANDO: Thank you.

DR. ROBERT CHAPIN: Remember to give your name.

DR. JAMES BLANDO: Yes. Jim Blando. My question is, you guys presented a lot of great information, in particular, showing that your belief that the in vitro tests do a better job of predicting human toxicity. In particular, you talked about the mechanistic studies and the 50 or so cancer mode of action studies in the mouse local lymph node assay.

I was just wondering if any of these in vitro studies have ever been compared to scenarios where people have looked at actual human populations under actual exposures, like epidemiologic studies or clinical studies, as further verification that these in vitro can accurately predict the risk that may be faced by human population?

DR. ANNA LOWIT: I think we have to be careful with this idea that we can use epidemiology studies to help validate in vitro studies. Most epidemiology studies have focused on cancer endpoints and reproductive endpoints and the effects on a developing brain. And in the case of where the in vitro assays are ready for regulatory use, is in the contact effects,

the eye irritation, the effects directly on the skin; in this case, the surface, where Chlorothalonil interacts with the surface.

At this point, I'm not aware that people in the regulatory community are ready to use an in vitro study in lieu of a cancer bioassay. We're very comfortable using in vitro data to look at a key event, and a pathway leading to cancer, but that's not the same thing as using it to establish for cancer.

So, to answer your question about to the degree to which the in vitro studies have been looked at with human data. In the skin sensitization arena, there are a couple of publications, notably, by Nicole Kleinstreuer, from NIEHS, who has looked at the worlds existing skin sensitization data and compared that to the in vitro assays; and how they're put together, and what's called defined approaches, in how they predict versus the degree to which the mouse LLNA study predicts. And if you read Nicole's publications, you'll see that actually the in vitro studies combined together, and defined approaches, actually do a better job of

predicting the human experience than does the mouse; which makes a lot of sense because it's human tissue. That sort of is the best case that's out there that's been done systematically.

I will make sure it's on the record that in the case of EPA's use of those data for the skin sensitization policy that we publish in April, we have not relied on those human data, largely because of issues around the human studies review board. So, our skin sensitization policy focuses on the relationship between the in vitro studies to the LLNA; because we require the LLNA in the guinea pig as part of our regulations. And that human studies review provides some barriers that we just didn't find useful.

So, in the case of the skin sensitization, we would have had to take all 150 individual studies to the HSRB. And I think there's at least one member of this panel who is on that; and would realize that 150 studies to the HSRB would back that road up for several years. So, the value added of doing that, we

determined really wasn't useful. But that is a well-documented publication that you can look at.

DR. NIKAETA SADEKER: Hi, Nikaeta Sadeker.

DR. ROBERT CHAPIN: Nikaeta, just move that mic. Thank you very much.

DR. NIKAETA SADEKER: All right. Nikaeta Sadeker. And I just want to ask for a clarification. This study is looking for irritation via Chlorothalonil exposure or local effects in the respiratory?

DR. MONIQUE PERRON: Can you repeat that?

DR. NIKAETA SADEKER: So, the focus for this case study, is it irritation via Chlorothalonil exposure in respiratory, or local effects of respiratory tract?

DR. MONIQUE PERRON: So, this is Monique Perron. I think those are sort of intertwined because of the biological understanding of Chlorothalonil, that you have this initial contact that causes the cell death; and then that leads to the subsequent effects that you're going to see.

So, in the two-week studies we saw epithelial degeneration and all those other things; but what you're seeing here is that there really isn't a time component. So, there's this initial contact and the damage will happen if you've had enough deposition of the chemical. I'm not sure if that answered your question, hopefully yes. Thank you.

DR. STEPHEN GRANT: I have some comments. Stephen Grant. First of all, with regard to use of epidemiology. In the cancer area, despite the fact that it takes a long time to do animal studies, the designation of chemicals as known carcinogens is much more held up by the lack of supporting human epidemiological evidence than animal data.

So, one of the things that I say in that field is, do we have to have a Hiroshima for every chemical to go on that list; in other words, huge exposure with lots of different doses? And yes, we need to get pass the idea that only things that have been actually proven in human, to show a toxic effect, are the ones that we're going to regulate.

But going back to the question that was just asked, the adverse effect pathway leads to cancer, and we're trying to discuss irritation. Irritation isn't in the pathway; so we're asking, why don't we have an adverse effect pathway to irritation?

DR. MONIQUE PERRON: So, this current approach is being utilized for non-cancer inhalation effects for our risk assessment. We do a separate cancer assessment if we have that data. So, this is for the non-cancer portion of the risk assessment.

DR. STEPHEN GRANT: The adverse effect pathway, in the package, led to cancer. How is that relevant to the question that we're asking here about respiratory toxicology irritation?

DR. ANNA LOWIT: Anna Lowit. There might be some semantic challenges. Why don't we -- if it's okay with the chair and the panelist -- hold that question. Syngenta is going to come up and give some very detailed, lengthy presentations, and can maybe provide some clarity on their proposed adverse outcome

pathway. And if that doesn't answer the question, we can circle back.

DR. ROBERT CHAPIN: That sounds great. Yeah.

DR. KATHRYN PAGE: Kathryn Page. Thank you for that great presentation. I have a clarification question. You mentioned an ORD research project that's currently comparing 3D models. Is that comparing known irritants? And if so, how far along is that study, and is there any data that could be helpful to this panel?

DR. MONIQUE PERRON: So, they just recently got a list of chemicals from OPP and OPPT and are trying to narrow down to -- some of it will include like Chlorothalonil, where there's quite a bit known about it being a respiratory irritant. But it also will include chemicals that cause systemic toxicity as well. So, at this point, I don't believe that it would be helpful for the current deliberations. This is Monique Perron.

DR. CLIFFORD WEISEL: Cliff Weisel. Again, thank you for your presentation. One of the last things you said on charge five

was you wanted us to give you some thoughts on how to take the case study and maybe think about other issues. That's actually very broad as you're quite aware. If you can give us some guidance in particular.

One of the things I'm thinking about is you talk about three different models, one was charged with this case, the others weren't. Do you want us to look at the pluses and minuses of that, or do you want us to be narrow? If you could give us some guidance so we can give you something concrete, rather than a theoretical goal that's going to take you a decade.

DR. MONIQUE PERRON: Thank you. This is Monique Perron. From a starting point, obviously, if there are any hurdles that you can see for using this approach for other chemicals, that are considered contact irritants, that obviously is not as broad. That's definitely more direct; where we can definitely say if you can reach a level of showing us that you have this type of biological understanding, that

underlies the respiratory irritation that we're seeing, then this approach could apply.

I think the harder one maybe is beyond that, to the pesticide chemicals that cause portal of entry effects that may not be consistent with contact respiratory irritation. I think giving us some guidance on what the best approach for us to attack that would be. It may not have to be as detailed, but if there are specific questions that we need to answer, before we can move into that realm, I think we need to know those. That would be really helpful for us as we move forward.

We might not be able to apply this immediately to a chemical, but maybe if we know what those hurdles are, and what are the scientific questions that need to be answered in order to apply the approach, that will be really helpful.

DR. ANNA LOWIT: Anna Lowit. Just to add a little bit to that. Question 5 is not a request for a ten-year research program. Just to sort of ground the question a little bit.

There's great interest in regulatory community, both in other organizations -- regulatory organizations -- but also in other companies, to be honest, of using something similar to this approach for their chemicals. Either for ethical reasons, or a lot of companies that want to move away from the animal. Or for similar reasons that Syngenta moved to this approach. That they realized that their chemical as a point of contact causes point of contact injury. And because the rat to human anatomy differences; we want to make sure we've moving to a more human-relevant approach.

So, as you think about that question, the questions that we're asking ourselves, in the next one to two years, is when does it make sense for this to apply to other chemistries? And how to expand the Chlorothalonil to the other pesticides in the industrial chemical space.

We acutely realize that this one case study doesn't answer all those questions. But we're asking for your feedback on what are some other questions we should be asking?

For example, Dr. Perron, very briefly, mentioned the collaboration we have with ORD to compare that the two three-dimensional with the two-dimensional assay, using some chemicals of interest to cross our two programs. So, that's one space where we do a systematic look across a couple of different assay systems, to look at their differences and whether or not they provide equivalent information or not.

And the grant that were working in under ICCVAM, it's an SBIR grant, with the steering group of people across the federal government. And MAT Tech is actually going to test a lot, up to 50, 70 plus. It's an expansive list of chemicals that have been recommended across the federal government, that represent a broad swath of chemistries. And both irritants and non-irritants.

So, we'll have one system where we look at a lot of chemicals of interest across the government. And then another area, we're going to systematically compare some systems.

So, we know that those are necessary steps in this. We really like your

feedback on are there others? You know, are we missing something? How do we make that decision tree of when to move to the alternative, versus asking for the traditional animal?

DR. ROBERT CHAPIN: This is Bob Chapin. Before we sort of metastasize off into broad, enthusiastic discussions about all the things that we could do, I'll just sort of remind the panel that we want to kind of keep the questions focused on clarification for the current presentation, and anything that we need to know to go forward. And these questions are standing between us and a bio break. With that, Steve, did you have another one?

DR. STEPHEN GRANT: Yeah. Steve Grant. You brought up, once again, the idea that there's an anatomical difference between the airways of the mouse and human, which of course is a theoretical concern. But has there actually been cases in which that has affected the applicability of the results to human?

DR. MONIQUE PERRON: This is Monique Perron. You're hitting with the hard question there. So, at this time, Chlorothalonil

is a case where you can see that utilizing the animals, if you keep going, you're just going to keep killing animals and moving the dose lower and lower and lower. And rather than trying to figure out, you know, where that tiny bit can be for the rat, we really think that we should be moving to the more human relevant. So, there really shouldn't be that question of -- if we're moving to an approach that uses human tissues and human relevant exposure conditions, then we shouldn't be trying to move backwards to the whole animal testing.

DR. STEPHEN GRANT: Further comments. Stephen Grant. Again, in genotoxicolgy there's human geno and mouse geno. But I can show you -- I used to work in the mouse geno project and I said, let's just do a really detailed mouse geno, because it all extrapolates to human anyway. So, let's not overstate the idea that since we're using human cells that magically there's going to be a much more direct approach.

In fact, one of the questions that I'm going to bring up here -- and you might want

to decide, could be another charge question -- is that we clearly have metrics to extrapolate from animal to human. I think one of the things we have to consider, is that we need to consider various metrics to extrapolate from an in vitro system to an in vivo system; because the in vitro system cannot be as complicated as the in vivo.

DR. ROBERT CHAPIN: Dr. Sullivan.

DR. KRISTIE SULLIVAN: Kristie Sullivan. I just wanted to briefly follow up from the clarification of question five. Just to ask, we talked very specifically about pesticides, but are you considering that question to also include industrial chemicals, given the involvement with OPPT?

DR. MONIQUE PERRON: This is Monique Perron. In case you can't hear the nodding, we said yes. Thanks.

DR. ROBERT MITKUS: Rob Mitkus. Just a follow up -- and sorry to keep from the bio break. Just following up Stephen's comments there. I think there's sometimes, you know, a risk of overthinking things. And when I read your issue agency paper -- which I thought was

great -- you kept saying over and over refine, refine, refine.

So, it's my understanding -- and I want to make sure my understanding is correct. A risk assessment has already been done using the in vivo rat data; and this particular approach that's being proposed as really meant to refine the current risk assessment? Not reinvent risk assessment using a human model, but to refine the current risk assessment for this particular product? Is my understanding correct?

DR. ANNA LOWIT: I think we have to be careful to dissect the different pieces of what we're doing. So Chlorothalonil is in the registration review schedule as per the requirement to make a risk safety determination by 2022. So, there is a risk assessment on the books for Chlorothalonil; and that assessment will need to be updated with the most current information, prior to the Reg. review deadline.

In the case of Chlorothalonil, there are some risk challenges associated with worker exposure that Syngenta clearly has an interest of refining, to move to a more human

role and approach, that will provide a more accurate view of the margins of exposure.

So, that's the Chlorothalonil situation. But if you think about the case study that's being proposed for using an in vitro assay linked to human dosimetry, the idea is that there may be cases where that would be the approach to use from scratch.

So, another chemical, for example, Chlorothalonil has some inhalation testing that Syngenta had conducted and that inhalation testing led them to this refinement. The long-term goal here would be to work through the decision logic of when you would just avoid the rat completely and go straight to this approach.

So yes, in the case of Chlorothalonil the idea is to refine the margins of exposure for purposes of risk evaluation. But in the big picture, we're really looking to move towards that reinvention of toxicity testing towards a more human relevant approach where it applies. Does that help?

DR. ROBERT MITKUS: It's helpful. I think it's important to understand kind of the

weight, in the panels approach, how much effort were going to put into evaluating Chlorothalonil as opposed to where maybe in conjunction with putting a lot of weight in evaluating the new approaches in itself. So, just trying to get the handle on that.

DR. ROBERT CHAPIN: I think we're kind of doing both, but with a longer term view of the extrapolation of this method; if we find it satisfactory for Chlorothalonil to use for it, to say, okay, this looks like it worked for Chlorothalonil, these are some things to think about as you go ahead and use it for the next batch of irritants on your list. So, the source is carrying two riders. Steve?

DR. STEPHEN GRANT: Steve Grant. One of the problems with this is that we are trying to do two things at the same time; establish something for a particular agent, but then using a new methodology which hasn't been established. As Ray said, when you say we're refining this over and over, a couple of in vivo studies were done but they didn't reach LOAEC or NOAEC. And the refinement was to go into an in

vitro system and find the LOAEC and NOAEC and assume that it's the same one or that it can be used to establish that.

Again, from what Ray was saying, we have chemicals where we have LOAEC and NOAEC's from the in vivo; and it would have been very interesting and very supported to have done this system on those agents, established the relevance, and then apply it to a new chemical.

DR. JON HOTCHKISS: Jon Hotchkiss. I just had one question for clarification. You mentioned using the HSRB. So, under conditions -- like, why is that necessary for this work?

DR. MONIQUE PERRON: So, our human studies rule, depending on the data being utilized and relied upon, we must take the data to them. In this case, it's not necessarily just Chlorothalonil, it's the use of the CFD model. There is human data that was utilized to develop that model, and also if you wanted to look at data to possibly validate it as well. So, any of those where we have flagged them or they would be needed to go to the HSRB rule, we take in those.

DR. JON HOTCHKISS: Okay, thanks. Since I'm on a roll here. So, this test material seems to be a special case in that you already have acute hazard data available. And so, you're able to leapfrog and estimate a repeat exposure, or at least identify a point of departure. There are going to be many materials, especially things that come in through the PMN process, where that data is not available.

And so, there are options using chem informatics in order to assess potential hazard effects. And then they can be sort of double checked initially in vitro. But I just worry that kind of hopping over hazard, to get the rest, which we've been arguing for a long time, but now I'm going to sweep that to the other side of my mouth.

DR. ANNA LOWIT: Anna Lowit. Thanks, Dr. Hotchkiss. We're keenly aware -- and if my toxic friends want to get up and answer, they can kick me under the table. The agency's keenly aware, in the PMN's space, that often if not frequently the chemicals come in with not a lot of hazard information. We are also aware

that American Chemistry Council is actually beginning efforts to think about a framework in the PMN's space; where you would actually begin with a QSAR or bioinformatics kind of approach, moving to high throughput. And then something like this will be the last step, if not an animal study would be the last step.

So, there are people thinking about what you just inferred for that PMN's space. Certainly, there are a lot of questions there to answer that we don't know right now.

DR. JON HOTCHKISS: Okay. This is picky.

DR. ROBERT CHAPIN: Identify yourself.

DR. JON HOTCHKISS: This is Jon Hotchkiss. This test material is a direct acting toxicant. You keep on calling it an irritant. It happens to be irritating at some level. But the only endpoint in the in vitro system, that might roughly align with irritation, is tear. So, there are other endpoints that could really address the irritation space. And perhaps even a different model, looking at sensory irritation,

if you want to use that as a point of departure for risk assessment.

I think linking irritation with toxicity, that's kind of a jump. It's a direct-acting toxicant, and it just at some level happens to be irritating before it kills its cells.

DR. ROBERT CHAPIN: Is there a question there? Or are you just giving them a whack.

DR. JON HOTCHKISS: Yeah. So many times, we sort of smear the distinction between irritants and toxicants. And so, irritation really has a different sense in respiratory toxicology. So, you can have irritation where you get a minor inflammatory response, or you get some other modification like up regulation of mucin gene expression, without cell death. And so, I just don't want to blur that distinction too much.

DR. ROBERT CHAPIN: I think there will be time to beat this horse later. Dr. Cavallari.

DR. JENNIFER CAVALLARI: Hi, Jennifer Cavallari. Thank you for your presentation. My question is about the uncertainty factors. So, in the explanation of the uncertainty factors, you explained that both the toxicokinetic and toxicodynamic interspecies factors are both reduced to one due to the way the human-relevant data has been used. My question is, have you considered other uncertainty factors to account for some of the unknown, the uncertainties in the model assumptions that underlie this new approach, that go beyond the intraspecies factor that's already applied?

DR. MONIQUE PERRON: At this time we are not considering any additional uncertainty factors. What we were presenting was just the potential for the reduction of the interspecies, given that you're accounting for both the toxicokinetic and toxicodynamic portions there. So, at this time we are not considering any additional uncertainty factors. Those are an agency policy decision that we'll have to

determine as we move forward with these approaches.

DR. ANNA LOWIT: Anna Lowit. Just one thing to add. There's a little bit of a gray line between where the science ends and the policy start. So certainly, if there's a charge question where it makes sense for you to provide some science feedback on how we might assess that, we would welcome that. Understanding that at the end of the day it's the agency's determination of what the values will be. But certainly, the science that underlines those is within the purview of this panel. I don't think we're asking a question about the factors, but that doesn't prevent you from making a comment on it, if it's something that you have views on.

DR. JAMES BLANDO: Jim Blando. Just one quick clarifying question. The presentation that you gave, you mentioned the MOE. And I just want to clarify, if the MOE is computed as being greater than 10 or less than 10, in which case is that considered level of concern?

DR. MONIQUE PERRON: So, risks of concern are those below the level of concern. So, in this case, say you were able to reduce it down to 10, any MOEs less than 10 would be a risk of concern.

DR. ROBERT CHAPIN: Okay. Anything else for this round? Nope. All right. I've got 22 of; let's reconvene back here at 10 minutes of, gives us 12 minutes. All right, so we'll take a bio break until 10 minutes before 11:00.

[BREAK]

DR. ROBERT CHAPIN: All right. Let's do this. Okay. Next up we've got the full presentation from the Syngenta group. So, I'll just let you guys introduce yourselves. Thanks for being here.

SYNGENTA - WOLF

DR. DOUG WOLF: I'm Doug Wolf with Syngenta Corp Protection. We'll go through the

four Syngenta people and thank you for the opportunity here.

DR. SHEILA FLACK: I'm Sheila Flack from Syngenta, Operator Consumer Safety, focusing on human health risk assessment.

DR. PAUL HINDERLITER: Paul Hinderliter from Syngenta. I do modeling.

DR. ALEX CHARLTON: Alex Charlton from Syngenta. I'm a toxicologist.

DR. DOUG WOLF: So, the way we've structured the presentation today is I'll give the first part, which is really how did we get here. Looking at some of our approaches and frameworks that we used within the company to evaluate issues, come up with potential solutions to problems, and move ahead.

So, I'll kind of lay out the how we got here. And then I'll hand it off to Dr. Flack, who will cover some of the next topic of exposure in the morning. And then I think we break for lunch; and then after lunch we'll cover the modeling and the in vitro assay, and then close it out with the risk assessment.

There's a natural break between each section. And so, there will be an opportunity, before we hand off, to ask clarifying questions of what was presented. And at least for me I don't get upset if someone interrupts and says, what does that mean? But as I say, we'll stop for questions along the way.

So, to give you a bigger picture of how we approach problems to solve, and issues within our risk assessment evaluation strategy, we have adopted and adapted the health environmental science institute risk assessment, a 21st century approach to evaluating do you have enough data, in order to support whatever decision construct you're trying to make a decision about.

And so, the first step in this risk 21 framework approach is problem formulation. So, what is the problem trying to solve? And then, in the context of chemical risk assessment, the first step is to understand the exposure. Because without exposure, there's really no hazard and no risk. And so, understanding the exposure is really critical.

And the exposure is driven by the use and the physical chemical properties.

And then, once we have understood the exposure scenarios and concerns there, moving into the hazard characterization. Sometimes using an approach such as a threshold of toxicological concern is sufficient, and you don't need to go beyond that because the exposure is not very high. Sometimes an in vitro assay is sufficient, as was described by Dr. Lowit and Perron, about some of the modes of action, identifying a key event. And when that occurs, that might be sufficient.

And sometimes you have to go into whole animal studies; and sometimes you have to do very extensive studies in understanding the entire biological construct from exposure all the way to a long-term adverse outcome, such as cancer.

And then using this particular framework tool, the graph on the right allows us to visually represent what we're trying to understand. And gives us a first inclination, as a communication tool within our project teams,

within the company, and then expressing it to other interested parties, of what information we've used.

Now this risk 21 framework, you see an X-axis of numbers around the estimates of exposure. And you'll see the typical low numbers on the left and high numbers on the right. But on the Y-axis is the estimates of toxicity. And the high number is down at the bottom, and the low numbers are up at the top. So, a high number there means low toxicity; obviously, thousands of milligrams per kilogram as low toxic, and the low numbers is high toxicity. So that's why it's graded from green, in the lower left, up to red in the upper right.

So, the lower left, very low toxicity, low exposure; upper right, high toxicity, high exposure. So, the opportunity there is to evaluate do you have sufficient information to then go ahead and move forward to doing a risk evaluation; or maybe a business decision, depending upon what conclusions you're trying to make?

So that's our communication and evaluation construct. And then we always start with problem formulation; what is the problem you're trying to solve? Frequently, as scientists you want to get to the experiment really quickly, so we have to slow ourselves down to do that.

Now with the particular active ingredients, such as Chlorothalonil, for those of you who are not familiar with the legislation that the EPA works under, crop protection products active ingredients need to be reregistered on a regular basis; I believe it's every 15 years. So Chlorothalonil was first registered in the early 1960's. It's been used successfully for many decades.

Overtime, more and more crops have been added to more and more uses; so, it's even used in paint and wood protectants to prevent mildew and other fungus from growing. It's used on food crops as well as in the lawn and garden sector, such as protecting golf courses from fungal infections.

We're also very aware, over that same time period, that the product has been registered for use. Our lives have changed dramatically. The top left here is representation of what's the encroachment of the built community's neighborhoods around what used to be strictly agricultural properties.

And so, concerns continually change and adapt; and we have to be able to understand that the people that live next to those fields that are being sprayed have justifiable and valid concerns of what's drifting over to their yard. Should we be worried about our children in the background? So, this is where some of the requests for studies come from, new studies. Even for a product that's been registered for a long time.

And of course, there's different communities, such as the Pesticide Action Network, that point out different exposure scenarios that we need to continue to monitor. And gets us to, well what are the methods that the agency uses to address some of these concerns and ask the registrant community to respond to

them? And that's through a data call in. And in this particular case the request was to perform a 90-day sub-chronic inhalation study.

So, the other thing we have done within Syngenta, is develop a framework for staying focused on the problem formulation. It's a very critical step in everything we do. And we created this framework, which we've just recently published in Regulatory Toxicology and Pharmacology, to keep us focused on responding to the issue, or the problem that was presented to us. So, the problem we were presented is not we need to do an inhalation risk assessment, but you guys need to do a 90-day sub-chronic inhalation study.

And so, the first step in problem formulation is really to understand what is the problem statement we are trying to address? What are the concerns? What's the key question? And then frame that. And so, we took the problem that we received, we had internal discussions; we came to the agency and had further discussions to go through and find out, well what is it we really want to address here?

And then once you've done that step, the next step is to look at that problem statement and explore the problem. What do we know? What do we not know? What additional questions need to be answered? What hypothesis can we come up with? And then finally, once we exhausted that as best as we can, with a desperate group of people with different skills and understandings and expertise, we then finally map the approach.

So, the structure we're going to have today, is I'm going to first relay our problem statement and how we got to that. And then do some background exploring the problem; and then, hand it off and the rest of the team will map our approach for you of how we tried to solve this problem.

One of the key features, which will be our touchstone throughout the presentation, is at the end of exploring the problem, we typically try to develop a visual representation, our conceptual model of what we're trying to accomplish. And that's really key here.

And so, in describing the problem, coming up with a problem statement, we were given the charge to do the 90-day inhalation study. In our discussions with the EPA, we didn't really feel how that particular study would provide additional information that would improve a safety of risk assessment. True, these are very valid studies for hazard identification. But to really understand a risk assessment, you need to understand the exposure context -- the exposure and the internal exposure, and how that relates to any potential hazard that could occur, even in the rodent part in the human situation.

And there are no additional systemic risks. So, because the nature of the Chlorothalonil in this particular product, it really is a contact irritant. And as pointed out, irritation in my -- I'm an old cow doctor. So, for me, irritation is really a clinical manifestation of something that is harming the surface. So that's a clinical response, you're irritated.

But what was pointed out, is the concern we have in this particular situation, the

specific thing that happens, is as the cell is exposed to the chemical, it dies. So, it's really the point of contact cytotoxicity is what we're looking at. And as I pointed out, this product has been on the market for many years, used in lots of different scenarios and has a long history of safe use.

Now the other thing that is a driver, is the way that rodent studies are designed, and as we think about the exposure component. And so, the OECD guidelines -- and this is a category one irritant -- we try and maintain the same amount of aerosol droplets in the air, so that's the gravimetric. So, the amount of exposure, the volume, or the amounts of droplets that the rat is inhaling, stays constant. The target dose on the left-hand side increased, is what we would increase. And so, you see in the analytical chemistry column, there is an increasing dose.

But the other thing that's really important in the rodent -- and this will come back to use later -- is that the size of these aerosol droplets is very small, 2 to 3 microns.

And so, it may be relevant for respiratory toxicity in the rat, but not necessarily in the human situation.

And so, taking what we are presented, working internally with a large group of people, and externally we came up with our problem statement. And the problem is then we want to develop a new approach method, that would be suitable to inform the inhalation toxicity, in lieu of a sub-chronic whole animal inhalation study.

So as Dr. Perron mentioned, the USEPA has the flexibility to waive a specific guideline study, in lieu of other information that sufficiently informs the decision context that the agency has to fulfill. And if we can come forward with an alternative source of knowledge and information that is equivalent to that guideline study, we can waive that specific study, and submit an alternative study.

And so, that's what we were focusing on. Is there a way to come up with a sufficient amount of information that would inform the human health risk assessment for

inhalation exposure, in lieu of doing the whole animal study? And if that's the case, would it be adequate, then, to waive that 90-day study, and provide the information that the agency needed to do a health protective risk assessment?

So, the next part of problem formulation is to explore the problem. And that's really about what do we know? And it turns out, in most cases, we know a lot. We don't think that we do sometimes, we think each case is unique. But in fact, if you think about inhalation and spraying materials on crops, as -- Chlorothalonil is not the only fungicide in the market. This is not the only in vitro assay system. So, we do know a lot of information.

So, the first place we start -- and as a veterinary pathologist, it's where I always like to start -- is on the pathology. And this really helped us clarify, again, in working back and forth with the agency, on what was the problem we're trying to understand? And how, then, do we move forward to understand that specific problem?

So, if you look at the acute tox -- and I just pointed out for those of you who aren't necessary well versed in crop protection products -- every new product -- and you heard mentioned this morning that we're moving away, on a lot of these acute whole animal studies, to in vitro assays. But each new product, each new formulation, has what was called a six pack of animal studies. And these acute tox packages -- and we do typically hundreds of these, every year -- really detail the acute exposure and the expectation of what you might find in acute toxicity.

So, these studies are done. What you see here is the dose response, in both male and female, and a time course, single exposure. After two hours you start seeing necrosis. In this particular example, we're looking at the larynx, although we did look across the entire respiratory tract. So, in this particular example of the data it's just the larynx.

By two hours you see necrosis; by four hours it's about as severe as it's going to be. So, the information in the parenthesis is a

severity score. The pathologist would have scored this from 1 to 4, or 1 to 5, on mild, moderate, marked severity.

And so, you see by four hours it's as severe as its going to get; six hours their cells are still dead. And so, what this information was able to tell us, is what we see is a very acute toxicity. As soon as there's sufficient exposure to the epithelial cells over sufficient time -- in this case two hours for the higher doses -- the cells die. And they don't get any more dead over time.

In considering the fact that there's also worker exposure, and their exposed more than acutely, and so we looked at six hour per day exposures in rats. Again, in a dose response manner, over five days with the product -- with the highest concentration of Chlorothalonil in it, followed the typical test guidelines, looked at all the traditional endpoints, and found effects across the respiratory tract, even into the lungs at the highest dose.

But after two weeks of no exposure -- so the recovery period -- all the alterations went away, except what we found in the larynx. And so, this was the reason we focused on the larynx as the model location for all of our dosimetry; because that is the place where the lesion stays. So, if you look at the larynx data, you will see, again, that there was recurrent damage that got more severe with dose. And then, after recovery, it did start to resolve.

I'll go into more detail on the particular alteration of squamous metaplasia in this site in a little bit. But that was the diagnosis.

And what that is, in this particular context, is whenever you have a recurring irritation, a recurring toxicity in a respiratory and mucus epithelium, over time it wants to protect itself. So classic recurrent irritation and associated necrosis -- I'm trying to stop using the word irritation. Associated cytotoxicity at the site of contact.

If that reoccurs repeatedly over time, whether it's formaldehyde, glutaraldehyde, ozone, chlorine, and in this case, Chlorothalonil, the tissue responds to try and protect itself. And it moves from a respiratory epithelium, which is a very sensitive epithelium, to a more stratified squamous epithelium like skin.

And this is just an adaptive response to repeated damaging exposure to a corrosive chemical. And so, that is a response that we see in the respiratory tract when repeated exposure to the Chlorothalonil, which is causing repeated cytotoxicity, leads to the squamous metaplasia, which in this particular study did not fully resolve. So that became a concern from the agency.

The other alteration that we saw, is in this particular place -- in the larynx of the rat is a piece of U-shaped cartilage, because it's U-shaped. That because of the severe corrosive nature of the chemical, it went through, ulcerated, and damaged the cartilage. And so, there we got cartilage necrosis as well.

And so, while this particular feature in anatomy is not present in humans, we do have cartilage. So again, the agency said, well this is a concern. What you see here is it's associated with the acute toxicity. And over persistent exposure, it stayed and there was no recovery. And so, we don't know if this would have fully recovered but in the context of the 14-day recovery, we still had the same evidence of cartilage necrosis, although the squamous metaplasia was recovered.

So, as Dr. Perron mentioned earlier, the rat respiratory system is different from the human respiratory system. And it's not that rodents aren't good models for identifying hazard, for detailing the pathogenesis of developing of a disease, whether it's an infectious agent or a chemical; but when you're starting to talk about dosimetry relevance for risk assessment, in both the external and internal exposure, the anatomical differences become important. And Dr. Hinderliter, after lunch, will expound more on the relevance in these anatomical differences.

So, on the left is the rat larynx. And this rat, standing on his butt looking at the ceiling, not the normal way, but it's to be able to more directly compare to the human larynx on the right. And what we see is some changes in the direction of the lumen, which of course would impact airflow if you have things floating in the air.

But the other, what you see in the middle there, in the red circle, is the location of where this lesion occurred that we're describing in the graph. The U-shape cartilage and the associated epithelium over it; around and into the pocket of the U-shape cartilage is where you see the squamous metaplasia and the cartilage necrosis. And there's no comparable anatomic feature in the human.

In the human, while we have the laryngeal folds, just like in the rat, in the rat there was very minimal. There's, again, necrosis and metaplasia, but it resolved on the larynx; but we don't have the same features in the human. So, again, anatomically the human is different.

So, what were our conclusions from the pathology? We had the squamous metaplasia and the U-shape cartilage necrosis. They're still present after two weeks; so again, this got to be a concern to the agency. The squamous metaplasia was mostly resolved. And, according to the literature, would be expected to completely resolve over time. Now I know some of you are well aware of a lot of the literature on the pathogenesis of cancer, with various respiratory cytotoxicants, such as formaldehyde. And cigarette smoke in human respiratory system. With smoking you get squamous metaplasia as well.

In those situations, with persistent exposure over long periods of time, those cells will transform and can become tumors. However, in the early stages, the reason they become present at all, is because initially it's an adaptive response to that persistent irritation. And so, at this point, after a couple weeks, this is an adaptive response to protect the surface from this corrosive material. And it's not, at this point, a pre-neoplastic lesion.

Again, there's a lot of literature in the formaldehyde world on what happens when the cells transform, and mutations appear. It has been documented for different genes, and that's a later process.

But in this early stage -- and so, where even earlier, what we see is if you have the acute toxicity for over long periods of time, two weeks, you get the squamous metaplasia. We're concerned with that initial part of preventing that acute toxicity.

So, the squamous metaplasia is an adaptive, nonspecific response to any corrosive irritant product or material. And the literature shows that this level of squamous metaplasia is not considered an adverse effect, but an indicator of response.

Some literature suggests that the cartilage necrosis could resolve. Those of you who have bad joints, like I do, know once the cartilage is gone, it's gone; so, that's debatable. But the more important point is the reason you have cartilage necrosis is because of corrosivity of the chemical on the respiratory

epithelium, moving through and killing the cartilage cells. And so, again, it is a secondary response to that acute toxicity. And so, if we can prevent that acute toxicity, then we can prevent the rest.

So, when you think about the specific adverse outcome pathway, our mode of action for the specific endpoint that we're looking at, which is squamous cell metaplasia -- we're not going to cancer, we're not going to any other effect; we're going to the earliest, quantifiable, measurable, histologic change. Then the first event is killing that respiratory epithelial cell.

So, you have to kill that cell. But just killing one isn't going to make any difference. You have to kill its daughter, and its granddaughter, and its great granddaughter, repeatedly, over time. Repeated injury to lose that epithelium, so that you stimulate those basal cells, to say, hey, I've got to change, I've got to protect, I've got to become a different kind of cell type. And that's where you get the typical skin-like cells, and you get

the stratified squamous epithelium to protect that surface from the recurrent.

We're still exploring the problem, what do we know? If that first initial step is the critical initiating event in the adverse outcome pathway, then can we model that first step in an in vitro system?

Now this slide is just to describe the process we use to pick the particular in vitro system we settled on in MucilAir. It wasn't about whether if one system was better than another, or inherently great or inherently poor; it's whether it was fit for purpose for the questions we were answering.

And in fact, what Dr. Charlton will show later is, actually, the fact that we were already using the MucilAir to answer some questions for us in another project; we already had experience with this assay system and adapted it for this purpose. So, for the uses we were using within Syngenta -- and I think one of the comments earlier, well, how are going to move this out? Well quite frankly, within our company and other companies, we're using these types of

tools all the time to make business decisions, to make project decisions.

So, we are hoping that you'll see the value of moving this out into the regulatory world. But in fact, that won't have any impact on whether we continue to use these models, because they're a great utility in helping us. And you'll see that a little bit later today, the value of these kinds of models.

But for us, we asked some very simple questions to see which would fit our purpose. Is it easy to use and maintain? Our Syngenta model of gathering information is outsourcing. So, we need to make sure that the tools we use are well understood, and easy to use, in various different contract research organizations. We don't have an internal lab anymore, so that's important.

We're able to model the cell to cell interactions with it. Because that was critical for some of the questions we were answering in the different projects we were going to use the tool in.

Is it representative of in vivo tissue organization? So, when you think about the cell type of target, it's a pseudostratified ciliated epithelium goblet cells, and basal cells regenerating. It needed to look the same to the pathologist. It needed to react the same to chemicals, and it needed to respond the same, including moving cilia.

So, we wanted to make sure it simulates the mechanical action of the respiratory system. If we're putting particles, or other types of materials on products, we want to see how they move the crops and did they have impact on the cilium.

And is it suitable for long term testing? Now, in our way we've been using it, we haven't treated the cells for more than 24 hours as represented in this particular example, you'll see later. But it has the potential. So, if you wanted to do repeated exposures, if you wanted to find out what happened after 5 days, 7 days, 28 days, it's possible. So, that was an important criterion for looking to the future.

And then was it applicable to the in vivo situation? Did it respond like what we see in vivo? And again, for us this ticked off all our personal needs. Again, not to say some of these others wouldn't be equally good, but for what we were looking for, this was the best one.

So, having decided on in vitro, what we said was well then, we can actually model that initial step in the adverse outcome pathway, that initiating event. So, if you done any looking at the OECD adverse outcome pathway wiki, the first step there is the molecular initiating event. Well, in our case we don't have a molecular event, we have exposure to corrosive material, and it kills the cell. So that's our initial event. And if you repeated that, then eventually you get the outcome.

So, we know this stuff because there's literature on it and we have the information. We know what we have about Chlorothalonil. Again, we're still exploring the problem. The other thing we know, from the literature, is the structure of the human respiratory tract. There are also many people

out there, that have developed mathematical models for how things flow through the respiratory tract.

So, the folks we collaborated with, Pacific Northwest National Laboratory, Rick Corley, and his team, have published extensively on diesel exhaust on radon, on plutonium, on cigarette smoke. And so, a lot of different materials look at how does it move through the different structures in the respiratory tract in rodents, primates, and humans; and so, they have those models.

We worked with them to say, well what about aerosols, can we adapt? And so, we understand that models exist; and can we then adapt those models for the aerosol droplets that we are concerned about, for Chlorothalonil containing sprays?

So, putting all this together, and looking at it from a risk 21 point-of-view, we have identified the problem. We start with exposure, but what's the source? It's a spray in an agricultural setting; that's what we focused on. So, it gets sprayed out there. And then

that gets to the operators face somewhere. Can we measure that?

Once it gets there it's inhaled, moves through the respiratory tract to get to the target site. Once it gets to the target site, it kills that cell. So, the source exposure dosimetry outcome pathway was how we parsed this out so we could look at the separate different things.

So, the traditional conceptual model that we have always used in risk assessment, for human inhalation risk, was to poison a bunch of rats; hopefully, find a level that didn't cause harm in the rats. Skew a bunch of mathematical extrapolations to get to a human equivalent concentration, and then do your risk characterization assessment. And that's worked very well for us for decades. But that's not really the question that I'm trying to answer.

So, we changed the conceptual model and said, well, thinking about this from a risk 21 problem formulation-based approach, what is it we're interested in? We're interested in those people that are working in agriculture,

that actually use our products in the way they're supposed to be used. The spray comes out of a nozzle at some range of particles; some subset of those particles can get to the operator, they inhale them, gets to the site of contact in the respiratory tract, and can cause damage.

So, what is that dose at the site in the respiratory tract? How do you back calculate what you could be exposed to? If you can find a no effect level in in vitro testing, calculate how much you would have to inhale to get that level in the respiratory tract, and then back calculate that to get the human equivalent concentration. Then we can use much less mathematical manipulation, from rat to human, to say, okay, well that's the human situation, it's human exposure, it's human dosimetry, and its human cells, to then calculate the human-equivalent dose and feed that into the risk assessment.

So, that was our conceptual model that drove the project. And now, we're going to move into mapping the approach, which is the rest of my colleagues here who managed the science

part of this. They don't let me do science. And so, I'll stop here before I turn it over to Dr. Flack. And if there's any clarifying questions for this part.

DR. ROBERT CHAPIN: Nice presentation, Doug. Let me just clarify. It looked like the inflammation was at a low or -- almost background level at all time points in this, but you've looked at the histology of the tract, is that right?

DR. DOUG WOLF: Initially, it was present there. And then the inflammation that was induced by the chemical did resolve, over time, to be less severe. But as you know, background inflammation is always there. Yeah.

DR. ROBERT CHAPIN: Other clarifying questions? Ray.

DR. RAYMOND YANG: Let me ask you this question and please tell me if I'm thinking wrong, okay?

DR. ROBERT CHAPIN: Clarifying question.

DR. RAYMOND YANG: To me, I'm not too worried about mixer and operator, because

these occupational workers they could wear protective devices, protective clothing. What I worry about, is this chemical is incorporated into paint, into the wood. Do they vaporize, have off-gassing?

DR. DOUG WOLF: This particular chemical is not volatile. So, that was not a concern with this chemical and these products. So, no, Chlorothalonil is not volatile, and so that's not a concern.

We are required to do evaluations and predictive risk assessments, and the agency's required to do risk assessments, for all the different ways and scenarios. So, the applicator, the mixer/loader, bystander, however the product is used. You think about all the different kinds of people, in the factory where the products are made, we had to address those exposure scenarios. So, all the different exposure scenarios, we're required to evaluate those and predict those.

It is true that for those of you who work in formal laboratories, and you wear all your protective gear and face masks and hoods and

everything, that makes total sense. But in the agricultural world of how these things are used, we have to consider the comfort and the safety of the individual. So, while you might say, well, you know, if a guy is spraying this on a golf course, he really should be in Tyvek suits and hoods. But its 85 degrees with 95 percent humidity, is that really how you want him out there for several hours spraying a golf course.

So, we try and create products that are safe and fit for use, and under the circumstances in which they are best used; both for the safety of the operator, safety for the bystander, and also the practical concerns. And so, if we can't get a product that can be used in the way that people need to be able to use them, then it's not a registerable product. But that's a very good point.

DR. RAYMOND YANG: Thank you.

DR. ROBERT CHAPIN: Other clarifying questions?

DR. SHEILA FLACK: Okay. So, this portion of the talk, I'll start --

DR. ROBERT CHAPIN: Sorry Sheila, just give us your name.

SYNGENTA - FLACK

DR. SHEILA FLACK: Oh, I'm Sheila Flack, sorry. I'll try and remember that. So, here we have our conceptual model. And what I will be talking about is to the left of that model you see particle size distribution of inhalable particles.

I'll be talking about how we derive a human relevant particle size distribution that we can use. In the discussion, later this afternoon, we'll see how we use that information integrated with our CFD modeling, inhalation dosimetry modeling, to generate that human equivalent concentration.

So, it's important to keep in mind that in exposure-based risk assessments, inhalation exposure for low or relatively nonvolatile pesticides, like Chlorothalonil, is to particulates for aerosols. And by definition, we use those terms; but what it is, is it's a

water droplet, and within that water droplet is a solid particle. And as been mentioned before, the focus of this case study is on applicator spraying a dilute formulation of Chlorothalonil.

And as been mentioned, current alternative data generation that we'll be talking about here, can provide alternative approaches that are suitable to inform inhalation toxicity in lieu of an acute or sub-chronic inhalation study.

So, exposure data is commonly collected from agricultural workers using an OSHA versatile sampler, OVS tube. What this device does, is it's connected to an air-sampling pump. The device is worn in the breathing zone of the worker, that you see in that picture to the right. And as air is pulled through the device, the aerosols and vapor, whatever is in that breathing zone of the worker, is going to be trapped onto the filters and absorbent material in that tube. And then the material in that is taken out, and extracted, and that provides an estimate of inhalation exposure. And this method is used by the agricultural exposure task force

to generate exposure data that is used in risk assessments.

So typically, the OVS tube data is reported as total concentration without consideration of particle size. We know particle size is really important in how things are deposit in the respiratory tract. Part of the goal of this work was to understand what the particle size distribution is being captured by this device. And so, at Syngenta, we undertook some studies of spray particle size characterization to compare the OVS tube data with standard sizing methods, to better understand the particle size distribution.

In order to answer this question, of what is the particle size distribution captured by OVS tubes, we did some side by side OVS versus Respicon air sampling method. And so, you'll see on this picture to the left is a photo of our experimental setup. This is conducted in a laboratory spray chamber, and so the devices were positioned about two feet away from the nozzle. We used different types of nozzles, different spray quality nozzles, applying Bravo

Weather Stik, diluted formulation of Bravo Weather Stik, which was about 5 percent Chlorothalonil.

And to the right here we see just a schematic about the Respicon air sampling devices. It's basically a multistage virtual impactor, consisting of three different stages. And as the air moves through the device, it's connected through a sampling pump as well. And as the air is pulled through the device, the particles are separated according to size. Particles with a larger mass, larger inertia, will impact on the bottom of the Respicon. And the smaller particles will settle on the top filter, at the top stage.

So, by analyzing those different stages, we can get an estimate of the inhalable thoracic and respirable size fractions, which the current definitions -- criteria definitions that's been established for those three fractions.

This is a summary of those results, from that side by side comparison of the

OVS tube and the Respicon sampling of the inhalable fraction.

What we have on the Y-axis is the total Chlorothalonil concentration that was measured in that spray chamber. And this was done from various spray quality nozzles, as I mentioned before. We have extremely coarse, which means it's applying much larger, coarser droplets. And then, to the right, we have medium and then very fine, meaning that it sprays much finer droplets.

As you can see, when you compare the OVS versus the Respicon, the concentrations are very similar for these different spray quality nozzles. What we can conclude, the main conclusion, is that the OVS tubes capture the inhalable fraction. What we did note, however, was that we did see difference in Chlorothalonil air concentrations by spray quality. So, with a very fine nozzle, we see a much higher overall concentration, compared to the extremely coarse nozzles.

With this information, the OVS tube sample the inhalable fraction, we derived a

distribution based on the standard definitions set forth by the ISO/ACGIH/ECEN sampling conventions for the inhalable thoracic and respirable aerosol fractions. We wanted to maintain that cutoff of 100 micrometers, so we're not considering anything above the inhalable fraction. And so, by binding it to 100 and using those sampling conventions, we can mathematically derive a representative particle size distribution, with the mass-needed air dynamic diameter at 35 micrometers and a geometric standard deviation of 1.5.

This is just to really illustrate and point out that spray applicators are exposed to an array of particles. And some of these can be very large, up to the human-inhalable size here, bounded by 100 micrometers. And this is very different from some aerosols that are used in the rodent study, if you compare that to a 2-micrometer particle size, which is a very small relative.

We're trying to bring more of a human-relevant exposure situation into this

study. With that, I'll pause here, and I can take any questions.

DR. MARIE FORTIN: Marie Fortin, I'm with Jazz Pharmaceuticals, and the questions are my own. So, on slide 31 --

DR. ROBERT CHAPIN: Marie, move a little closer to the mic so the rest of us can hear you.

DR. MARIE FORTIN: On slide 31, that's the measured of particle size distribution? Is that right?

DR. SHEILA FLACK: It is measuring total concentration in the spray chamber. This doesn't show the different respirable thoracic fractions, this is the total available. What we found was that -- actually, if we go to next slide here. Those numbers at the bottom of that graph, actually, show the percentages that we did measure, if you were to fraction those off in those different stages. So, about 5 percent were in the respirable, 40 percent was in the thoracic, and 60 percent in the extra-thoracic.

DR. MARIE FORTIN: That's exactly my question. Was this measured or modeled?

DR. SHEILA FLACK: Well what was modeled was the derived distribution. We didn't take the actual data from what we analyzed. What we understood from our analysis was that the OVS tubes are sampling inhalable fraction. We wanted to make sure we encompassed that whole distribution, in our particle size distribution, that we were deriving.

DR. MARIE FORTIN: Okay. So, if you go back to the previous slide. You attribute the higher concentration to the particle size, or do you attribute that you have very fine particle size, and therefore a greater amount?

DR. SHEILA FLACK: Right. So, with the very fine spray nozzle, you're creating more of the smaller particles. So, there is going to be more particles in that inhalable fraction, and that's what we're capturing here.

DR. MARIE FORTIN: And yet you utilize the model distribution that's based on general values?

DR. SHEILA FLACK: Exactly.

DR. MARIE FORTIN: Despite the fact that it changes from another to another?

DR. SHEILA FLACK: Well, from one nozzle to another, we didn't see a difference in the relative proportion of the respirable thoracic and extra-thoracic. What we did see was the overall concentrations would change. But what we were trying to do is derive a distribution that we can use.

So, we're saying that the distribution itself doesn't change according to nozzle type. What does change is the overall concentration; but that's not really what we're using to derive at distribution. What we're trying to understand is, what are the relative proportions within that inhalable fraction? Does that answer your question?

DR. MARIE FORTIN: Well that's all right. But if you change a nozzle, you change the flow rate, you change the excipient, all of this is going to impact the particle size distribution. It doesn't matter what it is, but it's going to impact it. And then you described a model distribution for the complete unknown, when at the capacity, of measuring the actual particle size distribution.

I'm confused as to whether you would just use a model and, therefore, get to a larger size than is actually possible than what you're actually measuring.

DR. SHEILA FLACK: What we measured, in our study design, was trying to understand what was being captured. Are we looking at just a respirable fraction, are we looking at the thoracic fraction, are we looking at the inhalable fraction? Because what we were trying to do, is come up with a size distribution that we could use in our model.

DR. MARIE FORTIN: Yes. So, my point is that you can't measure that.

DR. SHEILA FLACK: We can measure that, but we can't derive that from the work that we did. We can't derive an actual distribution from the work that we did.

DR. MARIE FORTIN: All right. Thank you.

DR. JAMES BLANDO: Jim Blando. And I have a follow up question. How did the laboratory-generated aerosols compare to what you would actually observe in a field? Because as

she mentioned, many of the operational characteristics that someone would use, when they're actually out in the field, are going to impact the particle size distribution.

So, for example, things like pressure, pressure drop across the nozzle, and so forth, are going to drastically impact the particle size distribution. Your assumption is that these particles generated are very large, 35 micrometers MMAD. But I'm just trying to, in my own mind, compare how that large size would be to something that someone might actually encounter if they were actually in the field.

And in addition, you could look at -- not to get into too subtle details, but if someone is applying -- you mentioned this is a solid particle in a water droplet? Or is it dissolved in the water droplet?

DR. SHEILA FLACK: It's a suspension concentrate; so, within that water droplet it's a solid particle.

DR. JAMES BLANDO: Okay. I also wonder if the particles are drying out, because say you're in a dry atmosphere. And just trying

to think about what you would actually encounter in the field, versus what you actually generated in the laboratory.

DR. SHEILA FLACK: Yeah. Our data was done under laboratory conditions. We did use pressures that you would typically see in an operator scenario, so that condition was probably comparable. But in terms of temperature, humidity, and things like that, we didn't alter any of those types of conditions.

DR. ROBERT CHAPIN: Jen.

DR. JENNIFER CAVALLARI: Hi. This is Jen Cavallari. Thank you for your presentation. I have two questions. The first with respect to how relevant your laboratory scenario was to the field. I was confused; did you at all look at pressure, and changes in pressure, and how that affected the particle size?

DR. SHEILA FLACK: Yeah, we kept the pressure constant; it was about 40 PSI, which is what a typical applicator would apply. We didn't attempt to vary any of those spray pressures.

DR. JENNIFER CAVALLARI: Okay. Thank you. And Jen again, this is my second question. I'm trying to understand the parameters that were inputted into the model, that you used to derive the 35 micrometers with the geometric standard deviation of 1.5. What test data were used in this model? I just need some more clarity around how that 35 came about?

DR. SHEILA FLACK: So, the 35 came about by using the -- the mathematical descriptions, for each of these size fractions, are published in the literature. They've been well described and established. Their probability density fractions have already been defined. And so, we took the description for each of those factions, and applied that same mathematical function to derive our distribution.

DR. JENNIFER CAVALLARI: Did you use the percentages below at all?

DR. SHEILA FLACK: We didn't use those percentages at all to derive our 35. The only information we really took was that we're capturing the inhalable fraction; that anything that we're capturing is between 0 and 100.

And so, we derived this distribution based on the already known, well characterized and established distributions that have been published. All we did was bound it to 100; because that was what our data showed, was anything above 100.

DR. JENNIFER CAVALLARI: Okay. So, that data inputted it to this deprivation was the bounding of 100?

DR. SHEILA FLACK: Exactly. Yes.

DR. JENNIFER CAVALLARI: And you used the previous studies to confirm that 100 bounding?

DR. SHEILA FLACK: Mm-hmm.

DR. JENNIFER CAVALLARI: But no addition data for including?

DR. SHEILA FLACK: Right.

DR. JENNIFER CAVALLARI: And how about the geometric standard deviation?

DR. SHEILA FLACK: That was also part of the mathematical description for each of those distribution. So, the 1.5 comes from the definitions of the respirable thoracic that have been well established.

DR. JENNIFER CAVALLARI: And did you do any sensitivity analysis around those parameters?

DR. SHEILA FLACK: No. We didn't do any sensitivity.

DR. JENNIFER CAVALLARI: Okay. Thank you.

DR. ROBERT CHAPIN: Kathryn.

Dr. KATHRYN PAGE: Kathryn Page. Similar lines to what James touched on previously. EPA typically does consider evaporation for the particle to determine final size in the inhalation zone. And agglomeration, obviously, is also known to effect particle size. Were there any considerations to account for this during exposure? And were there solid particle sizes taken to account to the total as well?

DR. SHEILA FLACK: We didn't look at evaporation of the particles. We were just simulating a condition, that we tried to mimic what would occur out in the field, using an appropriate spray pressure, different nozzles that a worker would use. And no, we didn't

attempt to look at that. The OVS tube data reflects the actual human exposure.

DR. KATHRYN PAGE: But it's not looking at the particle size, it's just looking at the size under microns? I mean the volume under micron? Sorry.

DR. SHEILA FLACK: Yes.

DR. KATHRYN PAGE: And sorry, just one more point on that. When you were looking at the Respicon.

DR. SHEILA FLACK: Respicon.

DR. KATHRYN PAGE: Respicon, sorry. I understand that you're increasing the airflow to try make the conditions more realistic in the outdoor environment; would you say that the spacing between the nozzle and the receptacle would represent a standard exposure for somebody?

DR. SHEILA FLACK: Perhaps for like a handheld -- someone who's applying via handheld, the distance would be representative of that. I think for like an air blast or ground bloom, there would be a greater distance separation, which would likely impact the overall air concentrations; that farther away, those

particles are likely getting deposited, falling out before actually reaching the worker. So, in terms of measuring air concentration, this might be like a worst-case scenario because of the shorter distance.

DR. CLIFFORD WEISEL: Cliff Weisel. You said the short distance. Can you give us a time frame, you think, from the admission of the nozzle to your sampling, and how long you did the sampling for?

DR. SHEILA FLACK: We did the sampling for several hours. Or, actually, I'm trying to remember. No, it was less than an hour. We did kind of a standard amount of time for each sampling. We started the sampler, we let it run for -- pretty much, as soon as we started spraying, we started capturing.

DR. CLIFFORD WEISEL: Okay.

DR. SHEILA FLACK: Set that pump flow going. So, it was pretty much right at the same time. And then we captured that as the nozzle was spraying, it was about 40 minutes, I think, we were capturing.

DR CLIFFORD WEISEL: And the distance, you think, from the nozzle too -- you said was short? I'm just trying to get a sense of what you --

DR. SHEILA FLACK: Yeah. It was about two and a half feet.

DR. CLIFFORD WEISEL: Okay. Because that picture looked like a small box.

DR. SHEILA FLACK: Yeah, yeah. It's much larger.

DR. CLIFFORD WEISEL: Okay. That's helpful. All right. The other thing is you had -- the impacted had different size. What did you use that data for? I'm confused. Because you said the distributions are purely mathematical modeling. But you did collect an impacted system that gave you different amounts and different size ranges. How well did that data fit in with your modeling?

DR. SHEILA FLACK: Yeah. I think that there is some confusion.

DR. CLIFFORD WEISEL: Yeah, I'm confused. That's why I'm asking.

DR. SHEILA FLACK: How we are using this information. Yeah, I see your point.

That really is just more information, that was helpful to us, to show that we needed to consider particles within what we called an inhalable distribution. If were only capturing a respirable fraction, then maybe we would fit the model to look at the smaller particle size.

Really, it was just an exercise to help us confirm that what we were capturing, in that comparison, on OVS tubes, in a real-life scenario, is the inhalable fraction.

DR. CLIFFORD WEISEL: But you do have data that tells you the mass in those three-impactor size, right?

DR. SHEILA FLACK: Yeah.

DR. CLIFFORD WEISEL: And did you compare that data to your model?

DR. SHEILA FLACK: No, we didn't.

DR. CLIFFORD WEISEL: Okay. So that's something that I think we would like to see at some point.

DR. ROBERT CHAPIN: Okay. Last one, Ray. Name please.

DR. RAYMOND YANG: Ray Yang.

DR. ROBERT CHAPIN: Thank you.

DR. RAYMOND YANG: Am I correct, that when you spray, it's polydisperse, meaning different particle size. Whereas, when you do CFD modeling, it's monodispersed. Could CFD modeling be done with more than one size?

DR. SHEILA FLACK: The CFD modeling was done at different monodisperse-sized particles. And we'll go into that in our later discussion.

DR. RAYMOND YANG: Yeah. You didn't answer my question. Can you do two different sizes or three different sizes in one model?

DR. SHEILA FLACK: Oh, at the same time?

DR. PAUL HINDERLITER: Paul Hinderliter. Yes, you can. It gets a bit complicated. It is feasible to do. We'd have to think pretty hard what the setup actually looks like, and what the results would mean.

DR. RAYMOND YANG: If that's true, then the individual simulation may not represent the real impact of deposition.

DR. PAUL HINDERLITER: Paul Hinderliter again. I'm not sure in what way you think it would be different. We'll get into the CFD in datil after lunch, but the particles in the CFD models are assumed to be non-interacting.

DR. RAYMOND YANG: Okay.

DR. PAUL HINDERLITER: So, including a variety of particle sizes would give you the same answer that you would get from summing up the individual model disperse phase. Summing them up, you would get the same answer that you did if you would put them together and do that same CFD. We can come back to that in detail later.

DR. RAYMOND YANG: Thank you.

DR. ROBERT CHAPIN: Last one.

DR. HOLGER BEHRSING: Holger Behrsing. The particles contained in the droplets or spray. So, the particle size there really just doesn't change at all? I mean,

there's no solubility, there's nothing that occurs over time?

DR. SHEILA FLACK: You mean as you spray the particle, is it changing over time?

DR. HOLGER BEHRSING: That's correct. The material that's contained in the droplets?

DR. SHEILA FLACK: Well, I think, over time what you're probably seeing is droplets might be coming together. And if you think of an atmosphere of different droplets, what's changing is you might have something smaller, some particles are coming together. The components of that actual particle would be the solid particle in that droplet. The behavior itself isn't changing; it's just maybe the dynamics of that droplet might be changing. The sizes might be changing.

DR. HOLGER BEHRSING: Okay.

DR. JAMES BLANDO: Can I just ask one quick question, please? I promise it's a quick question.

DR. ROBERT CHAPIN: Yeah. Turn on your mic, identify yourself. Speak into the mic so the people online can hear you.

DR. JAMES BLANDO: Sure. Jim Blando. My question is actually for Dr. Wolf. It took me a few minutes to digest your presentation. When you discussed the metaplasia and how it would resolve after the recovery period, just thinking about what you described. It sounds to me -- correct me if I'm wrong -- a really important parameter to think about, when you're interpreting this data, is the length of time of the exposures.

DR. DOUG WOLF: That's absolutely critical. Yeah.

DR. JAMES BLANDO: Thank you.

DR. ROBERT CHAPIN: Is this the natural break point for lunch that you guys were planning on? Okay. All right. I'm looking at our DFO. Shall we break for an hour? Return at 1:05. Okay. Remember to leave enough time to get through our friends with the scanners at the front door.

[LUNCH]

DR. ROBERT CHAPIN: This is Bob Chapin, for those online. Let me remind the panelist, please, that the microphones are to broadcast our voices through a webcast. And so, people who speak like this do the folks online a real disservice. I was asked by the AV guy, one of the technical support specialists here, to make sure that we're within a couple of inches of the microphones, especially our soft-voiced colleagues. If we'll do that, that would be appreciated by all online.

We're going to start off with a brief recap of something from Dr. Perron.

DR. MONIQUE PERRON: Thank you. This is Monique Perron. I actually just wanted a quick moment to remind people. I kind of went over it very quickly at the end of my presentation, about some ongoing work that we're doing.

We really appreciated the conversation that you guys were having prior to lunch, on the particle size distributions. And you'll notice that there wasn't a question on the

particle size distribution; because that work, we've been working with Syngenta and the Crop Life America representatives to try to figure out the most appropriate particle size distributions.

So, we've been working with them to try to start mining data on -- we have a lot of spray-drift data out there. We're just trying to identify all the available information out there. And also, possibly determine if some additional data needs to be conducted in order to support appropriate particle size distributions for each exposure scenario.

We really do appreciate that feedback that you guys are giving. We're not sure where it will fit in under the charge questions, but if you can figure out the most appropriate place that you want to give us that feedback, we do appreciate it. I just wanted to add that quick clarification.

DR. ROBERT CHAPIN: Thank you. Okay, back to our colleagues from Syngenta. Dr. Wolf, I'll let you hand things off.

SYNGENTA - HINDERLITER

DR. DOUG WOLF: Before we move on to Dr. Hinderliter and the computational fluid dynamics model, I just want to provide specific clarification on the adverse outcome pathway. In this particular case, Chlorothalonil, as a fungicide, is a direct-acting fungal toxicant. So, it kills -- it's a highly chlorinated compound under hydrolysis. It gives off chlorines. It enters into the fungal cell and kills it.

In a similar manner, when you think about respiratory cells with lipid membranes, once it comes in contact with that lipid membrane, hydrolyzing in the seromucous layer, overlining the respiratory epithelium. It would, again, give off chlorines acidify that enter into the cell and kill it.

For those of you who worked in modes of action, adverse outcomes pathways, there's many different kinds. Those of you in the pharmaceutical industry, developing drug targets to receptors and that, there's a lot of

nuances sometimes. But once in a while, you have one that is pretty straightforward. It's a bullet, shot to the head, and kills the cell. That's the model we're dealing with in this particular situation.

If you're looking at bigger tissue response, then there might be some nuances you want to look at. If you're trying to develop treatments in the respiratory tract for someone who's exposed, that's a different issue. But for us, for the risk assessment, risk characterization, developing a particular number for the human equivalent concentration, we focused on this simple mode of action of exposure, death, and then the subsequent response in the tissues with repeated death in response to trying to repair that.

It's a very common cytotoxicity regenerative proliferation mode of action, which you see with a lot of different corrosive chemicals: formaldehyde, chloroaldehyde, acetochlor, and many others; cytotoxic chemicals in the liver, chloroform, carbon tetrachloride. They all do the same thing; get into the cell,

kill it, and then you get that regenerative proliferation; and in this particular case, leads to squamous metaplasia.

I just want to clarify that point because, of course, there's a lot of other testing we could do; but in this particular approach, we were trying to focus on what is happening in this particular case with this chemical.

DR. SHEILA FLACK: There was a previous question about whether or not the presence of --

DR. ROBERT CHAPIN: And your name?

DR. SHEILA FLACK: Oh, I'm sorry. Sheila Flack. There was a question about whether or not the presence of Chlorothalonil, in the solution, will have an effect on the particle size distribution compared to water.

We had done some initial work regarding particle size distributions coming from the nozzle. We did a comparison, looking at five percent Chlorothalonil formulation, versus water, and did not find a significant difference between

those two. I just wanted to point that out because that was a question that had come up.

DR. PAUL HINDERLITER: That would be me. Paul Hinderliter from Syngenta. I'm going to take us through -- my colleagues, so far, have taken us through the external part of the distributions and the exposure. Later on, we'll look at a bit about our in vitro endpoint. Where I'm standing in all of this, is the kind of bridge in between what does it mean to be exposed to an atmosphere of particles, or aerosols, or some sort of inhalation atmosphere? And what actually winds up on the surface of the respiratory tract. Because, after all, what is our in vivo system? It's a representation of a piece of the surface of the respiratory tract.

There's another study that we haven't actually mentioned in the work here. But we did do some early work on some pharmacokinetics, comparing the oral and inhalation route for Chlorothalonil. We had some oral data that was part of our registration package. And in one of the acute studies that Doug Wolf had mentioned earlier, we did collect

some pharmacokinetic data, some blood samples, during and after inhalation exposure. We showed that the systemic exposure was pretty similar between the oral and inhalation route. So, we could establish an equivalence between an oral dose and finding an equivalent inhalation dose.

We kind of took the systemic toxicity issues off of the table, that we can get what the exposure would be for that. So, we're focusing here solely on the portal of entry, contact effects.

We've been through this a couple of different ways, this morning, with external particles. So, what is a human actually exposed to, versus what are rats exposed to in our inhalation guideline studies?

It went by kind of quickly on one of Doug's earlier slides, but the rat studies were standard guideline studies. And, in average, on the ones that we'd done in the two-week study, we had a mean diameter of about 2.7 microns, within the guideline size of that.

You see on the small table on the right-hand side there, if you were to look at

some seemingly arbitrary -- these are based off of the impactor sizes. You see that by the time you get out to eight microns in size, you've accounted for about 94 percent of the mass that the rats are exposed to. The predominate portions of it, actually, are in the sort of one to five range around the MMAD.

If you take what we've been talking about for these reference nozzles, and if you use a 35 micron, or a hundred micron, or whatever appropriate aerosol size you're looking for, you'll see that the sizes don't overlap very well with what's actually in the rodent study. Only about one percent of a 200 micron-ish particle size is down in the range that overlaps with the rats. So, these are quite different exposure scenarios.

Now that's initially a bit confounding, because if you're not exposing to the same thing, then what can you actually say about exposure? Well, the answer is quite a bit.

There's a model that's been in existence for quite a while called MPPD. They just released version 3.0 sometime within the

last year. This a deterministic model that models rats and humans. They have mice. I think in the last version they have expanded to include rabbits, and monkeys, and hamsters, and wombats or something. I'm not sure what all of the species are; but most of the species of interest are available.

We ran this in some of our scoping work to see, well, where do these particles actually go? What does the size difference make in terms of exposure? Keeping in mind that we're talking about exposure as the contact on the respiratory surface. The slide's a bit busy, and I apologize for that, but I wanted to lay the lines on top of each other.

So, in this slide, the solid lines are human simulations and the dotted lines are rats. If we look at the rat, the two dotted lines, that kind of peak out around three or four microns, the purplish one is what MPPD calls the head, and that's the upper parts of the respiratory tract. Then, down near the bottom, you see in the red and the, of course, in the other shade of blue, those are the conducting and

alveolar depositions in the different sizes. Then the pinkish color, the highest rat number, is the total deposition.

Now, if you remember from the guidelines, the peak -- the guideline size of these aerosols is around three or four microns. Not coincidentally, that's about where the peak of the total exposure is, because the guideline studies are, by design, largely a hazard identification study. So, if you're looking to say, what's the most of a material that I could get in by the inhalation route, to elicit a response in the rat system, it would be about three or so microns.

Now, you see from the conducting and alveolar curves, the amount that gets down into those lower regions, even down as low as half a micron or so, is still less than ten percent in these different regions. It's not until you get down into the submicron, down into the sort of nanoparticle range, that almost the exposure becomes sort of more widespread in the lowest parts of the respiratory tract. Not saying that there is an exposure, but we sort of

lose track sometimes with how much is in the upper part of the respiratory tract compared to the lower.

So, if we look at the humans, the solid lines, humans are larger than rats. And that's one of the few things I always think that I'm pretty sure of in my science theory, rats are smaller than humans. So, all of the dimensions are also larger in humans. We have a larger airway, we have a larger nose, the airflow's larger. All of the things are larger.

And so, if you look at the optimal size for the deposition, it's actually -- according to the MPPD simulations -- around ten microns for what gets into the body at all. And then down around the three or four microns, for what's sort of the best size for getting things into the lower parts of the respiratory tract, until you get down again into the very low portions.

So MPPD was a very useful tool for us to sort of scope out this problem. The difficulty we found is this; if you look at for the humans, it lumps everything into this head

compartment. And actually, in the head around ten microns, almost everything is there. It's not until you get into the larger ones where this curve starts to drop off, that you start getting lower and lower fractions deposited. Actually, that fraction deposited is not lower because these larger particles wouldn't deposit in the head, they become lower because it's just very difficult to keep a hundred-micron particle entrained in an air stream long enough to get it into the nose.

That's some of the work that Dr. Flack had shown before, when you're talking about what does a sampler actually measure. If you've got like an OVS sampler, and it's the same dimensions and breathing rates as a human, it has a hard time getting those large particles to even be sort of sucked up into the OVS sampler. That's why these things, as they get so much larger, they're of less concern because it's just so hard to get them into the system with the breath.

To put a couple of numbers around some of the particle sizes that we've seen, using

the MPPD for the rat of the guideline, we get about half of it being deposited in the head, about a percent of the tracheobronchial, and about three percent in the pulmonary, with about half of it being absorbed in total.

One of my other colleagues asked me, well, where does the rest go? It's a combination of back out with the breath; some of the smaller particles stay entrained in the airstream and go back out. Or some of it just never made it into the nose to begin with. So, it's a combination of those two.

For the humans, we get -- for the 35-micron, we get about 35 percent in the head, and fractions of a percent in the lower respiratory tract. And in this case, it's actually, by and large, the larger particles at a 35-micron distribution, you're starting to get a fairly significant population of 50s and 100s and larger things. And they're having a hard time, again, getting in.

Even more extreme, if you had a 100-micron particle, you're only going to get about three and a half percent in, and you're

going to get functionally nothing past the very upper reaches of the respiratory tract.

In order to get a bit more useful description of the upper airway of the nose, the nasal cavity essentially, and down into the larynx, we had to move to a different tool. What we've moved to is a technique called computational fluid dynamics, or CFD is the acronym around it.

It's a tool that, actually, in my days as an undergraduate chemical engineer, we used it in designing reactors and doing modeling of those sort of things. It's very common, in the nice pictures that Dr. Corley sent, for simulating air flows around hard bodies like racecars, airplanes, wind turbines.

It's a very common technique to use. And it basically takes your system and describes it using the Navier-Stokes Equations that describe the flow of a viscous fluid. A viscous fluid, in short, is pretty much any fluid that we have to deal with in a biological or environmental situation. There are non-viscous fluids, but they're not our problems.

So, you can describe any moving fluid using these equations, and it gives you what we call a flow velocity field over space and time. So, at any given point and time, you can describe what's there and where it's going; which then is solved using a 3D computational mesh and boundary conditions. And the boundary conditions are things like shape, fluid characteristics, pressures, and things like that.

As I mentioned, they're used across a variety of sort of hard physical sciences to develop a lot of things without actually having to go and build physical prototypes. The biological community, at some point in the -- I think, they started some of this work even back in the '80s and '90s said, well, that's not that different then what we do. Airflow into a respiratory system is just another viscous fluid, flowing into a defined sinus region. It's also used for -- I've seen simulations in things like aneurysms and other sorts of blood flow things. It's a very common technique that gets used.

Where do you actually get the data though to generate the airways? So, there's been work -- Julie Kimble (phonetic) in North Carolina was one of the pioneers of some of this stuff. They take basically high-resolution MRIs and CTs. And if you've ever seen them from your own medical experiences, basically the images will sort of slice you in the horizontal and then the vertical. And from that, you can sort of look down.

They're kind of cool if you look at the head ones; you go down and start to see the brain appear. And then there's eyes, and tongues, and teeth and all these sorts of things. So, it gives a very good view of what's going on. And from that you also -- this wasn't the original purpose, you can see in the negative space -- you can see the airways.

So, in the good old days, they would sit down with these MRIs and all the computers, and they'd have to manually trace out the airways. And then take each of these, and digitize them, and get a very rough description -- and I'll show you the surface elements in a

minute. It was a very sort of low resolution. Almost if you think about the video games that, at least, people in my generation used to play when we were kids and the little eight-bit guys moving along. It was kind of that analogy that it was a bit crude.

But, now with the advances in both digitization, imaging, computer storage, all of the sorts of things that go into it, the images are remarkably high definition. And the task of creating a representation of the airway is very largely automated. It used to take months, now you can go it in days. And sometime, in the not too distant future, you would probably be able to generate enough of these that you could do almost an individualized model of everybody of concern.

So, once you've got these MRI's and CTs, you take them, image them, segment them. Construct this surface representation there in this sort of purplish color, and then take it down to a representation of the airway, down in the lower end and then run the CFD.

Now one thing to note is that when you get to these CFDs, there's sort of a cylinder

hanging off of the front. These models are what are called stochastic models. The MPPD was deterministic. If you put in a set of conditions, you're always going to get the exact same answer out. The stochastic models, in that cylinder on the front, they introduce a number of particles. And depending on how long you want the simulation, thousands or tens of thousands, the particles are introduced in the airway in that cylinder, and then they go into the breathing zone and are subject to the models of the inhalation.

They are stochastic, so you won't get the exact same fine distribution every time. But that's the whole point of running the large numbers of particles across these, is that with a large enough number, the answers on a sort of more macro scale will be the same every time that you run through them.

A little bit more here on what they've actually done and what the structure of the CFD model looks like. The large gray blob -- for lack of a more scientific word -- in the middle is a representation of a human nasal

cavity. You see on the left side of it, there's a sort of a horizontal grayish-pink surface. That's the nostril. And then you go from left to right down, where the bend to go down the airway is, and then the lower middle airways would be hanging off the bottom right there.

If you look at that A to A slice, it gets magnified on the top right, and again that pinkish-gray color. And that's a negative view of the airway spaces where the tissue is in white and the pinkish-gray is the actual airway. And it would be looking as if the air would be going into the screen; so, you see all the turbinates and the structure of the nose is intact. And given that these are all taken off of individuals, you see that it's not an idealized structure. The left and right turbinates are different, and that's what they are in an individual.

It's a bit difficult to see on the screen here, but that airway is full of -- it reminded me kind of like a bubbly foam if you actually put laundry detergent in your dishwasher -- that you get these discrete elements -- and

this is where the CFD part of this comes in. Every element in the airway is described as a three-dimensional chunk of airway; that they're all polyhedrals that mesh together and describe the entire airway.

You see also, in the gray, on the lower right there, the magnification is that the surface is also covered in a polyhedral representation, to give you the resolution to capture all of the surface features, all of the turbinates, all in the rest of the nasal cavity and the whatever portion, the respiratory tract you're modeling, so that you can actually get a good fine resolution of what this surface looks like. Then you could describe the airflow with your Navier-Stokes in your CFD models.

So, these models are not new to biology. They've been used extensively over the past 20 to 30 years in the assessment of environmental particulates, particularly cigarette smoke, diesel exhaust, bacterial spores. There's been anthrax models that have been done with these.

But generally, they focused on things that hit -- as I showed in the MPPD simulations -- things that hit the sort of sweet spots for inhalation. The inhalation community's been less interested in our sort of ag-chem (phonetic) problem because they look at these larger particles and they're like, that's not very interesting. It isn't going to go into my models, so I don't really care. It's been a different problem.

For those of you in the pharmaceutical realm, the problem is a bit reversed in the optimization of drugs that are delivered by inhalation. And that isn't just anymore sort of drugs for asthma and other respiratory diseases. Inhalation is becoming a very prevalent route for delivering all sorts of drugs, because you can then -- from the alveolar and the lower respiratory tract, you can dump it, essentially, straight into the bloodstream without having to worry about the first pass liver effects or all the issues that come along with needles, and injections, and those sorts of things.

So, it's a technique that's been used quite extensively for inhalation, just not in agrichemicals or chemicals, in general, this far.

So, we went back and said, well, we've got this nice rat study, let's go back and simulate it. We took the CFD model that Rick Corley's group, at PNNL, had already assembled, and ran it for the conditions of the rat study that we had. The body weight of 315 grams from the study, all of the particle characterizations, the density, the tidal volume, everything that was measured, and checked to see what actually wound up being inhaled in this study.

If you notice between here -- so this was our full model of the rat respiratory tract, several branches down into the lungs and the bronchioles. I've cut it off here at the trachea, because if you look at the -- these are percentages deposited in each of these regions.

On the far right, what lit up like -- and since it's the holiday season -- Rudolf's nose, is the dry squamous. This is the reason that we were struggling with the MPPD model.

Because the bulk of the deposition, over half, at least 2.7 micron particles, was deposited in this dry squamous region.

And as Dr. Wolf has been educating me the last couple of days, that the reason that this doesn't make as much difference for the inhalation scenario; is the dry squamous is not, sort of, regular respiratory tissue. It's more like a dermal exposure.

And the things that wind up in the very front, in the dry squamous tissue, are also generally moving out of the body, not things that are deposited in the rest of the nasal cavity, likely to be taken in and either wind up in the respiratory tract. Or as an oral dose, the dry squamous is sort of moving in the other direction.

So, I digress. About half of our exposure mass is deposited in the dry squamous. You see about almost five percent in the wet squamous right behind it. And then, fractions of a percent, down the rest of the upper respiratory tract, and less than that down into the lower parts below the trachea.

A couple of interesting things to note, these are not vapors. So, the typical spot of interest in respiratory dosimetry is the olfactory region of the rats; because it's got that huge surface area with all the respiratory turbinates, much more complex than the humans. There's just an enormous amount of surface area in there. That's where, if you're doing vapor dose imagery, that's where you typically wind up with issues.

Since we're talking about aerosol particles, we've only got .02 percent of these 2.7-microns particles making it all the way through the airway, and then other parts of the airway, up into this olfactory. You see actually a bit more coming down through the respiratory and transitional tissue. About .32 percent in the larynx, which, as we've mentioned before, is actually for the rat, our sort of target site. And then only a very small fraction of a percent making it down into the trachea and beyond that.

These all make sense. And I've kind of taking a note here to make sure that I mention that, if we think about the main modes of

deposition, this kind of makes sense for these particles; that the very small ones tend to be traveling entrained in the airflow, and you get -- diffusion is sort of the main mechanism for these particles to be delivered to the surface.

For the larger ones, you get a lot more of the impaction, interception, and particularly for the very large particles, sedimentation. We'll come back to sedimentation when we get to the humans and the large particles, because it's a very good demonstration of the influence of sedimentation on these particles.

We simulated, then, the rats and the humans at this 2.7-micron particle. Now, remember from the MPPD, that 2.7 was pretty close to the size range that was the optimal for delivering mass into the respiratory tract.

So, you get it fairly spread out. You see most of it up at the front, as we predicted from our wet and dry squamous. But it's kind of fairly well distributed. You see on the left side there, you see the larynx. Again,

that sort of higher number of red particles deposited on the left-hand side.

On the right -- I'll never forget one of my colleagues from college calling it the emu, because I can't unsee it. Is that that's a representation of the upper part of the human respiratory tract. You see for the 2.7-micron particles, these are actually fairly small for humans, and they're fairly well distributed all over the nasal cavity. Some of them had impacted in the back of the throat. And there's a bunch of them around the larynx in the human as well. This would be sort of a typical simulation that someone would have done if we were looking at environmental things, like spores or smoke or particulates of soot, and things like that.

To come back to the rat, quickly -- I apologize for the size of the table here. It's included in your materials. The CFD tends to also generate a copious amount of output, which then takes us little while to filter through.

So, what does this mean? Because the CFD says, at all of these surface elements --

and there's thousands, if not tens of thousands, depending on which model we're talking about -- each of them, at the end of the simulation, has a certain mass that was deposited at each of the elements over the exposure time that we've simulated.

Then, to take each of these surface elements and turn them into something resembling a surface concentration, takes the adjustment that we have to do here. We're modeling the deposition in a single graph and making the assumption then that the rest of the breaths, across the time, have a similar performance; and we modify it by the number of breaths per minute.

So, it's about 36,000 for a six-hour rat exposure. Which gives us a surface concentration of about seven times ten to the minus three milligrams of Chlorothalonil, per square centimeter for our six-hour exposure.

I'm going to tease the in vitro work that Dr. Charlton is going to show soon, that our MucilAir-derived point of departure is also in that seven times ten, to the minus third,

milligrams of Chlorothalonil per square centimeter. Now that is a human endpoint, but as Doug has mentioned, it's a relatively non-specific effect that Chlorothalonil is causing, so we don't expect there to be a huge species difference in the response. So, the fact that these are extremely close in their magnitude, gives us a bit of comfort for the use of these models.

We did go back and there's a bit of, as I mentioned, the CFD is quite complicated in terms of how many surface elements there are and what you actually use as the dose metric.

If we look here, the black bars are, what if you just took the concentration of the particular elements that had deposition? Well, that doesn't actually include all of the neighboring elements. Remember these are stochastic simulations. So, in one simulation, this one particular element might have deposition and his neighbor doesn't. In the next simulation, they could be switched.

So, including all of the elements in a representative slice of the tissue, or an

area of the tissue, in this case like the respiratory transitional, gives us a better estimate of what's actually going on. We took the 75th percentile of that number, just to make sure that we actually had a good conservative representation of what was being deposited.

Let's look at a little more detail of the human's now. So, across the bottom of this slide is a variety of human simulations of 1-, 3-, 5-, 10-, 15-, 20- and 30-microns particles. Now each dot on here -- and I should have said this in the rat simulation we were looking at before. Each dot represents a surface element that has some deposition on it. So, it's a bit like a precipitation map; that wherever you see the higher concentrations, that's where the deposition has occurred.

So, in the one-micron particles, and the three-micron particles, kind of like we showed somewhere in our preliminary work there, they're fairly well distributed. The ones, they are just defusing everywhere. And the threes are pretty well distributed. You can start to see a

little bit more deposition on the bottom of the nasal cavity up there at the top.

When you move into the fives and the tens, you're starting to see those real focuses on certain areas. So, if you're looking at ten-micron, right there in the middle, you're seeing most of the deposition being along the floor of the nasal cavity. Then they hit the bend at the back of the throat and kind of fall down towards the larynx, where they're getting caught up in the complexity of the larynx right there in the middle.

And you see that, sort of, as you're working through the 15 and 20 microns, that you're still getting some around the larynx and some on the floor of the nasal cavity. But you're starting to see more and more captured at the front of the nose, sort of in that vestibule in the dry squamous.

And by the time you get out to 30, not much of it is actually making it past the vestibule. It's getting stuck there, but does make it past, winds up on the floor of the nasal

cavity. And the little bit that gets past gets, kind of, hung up in the larynx.

To put some numbers to the pretty pictures -- and by the way, I would highly recommend, that if you ever get a chance to see some of the movies that they put together of these simulations -- we weren't sure that we would actually be able to make the technology worksite. I skipped them for today, but they've got movies of these, from Dr. Corley's lab, where you can actually see a time series of the particles coming in. And they kind of tumble through the airway. Then you can see the development and the spread of these depositions. It's just fascinating to watch. Well, I find it fascinating. That says more about me, I guess.

So, the regional deposition in the humans. If we put some numbers onto these things, you'll see on the left-hand spot here, again, the vestibule being the highest line here. There's a blowup of the other spots in the upper respiratory tract in the documents that we've prepared.

As you get up to the 15-, 20-, 30-micron particles, it's all, essentially, as we would have expected from the graphical representation being captured in the vestibule, you're seeing smaller amounts in the upper parts of the respiratory tract. The peak exposure of the regional airways being around the 10 to 15 microns -- very consistent with what we were seeing from the MPPD simulations; but again, we needed the resolution in the upper parts of the airway.

The graph on the right-hand side is actually the fraction of the surface area. So, if you think about all of those little elements that the respiratory tract -- the surface was carved up into, what fraction of those actually have any deposition? If you look at the ten-micron ones, if you look down at the larynx, and that sort of light blue color, that's the one where that sticks out that you're getting about 20 percent of those elements would have some deposition, some amount of an exposure, and then sort of decreasing as you get to the larger sets of particles.

In any case, in any of our simulations -- and this is something, again, remember that as you consider the entirety of the sort of respiratory tissue, is that the deposition can be a bit focused; but since it's a stochastic process, and all these surfaces are covered in a liquid interface, that it kind of smooths out these depositions.

We've done a lot of work with the CFD model in terms of trying to answer some of the questions that we were anticipating on. Well, how do you know this model works? How good is it? How dependent is it on the parameters? Because these are quite complex models and take some specialized software to be able to run?

We've stuck to the basic physics of airflow and aerosol transport, which are well understood from the physics that have been established for many years. And then the equations that have been well established for fluid flow.

In the current study, we've done a fair number of validation-type studies to determine whether or not -- you know a mesh

independent study. So, how dependent is the answer that you get on the sort of artifacts of the way the model is constructed? We found that changing the mesh density, and moving things around, didn't change, appreciatively, the answer to what we were showing for the deposition.

We confirmed the conservation of mass flow and energy. It's always good not to violate the laws of physics. And checked a variety of exposure conditions, aerosol sizes. To go back to Dr. Yang's question, from earlier, we have assumed that there is no particle-particle interactions. So, that does allow us to calculate the polydisperse aerosols -- and I'll show that in a minute -- based on the series of monodispersed ones.

I did ask Dr. Corley, during the break. He is on the phone, but I don't know that he'll be able to directly answer questions. That they can, in fact, feed polydisperse distributions into the model; but under the assumptions that we've made so far that the particles are interacting, it would give you the

same answer that you would get from the series of monodispersed simulations.

Again, the biological basis of these models, as I went through before, they are based on the 3D structures of actual individuals. And the physiology is standard literature-based physiology for things like resting body breaths per minute and things like that.

It is also consistent with the published CFD models that predict airflows. There's a few references listed. The deposition results are consistent, as we showed with the rat, and matching up well with what we see from the human in vivo, which Dr. Charlton will show.

These models, I mentioned before, the reactive vapors also went through a similar type of validation exercise, which Dr. Corley and his colleagues published back in 2015. Consistent with the experimental data sets, and consistent with the deterministic MPPD model. So, overall, we feel that we've got a pretty good understanding of what's actually going on in the respiratory tract using these CFD models.

Just to sort of touch base one more time here on the questions of, what are we actually doing with this? I just wanted to show you the rat model one more time. But really, I wanted to come back to the human a little bit.

So, our design in these dosimetry models, since we are focusing on large aerosols, and as the question as Dr. Perron mentioned, there's still some work ongoing to determine exactly what the aerosols look like. But these aren't smokes and bacterial spores and things like that. These are larger particles, larger aerosols.

In these cases, there's not a need to simulate the lower respiratory tract, particularly in the lungs. And that actually has given us, it seemed, an enormous amount of computational time to be able to do that. It allowed us to do some additional simulations in the same amount of time and get a better variety of data.

Now, the other thing that I want to mention with this, is that these simulations are -- the products that we're simulating here

are aqueous suspensions of fairly dilute amount of Chlorothalonil. That's actually the way many agricultural products are used. So, if you had a dilute solution of another agricultural chemical, if you wanted to do a risk assessment with a different chemical, the CFD and deposition work that has been applied here is also applicable to those types of situations; provided that you stay within the bounds of knowing the size of the particles and essentially a unit density solution. It's not something that we're going to have to go back to PNNL, or a lab that has the capability to do CFDs, if we want to change something in this.

When we're looking at these polydisperse distributions, remember that we simulated a range of eight or nine different particle sizes; but all of our real exposure scenarios are going to be polydisperse. There's no such thing in the environment that monodispersed exposure.

Given that we know what the deposition looks like for each of these individual sized particles, we have some

techniques. Dr. Flack will come back, again, in the risk assessment portion and show some applications of how we do this. But there are ways of putting together a polydisperse distribution from our monodispersed simulation.

So, if you were looking at our friend, the 35-micron particle -- you see down in the bottom left, that's the sort of cumulative distribution in the yellowish color, and the point distribution in this sort of typical bell-shaped curve -- is that from what you know about a standard size distribution, you can reconstruct, based on the percentage of each of these monodispersed things, you could put that distribution back together. So, for a 35-micron, you wouldn't need essentially any 1-, 3-, or 5-micron monodispersed; but you can take a significant chunk of the 20- and the 30-micron particles to reconstruct that.

Now we could have gone higher and done 50, 75, and 100; but, since those are all lower deposition, lower availability to even get into the nose, the 30 is at least a sort of protective number that the number would not be --

the deposition exposure would not be higher than that. So, we have a way, from our monodispersed exposures, to be able to put that together.

We're back to our paradigm here, and I hope that I've given you a reasonable overview of the exposure modeling that we've done. I'll turn it to Dr. Charlton here, in a moment, to go through the in vitro testing. But if I could pause here to see if there are any questions or clarifications necessary on the current exposure models.

DR. ROBERT CHAPIN: Clarifying questions.

DR. ROBERT MITKUS: Rob Mitkus. Thanks a lot for a very extensive presentation. I had a question for you just about transparency, just modeling in general. I think, as you alluded to, MPPD software is available publicly. It's free. You know, it would probably be an improvement of the current RDDR software that the agency uses.

You talk about CFD models and different individuals or groups making models. If you look at it from the point of view of the

agency's perspective, in terms of transparency of models; so, if a company wants to come in and propose a particular model, would it be better, do you think, from the agency's perspective to have one particular type of software that they could use and go to each time, as opposed to review a lot of different CFD models that are being produced by various individuals?

DR. PAUL HINDERLITER: Okay, this is Paul Hinderliter, again. You've kind of touched on my day job in PBPK modeling. What's the easiest way to do a model, such that a regulatory agency can do something with it and have some confidence in it?

For CFD, there are a few different software packages. It's always an issue of picking one particular one and then having, for the agency's needs of transparency and accessibility, how do you actually get to that point where they can think this model is reviewable, like BNDS (phonetic) and those sorts of things?

These models aren't necessarily complex; so all of the source code that goes into

the description is available from our colleagues who have developed it. I'm not sure that there's a sort of straightforward simple way. So, in the PBPK models, there's -- depending on how many compartments -- a few dozen differential equations; so, the code is actually fairly concise and easier to review.

For the CFD, there aren't that many equations, they're just repeated for each of the surface elements. You would have to have someone who had a level of ability to review this sort of code. I think I'm going to have to leave it to the agency as to what they would feel about different software packages; but it would obviously be good if there was at least a short list of packages that were applicable for that.

DR. EMILY REINKE: Emily Reinke. Thank you for the very nice presentation. Just a couple of questions about the assumptions that were made in terms of the input. You said you were doing standard lab: about 20 degree Celsius, x percent humidity. Have you thought about -- this kind of goes back to the particle size distribution question too, with the different

humidity and different temperatures and trying to model in a more, I guess, applicable scenario.

DR. PAUL HINDERLITER: The humidity and things like that. So, we're not actually -- in these CFD models, we're not modeling the external environment. We're taking it as a presumption that however this particular aerosol is generated, we have some idea of what it is when it hits the nose.

There are models that, depending on the environment in which the individual finds itself, the air inside the nose can have a different humidity or temperature. Generally, the nose is pretty good at both humidifying and temperature control, and fairly quickly to the nasal ambient. I'm not sure of the right word to use for that.

So, it is possible to have the particles -- generally, they would gain a bit of water, but not necessarily. It is possible to have them grow or shrink, but we do not have that in there.

DR. KATHRYN PAGE: Kathryn Page. I've got a clarification. If you could go back

to slide 38. You mentioned that all liquids that you do with the viscous; so, are you considering water to be viscous in this instance?

DR. PAUL HINDERLITER: Yes.

DR. KATHRYN PAGE: Because that wouldn't meet EPA's definition of a viscous liquid.

DR. PAUL HINDERLITER: Okay, I'm not aware of that definition. In this case, we're considering it to be viscous in terms of there are non-viscous or non-Newtonian fluids that have completely different types of flows. I didn't mean this to be a description of -- if you had a solvent and it might have a slightly different viscosity. In this slide, what we're just talking about was that it's a Newtonian-type fluid that has predictable flow characteristics.

DR. ROBERT CHAPIN: Are you good?

DR. KATHRYN PAGE: Yeah.

DR. ROBERT CHAPIN: Keep on. Cliff, you're next.

DR KATHRYN PAGE: I noticed that in the study you used sedentary calculations, and it was noted that that could be altered to

predict an active situation. Can you describe how that would change, or if there's any data that you guys collected that did look at the adjustments made for activity, as it may apply to some of the uses?

DR. PAUL HINDERLITER: Okay. Yes. And some of the EPA risk assessment scenarios do involve workers actively applying things. And so, the assumptions are that the breathing rates do change.

So, we did do some work -- we, the PNNL group -- did some work to determine what the impact of the airflow actually is on this. And the majority of the difference, based on the different airflows, was not as much in the locations of the deposition; but by having more breaths you would have more mass per time.

So, it was largely just a static adjustment factor. That if you have ten breaths instead of eight breaths over a period of time, that you would have a larger deposition. But it didn't largely change the patterns of the deposition. To a fine number, yes, but on the larger scale, not much.

DR. CLIFFORD WEISEL: Cliff Weisel. So, my question is, after the follow-up is, if I read it correctly, you did nose-only breathing for the CFD model?

DR. PAUL HINDERLITER: That's correct.

DR. CLIFFORD WEISEL: As people move more, exert more, they shift to mouth breathing. Any thoughts of how that might affect -- I know that the CFD models have looked at both of them individually or together; and from what I've seen, they are different.

DR. PAUL HINDERLITER: Yes. So, you can from the mouth-breathing scenarios get a bit different exposure. In the mouth scenarios, kind of like the nasal-exposure scenarios, with these larger particles, you would see the bulk of the deposition being in the mouth and in the back of the throat.

So, it wouldn't change our presumptions that the lower respiratory tract is not the target. You could, if you had a mouth scenario, you could potentially have a target site in the mouth. I don't know that that would

give you much of a different answer than what we're seeing with the larynx, but we have not extensively explored that.

DR. ROBERT CHAPIN: Lisa.

DR. LISA SWEENEY: Lisa Sweeney. We had a solution to the premeeting comments from some of the other people on the same questions as me. A number of us did have questions about the use of the single individual as the model. And hearing that where Corley and his team did do some of these sort of sensitivity analyses, it really would have been nice to have seen that in the package. Because a lot of us had questions about, geez, one-person, particular rate; and rates didn't necessarily match up with scenarios.

I think that's the sort of up-front information that some of us really would like to have seen. Because instead of trying to puzzle them out, well, how did you pick this number? And the question of the oral breathing was also something that was brought up by a couple of people. So, you did the work; it would have been nice if you'd shared it with us up front.

One of my questions was that a lot of mass did hit the early parts of the nose, so it sort of doesn't matter. So, you're saying that the toxicity kind of hangs on the larynx, which was a very small fraction, actually, of the total that was inhaled.

But then we have this sort of missing part of, okay, it didn't get absorbed anywhere in the upper respiratory tract, and it went to the lung, which was a site of toxicity in the rat; so why did you sort of stop in terms of the localized dosimetry calculations at the upper respiratory tract? Why didn't you at least sort of track what was left going into the lung; and see, gee, even though it's a smaller fraction of it, if it's all in the same place and someplace important, why'd you stop there, basically?

DR. PAUL HINDERLITER: Okay, so this is Paul Hinderliter again. From the simulations, there wasn't enough going down into the lower respiratory tract to be worth tracking. For the larger particles, it was essentially zero. It wouldn't have changed our answer very

much. And, Doug, correct me if I'm wrong, there wasn't lung toxicity noted.

DR. DOUG WOLF: There was only at the very highest dose.

DR. PAUL HINDERLITER: Okay.

DR. DOUG WOLF: And it resolved. So, at the low concentration, there wasn't. We were talking, looking at -- trying to relate to no effect levels of the distribution. So there really isn't -- I mean, again, it's the risk, so it's sufficient exposure to cause the hazard. There might be exposure in there, but there's no effect.

DR. LISA SWEENEY: Yeah, well, that's part of the thing that the rat is an obligate nose breather, where the human is not. So, accounting for the nonnegligible portion of the human population, especially at the higher exertion levels, that's going to be doing the mouth breathing; it's like, well, we know exactly how much is going to be lost in the mouth before it gets to the lungs.

So, I think this is a little bit -- it makes sense that it probably doesn't matter,

but you can't say that MucilAir is representative of all these other tissue doses; so, you don't have to go back to the lab to test another tissue type. At least, it's extending the computational. I think, especially for a demonstration chemical, to at least show the math. Because the first time you'd like to be especially cognizant of dotting the i's and crossing the t's.

As much as I'm a fan of doing less animal testing, some of the animal testing has already been done. I'm still a fan of what's called the parallelogram approach; where before you apply the in vitro approach to the human, you see how it works in the rat. I would like to have seen a little bit more of that.

For example, with the in vivo, the computational dosimetry, you see similar per area doses for the -- I think, it was the larynx and the transitional. Did you see effects in the transitional epithelium? So yes, your key tissue is the larynx and you saw relatively high doses computed; but you also saw similar levels computed for transitional. Did you see effects

there? So, yes, you got the top one, but did you see sort of a similar ranking across the other tissue areas?

DR. DOUG WOLF: So again, the focus came to the larynx because that's where we didn't get resolution of the lesion over time. There's no recovery. So, there's was an effect in the upper respiratory tract and the other epithelium in the rat; but once the exposure stopped, it resolved.

But to your point of the different scenarios, yeah, it makes sense. Because, perhaps with this particular chemical model, first pass to get to this point, it was adequate. That's part of the reason you have these broader discussions to expand the problem formulation discussion and say, well, what about these other scenarios?

DR. LISA SWEENEY: Right.

DR. DOUG WOLF: We had discussed -- to your point -- about exertion. And when you think about a person with a backpack sprayer, going through a citrus orchid spraying these products, yeah, there's a lot of exertion. He or

she is breathing harder, and so that could change airflow. And these are all additional iterations of the model.

As Monique mentioned earlier, that's part of this expanded evaluation strategy that we've been discussing within the Crop Life America community, with EPA, and others to say, well, what about all these other scenarios? What additional work needs to be done? What additional modeling needs to be done?

I think Dr. Sweeney, you're absolutely correct on that. And we had considered it, but we kind of focused on the one scenario to get to this point.

DR. LISA SWEENEY: The acute effects are still effects. They're not as much of a concern when you're thinking about replacing the 90-day exposure, you're thinking more about the things that don't resolve. I understand that that's the mode of action that's most relevant to replacing a chronic or sub-chronic test, but for other scenarios that might matter. Thank you for the clarification.

DR. JON HOTCHKISS: Oh, that's great. Jon Hotchkiss. One of the reasons that the guideline studies specify one to four-micron range is so that we don't pre-suppose what the most sensitive site's going to be. It's designed to give a dose to the entire respiratory tract.

I'm just wondering, by selecting 35 microns with a tight GSD, if you're not kind of skewing the results to the upper respiratory tract. That's almost a moderate dispersed aerosol, right? If you look at a realistic aerosol, that had a wider GSD, would you then get any dose to the lower respiratory tract?

DR. PAUL HINDERLITER: Before I talk, I'll try to get back to the slide that I'm thinking of. It's near the end. If you look at what it takes to get to a 35-micron particle with -- or polydisperse distribution with a GSD of 1.5 -- and yes that is a bit tiny. But you see that even at that range, you only have about half of a percent being at 10 microns, and essentially none being smaller than that.

So, if you were to widen that GSD to some larger value, you would bump up that ten,

and then potentially have a contribution from the three- to five-micron particles. But it would be still a -- particularly, the five micron would be a fraction of a percent of the original. So, hypothetically, you could. I don't know how much functional inputs it would have.

DR. JON HOTCHKISS: Jon Hotchkiss. Did I miss it? Did you compare dose per surface area between your rat studies and the CFD modeling in humans?

DR. PAUL HINDERLITER: I did not directly compare it with the numbers, but we did show both the rat and the human numbers. One of the earlier slides, where we did do the 2.7 for both the rat and the human had the most; but I don't think I have the numbers in front of me to show what the relative deposition was.

DR. JON HOTCHKISS: The rat has the disadvantage of that. Their larynx is like the biggest rock in the stream. And so, that's why it keeps on getting hit so hard. That's just life. And that's part of the revised methods for sampling that tissue. It's in those guidelines.

That's why everything looks to be an irritant or it injures the larynx.

DR. JON HOTCHKISS: From the CFD standpoint, it just sticks out and blocks the airflow. So, even things that are well entrained in the airflow just crash into it.

DR. ROBERT CHAPIN: Steve.

DR. STEPHEN GRANT: Steve Grant. Now forgive me if this is a naive question because it's not my area of expertise; but you've done a great job in mapping out initial deposition. But I'm still concerned with the effect of exposure until they're cleared. Is there further evolution of exposure? First of all, there's further exposure if there are multiple exposures, or you simply stopped what happens to the previously deposited area, correct?

DR. PAUL HINDERLITER: So, we don't have clearance in this model. I know that the PNNL group has looked at models that have clearance. You know, either macrophage or mucociliary clearance. But, given that we don't

have that clearance in there -- this is sort of the worst-case scenario.

So, we take all of the mass that's deposited in a certain region and basically multiply that by the number of breaths. So, you don't get any credit for any clearance mechanisms that might actually happen. This is all of the deposited masses still at that sight, and available, for toxicity or whatever other sorts of effects would happen. So, if you were able to build clearance in there, the numbers would actually be lower. There would be less mass left to cause effects.

DR. JAMES BLANDO: I think you may have already answered this. I guess that the argument is that the in vivo model, the two-week animal study that was done, is inferior to the CFD model that you've done. I guess the sort of apples to orange comparison problem that I'm having, with thinking about the CFD and the two-week study, is that you did you did use two different particle sizes.

Is the reason that in the two-week study you used the smaller particle size is

because you were required to that by EPA protocol? So, you could not do a 35 MMAD animal study with -- because otherwise it makes it very hard to kind of compare.

The argument is the in vivo animal study doesn't really tell us anything, and it should just be CFD. It's really hard to compare, then, because it's an apples to orange comparison. So, I guess, that's just a difficulty that I have in sort of evaluating the argument about the CFDs.

DR. PAUL HINDERLITER: Let me see if I can tease a little bit of that out. So, yes, we did use the smaller particles because that is the guideline. So, that is the guideline size and, as Dr. Hotchkiss mentioned, that's designed to give you sort of the optimal deposition, and then exposure in the respiratory tract, to do kind of hazard identification.

Now, if you remember from the -- it showed it most clearly on the MPPD slides, that actually isn't even necessarily -- if you were to do the same study in humans, because humans are larger, if you were to have sort of

the ultimate depositing particle, it would actually be larger in humans, just because of the difference in the physiological size. So, it would actually be at the highest deposition in humans is more like eight to ten microns.

If you were then to take a rat and expose it to a 35-micron particle -- and we did have some abortive thoughts in this direction, that we quickly realized was going to be a disaster to try to do this study. Was that those are very difficult to handle experimentally. Most of the inhalation labs are not designed to generate or measure those types of particles.

And then that's also not the appropriate particle size to expose a rat to because that's the relative size for the human exposure. For the rat, that particle would be even comparatively larger, because that's -- with the scaling down to the rat size, that wouldn't be the relevant particle size.

One thing to remember now, and we've actually clarified this, is that this is not the sort of deposition a solid particle that causes a toxicity because of its nature as a

solid particle. Some of the nanoparticles and things like that deposit, and their toxicity is driven by the fact that they are recognized by the body as a particle and something happens. The macrophages get to them. Or they, in some manner, cause a toxicity due to their physical nature. The toxicity due to Chlorothalonil, in this case, is actually due to a chemical response, the Chlorothalonil molecules interacting with the cells.

It's not the same type of system that you might be thinking of, where the size that's delivered determines the toxicity. The size is the delivery vehicle, which determines how much mass is available. How many molecules of the chemical of interest are available at the site of deposition?

DR. JAMES BLANDO: One final question.

DR. ROBERT CHAPIN: And your name, please, for the record?

DR. JAMES BLANDO: Jim Blando. The particle size test that you have up there in your model, they don't match, if I remember, the

Respicon impactor that you used. How did you come up with those size cuts?

DR. PAUL HINDERLITER: These size cuts were nicely spaced to give us sort of a good sampling across the sizes that we were interested in. We started at one-micron particles, sort of near the lower end of what we had expect it to be, relevant to this exposure scenario. Then we stopped up around 30 microns, because that was where we were really starting to get into the particles, which don't get into the system very well. They are arbitrary decisions, just based on the spacing to give us a good representation of the possible particle space.

DR. CLIFFORD WEISEL: Cliff Weisel. So quick question, I think. We'll see what the answer is. You said that you used 75 percent deposition to be conservative. Could you just clarify, 75 percent of what?

DR. PAUL HINDERLITER: Yeah, if you actually go through the raw data that comes out of the CFD -- so that's not assuming that 75 percent of the particles deposit. If you finish the simulation, the surface of the respiratory

tissue, based on the CFD, has all of those elements; and that is the 75th percentile of the concentration of those.

DR. CLIFF WEISEL: Oh, so it's just -- of what's been deposited you -- instead of taking the average concentration across the area to the 75th percentile --

DR. PAUL HINDERLITER: Correct.

DR. CLIFF WEISEL: -- and that's what you multiplied by the area associated with the -- to get to your total?

DR. PAUL HINDERLITER: Correct.

DR. CLIFF WEISEL: Okay. Thank you.

DR. ROBERT CHAPIN: So, we're running -- this is Bob Chapin -- we're kind of dragging this out. Questions for clarification? Ray, is this for clarification and to help your understanding?

DR. RAYMOND YANG: You have some doubt?

DR. ROBERT CHAPIN: We just need to stay focused on clarifying.

DR. RAYMOND YANG: Ray -- yeah. In earlier studies, by Rick Corley and colleagues, quoted in the report -- this is 2012 and 2015 study. They integrated CFD with the PBPK model. Have you folks talked about doing the same thing? And if so, was it rejected, and for what reason?

DR. PAUL HINDERLITER: I think it is a fascinating idea and I would love to do it. But for the mode of action that we've shown so far, these are presumed to be direct-acting compounds on the tissue on which they are deposited. We didn't think there was enough of a benefit from trying to describe -- from a PBPK standpoint, trying to describe the kinetics of what the deposited material is actually doing.

Kind of like the same thing that we, in theory, could have done some clearance calculations, but we didn't think it would materially change the answer that we had done, and would add quite a bit to the complexity in what we were doing.

DR. RAYMOND YANG: Quick follow-up clarification. You and Dr. Wolf use the term

direct-acting. Whenever I hear this, I'm thinking about reactive species. Does this chemical create reactive species? And is there a possibility of adduct formation?

DR. PAUL HINDERLITER: I think I'd like to let Dr. Wolf handle that one.

DR. DOUG WOLF: There's no evidence of adduct formation. At least in the fungus, it's an oxidative -- it inhibits glutathione mechanisms, so it would cause alterations and oxidative stress. It would be similar in any cell system, because, again, you have these chlorines that come off with hydrolysis. So, it would be similar to a lot of other potent cytotoxic chemicals.

I suppose, if it got far enough into the cell, it's possible. But these are very direct-acting toxicants, chloroform, carbon tet., those types of things; so, getting to the DNA's unlikely. The only tumors you see with Chlorothalonil are in the kidney, and it's, again, a cytotoxic mode of action in the kidney as well.

DR. RAYMOND YANG: Thank you.

DR. ROBERT CHAPIN: Okay, I want to get to Alex's -- Dr. Charlton's presentation; but I know that the tailbone is connected to the head bone, and I want to make sure we're all awake for it. So, I'm going to give us -- I'm going to watch my watch -- I'm going to give us 60 seconds to stand up, get the blood moving and then we'll sit back down and start again. Okay. Sixty seconds.

DR. ROBERT CHAPIN: All right, that's our 60 seconds. Dr. Charlton, you're up. We're online.

SYNGENTA - CHARLTON

DR. ALEX CHARLTON: Hello, I'm Alex Charlton. I'm going to be talking about the in vitro component of this work. I'm going to be focusing on the three key areas: the model itself and the endpoints we're using; some of the historic work we've done, and I'm trying to explore these endpoints and what they mean in a biological setting; and the study that we conducted for the Chlorothalonil itself.

The method we've used here, as

we've said a few times in this presentation so far is the MucilAir model. Mucilair is a 3D organotypic model of the human respiratory epithelium.

The model itself is derived from primary cells, taken from human volunteers, by a company called Epithelix who make and sell the model. Essentially, what they're doing is they're taking these cells, they are freezing them down when they first get them. And then in order to construct the tissues for use they are unfreezing, allowing the tissues to differentiate. And then when the tissues are fully differentiated, they are then shipping those to a contract research organization for our use.

For those on the phone, I'm trying to laser point. At the top, this is not quite as clear as I was hoping it was going to be, but on the top left, we're trying to show how we -- so on the top right, you can see the tissues itself. This is how they are shipped. So, in a 24-well plate into a tissue culture insert. It's not as quite as clear as I was hoping it was going to

be, but, essentially, the tissue themselves are cultured in the air-liquid interface.

So, the top of the MucilAir tissue is exposed to the air with an incubator, and the bottom of it is submerged within the culture medium. And they take their nutrients up through the base of the membrane just like a respiratory epithelia tissue would.

So, the tissue itself -- and I'm going to get my left right this time. We're going to start with the figure on the bottom left here. This is a histological section of the MucilAir tissue that's been taken. Now, it's a pseudostratified columnar epithelium, which is fairly familiar, I think, to most people who are used to seeing histological sections of the respiratory tract.

We can see these darker stained cells. These are goblet cells. They're stained slightly darker, obviously, because they contain some mucus. You can see across the bottom of the tissue. At the bottom of the construct, there are these basal epithelial cells sticking onto the plastic insert that’s taking the place of the

baseline membrane.

Just visible on this figure are the cilia at the very top, which you can just about see. The two electron micrographs, that are above that, are top-down views onto the cilia themselves. It's unfortunate they are static images, because it's quite impressive to see these things live because they waft. So, they are doing what cilia should be doing. They are functional cilia of the heart beating as a cilia should.

As we've talked about a bit today, we're primarily concerned with trying to model endpoints that are related to the Chlorothalonil mode of actions, this direct acting toxicant. We've used 3N.7. Obviously, you can scale that in and out as you need to.

So, we're looking at transepithelial electrical resistance, which, I think, most people who are familiar with assays, looking at cytotoxicity and irritancy, are familiar with. So, an intact tissue with good tight junctions between the cells acts as an electrical barrier. Whereas a tissue that's

starting to break down and start to lose cohesion, loses that electrical resistance as such, and you can pick that up with an electrical probe.

We're also looking at LDH release about the agent enzyme that's supposed to -- that's contained within most cells. As you start to damage the cell membrane, you start to get leakage of LDH from the cells into the tissue culture medium; and that's, again, something we can pick up.

The third endpoint is a fluorescent dye. This is oxidatively reduced in the presence of functional mitochondria. So, everyone, say familiar with the MTT assay, for example, this is exactly the same thing. So, a colorimetric and fluorescent change as a result of oxidative reduction of the dye.

All three endpoints here are measuring slightly different parameters. LDH is really the only thing that's measuring direct cell death. Everything else is measuring kind of secondary parameters that are precursors in many ways to that cell.

We have three mutually supporting endpoints, all look at different but related parameters. With those three parameters, we're quite confident that we're picking up any cell death that's going on within that system.

Before I start to get into the data here, that we've done for this data call in, I'm going to take us back in time to a year or two before we started working on the project that we're presenting here today. This is some work that was done for slightly different purposes within Syngenta. I think those of us who work in industry might be familiar with this kind of scenario.

Someone from the business came to us and said, we like Chlorothalonil. We'd like to think about a product that would enable us to keep its biological functionality, but would reduce its acute inhalation toxicity, something more marketable. We said, okay, fair enough, let's start to explore that.

But, obviously, when you're talking about formulation development from the very beginnings, it's not really practical to

start saying, well, we'll use the acute inhalation study in vivo, and set a marker for how well we're doing. So, as part of that, we started to try and validate an in vitro model, and the model that we selected was MucilAir, for the reasons we spoke to earlier in this presentation.

In essence, the technology we were exploring here was encapsulating the Chlorothalonil, reducing its bioavailability, and thus reduce its effect on the respiratory membranes when inhaled. So, it reduces its toxicity through that mechanism. The goal here was to try and reduce its acute lethality. We're often worry about histological lesions that we see in other studies with Chlorothalonil.

You can see here, we've used, essentially, three different levels of encapsulation for our Chlorothalonil. We have no encapsulation, which is this blue line. So, the transepithelial electrical resistance is fine, and then you reach a certain threshold and it falls off a cliff. And essentially, you go from a point -- from a dose level where everything's

fine to a dose level where everything's basically dead.

And then, as you move up through these levels of encapsulation, from low encapsulation to medium to high, you start to see a change in the response profile. So, a little bit of encapsulation softens that initial drop. And then as you go up through the levels, you start to see a reduction to the point where the very highest level of encapsulation gave us a result that was actually no different from a formulation that just didn't contain any Chlorothalonil. So, that's where that blank formulation is.

What we did then was to try to relate that to what we see in vivo, in short-term studies with these formulations. You can see that with no encapsulation, at a one mg per liter concentration, more than 50 percent, again, died at that level. And those that died had a fairly severe clinical observations, consistent with respiratory irritation. So, we're talking here things like wheezing, and labored respiration.

As you start to go upwards through

the levels of encapsulation, you see less and less lethality. It's almost entirely -- so it's completely gone by the time you get to a medium level encapsulation, and you see less and less of the respiratory irritation, in clinical observations, as we go up through our levels of encapsulation.

So, we were really excited by this, because this seemed to show us that the MucilAir model we're using is predicting the outcome of our short-term studies. Which is exactly what we wanted, to be able to try and guide formulation development without having to rely heavily on excessive animal testing. Next slide.

This is some other data we've been generating as part of a similar project. This is not Chlorothalonil this is a different active ingredient. What this was, was an exploration of how our transepithelial electrical resistance endpoint matched against a microscopic evaluation of the tissue. What would a pathologist see at the various levels of disruption of the transepithelial electrical resistance?

You can see for our two test items, we explored a fairly broad concentration range; and as you get towards the top end of that range, you start to see a fairly marked drop-off of electrical resistance indicating that something's disrupting the model. Then, in the bottom table, you can see that -- sorry, I should say, the scores given in the bottom table, these are scores assigned by the pathologist, they're very standard one to five classifications. So, it runs from a very mild observations up to a severe observation.

This is the pathologist's microscopic evaluation of the tissue disruption and tissue degradation. You can see it that actually it matches very, very well. So, you see almost nothing as you go up through the concentration levels, until you get to the very highest two levels. Where the TEER is, you actually start to see severely significant disruption of the membrane. And then that's exactly what you see microscopically as well. I've given two -- which I think are just about visible here. So, the two examples of what the

pathologist was recorded as being mild disruption and fairly marked disruption there.

So, again, we were quite excited by this. We were happy that the transepithelial electrical resistance measure we were making here, was correlating quite well with what you might see microscopically if you looked at these tissues.

The work that we've been doing with MucilAir historically was trying to, essentially, rank formulations; trying to say, well, if we're going to take one of these concepts through development, which would it be and why?

As part of that, we ended up using quite a wide dose spacing. And as you saw earlier, we saw a fairly binary response as something goes from fine at one concentration to complete dead at the next concentration. Which is not exactly what you want, if you want to try and come up with a point of departure for the risk assessment.

But we did have quite a lot of data and we were determined that there was a use

for this. So, we contracted RTI, who are statistical consultancy, to take that data and essentially to look for where we started to see that drop-off. So, recognizing there's a binary drop-off, where did it actually happen? With the idea that we would use those values to try to produce a study to specifically answer the EPA's question? And now we know our concentration range so we're looking exactly where we expect to see something interesting happen.

There's about 15, I think, 10 to 15 studies that went into RTI statistical analysis, as we've been using the MucilAir model for quite a while at this point. RTI said that, if you look for the point of departure, you often see that between two and four milligrams of Chlorothalonil per liter. Also they looked at its insensitivity analysis to try and give us an indication of a replica number we would need, in order to be confident that we see a confidence analysis in the study.

So, historically we'd be using four replicates per concentration. They looked at that, and they said that four was probably not

enough; six is a good level. If you go beyond six, you get a little bit more confidence, but not very much more. It's really not worth the extra effort to take it to eight when six is perfectly good.

All of this went into our study design. We used the same endpoints that we've discussed previously. We used five MucilAir tissues derived from five sets of donors. Now, not going to say that this fully encompasses all the variability that sits within in the human population, but it was done. We used several different doses to try and give us an idea of what that variation might look like.

We used a 24-hour topical exposure, so that's a lot longer than a human -- I'm sorry -- the rat studies we've done earlier, which went up to about six hours, and obviously far exceeds a normal human workday. We did that to try and maximize our ability to see a hazard endpoint in our in vitro system.

The Chlorothalonil was applied as the Bravo 720 formulation, which is also called Weather Stik, and it's the subject of the data

call in. We used ten concentrations per donor. You can see here between the 2 and 200th range, the milligrams per liter range recommended by RTI. We used six concentrations per donor, again, as recommended by RTI.

I'm just going to give a few example output plots. This is transepithelial electric resistance from the first of our donors. You can see here, obviously, you've got a good few concentration levels where not very much interesting happens. And then once you start to get towards the top end of that curve, you pass that threshold. You start to see this drop-off in electrical resistance, indicating a tissue has become disrupted. You can see on that plot of the BMD, the BMDL values, which were calculated by RTI using the standard methodology.

You can also see the data here has been fitted to a hill plot. And that, just by eye, looks quite good, and that its statistical measures look for plot fitness, which also indicate that's a good model fit.

This is the LDH data from our first donor. You can see, again, very similar to

the TEER, nothing particularly interesting happens with the first few concentrations. And, again, you exceed this threshold and you start to see this rapid increase in the output of LDH, into the tissue culture medium.

Again, something very, very similar to the resazurin metabolism: not very much happens. You pass the threshold and then you see a fairly rapid drop-off as the cells start to die.

I've plotted out all of the endpoint data in the table below. But I think, to me, it was quite encouraging. That when you look across donors, of course, endpoints across donors, while there's some differences in donor sensitivity to Chlorothalonil, there's nothing particularly pronounced going on here. The biggest difference here is probably just under the two-fold difference.

Very similarly, if you look across the transepithelial electrical resistance, the LDH, and the resazurin, these endpoints are very, very close to each other, indeed, across donors. As a result of that, we think taking this overall

geometric mean of 0.0073, which is what Paul was presenting earlier, is the (inaudible) for Chlorothalonil, clear with the in vitro system.

In conclusion, given our understanding, given our view of the direct-acting effects of Chlorothalonil, we were quite confident that this is something we could model in vitro. We designed a study on the basis of historical data that we had to try and maximize our ability to pick up the point of departure and robustly analyze it.

When we saw the output of that study, there was good concordance across endpoints, good concordance across the elements. We can derive the in vitro benchmark dose level of 0.0073 milligrams per centimeter squared of epithelial tissue.

So, I think it's a good point to pause and ask any questions.

DR. ROBERT CHAPIN: Questions for clarification? George is positively quivering. We'll let him go first.

DR. GEORGE CORCORAN: This is just a clarification. I was amused perhaps or, at

least, I couldn't understand some of the LDH data, and the LDH release of values, were more than 100 percent of maximum. Some values were as high as 230 or 250 percent of maximum. And I just didn't understand that.

DR. ALEX CHARLTON: Okay. LDH values are calculated against a positive control compound. So, where you can see the 100 percent of the supposed maximum -- is not actually a maximum, it's 200 percent of the positive controls. So, that's how that happens. Essentially, greater LDH release than the positive control.

DR. ROBERT CHAPIN: So, it's not just a little dead, it’s really, really dead.

DR. GEORGE CORCORAN: You (inaudible) the cells to get maximum release, right? You treat it with a detergent.

DR. ALEX CHARLTON: Yes, I would treat it with -- I'm trying to remember what detergent, I think SDS.

DR. GEORGE CORCORAN: I just don't understand how you can get more LDH release, and presence of Chlorothalonil, than you can when you

essentially lyse the cells with a detergent.

DR. ALEX CHARLTON: Well, I'd need to go back into the data; but I think what may have happened there is there's potentially been incomplete lysis, which is why we end up with a maximum that's actually perhaps less than the true maximum.

DR. GEORGE CORCORAN: And the second point of clarification is, resazurin is used actually in two different assays. One is a coupled LDH assay with diaphorase. And you use the same reagent to look at the reductive capacity, and therefore the vitality of cells. Is that as you understand it?

DR. ALEX CHARLTON: I'm really only familiar with the second use.

DR. GEORGE CORCORAN: Thank you.

DR. ROBERT MITKUS: Rob Mitkus. I enjoyed your presentation, and I particularly enjoy your historical perspective. I think that's sometimes lost on non-industry folks. Some folks might think, hey, one day I wake up and I'm going to do a MusilAir study, but no, there's the whole business model and the

procedure.

As you're probably aware, big tobacco's undergoing -- or performing harm reduction, and looking at in vitro models to reduce harm and develop products. Is MucilAir used by other companies that you're aware of in the tobacco industry? Or SmallAir, or any other types of models?

DR. ALEX CHARLTON: I know they're certainly used by other companies. I'm not sure whether it's used by big tobacco companies. I'm sorry. I don't know if it's used by big tobacco companies.

DR. ROBERT MITKUS: Even though they're other industries besides tobacco?

DR. ALEX CHARLTON: I believe that the laboratory that runs our MucilAir studies also runs other pharmaceutical clients.

DR. ROBERT MITKUS: Okay. Thanks.

DR. ROBERT CHAPIN: That may be better for the next presenter, who's going to be talking about the model. Kathryn.

DR. KATHRYN PAGE: Yeah, great presentation. It seems like you've done lots of

work to support TEER, with new phenotypes. What are the other endpoints that have been assessed; particularly, if you can talk about the variation that you see with res-, I can't say that word?

DR. ROBERT CHAPIN: Resazurin.

DR. ALEX CHARLTON: I was worried about who was going to try to say it.

DR. KATHRYN PAGE: In order to see an effect, you have to combine the lower doses with the control, in order to produce a significant difference. There's two parts.

DR. ALEX CHARLTON: I think the first part is that, generally, when we're presenting the data here, you tend to use the TEER because TEER correlates quite well with LDH and resazurin. It's the endpoint that we tend to put the most faith in; but the whole point of using the three different endpoints, is that when one starts to vary a little bit, we tend to use the other two to try to interrogate that and figure out what's going on.

TEER is the one that tends to give us difficultly to interpret the results the least. So, it just tends to be what we use for a

comparator. And so, your second question?

DR. KATHRYN PAGE: Specifically, about -- I think you talked about it a little bit just now. About the variation that you see with the other endpoints that you looked at. Specifically, where you have to combine all the lower doses with the control to produce significant difference at the higher two concentrations.

DR. ALEX CHARLTON: So that's the resazurin data, isn't it? Yeah. So resazurin can be sometimes problematic at the low end of the dose-response curve. And the reason for that, we think, is that a very small amount of resazurin results in the cells having to slightly upregulate their metabolic rate in order to try to clear the stuff, clear the Chlorothalonil. So, we end up with the low concentrations, apparently showing an improved level of health relative to the negative control; which is why we had to put everything together like that.

DR. KATHRYN PAGE: Is that typically done?

DR. ROBERT CHAPIN: That's a

common response. That sort of U-shaped kind of dose response.

DR. KATHRYN PAGE: No, I understand that. I'm just saying that I personally have not seen, when you're looking at dose response, combining a lot of low doses of your chemical into the control, in order to show that you got a response at high doses.

DR. ALEX CHARLTON: Yes, I see. So, we had processed that data of a few different ways. That was our initial way of looking at it. Subsequent to that, we had some conversations with EPA about how that data gets processed. We've adopted -- and essentially, we did a more direct comparison against the control.

DR. ROBERT CHAPIN: Cliff.

DR. CLIFFORD WEISEL: Cliff Weisel. I appreciate what you ended up saying you did was a 24-hour exposure. One of the things I'm trying to do is understand chronic, sub-chronic, repeated exposures. Does the MucilAir model have any recovery, if you were going say put a dose on it, and then put another one so the cell would somehow revitalize as you

would in a human system?

DR. ALEX CHARLTON: We've made some attempts to explore that, but we have not fully got into it. As part of this formulation development work, we were trying to explore the idea of repeated dosing; giving a dose of one formulation, taking it away, and then giving a second different formulation, as a dose, of a specific question we were trying to answer. I think you do see some degree of recovery after you've administered the dose and taken it away; but you don't see a full recovery within 24 hours.

DR. RAYMOND YANG: Ray Yang. I'm particularly interested in this T-E-E-R that's transepithelial electric resistance, right?

DR. ALEX CHARLTON: That's right.

DR. RAYMOND YANG: Please educate me a little bit. What is the electricity doing here? How is this correlated with cell deaths?

DR. ALEX CHARLTON: Okay, so the intact MucilAir constructs have very tight junctions between the cells; and as a result, they tend to impose a reasonable degree of

electrical resistance on. So, TEER, the way you measure it is you take a probe, apply it to the top surface of the cells, and then a second electrode into the culture media. Essentially, you are monitoring electrical resistance across the tissue construct.

As the construct starts to lose its cohesion, because the cells are starting to lose their viability and starting to die, that electrical resistance drops; and that's what the TEER is measuring.

DR. RAYMOND YANG: These cells are from a piece of tissue. Before the cells are dissociated, are the electrical resistance of a piece of tissue is different from the cell and cell? In other words, in your in vitro system, do you retain the original electrical resistance of the tissue, which are multicellular?

DR. ALEX CHARLTON: Retaining the electrical resistance of the tissue, in comparison to the actual resistance might look like, in an in vivo situation?

DR. RAYMOND YANG: In vivo situation.

DR. ALEX CHARLTON: We've not done that comparison.

DR. RAYMOND YANG: Thank you.

DR. ROBERT CHAPIN: Marie's been apparently very, quietly, desperate to ask a question. So, we'll just let her go first and then you're up next.

DR. MARIE FORTIN: Thank you.

DR. ROBERT CHAPIN: And I'll remind everybody that sort of doing this the way Katheryn has done, it has been really useful. Sorry. Dr. Fortin.

DR. MARIE FORTIN: All right, so, Marie Fortin. I just want to clarify, I guess, the approach. I've always seen benchmark modeling done with animal data. What they have is the data, at each dose, for the group of animals and the variance for the specific endpoint. I just want to make sure that I've captured, summarize what you did.

So you did the benchmark dose modeling, within donor, so, per individual with different replicates. Then you did the geometric mean of all the donors, then the geometric mean

across.

DR. ALEX CHARLTON: Yeah. Well, in perhaps a more conventional way of conducting benchmark dose modeling, you would be using animal groups for your modeling. Obviously, we used cell populations here, so replicates from each donor. Then we looked at geometric mean across donors and geometric mean across endpoints.

DR. MARIE FORTIN: But really they're replicated, right, just they're some same donor. So, each will really represent the same individual?

DR. ALEX CHARLTON: Yes, yeah.

DR. MARIE FORTIN: Okay. I'm sure you've seen the Civar (phonetic) et al. paper 2018. They use this very specific model. And they calculated the method detection limit. I was wondering if you guys have done that as well?

DR. ALEX CHARLTON: I don't think we have, no.

DR. MARIE FORTIN: Okay. That would have been interesting looking at that. Because basically, what they did is they used the

exact same endpoint, so TEER/LDH and the resazurin. I got that one. And they applied a bunch of different toxicants to this very specific model and they calculated the method detection limit, which is a way of looking at the viability of your assay and detecting -- and analyzing what threshold that you can detect within your assay system.

In that context, what you've done is you look at the viability and use, basically, one as the -- and the BMDL in the benchmark dose modeling as what you see as a threshold for response. But it's unclear whether or not -- what's the relevancy of that in terms of actual response.

The flip side to that is that, obviously, phytotoxicity is a very overt response. But the system that is used and those endpoints are not very sensitive. So, you need to create a lot of damage to that specific plate to able to pick up anything with that system.

DR. ALEX CHARLTON: Yeah, so this is about the biological relevance of the single standard deviation benchmark dose response. This

is a conversation we had with the agency when we generated our data. Through that discussion, we agreed that we would use their standard calculation for benchmark dose response.

DR. MARIE FORTIN: Okay. I guess, to follow-up for that is, did you consider using another type of assay with respect to viability like the lysis assay, for example. And backtrack the value to assess what level's damage is really occurring in the cells.

DR. ALEX CHARLTON: We didn't, no. We didn't do that.

DR. MARIE FORTIN: Thank you. Okay, one last point. So, you did the geometric mean for LDH, TEER, and resazurin. In the Civar et al. paper 2018, it specifically says that LDH is not very sensitive in that specific model. It may be different from one toxicant to another. Have you considered, that by doing the geometric mean, you're, basically, not taking the most sensitive endpoint?

DR. ALEX CHARLTON: Okay. I think one of the uses of taking multiple endpoints, in the way that we did, is to look at the endpoint

sensitivity. So, if we had done that and seen that the LDH was considered to be less sensitive than TEER or resazurin, then we could have a conversation about whether it would make sense, to set those risk assessment endpoints on the basis of the LDH dose, rather than to try and generate an overall mean.

Like I said, the endpoints actually kind of sat on top of each other, across TEER, LDH, and resazurin. So, we didn't see any evidence the LDH wasn't particularly sensitive relative to the other two measures.

DR. MARIE FORTIN: In your table, they should be actually significantly higher than the other two measurements. So, by using BMDL -- I apologize. By using the geometric mean, you're skewing the result and the endpoint you use for POD?

DR. ROBERT CHAPIN: Alex, can you go back to that table?

DR. ALEX CHARLTON: Yeah. When we looked at these data, we thought that there was a really good degree of concordance between TEER, LDH, and resazurin. We didn't think anything was

clearly more sensitive than anything else.

DR. ROBERT CHAPIN: Or less, which is her point. That it's less sensitive. Is that what you're saying?

DR. ALEX CHARLTON: More sensitive. Everything seems to sit on top of each other.

DR. ROBERT CHAPIN: Right. Holger?

DR. HOLGER BEHRSING: Holger Behrsing. So first, I had just a quick comment about the percent of LDH and how you can have more than the control. I suppose it's possible that some tissues may have greater biomass than others. And that's something that maybe Song Haung, when he's up here next, I can address. The question I have, is so you had a topical application of the material; and I mentioned that with the LDH release, that was done from a basal-lateral medium? Is that right?

DR. ALEX CHARLTON: That's right, yes.

DR. HOLGER BEHRSING: Wouldn't it make sense to have an assessment of LDH at the

site of exposure since, Dr. Wolf, he mentioned it a direct cytotoxic event when the material actually touches the cells. In this case, it actually touches the mucus layer, right?

DR. ALEX CHARLTON: Mm-hmm.

DR. HOLGER BEHRSING: It's the first site of the exposure; and then that mixture is what then exposes the cells. So, without an apical rinse, you wouldn't know how much LDH was there. And LDH being a release marker, it wouldn't necessarily be free to diffuse through all of the other cell layers that are beneath it, getting to the basal-lateral medium?

DR. ALEX CHARLTON: Yeah, I see. Yes, within this, there's an assumption the LDH release ends up in the basal-lateral part of it. We've not specifically tested that hypothesis. I think it's potentially something worth exploring in the future.

But, I guess, I'll bring it back to the endpoint data for the other -- for the resazurin and for the TEER. With everything kind of sitting on top of each other, we weren't too worried that we may have underestimated the LDH

release.

DR. HOLGER BEHRSING: Thank you.

DR. ROBERT CHAPIN: George.

DR. GEORGE CORCORAN: George Corcoran, Wayne State. This is a beautiful model. I think, everyone has the hope that it will reach the full potential that it has.

When you get maximum TEER disruption -- I'm looking at a photomicrograph on one of these slides that shows the destructive degradation score of four, which would seem to be almost maximum destruction and degradation.

But as I look at that -- and maybe Dr. Wolf might want to comment on this -- it would seem to me, if you were getting a hundred percent release of LDH, you'd get denuding of these ciliated cells completely, and severe damage to the more interior location cells. Just looking at the photomicrograph, to the right, I'm having a hard time making that connection.

DR. ALEX CHARLTON: Yeah. These images are taken from a different chemical, this is not Chlorothalonil. This is a set for other chemical --

DR. GEORGE CORCORAN: I would say that it should be chemical independent. If you're losing 90 percent of your TEER, that's an invocation that you're having 90 percent cell death.

DR. ROBERT CHAPIN: No, just cell separation.

DR. GEORGE CORCORAN: Oh, separation.

DR. ROBERT CHAPIN: Right?

DR. ALEX CHARLTON: Mm-hmm.

DR. GEORGE CORCORAN: Yeah. Okay. And then if you're going to -- well, let's go to the LDH then. That's why these are parallel but different measurements. If you're getting release of all your LDH, it would imply to me that virtually all cells are lysed?

DR. ALEX CHARLTON: Yes.

DR. GEORGE CORCORAN: I think about necrosis as the big leak. It's the big bang when the cell is alive and then all of a sudden it no longer has integrity.

DR. STEPHEN GRANT: Stephen Grant. Just to follow up on that, that looks like a

disorganization of the tissue, to me. And certainly, it would cause disruption of gap junctions or tight junctions. Clearly, I think that you can see that sublethal effects in this model are still going to allow TEER to happen; which is you're disrupting rather than destroying.

And there's going to be a bunch of chemicals. One of the things that's going to come up, later on, is that some people saw some sex effects in the Chlorothalonil live stuff. And the question would be, is this sex dependent, and would there be hormones having effect? And that might be something that affect the integrity of the tissue as opposed to killing it.

DR. ROBERT CHAPIN: This is Bob Chapin. I'm thinking that these two issues would be good to bring up with the next presenter, who's going to present specifically on this model; not the use of it, but the model itself, the model's construct and interpretation.

DR. GEORGE CORCORAN: Thank you, Dr. Chapin.

DR. ROBERT CHAPIN: Thank you,

George. Jon.

DR. JON HOTCHKISS: Jon Hotchkiss. What I'm going to ask you shouldn't be taken as me not liking this model, because I'm doing exactly the same thing. I'll be honest here. But these questions are what keep me up at night. And so, I was wondering why you chose not to include histopathology in this, in order to correlate the restructuring of the tissue with your measured values? I'm going to rattle them off here.

And then why on a single exposure, when what you really want to do is model a repeat exposure, in place of a subacute or sub-chronic study? And why no recovery? Because you don't know what the biologic significance say of your TEER value is. Do you have a bottom-line threshold, that you say, okay, it's below 100? It's toast. It'll never come back.

What we actually see is with recovery, TEER can shoot up way higher than it used to be. But if it was tight before, it's super tight now. And that has to do with the metaplastic response that we see with that

epithelium.

Dose rate. Okay. For a direct-acting material like this, I can say, so maybe dose rate doesn't make that big of a deal. But you're putting on, in a plot, all your dose; and so, the cells are instantaneously seeing that entire dose. Whereas, if you're applying it as an aerosol, it's like pitter-patter of raindrops.

And so, if you have any adaptive mechanism, whether it's upregulation of TSH, or mucus clearance or something like that, I'm just wondering if that can impact the dose response that you're seeing? These are all questions that I just don't know the answer to.

DR. ALEX CHARLTON: Well, I have thoughts. I'm not going to tell you that I know the answer. I think that maybe part of that is the exposure systems to this idea of topical application, versus aerosolized application. We've had conversations about this when we were setting this up originally.

I think the view was that, once the MucilAir construct themselves look like they should be capable of clearing some event --

they've got the kind of muco -- they've got the ciliary component to it. There's not actually anywhere for applied material to go. So, any kind of apparent clearance is not really being cleared. All it's doing is being shifted around within that tissue culture insert.

I mean, when we talked about this kind of aerosolization or applying it that way -- obviously, this is inhaled material. I think the concern we came back with, was one around dosimetry.

So, you can generate an atmosphere within the box, and then then allow that material to gravitate and settle onto a tissue culture construct. Or you can direct an airflow onto the tissue culture construct. But by doing so, you're adding a degree of randomness into your exposer system. With a topical application, we know exactly what's going onto that construct.

DR. JON HOTCHKISS: Or you might say that you're more realistically modeling the in vivo condition of an inhaled aerosol.

DR. ROBERT CHAPIN: A lot of the specifics about this might be what we can save

for Song Huang, who's going to be next. Allison?

DR. ALLISON JENKINS: Can you speak about the five donors, and any characteristics of donors that may make a difference? Then also, you mentioned that only the nasal tissue model was available. And so, any differences you would expect if the other models were available?

DR. ALEX CHARLTON: Maybe we can start with the nasal tissue model. When we talk about respiratory epithelium, we're talking about respiratory epithelium, you know, where in the respiratory tract the epithelium is actually coming from. It's the same sort of stratified epithelium, the same cilia, the same goblet cells, the same basal stem cells.

So, when we were talking about this, our view was, what's the dose? Was the MucilAir construct we used, did it actually originate from the nasal region of the human donors? The tissue that's produced as a result is the same respiratory epithelial tissue that's throughout the respiratory tract. I'm sorry. What was your --

DR. ALLISON JENKINS: About the five donors and their --

DR. ALEX CHARLTON: So, we don't have hugely detailed information about the donors, so it's not -- and we have only got a relatively small number of them. So, in terms of picking out what's important in driving a particular response, it's not very clear around that.

DR. ALLISON JENKINS: So, just from other studies you've done, no difference?

DR. ALEX CHARLTON: No. We've never -- you do see some degree of donor basal level response. But what you often don't see is a huge difference in the point at which you get that kind of infraction between tissue that's perfectly healthy and tissue that's largely destructed. Of course, that's where the majority of our data comes from, is those kinds of very widely dose spaced, quite binary responses.

So, yeah. The basal TEER, for example, does vary a little bit; but the point of infraction tends to stay very static.

DR. SONYA SOBRIAN: Sonya Sobrian.

I'd like to follow up on the donors. I noticed that of the five donors, there were three females around the age of 45, and there were two males at different -- one was 50 and one was 71. None of the discussion talks about the differences in gender, or the possible changes you might see in the aging organism. Can you address those?

DR. ALEX CHARLTON: I think the reason we've not discussed that is we didn't feel there was enough data here to form the basis of a discussion. Three females, two males, some older donors, some younger donors; there wasn't a huge amount of replica within those particular populations to enable us to be confident in anything we would say there.

DR. SONYA SOBRIAN: Not so much just the -- but the idea that those variables might impact what you're looking at. I'm going to sort of go back to the earlier discussion.

On your first slide, 13, you had both males and females in the two-week toxicity test. In slide 15, you just had recovery data, but you didn't indicate if that was from males or females. I think in some of the writeup it said

that females were more sensitive in some of the animal studies. And in some of the others, you said, males were more sensitive.

It's just an issue that was sort of glossed over. And it might be important to at least discuss in further studies, especially the age. Because if you look at slide 65, you see that donor 5 has -- it's really -- I don't know if it's significant, because I didn't do the standard deviation, but it's different. You can look at it and see that it's different; and that's the older male.

DR. ROBERT CHAPIN: Okay. Are we good for this in terms of clarifications for what Syngenta has done with this? Looks like we are. At least as good as we're going to be. What I'd like to do is, we all get to stand up and relieve the pressure for 60 seconds while Song Huang --

DR. ALEX CHARLTON: We're not done yet.

DR. ROBERT CHAPIN: You're not done? Sit down, Bob. All right. Keep going, guys.

DR. SHEILA FLACK: This is Sheila

Flack again. We have just one little section to go. Fast forward through these slides really quick. So now that we have all these different pieces, I'm going to describe, go through how we derived the human equivalent concentration.

So this slide just kind of outlines our approach. Then I'll go into this in more detail, but our approach to deriving our human equivalent concentration.

On the upper left, we start with our CFD deposition. This is our monodisperse of which we convert to milligrams Chlorothalonil per centimeter squared per breath; results that Paul had shared with us. That needs to be translated to a polydisperse deposition.

And then a total daily deposition, calculated for an eight-hour exposure workday for a typical worker. And then that is compared with the benchmark dose level that was determined from the previous section Alex has described and gone through. All this information, together, will give us our human equivalent concentration.

This table shows us the CFD deposition values for monodisperse across the

different respiratory regions for the discrete particle sizes that we looked at. These are adjusted for a 4.9 percent Chlorothalonil, which is the highest dilute formulation that a worker would be using in a spray mix tank.

Now, in order to convert those to a polydisperse, and this data shows an example for the larynx; but this was done across the different regions of the respiratory tract. So, to transform the monodisperse deposition, for discrete particle sizes, to fit with our continuous distribution that we identified earlier -- that mass median aerodynamic diameter of 35, GSD of 1.5 -- a probability mass function was constructed to determine the percent contribution for each particle size.

Another way to look at it, is if you have like a box with all these different -- with these discrete particle sizes, what is the probability you would pull one of those particle sizes given that distribution, that representative distribution you have? Those percent contributions are multiplied by the deposition, in the larynx, for each of those

discrete particle sizes, to give us the deposition in the larynx, that final column here. And then these numbers are summed together to give us our cumulative total deposition.

The next step is to calculate the total daily deposition. For example, for an applicator who's applying for an eight-hour workday, we're using a breathing rate of 12.7 breaths per minute; which is equivalent to 8.3 liters per minute breathing rate, which is for a sedentary worker.

That is calculated over that exposure period for each of the different regions, again, for the respiratory tract. And then the final numbers at the bottom of this table just give us the total deposition, in terms of milligrams of Chlorothalonil per square centimeter.

With that total daily deposition, we took our benchmark dose level and divided it by our total daily deposition, for one milligram per liter aerosol concentration, which was used in our CFD modeling. That was done to calculate our HEC values across the different regions of

the respiratory tract.

That is how we calculate our human equivalent concentration. And if there's any questions that you have, or clarification on how that was done, please ask away.

DR. ROBERT MITKUS: Rob Mitkus. The HEC calculation makes sense to me as you presented it. I had one question. Did you consider doing some BMD analysis of your in vivo rat study?

For example, in this case, I probably would have used -- since you're HEC in vitro is for eight-hour applicator exposure, maybe your six-hour acute inhalation tox study would be the most relevant to compare. So, you have an airborne concentration rat, convert that to an HEC, adjust for the six- or the eight-hour exposure, and then see where you come out. In other words, compare your in vitro HEC to an in vivo HEC that you can estimate using BMD.

DR. SHEILA FLACK: Just to see how they compare with each other?

DR. ROBERT MITKUS: Yeah.

DR. SHEILA FLACK: Comparing the

in vivo to -- we did a comparison but -- yeah. But, no, that's interesting. I think we actually had some discussions about doing that.

DR. ROBERT MITKUS: Okay.

DR. SHEILA FLACK: Thank you.

DR. ROBERT CHAPIN: Questions for clarification? Yes.

DR. JENNIFER CAVALLARI: This is Jen Cavallari. My question for you -- I have two. One is that you chose to use a resting breathing rate. Had you considered using an active breathing rate for that?

DR. SHEILA FLACK: We picked the breathing rate based on kind of the standard approach the Ag Handler Task Force used that same value in calculating their exposure. We were consistent with that.

And we could, for various activities, modify that breathing rate to account for more active scenarios, like a mixer/loader or a handheld applicator who would be moving around. In our situation, we were assuming a person sitting at tractor. So, it would be a lower breathing rate compared to someone moving around.

DR. JENNIFER CAVALLARI: My other question was your use of the 75th percentile. I saw that you used the 75th percentile to be conservative in the CFD models. But there are other calculations that kind of go into your calculation of the HEC. Had you considered other places where the 75th percentile might be appropriate?

DR. SHEILA FLACK: I'm trying to think if there's a situation where we could look at the 75th percentile to match that. We didn't look at that.

But that's something we can think about and keep in mind, if there are places we can -- to see the range.

DR. JENNIFER CAVALLARI: Continue through with that. Definitely. Thank you.

DR. EMILY REINKE: I'm Emily Reinke, Army Public Health Center. To go back to the sedentary, the choice you used in the sedentary; I would argue that driving a tractor, unless you're in a large production, is not a sedentary activity. If you don't have automatic steering, and you're actually having to fight a

tractor, and you're concentrating on keeping in your rows, it is definitely not sedentary. I would at least say mild activity.

DR. SHEILA FLACK: Thanks for that input. I don't have experience driving one, so I don't know, but thank you.

DR. MARIE FORTIN: Marie Fortin. So, it's with respect to the -- it was kind of brought up a few minutes ago, comparing -- I think it was Robert Mitkus. The question was to compare the HEC, the human equivalent concentration, that derived based on the in vitro assay, .037 mg per liter, to other values. But in fact, the in vivo value for a low effect level, in a rat, where they had clinical signs of hyperactivity, gasping, like we mentioned, was lower than your derived HEC by about 20-fold. I was wondering if you had any thoughts on that.

DR. SHEILA FLACK: I think in our discussions, we've been trying to move away from the rat study, to focus more on this new approach. And I don't know what value bring to really do those strong comparisons. I don't know, Doug, if you wanted to add anything.

DR. DOUG WOLF: Just that the whole point is to do the human situation. So, you would still end up having to do all the mathematical manipulation to extrapolate from the rat respiratory, the rat exposure, the rat particle size distribution, the rat aerosol droplet size to the human situation. Whereas here, we're modeling the human situation and trying to understand what's happening in human cells.

The assumption that you make in this is that the rat is accurate and representative of everything; and we don't know that either. It is a hazard indicator. But for the modeling part, I don't think it would add anything. It would just be another comparison.

We do have the comparison that Paul showed, initially, looking at the comparison between the CFD model and the exposure side. And the amount being exposed in the rat is comparable to what we're seeing in the human. So, we do have that.

The parallelogram we have here is the rat CFD, the human CFD, the rat in vivo, and

the human in vitro; so that was a parallelogram approach where we had the CFD models being able to go across the different -- extrapolate across species. That's how we looked at the rat to the human.

DR. MARIE FORTIN: If I rephrase it differently. It still means that the benchmark value that's derived, based on the in vitro model, is 20-fold higher than the value that caused overt toxicity in rats. So, what your saying is that based on your assessment, we could be exposed to a concentration that's 20-fold higher than what caused overt toxicity in rats and we would still be okay. Thank you.

DR. SHEILA FLACK: This is Sheila Flack. Oh, I'm sorry.

DR. ROBERT CHAPIN: One more question.

DR. CLIFFORD WEISEL: This is Cliff Weisel. One of my understandings of the HEC is to try to go from an animal to a human and try to understand it. What you're trying to do now is say the in vitro method is a human, and I'm not convinced that that's true. You use a

human cell; that's not a human.

Have you tried to do like a full sensitivity analysis to see which parameters in this calculation give the largest variability? And then we can use that to help understand what took place. And more efforts to understand should there be more -- other factors that should be put in, like you have in the animals' uncertainty factors.

Because I don't think that -- your cell system is beautiful, but it's not alive yet. And it's not us. And so, we need to make sure that we don't assume that it's us, which is sort of what you're doing right now.

DR. SHEILA FLACK: This is Sheila Flack. In terms of the sensitivity analysis, are you suggesting that we expand that out and look at more variables to include?

DR. CLIFFORD WEISEL: We heard one thing about breathing rate. There's a lot of different variables that go into that. And you're assuming you can use it to full value right now. We have no way of knowing whether that's correct. This a new methodology that's

being applied. And if you have a new methodology that's being applied, we need to understand which factors are the ones that are potentially most critical to making these jumps of assumptions.

I don't think animals is the end all. It's not that we already have, but we use it enough that we have some sort of sense as to where the pitfalls are. We don't have that with what you're proposing. I think what you're proposing is what we need to do. But until we get to the point of really understanding that well, I think we need to do some sensitivity analysis, we need to understand what are the factors going in?

Do we need some uncertainty factors until we have more control and understanding, so we don't run into a situation. Like Dr. Fortin just said, maybe that 20-fold percent, 20 times percent, is really important? You can't just make that leap until we come there.

DR. SHEILA FLACK: Thank you. We now have all the different pieces to do our risk characterization. We've done our problem

formulation, we've characterized our external exposure. We've calculated our internal dosimetry, generated our endpoints, calculated a human equivalent concentration; and so, now we're moving onto our risk characterization.

This is the final slide that I'll present, which shows a risk characterization, risk assessment for Chlorothalonil. We've identified the highest exposure scenarios for Chlorothalonil to show on our RISK21 matrix. And I'll just quickly explain what you're looking at here.

So, on our y-axis, we have our estimate of toxicity. So, the range is from high to low values or low toxicity to high toxicity. Then, on our x-axis, we have our actual real worker exposure values, running from low exposure to high exposure. We also identified a point of reference, a level of concern of ten, which is indicated on this -- oops, I'm sorry. What did I just do? I hit a button by accident. I'm sorry. Right at the end here. It wants to be done, I think. Can I go backwards? It's at the very end. Sorry about that folks. There it is.

So we have identified a level of concern here, which is indicated on this yellow line of ten, as a point of reference. Anything up here in the red region would mean high toxicity, high exposure, unacceptable risk. This area in the green region is low exposure, low toxicity, or acceptable risk.

What we've shown here, is plotted here for the spray applicators for Chlorothalonil, is our range of human equivalent concentration values, versus the actual real exposure measure values that are generated by the task force that are used in risk assessments. So, that is just a summary. It captures everything that we've actually done here in our slide.

DR. ROBERT CHAPIN: Okay, last round of questions for clarification.

DR. RAYMOND YANG: Ray Yang. This last slide 76; that spray applicator, do you assume they are not wearing protective gears?

DR. SHEILA FLACK: That's correct.

DR. RAYMOND YANG: Thank you.

DR. STEPHEN GRANT: I know this is

coming out of the blue when you've answered --

DR. ROBERT CHAPIN: This is Stephen Grant.

DR. STEPHEN GRANT: Stephen Grant. How would that box change if you changed breathing rate? Would it double, or slightly move, or do you have a sense of that?

DR. SHEILA FLACK: It would probably slightly -- well, in terms of the exposure, it would move slightly to the right. Because with greater breathing rate, higher exposure. In terms of the HEC, I think that would move up a little bit, because you're now getting higher, greater deposition.

DR. DOUG WOLF: The y-axis on the plot like this is dependent upon the range of toxicity, unless you're saying that the particle size distribution changes, then that would move it up. But, if the particle size distribution stays the same, that's what drives the y-axis, so that would stay the same. The exposure could move a little bit to the right or left, depending upon breathing rate.

DR. JAMES BLANDO: James Blando.

Just a point for clarification, you mentioned that the exposure values are based on a task force, I think you said. Does that mean that these are measures that are exposure measures collected in the field for people actually doing this work?

DR. SHEILA FLACK: Yes. So, going back to the earlier section, when I was describing how these workers are monitored using those OVS tubes, so that is how the Agricultural Handlers Exposure Task Force collects all this data, which then goes into the risk assessment. EPA does the risk assessment based on those numbers that are generated.

DR. ROBERT CHAPIN: What I'd like to do is without -- I want to give us a little bit of relief, but not too much, and keep the momentum going here; because I know we've got a lot of question about the model. What I'd like to do is move to Dr. Huang's presentation.

There are some slides associated with that. And I think it might be good if Alex and Doug stayed here, because there might be additional questions for how you use the model.

You're almost done, but not quite. So everybody can stand up while we've got the -- Andy (phonetic), do you have the slides loaded for Dr. Huang?

ANDY DUPONT: I'm working on it right now.

DR. ROBERT CHAPIN: And then, basically, as soon as he's at the table and we've got the slides, I'm going to start talking and we're going to get going again. We're going to go through this presentation. It's supposed to be 15 minutes, and then we'll take a bio break.

[BREAK]

PUBLIC PRESENTATION - SONG HUANG

DR. ROBERT CHAPIN: What I'd like to do is just do this because he's got presentations and slides and stuff. And so we'll do that. We'll talk about the model. We can ask a bunch of question about the model, and then we'll take a bio break. Dr. Huang, it's all yours.

DR. SONG HUANG: Good afternoon,

everyone. It's a great pleasure for me to be here, because Syngenta presented their results about their test of their chemical. I'm here because I know you all are in the project because we provide in vitro cell model for them to perform their test.

I would like to thank you for giving me this opportunity to present our company and the activity of Epithelix. Of course, I will talk also about this 3D in vitro model of human airway epithelia for inhalation toxicological testing of chemical.

Everyone knows that in 2007, NRC issued a report about the toxicity testing in the 21st century. It's a vision and a strategy. NRC was calling for a paradigm shift from in vivo animal tests to in vitro human cell and tissue-based testing of chemicals.

I will not get into the details of this report, maybe everyone had read it already. Since the application of this report, the landscape of the toxicity testing is virtually transformed actually. A lot of the investments in the in vitro models, a lot of the projects are

going on and especially EPA is driving this change.

As a small company, Epithelix is also trying to contribute to this paradigm shift. I will give a brief background about our company.

Epithelix was founded in 2006, located in Geneva Lake area. We have one site in Switzerland, Geneva and one site in France. It's self-financed, the company. We have about 15 employees.

The mission of our company was to promote, actually, the 3R principles. That means reduce, replace, and refine chemical test. This is written in our statutes of the company. Another mission, of course, is through business, is to develop and to commercialize relevant and robust in vitro cell and tissue models for scientific research purposes. We also develop relevant and reliable in vitro assays, based on these models for assessing the toxicity of chemicals. Our main focus is the human respiratory system, in particular, the human airway epithelia.

Everyone knows that the human

airways are very important, so there are a lot of functions. It is vital for human beings. So, they protect us against external insults as a physical barrier. They clean the air that we breath or inhale, through mucociliary escalator.

They play a crucial role in innate and adaptive immune responses against pathogens like viruses and bacteria. They carry out gas exchange in the alveolar region to oxygenate our blood. Adding perturbation of the airway epithelial structure and function, would lead to severe diseases, like asthma, COPD, cystic fibrosis, lung fibrosis, cancer, et cetera.

Unfortunately, since this is active bionic process, when we breathe, we uptake a lot of chemical particles in the air. So it's a main entrance into our body. That's why it's important to study the respiratory system.

Here is scaled to show the actual structure, morphology and structure of the upper and lower airways. Essentially, we can divide the airways into three parts: one is the upper airway, small airway and alveolar spaces. You can see that there are some structural

difference, but also in terms of composition of cells they're quite different.

In the upper airway, in the nose and the trachea, for example, you have three types of cells: goblet cells, ciliated cells, basal cells. When you go to the small airway region, actually, the goblet cells are replaced by the club cells, previously called Clara cells.

So when you get deeper into the lung, you get into the alveolar region. You find two other type cells: pneumocyte type one and type two. Actually, there's a lot of types of cells which is not shown here. It's the alveolar macrophage. It's a very important component also.

At Epithelix, we try to recreate this model in vitro. So what we do is try to isolate the primary human cells from the biopsies collected in the different centers in the world. Of course, with the consent of the family or at least the donors.

First, we isolate the epithelial cells. We amplify, but not too much. We store them in liquid nitrogen, whatever needed. We

just take the frozen cells out and thaw them, and place in this kind of transfer insert which has a semi-porous membrane between two compartments. That's why we can see the cells on top. Once they get confident, we can expose them to air, which simulate what happen in vivo. One side, the cells are exposed to air, and outside is (inaudible).

Under this condition, culture condition, after several weeks the cells are getting fully differentiated. You can see there are cilia cells, goblet cells, and also basal cells.

This is a picture you haven't seen before. This is a study performed by Charles River. You can see there the epithelium is fully ciliated. These cells are functional, because if you put some beads, it's functional.

That's a very important aspect, because air epithelium has an important function. It's the mucociliary escalator. Sometimes even if you don't see damage at the cellular level; but you can still get some trouble, because a lot of diseases like cystic fibrosis, if you look at

the epithelium, they are quite long. There's almost no difference. But the cilia -- the mucociliary clearance is nearly a zero. There's no room. That's why it's important to reproduce not only the morphology, but also the function.

So this is the summary about the main characteristics of MucilAir. It's a system very robust. It has a long shelf life. You can maintain them and use them for several months. That's why it's good for chronic exposure experiments.

We have epithelium from different pathologies. Maybe it's not relevant for toxicity testing, but for other purposes it's very relevant. It's easy to handle and maintain. The media we used is serum-free. So, we can ship everywhere in the world from Asia, to US, and Europe also.

Actually, to use the system, we developed a so-called immunity endpoint testing strategy, which I think Alex just talked about the resazurin test, LDH, and TEER measurement. So I'm here to answer, at the same time, some questions that you asked about this endpoint.

So this endpoint, why we use it is because this endpoint has no destructive, so that means you can measure the TEER. TEER is the transepithelial electric resistance. Epithelium is tight because they form tight junctions, gap junctions. But also, the airway epithelial is quite special because we have very active ion channel activity.

So you have, for example, the ion channel CFTR. It's a chloride channel. At one mutation you catch cystic fibrosis disease.

So actually, we have the means to not only measure just the resistance, we can also measure the current. That means you can put specific channel inhibitor, you can measure individual channel like -- it's a certain channel. You can put inhibitor for CFTR, and you can activate CFTR. So, quite unique.

So that's the actually resistance as Alex Charlton said, it's a very sensitive endpoint. Because it not only measures the cytotoxicity, it's also the toxicity which interrupts the cell to cell junction. So, that's the measurement.

We also monitor -- since the membrane is transparent, we can see clearly what is going on within the insert. And we can measure the cilia beating frequency. Of course, we can see the morphology of the epithelium. And we can also collect the (inaudible), measure the amount of mucus on top of that percentage.

So, all this information you can track it. So, we can perform every day. So we actually have the experiment going on for several weeks. It's really a robust system.

That's the endpoint. We need to apply actually the chemicals. We have different means to apply, as liquid, as solid, as a nanoparticle, as gas, as smoke, for example.

The answer for the question whether we work with the cigarette tobacco company? The answer is yes. We work with them. Why? Because they have a lot of research going on. They do a lot inhalation study using animals. We have a system here, so why should we use animals instead of the in vitro models, essential for this.

Of course, we can also use another

endpoint, which is LDH for the cytotoxicity. But since the airway epithelium meets a kind of immunomodulator. So what is amazing you see, they separate tons of cytokines/chemokines. So, actually, this step is also kind of drawback, because a lot of the disease is over secretion of cytokine. If you get asthma, for example, you get a lot of recruitment of the leukocyte.

The point is we can use these cytokine/chemokines as a marker to see whether a chemical has effect on the epithelial cell, or no. Of course, then we can extract RNA/DNA and protein.

I just give two examples because since during our twelve years, a lot of the study has been done using this model. Maybe thousands of experiments, hundreds of articles have been published. This is why it's very, very interesting, because they did some in vivo and in vitro correlation. There's a study, actually, published by AstraZeneca.

They looked for 15 different compounds, actually have in vivo data. They used the MucilAir model. They use different

endpoints. So they found out that the TEER is indeed a very sensitive and predicting endpoint. That's the article if you are interested in having a look. It's a relevant and predictive model.

A lot of study we have done with ECVAM in Italy and with Unige in Geneva. So what we did, we test actually a long list of compounds, primary compounds to see how these chemicals, if you apply it on top, across the epithelial cells. So it's a kind of a measurement of the permeability, is Papp ready.

So what is amazing is we did it in three locations. There are different batches of epithelia, so you get very, very -- it's not intangible but very similar results. So, this, for example, hope to convince you that this model is not only relevant, robust, it's also reproducible.

So the conclusion is that MucilAir mimics the morphology and function of a number of human airway epithelia. It is easy to handle and maintain. It's a relevant and reliable 3D in vitro model of human airway epithelia for

inhalation toxicological testing of chemicals. Thank you very much for your attention.

DR. ROBERT CHAPIN: Okay, now's the time. George.

DR. GEORGE CORCORAN: Thank you, Dr. Chapin. I'm going to restate a question I raised earlier about measurement of LDH as a measure of membrane integrity and indirectly being interpreted as cell death by necrosis. Would that be correct? Is that your company use that measurement?

DR. SONG HUANG: No, that's why -- Alex actually mentioned that we always correlate, actually, the TEER and LDH. Sometimes they don't correlate. So sometimes there are some reason why. Because if your chemical, which interfere with the LDH enzyme assay, you will not see the result. Sometimes you have TEER, which stops but the cell don't die, actually, just because the junctions are broken.

For example, if you're stirring the cells with (inaudible) gas. So if you put the amount of that gas, which will not kill the cell, but just initiate a signaling, then you can

see the cells get around it. but there's no release of LDH, but you can see the drop of TEER. So that's a -- you have to be very cautious about this.

DR. GEORGE CORCORAN: There are other ways to get full release of LDH besides a detergent though, so --

DR. SONG HUANG: Detergent -- that's why (inaudible) that we use --

DR. GEORGE CORCORAN: Hypertonic shock, there's a whole variety.

DR. SONG HUANG: Triton -- that's what we used. Lysis we incubate 24-hour, so one hour sometimes is not enough. So, it's a very, very robust test.

DR. GEORGE CORCORAN: So in the Chlorothalonil data that was presented to this committee, a number of measurements were reported as more than 100 percent of the LDH release. In fact, some of the numbers were over 250 percent.

It led me to scratch my head saying -- well, I guess, if a graduate student brought those data into my office, I'd be saying, you got to go back and do that again. Or give me

an explanation as to why I'm not seeing what I would have predicted. Can you help me why the LDH values would be outside of a boundary one would predict?

DR. SONG HUANG: Like I said, if you do a quality control, if it's not fully lysed, then you can catch trouble because another one is your experiment --

DR. GEORGE CORCORAN: So, I guess, I would say I would be tempted to go back and use another lysis method, until the release never exceeded 100 percent.

DR. SONG HUANG: Yes. Yes. So that's why we should be cautious about this. We also faced this phenomenon with another test. It's the alamarBlue test. It's your MTT. Sometimes you get over more than 100. The reason is that if your other chemical injured cells, but not killed the cells, injured -- damage the junction, alamarBlue gets -- it's a 3-deminisional epithelial. You should be aware.

Obviously, they are compact. So that's why you -- instead of kill the cells, we just open the junction. The chemical gets into

touch about to the basal cells, with the surface of the cells. So you get more the ability than your normal control. So that's also what quite often happens.

That's also one of the problem with resazurin test. Because it's a 3D model, you get a layer off, but they still have a surface because the resazurin transformed the enzyme (inaudible) of the cells. So that's why you can still get a lot of these transformations.

DR. GEORGE CORCORAN: One other more minor concern, but it could be elevated. And that's you've used resazurin for two different functions in the cell. One would be the ability of the cell to produce a reductive reaction; and the other instance is measuring LDH through the coupling with diaphorase.

So my concern here is your -- if resazurin has a liability, it's impacting two different, supposedly independent measures of cell integrity in cell function. And it would just increase my confidence in the methodology that resazurin was not used in two of these probative assays.

DR. SONG HUANG: I agree with you. We should have be really careful about the test, about the interpretation of the results. Everything should stick together, then we can draw a conclusion.

DR. GEORGE CORCORAN: Thank you.

DR. ROBERT CHAPIN: Holgar was next and then Stephen.

DR. HOLGER BEHRSING: Holger Behrsing. Song, thank you very much for the nice presentation. One of the topics that came up earlier was that only cells or sort of tissue derived from the nasal pharynx were available at the time of this testing. There are different regions from which the donor cells are retrieved from the respiratory tract. Can you comment on what differences there may be between tissues from the nasal pharynx, from the trachea, or other regions?

DR. SONG HUANG: I'll just say that these are cells, actually, quite often we get from the patient with nasal polyps. So that means nasal polyps have a (inaudible) of nasal tissues in the nasal cavity.

So these cells, actually, has a tendency to do pretty great. But we do have a contraindication which allow that these cells kind of form your study state. So, indeed they are more sensitive than trachea cells.

The trachea cells, actually, they are -- how to say -- these quite often they come from the kind of normal donor, so they have not had this tendency to over (inaudible).

DR. HOLGER BEHRSING: Thank you. So one last question. So that's a nice explanation of what may be different between the cells from different regions. So, if you had one batch of these MucilAir tissues -- I know that it takes four or five weeks to create them. There's expansion, then there's maturation, the pseudo-stratification of the cell layers.

That's happening in each individual tissue, culture insert, over the course of that time. What kind of variabilities might one expect, in terms of biomass or responses to the exposures along -- I'm talking about not from the same donor, on different batches, but within the same batch?

DR. SONG HUANG: You are right that from batch to batch, indeed there are some variations. It's a very tricky business to make exactly the same product, especially biological product.

What we do is we try to make a quality control. So, before shipping out our product, we do a morphology checking until we measure TEER. We look at the overall morphology. Sometimes some customers, they ask if we can we perform also the histology, which they see the, actually, the cross-section of the epithelia.

DR. HOLGER BEHRSING: Obviously, you've talked about the quality control that you do. But in terms of usually protein content, ranging in one batch. I know that when we worked with similar tissues, we've seen two-fold difference in biomass, based on protein alone.

DR. SONG HUANG: That could happen, actually, that could happen. Because sometimes we have also -- because the inserts we get from the company, they're not always the same data. So that's why we have sometimes the variation between the inserts. So now we

negotiate with them and try to get more high quality inserts. But they have a program for that.

DR. STEPHEN GRANT: Steve Grant. Some of your donors are not normal donors? They have nasal polyps?

DR. SONG HUANG: Yeah.

DR. STEPHEN GRANT: Okay. Great idea to have an in vitro test; and great because it allows you to do a lot of different kinds of tests. However, the problem is correlations are apples and oranges, and it's nice to see them showing the same thing.

But what you'd really like to do is at least start with some similar measurements in vivo and in vitro. And that kind of reduces you to something that you could do on a histological section from previous --

DR. SONG HUANG: Histology looks quite similar.

DR. STEPHEN GRANT: But, again, it's very hard to quantify histology; but you can quantify histological staining, for example. Have you done any studies in which you take

samples from either animal studies, or exposed people, and show that there is a good agreement, quantitatively, between endpoints in the two systems?

DR. SONG HUANG: That's one example I showed you. It's the batch performed -- - the test of the 15 compounds. You see a correlation in vivo, in vitro. But it's a possibility to do that. Actually, we have a collaboration with a company. They perform this kind of really detailed analysis. It's a molecule to see the (inaudible).

DR. STEPHEN GRANT: They did Papp in vivo? They did a Papp test in vivo?

DR. SONG HUANG: Yeah. We collect the tissue. We collect the cells, which have not been amplified. And then we re-conserve tissue. Then we send them out again.

DR. STEPHEN GRANT: But that's not in vivo.

DR. SONG HUANG: Yeah, they were comparing in vitro and in vivo. So, we fixed the histology also. So that's a project going on. So as a small company, we cannot do a lot of the

things --

DR. ROBERT CHAPIN: Jim.

DR. JAMES BLANDO: James Blando. I just have a few basic questions about the cultures. You mentioned, if I understand it correctly, that you have some models for people with different disease states, like asthma for example.

If, in the future, if somebody wanted to apply these types of tests to other scenarios, or other chemicals, and wanted to use this for sensitive subpopulations to predict the risk for, say, people with asthma, would this in vitro test provide a good model for that type of scenario? In other words, do you have in vitro cells that -- because people with asthma, I think, have a different cell distribution, maybe more goblet cells or something. What do you see as the applicability of this for a test with people concerned about sensitive subpopulations?

DR. SONG HUANG: You are right to ask this question; very good question. Actually, at the beginning we are concerned about the -- one, you isolate cells. You put into

(inaudible). You will lose all the phenotype or disease features in vitro.

But it turned out that some features are still in the kit when you amplify the cells, when you reconstitute the tissue.

We have a reason we perform a comparative study using five kinds of normal cells from normal donors, and six COPD donors. Then we compare them with -- constituting the same time, then measured endpoint, this impact.

So it turned out that a lot of features are still present. For example, COPD you have more goblet cells. That's the, I think, one we're waiting. I will document that.

They have also less the rate of the cilia clearance is reduced. So, that's also one feature we saw. And TEER compared to studies, statically significant (inaudible).

Sometimes we have trouble, actually, to really reconstitute the epithelial from the diseased (inaudible). It just look very, very bad.

That's true, yeah. There's sometimes you -- but we have some case where it's

successful, yeah. We have collaboration with the University of Virginia, where we study the difference between (inaudible), epithelia and asthmatic.

DR. JAMES BLANDO: I just had two other quick questions. You mentioned that these cultures are serum free. So, if someone took -- again, thinking about not necessarily this specific chemical, but other air pollutants and -- like fibers, for example. If you had a pollutant that caused damage because it ruptured a macrophage, or something, caused it to spill out all its enzymes or whatever, these cultures do not have any immune cell component to it?

DR. SONG HUANG: At this moment, no.

DR. JAMES BLANDO: Okay. Is there plans to expand that?

DR. SONG HUANG: It's a plan made up, yes.

DR. JAMES BLANDO: The last question I had is, if people were going to try to apply this in vitro assay to other -- I apologize for my lack of familiarity with some of these

cultures. So, these cells are immortalized cell lines and over time, so is there drift? If they are, is there drift? In other words, if somebody wanted to apply this to like a cancer study, is there --

DR. SONG HUANG: No, it's a primary, so it's not immortalized.

DR. JAMES BLANDO: Oh, okay.

DR. SONG HUANG: Some project would, but fundamentally, it's primary. We only amplify once. That means we get cells, once there we put into the petri dish. Once confident, we just move them.

That's also why it's a good point. Because why we have a better quality, because we push less the cells to become this direction. We know that the more you pass the cells, the quality goes down very quickly. Even some ion channels, if you measure the T1 -- to make the (inaudible) will have the very generating --

DR. JAMES BLANDO: So, some of these donors for the Chlorothalonil study, you said had nasal polyps. Does that have any bearing on the assay itself?

DR. SONG HUANG: Yeah, yeah. I said they have tendency, but for most of our epithelium they are fine.

DR. CLIFF WEISEL: Cliff Weisel. This is very impressive, and I think it has lots of potential. But you mentioned it doesn't have the alveoli macrophages. You mentioned it doesn't have some immune systems. I'm sure doesn't the microbiome that we're starting to learn more about.

One of the things that we've been asked to do is talk about the process of using this whole methodology, yours as well as others, in toxicological risk assessment. What do you think some of the limitations might be with the current system, and as you said you didn't follow the -- just clearly some feedback. Where do you think I actually might work well and where do you think it might not work well?

DR. SONG HUANG: For the regulation?

DR. CLIFFORD WEISEL: Not for reg -- to use it to get the toxicological data that we want, regulation or risk purposes? Do you

have any thoughts on where you think -- who you would advise sayings, yes, this is good for what you're trying to do? And who you might say, wait another five years before you tweed it out a little further?

The recruitment for macrophages, looking at the way ozone will come in and cause damage. If you don't have the macrophages, you're not really going to understand repair mechanisms.

DR. SONG HUANG: I think, actually, we tried to develop the -- I think for macrophage, alveoli macrophage, the relevant model is alveolar. Because we tried to put some of the macrophage derived from (inaudible) cells in MucilAir. We just removed the (inaudible). They don't attach.

So, I think the microphage, its function is to protect along in the alveolar space, against all these particles when you smoke a cigarette. Why you get macrophage from smoking? It's just the fact. So, they are really active to engulf the particles.

But once they engulf this

particle, they just move out, and go up, and clear away. I think they have new functions, real functions, once they get into the bronchi. Because they are just -- by the cilia beating, just (inaudible). So I think more relevant model is alveolar model, alveolar macrophage.

DR. RAYMOND YANG: Ray Yang. In one of your slides, you indicated you could use gas for the system. How do you dose that? Dose the system?

DR. SONG HUANG: It's not easy. It's not easy. Actually, for this, we have kind of a collaborate company. It's called Vitrocell. So they are very inventive, very active in develop the device for the in vitro models, actually, for all our models.

So, they have already worked with us to have all kind of device, which is very sophisticated for gas, for solid, and so, actually, we are testing a new machine they are developing.

DR. RAYMOND YANG: Early, Jon mentioned -- another panel member mentioned of a repeated dose. Could you actually do repeated

inhalation?

DR. SONG HUANG: Yes.

DR. RAYMOND YANG: No inhalation, but dosing.

DR. SONG HUANG: Yes, dosing, yes. That's where we routinely do, is like I presented before. We use a nondestructive endpoint to assess the toxicity over time. So that's why you can apply -- depend on you reaching the dosing, and you can apply, and just apply every day without washing out. You can also apply and remove it every time and do TEER measurement.

DR. RAYMOND YANG: Thank you.

DR. ROBERT MITKUS: Rob Mitkus. Dr. Song, are you aware of any, either in the US or in Europe, regulatory submissions or dossiers that utilize this particular method for any class right now?

DR. SONG HUANG: This is the first one, that Syngenta -- this is the first one. Another one is (inaudible) company. I think this has appeared to FDA regulatory.

DR. ROBERT CHAPIN: Bob Chapin. I've got a couple of questions. Could you back

up a slide or two, please? Just go back to the list of endpoints. Right there. Perfect.

This is why you guys are here. So, we've got cilia beating, monitoring, mucin secretion, soluble factors. Did you guys look at any of those as maybe other earlier markers of irritation before you get to frank cell death? Thanks. And into that microphone, please, so that people can hear you.

DR. ALEX CHARLTON: This is Alex Charlton. Not on this study, we didn't. We have evaluated some of those markers. We've looked at things like -- we have evaluated some other markers beyond those that we've used in this study right when we were setting out with MucilAir.

We found that there was quite a lot of variability in some of the measurements, and we weren't very happy with our making decisions on those bases. So, those endpoints didn't get taken forward in our MucilAir work. So, what we've used are the ones that we've used in the past and we're confident that we understand.

DR. ROBERT CHAPIN: So, a follow-up question. Were those other compounds, did they produce clinical signs and symptoms similar to Chlorothalonil? What I'm trying to do is I'm trying to understand if the cell death endpoint is real -- and measures of cell integrity, are really the best ones to use. And if, in this case, some of those indications, that cells might be in less dire situations might have been the golden spike for you on this one.

DR. ALEX CHARLTON: So I should be clearer. So, the work we've historically done when we were initially setting out to try and look at endpoints on MucilAir, that was all done with Chlorothalonil.

DR. ROBERT CHAPIN: So you've looked at these other endpoints, with Chlorothalonil, and they were noisy, or gave you difficult to interpret results?

DR. ALEX CHARLTON: I think we've looked -- we looked at cilia beating, and we looked at interleukin release as a measure of our inflammation. And they were fairly variable in our study, in our initial study; and, as I said,

we haven't taken them forward.

DR. MARIE FORTIN: Bob, can I -- it's Marie --

DR. ROBERT CHAPIN: I'm sorry. Yes.

DR. MARIE FORTIN: Marie Fortin. Can I jump in, please? My question is directly related to this subject. We know -- and again, even just looking at the chemical structure, that it's going to create oxidative distress within the cell. I think the point you're getting at is, obviously, the endpoint that you're looking at is subtle. It's somewhat distal on the AOP, and the proximal part on the AOP, you don't have it, right?

So the MIE (phonetic) which would be degeneration of oxidative stress and other endpoints like that, are not pictured in that AOP. And, therefore, you're looking at an endpoint that's kind of distal and towards -- you know, cell death is pretty final, right?

And that's what I meant earlier when I said it's not sensitive; is that other endpoints would be earlier on that AOP and would

recommend a more sensitive for our sentinel effect than what you're looking at.

I understand your argument that the hypothesis is that the cell death leads to metaplasia. I understand that. I guess I have a question in there for you, Dr. Huang. So, have you looked at the (inaudible) or those type of endpoints in that model?

DR. SONG HUANG: Yeah, we tried for some time ago we quit (inaudible). Yeah.

DR. MARIE FORTIN: Okay. My question to you guys, is would there be a value to looking at more sentinel endpoints, and to add a more sensitive model? Because that's what I've been kind of saying so far.

DR. DOUG WOLF: So it depends on the specific question you're trying to answer. This is Doug Wolf. The conceptual difference between a mode of action, which is what we typically look at in a chemical risk assessment, and the mechanism of action, which is what you're getting at. Trying to understand the specific molecular details, from the exposure to all the different effects, perturbation of glutathione,

increased oxidative stress, all those different mechanistic considerations.

The question becomes, will that be helpful, and will it help you to tease out a dose response, to select a point of departure, to do a risk assessment relative to the exposure situation that you're evaluating? At the present time, where we are today in the process, to get from where we started to now, that isn't a question that we felt was necessary to answer.

I think, if there is a valid reason to refine the dose response, and when that type of additional mechanistic data is helpful in the situation where -- because, typically, in this particular situation where we're using the highest exposure, what we consider the most health protective endpoints -- 24-hours exposure, frank toxicity -- that the site where you get the highest exposure; and move that to be as health protective, conservative in the numbers as possible, where we typically do what you're suggesting is when our risk assessments don't pass. You know, we need to refine the dose response and see if it can do a better job of

relating the exposure to the specific.

It might be something we have to do once all this is done and we see where the agency is. It might be that adequately describing the major key events in the mode of action might be sufficient. We've done that many times. Sometimes just describing hypertrophy in the liver is sufficient. Sometimes you actually have to quantify the amount of nuclear receptor agonism, binding to the receptor. We'll have to see.

But your point is well taken, if we need to go to that mechanistic level. From where we started, to now, we didn't feel that was necessary at that time.

DR. MARIE FORTIN: Okay. Thank you.

DR. GEORGE CORCORAN: Thank you. Dr. Chapin. This is to Dr. Huang. You mentioned the potential for repeat exposure in this culture system. Have you done it, and have you been able to demonstrate metaplasia?

DR. SONG HUANG: Metaplasia for the -- actually, a different kind of metaplasia.

You have goblet cell metaplasia at this time.

DR. GEORGE CORCORAN: Just to make sure that I did enough homework, when I Google searched and PubMed-ed the MucilAir terminology, I think I came up with maybe 35 publications. Is that the universe of publications out there at this time? Is that all the publications there are in the public domain? Around 30?

DR. SONG HUANG: Thirty-five, yes.

DR. GEORGE CORCORAN: At 35. Thank you.

DR. SONG HUANG: Some may be in other references.

DR. STEPHEN GRANT: Steve Grant. I want to get back to the idea of looking at cell death in vivo versus cell death in vitro. And in this case, you're kind of in between because traditional in vitro is the two dimensional. I was around the last 20 years where cell death turned into apoptosis.

Does apoptosis happen in your system? And do you have a way to distinguish it from other types of cell death?

DR. SONG HUANG: Yeah, we could

use a different --

DR. STEPHEN GRANT: I didn't ask if you could. I asked do you?

DR. SONG HUANG: No.

DR. STEPHEN GRANT: Because what I'm worried about is that all cell death is not equal. Apoptosis is a technique which tries to minimize damage to surrounding tissue. And what you don't want to do is look at it as something in vivo, that's causing necrosis, and use as an equivalent the induction of apoptosis in vitro.

DR. SONG HUANG: No. We actually, have CIO (phonetic) activity, but a lot of our customers they ask that. But establish this to a mechanism of cell death. It's interesting to know, actually, to find out which chemical.

DR. JON HOTCHKISS: Jon Hotchkiss. Just a follow-up on your ciliary beating. What did make it reasonable to use? Is there too much variability between individual cultures, or is it just not unidirectional? Like you don't always get a decrease when you get toxicity.

You know, oftentimes, say with ozone or other irritants, the first thing that

happens is they go crazy because they're trying to get rid of it. Then, if you keep on bumping the dose up higher and higher, well, game over. So you can see an increase and then a decrease.

I didn't know if you were having trouble distinguishing between the variability between the cultures, or the type of response you were seeing consistently.

DR. ALEX CHARLTON: This is Alex Charlton. I'm sitting here desperately trying to remember that study from about four or five years ago. I'm afraid I'm failing. I seem to remember it was difference in responsiveness between cultures, but I couldn't swear to that.

DR. KATHRYN PAGE: This is Kathryn Page. Sensory irritation is one of the things that we can obviously look at in vivo. Do you anticipate that this is something that would be of a concern with this compound? If so, do we know if there's a way that we could address sensory irritation in vitro?

DR. SONG HUANG: Sensory irritation is maybe -- if you can care to address in this model because -- sorry. Because the

sensory -- it's a sensory neuron (inaudible). In our culture, there's no neuron cells.

But we developed an assay, which it has not been validated, but for a detection irritation it's based on cytokine release. You use the (inaudible) as a macro. But it's not to -- actually, it's not just your (inaudible) getting irritated.

DR. DOUG WOLF: Just to respond -- it's Doug Wolf. With regard to sensory irritation, if you remember from the CFD model, the olfactory part of the respiratory tract, the aerosol droplets don't get there. That's different, obviously, since perturbation is important with chlorine and other vapors that get into the olfactory, both in humans and in rodents.

So, if it was a different type of volatile compound, yes, that would be really important. Maybe, if you can't do the in vitro, if that's the endpoint you're looking at, maybe at this present time in vivo is the best course. But for this particular set of aerosols, non-volatile materials, then the CFD model shows that

where it lands is associated with where the respiratory epithelium exists.

DR. JON HOTCHKISS: Jon Hotchkiss once again. Were you talking about sensory irritation mediated through TRP receptors or as opposed to injury or olfactory receptors?

DR. KATHRYN PAGE: Both. I guess it depends on what your compound is. My point really is just that thinking about future application. Even if it's not considered this instant, it's definitely something that we're going to miss out on by not doing the in vivos study. Especially, if you aren't triggering inflammation and it's just a neural response. You know, that's definitely going to be of a concern.

DR. JON HOTCHKISS: Jon Hotchkiss. Some groups are modeling molecular interaction with various TRP receptors and going to expression models so that you can validate the chem informatic predictions with calcium release.

DR. ROBERT CHAPIN: Okay, have we satisfied everyone in terms of questions about the status of the model? And clarifications

about what Syngenta has done, and our understanding of that? Are we good with that? All right. Gentlemen, thank you very much. Thank you very much. Dr. Song, thank you.

I'd like to move to the other two public commenters, please. Dr. Clippinger from PETA. The floor is yours.

PUBLIC COMMENTER - CLIPPINGER

DR. AMY CLIPPINGER: Thanks. So I'll be brief. I just really wanted to thank the EPA for the opportunity for the dialogue this week; and its commitment to moving away from the checkbox approach towards the use of nonanimal methods that are protecting human health and the environment. My organization is certainly supportive of science-based testing approaches, based on human cells and human-relevant mechanisms of action, like the one that Syngenta has submitted.

I'm really looking forward to what I'm sure will continue to be a lively discussion over the next couple of days; about this specific case study, but also considering how some of the

general concepts might be expanded to the testing of other pesticides and industrial chemicals in the future.

As Monique mentioned this morning, in her opening remarks, there are multiple groups from government agencies like ORD, to industry, to non-profits like my organization. A lot of different groups working on efforts to advance non-animal purchase for respiratory toxicity testing. It's, I think, a good time where there's significant interest and momentum for additional companies to submit similar proposals.

I think one of the key points highlighted by this meeting this week, is the willingness of EPA to meet with and discuss alternative approaches with registrants and with the public as well.

Again, just a thank you to EPA and to Syngenta for pioneering this space. Thank you.

DR. ROBERT CHAPIN: Great. Thank you, Dr. Clippinger. Dr. Roper, you've been preempted by renal biology. So renal biology. So, we're going to take a five-minute bio break,

and we're going to be back here at 25 of. And I'm going to start talking -- and he's going to start talking at 25 of.

[BREAK]

PUBLIC PRESENTATION - ROPER

DR. ROBERT CHAPIN: There has been a little bit of an additional schedule modification. So, Dr. Roper has some slides to share with us. We'll go ahead and turn it over to him. Dr. Roper.

DR. CLIVE ROPER: Thank you. My name is Clive Roper. I'm head of In Vitro Sciences at Charles River. We performed the experimental in vitro work. There were some questions that I wanted to clarify, so I just want to identify a few things with some slides. I wasn't prepared to actually speak, but I think they'll answer some of the questions that have come through on part of this New Approach Methodology.

So, this is what we're trying to remember. We're trying to take out the in vivo. We've now got some amazing new technologies. We've got a rat in vitro. We've got the human in vitro. And we're kind of thinking about this person here, in this case, an occupational worker. Now I'm going to jump around because it's not the right presentation for this, so you have to work with me.

One of the questions that came up was about reversibility. So, we've got a project here that shows reversibility. Another question was about the LDH release and why we've got 180 percent, and I'm going to explain that. So, just looking, it's exactly the same as what we've done for the chlorothalonil, but this time it was a 24-hour exposure and we had the same endpoints measured. But the difference was that we left a recovery period of 168 hours.

So, you've seen some of these pictures. And we didn't show anything beyond the 2.5, so we did 0 to 10 millimolar SDS. And you've seen this picture already that both Song and Alex have shown. But if you actually look at

here, we've got the cross sections versus the surface morphology. Now, this is an important part of -- someone was asking about how does it actually affect -- what actually happens in this model with this SDS? Ignoring -- that looks damaged. It's actually just the way it was cut. But pathologists have scored all these as intact, and then here is where the damage comes in.

Very interestingly, and someone mentioned it, what happens to these cilia, and they actually get ripped off. So, the cell isn't dead. It's just damaged. And then, at this next level, you can see there's no cilia. And actually, beyond that, there's just the membrane. So, there's no point in showing it.

The black lines on all these are the same. This is our pre-dose values. All these are pre-dose values. Okay? That's what the black line is. If we look at the 24-hour, and I think it's really important here that we used SDS as a positive control at 4 millimolar in the chlorothalonil experiment, because we knew that, from this experiment here, that it was going to knock it out in 24 hours.

We could also see that things do change around about the 1.25 millimolar all the time. That's where we're seeing a point of departure here actually, and we see no recovery. So, there's no recovery if we look at the blue line. They are definitely not recovering. Now, at an earlier time point and at lower concentrations, they are recovering, but not at all in the higher concentrations.

So, it's really important that we use this 4 millimolar number, and it's going to help us understand why LDH release is 180 percent. And I'm going to share this poster because this explains the whole process in more detail. And then, the other thing that we're very interested in is -- I believe these models weren't available for us, but we've actually -- someone asked, has this data been tested against known toxicants? And the answer to that is yes.

So, this is a similar model. It's MatTek's EpiAirway. We've generated a rat model and a human model, and what we wanted to do is to demonstrate what would happen. Can we start to predict known toxicants? So, we've actually

taken 14 test chemicals, and they are of known in vivo toxicity. What we were actually able to create was a complete disease pathway with injury and repair.

This is in the rat. Sorry. This is in the human, but we've also done one for the rat as well. And we are in the process of putting this paper together, but you can actually see how it starts off normal, and then there is actually recovery and repair. It's all through these different diseases. I think you've seen these and a lot of these examples before.

Then here was our -- so, we've got known respiratory irritants, and we've got also skin and eye irritants. So, we really wanted to look at things that we knew were going to be toxic. Then we've got these GSH categories. So, the smaller the number, the nastier it is, which goes in with them being known irritants.

I'm not going to go through the detail of all this. It's not the right --

DR. ROBERT CHAPIN: Right before you -- I'm sorry. I was told by Doug Wolf that

chlorothalonil is a category 2. Is that right, Doug? Or a different category?

DR. DOUG WOLF: That's a different category.

DR. CLIVE ROPER: These are GHS categories for -- so, this is chosen for --

DR. ROBERT CHAPIN: I was just trying to put some context around what we --

DR. ANNA LOWIT: So, to answer your question, the GHS category system and the EPA category system are different.

DR. ROBERT CHAPIN: Okay. Sorry.

DR. CLIVE ROPER: No problem. So, we were really trying to look at a proof of concept. Now, without going into all the detail -- there's too much here -- but if you take the top, they've got small numbers, and the bottom have got large numbers. So, these are IC75s from the in vitro data in the rat and the human. The big numbers demonstrate what is not damaged, and the little numbers mean that that's the toxicity of the IC75 level. So you can separate that out as being the toxic ones and the not toxic ones,

as predicted in these two models. The rat and the human were very similar.

Now I'm just going to jump ahead to the -- and I'll give you all of these. I'm going to jump ahead. Where is it? Oh, no. I've got the wrong presentation. Right. So, I'm going to answer your other questions.

So, why have we got 180 percent LDH release? Let's go back to that question. So, it's an assay. It's a kit assay. And as part of the assay, you apply a lighting solution. And the lighting solution is purely kit form. So, it's not optimized to fully knock out all of the cells in this model. So, that gives you your 100 percent.

The reason we're getting 180 percent is because we know from this study here that if we use four millimolar, we will certainly kill all of our cells. So, that's why we get 180 percent off the 100 -- the 100 percent is the kit control. So, in this case, it's clearly not knocking out all of the cells in the model. But we know that our SDS positive -- and if you look at the data in the SDS positive control and you

look at the 200 mg per liter data, they're both virtually identical for each donor. And that's because both of them are actually wiping out all the cells. Okay? So, that's why you get 180 percent.

DR. GEORGE CORCORAN: Wouldn't you, under those circumstances, want to go back and adjust for those conditions so you could release 100 percent of LDH and have this be considered by reviewers and others as a secure measurement?

DR. CLIVE ROPER: Yes. I mean, I think it's just that it's a kit form. It's just, clearly, this kit is not knocking out all of the cells. So I think that does answer -- I hope that answers your question.

DR. GEORGE CORCORAN: So, would you be tempted to modify the kit for this application so that I could look at the LDHs and be very comfortable?

DR. CLIVE ROPER: I think that's something that we would need to go back to the manufacturer and actually explain, could they provide a different control. Or there's many

things that we could do to it. So, that is why we're seeing a bigger number, a bigger percentage, than what's there.

DR. GEORGE CORCORAN: It's an appropriate explanation. Thank you.

DR. CLIVE ROPER: Okay. Thank you.

DR. STEPHEN GRANT: Well, it does bring up another issue, is that it's a kit. But kit for what? I mean, is it a kit for 2D culture and basically what you're seeing is an inappropriate application to 3D?

DR. CLIVE ROPER: No. It's an off-the-shelf kit. It's an LDH release kit that's used for 2D tissues, 3D tissues. I think if we use that on the much more sensitive models, such as the ocular, I think we would find that that would quite happily provide you with a full destruction of that --

DR. STEPHEN GRANT: Well, a 3D model can be many -- I mean, we talked about biomass earlier. So, basically, one of the problems with simply applying it would be you simply don't have enough detergent in there to

wipe out all of the cells. Because, again, it's based on an assumption of the number of cells there. And I don't want to argue about this. It's just one of those cases where, when you have a new model system, I think you have to be careful in terms of using things like kits, because they don't apply directly.

DR. CLIVE ROPER: And that's why we've got our positive control. That's why we have this original data, to choose our positive control correctly.

DR. STEPHEN GRANT: Okay. Now I'm going to ask an important question. There's a made assumption here that human cells are better for modeling humans. And you said the rats and the humans look pretty similar. So, from the point of view of putting a mammalian cell in culture and then killing it, is there a big difference?

DR. CLIVE ROPER: So, for most of those examples, for those 14 compounds, there was very little difference between the sensitivity in the rat and the human. However, there were two which were different, and the rat was more

sensitive. Okay? But if I went through all that data, it would probably kill us all.

DR. STEPHEN GRANT: The rats that you used, were they random-bred or were they inbred?

DR. CLIVE ROPER: They were Charles River inbred animals, which --

DR. STEPHEN GRANT: That might be a reason why they'd be more sensitive innately because they --

DR. CLIVE ROPER: Which is also one of the usually-chosen rats for the in vivo. So, we chose to use the same animal that is a primarily used animal in the in vivo test. I think we even used the same age animals that we took it from.

DR. STEPHEN GRANT: Because that's -- and one of the things we can't really get from the human is because we don't have a wide range of donors. Are there effects of age -- I don't know, nutrition status, things like that? Can you see systematic changes in the system?

DR. CLIVE ROPER: So, that's why we want -- that's one of the reasons why we've

chosen to produce a rat model, because we want to be able to fill in that full square. At the moment we've always gone in vivo, in vitro, and we're not actually asking the right questions. What we should be saying is, in vitro rat, in vivo rat, in vivo human, in vitro human. And all the time that we're talking about in vitro, in vivo, we're not remembering that we're two steps. We're actually in vivo, in vitro and human, animal. Two steps. And that's why we've created that.

The other thing that someone mentioned was about the five donors. Just trying to look around, who said five donors.

DR. ROBERT CHAPIN: Sonya.

DR. CLIVE ROPER: It's fine. So, that pool of five is a random pool of five, which you would do in any human experiment. Any human, you would take a random pool. So, we've got a random pool there. The pool is too small to say that the female or the male or the age is too small a number to have picked any information out there.

Now, it would be great to have had any, 20 or 10 female, 10 male, and then you could do all of your statistics on your age groups then, age and sex. But at the moment, that pool is just too small. And we know that interindividual variability is huge.

One of the things I like to say is look around the room. We're all really, really different. But actually, those differences might well be that that part in the room is actually more similar and that's more different, rather than actually saying that it could be an age thing or sex thing.

DR. SONYA SOBRIAN: I just wanted to say, you say that your human donors are -- it's a small sample. And I agree. But somewhere along the line, if somebody had to make the decision about using sex as -- two sexes, because the last experiment they talked about, six-hour exposure, was only done in males.

DR. CLIVE ROPER: Yes, and that was in the male rat.

SONYA SOBRIAN: Right.

DR. CLIVE ROPER: And I think if you look at -- without knowing the data off the top of my head from Syngenta with their rat models, I would suggest they're probably in exactly the same -- they're a fixed age, and they're probably quite young, and they're probably quite small. Because they tend to be -- don't they? Jon, they tend to be quite young, quite fixed age, right?

DR. JON HOTCHKISS: Yes, sir.

DR. CLIVE ROPER: So, again, if we start to criticize a lot of the in vitro model, let's have a look at how we're going to criticize the in vivo model, because I don't think that any of those rats relate to someone spraying, because they're probably quite juvenile. Probably. Maybe.

So, we just wanted to focus a little bit more on the actual experiments that we've done and how they relate to the toxicology of SDS, because it's critical as our known and positive control that has been designed to be a positive control versus the results we're getting for chlorothalonil, and just trying to explain

where some of these numbers do come from or why we get these bizarre numbers. So, yes, the kit does its job. The LDH kit does it.

I think there was another question. We'll just wait for Anna to take that very important call. Can I just remember everybody to switch your telephones off, please? So, I should go back again.

And another thing -- so, you were actually talking correctly about the assay, that they were both very similar assays.

DR. GEORGE CORCORAN: To be exact, they use the same beginning reagent, but for two different purposes. One was coupled with another enzyme to measure out maximum LDH release, and release under exposure, and the second assay was to deem the reductive capacity of the cell.

DR. CLIVE ROPER: Correct. However, one of the things that we need to focus on is where did those samples come from? So, the LDH, we can take serial sampling for because it's nondestructive. It comes from the media, whereas the metabolism is a destructive in the tissue itself.

So, although, yes, they might well be on the face of it, using very similar mechanisms to measure something -- sorry. But what they're doing it is they're focusing on very different areas. So, we can do serial sampling with the LDH. And yes, it is that colorimetric assay, but we also have a destructive assay with the tissue. So, actually, it doesn't really -- they're measuring two different endpoints, but they're totally unrelated, where they're coming from.

DR. GEORGE CORCORAN: The only reason I brought that up is, if there is a liability in using this chromophore, or this chemical that's being reduced, and if it carries across to a second endpoint evaluation, you've lost that diversity in probing those two different elements of measuring the health of your cells. And so, I would just, in terms of constructing the experimental plan, I'd be much more comfortable if the same reagent was not the driver of two independent assays.

DR. CLIVE ROPER: Yes. So, if we go to these other assays -- and I think we did

mention very earlier on about the other in vitro assays that are there using 3D-tissue models. So, for example, the skin irritation and the ocular irritation assays, part of the new -- five pack? Am I saying that right? So, part of the new five pack.

So, if you take the skin and eye irritation models, part of that guidance to do that is to measure that you don't have colorimetric effects and you don't have chemical reduction. So, actually, we do know that these assays don't interfere. We would actually be checking -- we do check that they don't interfere with the actual assays. So, hopefully that sort of directs us a little bit more onto the confidence that we have on these assays.

DR. GEORGE CORCORAN: Thank you.

DR. ROBERT CHAPIN: Other questions?

DR. STEPHEN GRANT: So, it's strange I'm asking the animal guy this, but --

DR. CLIVE ROPER: I'm the in vitro guy. I work for Charles River, we're three yards all the way.

DR. STEPHEN GRANT: Okay. I'm just challenging the assumptions in a lot of these models. And one of the assumptions -- well, again, human is better than rat for human. Mammalian may be good enough. But now, we're talking about -- at least when we go from rat or mouse, we have strains; so that when we put the cells in, we know that they are the same cells. In fact, they are so similar that they don't exist in nature. Right? The inbred strains.

Why do we have to create models of the single individual and have the individual variability translated into the in vitro case? I'm not exactly sure how they seed the cells into the plate, but why can't we put an equal mix of 20 people?

DR. CLIVE ROPER: Okay. I'm going to answer that one for you. So, there is a model from MucilAir, and I believe that there is also a model from EpiAirway. So I believe that Epithelix and someone else, both create, also, multi-donor models. So, some of the things that Alex was saying about was, as he said, this is part of a large program of work for internal

decision-making initially. And what we were doing was -- is it okay to say about what we were decision-making over? We had different donors. I'm going to just say it. He can just tell me after.

So, what we were interested in, to start with, was that we were able to only buy single donors. So, you buy single donors. And we were interested to see which formulations had an effect on the tissues. But we put a drift in. We put in a compound -- a formulation.

Every different formulation that we tested, we stuck in a fixed controlled formulation, which allowed us to look for drift. And indeed we did see drift, but we could always see where that controlled formulation was. And you could see, with your test formulations, where they were and relative to your controlled formulation.

So, there was a lot of fixed there. And then we found out that Epithelix could create a multi-donor version. I can't remember how many donors it was. Song, can you remember how many it was?

DR. SONG HUANG: Fourteen.

DR. CLIVE ROPER: Fourteen. So, it was a 14-donor MucilAir, and we tested that exactly the same again. And of course where did our fixed control go? Yes, ends up in the middle of all of our drift.

So, again, you could do that. But we thought, with this experiment, it was important to put in the donor effects. But you could run the experiment with the multi-donor. And exactly as we do if we look at in vitro metabolism. When we're doing in vitro metabolism studies, we use hepatic multi-donor derived enzyme microsomes. Yes.

DR. STEPHEN GRANT: Do you have enough data now to say that 14 is enough to account for variability, or was that all you had?

DR. CLIVE ROPER: I think they use 20 in regulatory metabolism. I think it's usually 15 to 20 they use in this type of --

DR. STEPHEN GRANT: Right. I'm just -- is this a calculated number? Or is this, "Let's use 20, that's enough"?

DR. SONG HUANG: Actually, the idea to make the four donor, actually, it's two reasons. One is to try to reduce the donor variation. And the other reason is that we can have a big stock upstairs you can use for years, the same modeling. So that's the reason for this.

So, we make a calculation. Fourteen is good enough for five years, for example. Projection. Maybe we can put more. So, we have to consider whether it's a bigger advantage or not. Because why put more? The reason is you take one, you make a bigger production. So, it's getting very, very big if you put in too much donors.

DR. ROBERT CHAPIN: Song, could you just stay here for the rest of the questions?

DR. SONG HUANG: I can.

DR. ROBERT CHAPIN: Wonderful. Thank you. You can turn your mic off. Yes?

DR. HOLGER BEHRSING: I was aware of the mixed donor tissues that one can get. Again, going back to the five-week maturation period, if there are any differences in doubling

times between those donors, you can have a skewing of whatever you end up with after those five weeks. Has that been addressed or looked at?

DR. SONG HUANG: Yes, that is a good question. Because what we do is we preselect cells. We look for the proliferation rates. Already in 2D, for example, you put in petri dish, the same amount of cells in the beginning. And you see if within three or five days you can get a confident modeling or not. So, yes, we select actually a donor for this capacity of the proliferate.

DR. ROBERT CHAPIN: Jim?

DR. JAMES BLANDO: I guess, just the one comment that I would have with regards to talking about variability, versus human, versus rat cells and so forth. I think it's important to keep in mind that -- my understanding is that if someone's using human cells in vitro testing, the request is to have a reduced uncertainty factor.

So, I think that's important to keep in mind when you're comparing and thinking

about, you know, we have inbred strains of rats versus using human cells. My understanding is that the uncertainty factor that would be used in the models would be lower. So, I think it is relevant to ask yourself how representative are the human donor cells to people that are actually going to be exposed?

DR. ROBERT CHAPIN: Kristie?

MS. KRISTIE SULLIVAN: Kristie Sullivan. But actually, I have a quick comment, which is that the intraspecies variability, there is still a proposed 10x factor to account for that. Just to remind everybody of that.

DR. CLIVE ROPER: You did say interspecies?

MS. KRISTIE SULLIVAN: Intra.

DR. CLIVE ROPER: Intra. Sorry.

DR. ROBERT CHAPIN: The EPA is nodding in the affirmative.

MS. KRISTIE SULLIVAN: The other thing is that it's my understanding, in some cases, that males are considered more sensitive, in general, in the respiratory system because they have faster breathing rates. Again, very

general. So, is that maybe the reason why those male rats were chosen for that study in particular? Or --

DR. ROBERT CHAPIN: Doug or Alex, we're going to ask either one of you guys to --

DR. DOUG WOLF: This is Doug Wolf from Syngenta. We'd have to go back and look. Those studies were done quite a long time ago and, actually, predate me coming to Syngenta. So, sometimes those decisions are not made for that kind of reason, but for other reasons.

If you look at the response between the male and female, in a specific study, you might detect difference in numbers; but the frank response we're seeing isn't qualitatively different. So, we may have just decided to do males because they're easier to deal with.

MS. KRISTIE SULLIVAN: Yes. I wasn't trying to --

DR. DOUG WOLF: Well, we have -- I mean, you know, the issues around the male rats are a little -- a little cheaper, whatever. So there's a lot of reasons why we may have designed that study that had nothing to do with gender.

MS. KRISTIE SULLIVAN: I just wanted to clarify. I wasn't implying something specific about chlorothalonil. But generally, in respiratory toxicology, in the past, people -- I've heard that as a reason.

DR. JON HOTCHKISS: If there's a clear gender difference between toxicity, that gives you an opportunity to reduce the number of animals, so that guideline allows you to go ahead and select the core sensitives.

DR. DOUG WOLF: And sometimes, in this case, with the acute we didn't see a dramatic difference, so we just pick one sex over another because it's less expensive. We just do one sex and not two, because we get the same response.

DR. ROBERT CHAPIN: Okay. Other questions about the -- yes?

DR. NIKAETA SADEKAR: Nikaeta Sadekar. So I just have one question. Do you have similar micrographs for CTN exposures?

DR. DOUG WOLF: For the --

DR. NIKAETA SADEKAR: MucilAir --

DR. DOUG WOLF: For the histology?

DR. NIKAETA SADEKAR: Yeah, histology. Chlorothalonil.

DR. DOUG WOLF: Oh, in the in vitro?

DR. NIKAETA SADEKAR: Yeah.

DR. ALEX CHARLTON: This is Alex Charlton from Syngenta. The answer is, no, we've never taken histological sections of MucilAir tissues exposed to chlorothalonil. We showed some -- I showed, in my presentation, some histological sections that we'd taken with another active ingredient. But we've never actually used chlorothalonil this way.

DR. NIKAETA SADEKAR: Any particular reason for not doing that, specifically with this case study?

DR. DOUG WOLF: I guess hindsight being 20/20, and we did discuss this to repeat the study, but it would have required repeating the study to do that, and we had sufficient information to move ahead with this. So, it might be worthwhile, if we end up having to do more, to do some histology. But all you would -- I'd question, as a pathologist, if that would

actually add anything to our decision construct. And Clive probably can --

DR. CLIVE ROPER: No, I don't think it would. I think adding in the pathology is really interesting. But if you're looking at the sensitivity of the model -- if we're looking for a -- when you're calculating your point of departure, you're going to take your most sensitive models. So, your most sensitive model are the ones that we've actually measured; because you're going to see those first before you're going to see what occurs in the pathology.

But we're doing that a lot now. We're doing a lot of pathology with these models now, because they do give you a little bit more information. But it won't give you that information a little bit earlier on, because you're still going to go back to your more sensitive model, which is your first step, which is your LDH release.

DR. ROBERT CHAPIN: More sensitive endpoint? Or more sensitive model?

DR. CLIVE ROPER: More sensitive endpoint.

DR. ROBERT CHAPIN: Endpoint.

DR. CLIVE ROPER: Your most sensitive endpoint you're going to get, because they have to go first before you see the visual damage.

DR. ROBERT CHAPIN: Okay. Other questions about the model? George? Sorry, I'm sorry. Nikaeta?

DR. NIKAETA SADEKAR: I only ask this because we don't see a dose response with the chlorothalonil exposures. And it's just a curiosity as to maybe loss of cilia or something that's probably happening, and it's not indicating the LDH or TEER.

DR. CLIVE ROPER: The likelihood is you are seeing something first. But --

DR. ROBERT CHAPIN: You mean by histology?

DR. CLIVE ROPER: I just want to clarify a point. You said that we don't see a dose response in the chlorothalonil phase, when I think we do. Which endpoint was you talking about there, specifically?

DR. ROBERT CHAPIN: Or what do you mean by dose response? Because there is. I mean, a lot of it's flat, and then it goes nuts. Is that what you mean? There's no linear change?

DR. NIKAETA SADEKAR: Yes. So, the concentrations that are used for chlorothalonil -- the highest two concentrations, 200 milligrams per liter and the one above it, they are the ones that actually show cell death parameters that you can actually measure. But above that, you don't have a trend.

DR. ROBERT CHAPIN: Below that. Below that.

DR. NIKAETA SADEKAR: I'm sorry. The lower concentrations, yes. Below, yes. Sorry. Yes.

DR: CLIVE ROPER: Yes, a very flat threshold, plateaued phase before you start to see that kind of fairly rapid tail-off -- well, fairly rapid onset of toxicity, but there's a tail-off in TEER or increase in LDH.

DR. ALEX CHARLTON: It's a very steep dose response --

DR: CLIVE ROPER: It is a dose response --

DR. SONG HUANG: Actually, the TEER is -- it's very sensitive here. It drops suddenly, dropped very suddenly. And sometimes, if you narrow down your dose range, you can see a response curve. But you should really get a very, very, small concentration then.

DR. ROBERT CHAPIN: So, are you good?

DR. ANNA LOWIT: So, if I could add, just from a risk assessor's point of view, to make sure we sort of follow up on that point? Anna Lowit from EPA. From a risk assessor's point of view, I'm much more interested at the low end of the dose response curve. I'm not interested in a bunch of concentrations where there's 100 percent lethality. I want to see where you get that dip and where it's flat and where you begin to get that dip. Because, from a risk assessor's point of view, I want to make sure my point of departure is on that line or right as it starts to dip over.

So, a lot of those, the concentrations they picked in the values that you see in the earlier presentations were actually based on conversations that we had with Syngenta as they were designing the experiments, because we wanted them to be able to calculate, reliably, of the MDL, using a very low benchmark response. And the one standard variation is a very low response.

So, that's, to some degree, why they did what they did, because that was based on feedback with us. But from a risk assessor's point of view, that's where we're much more interested.

DR. STEPHEN GRANT: Just to comment on that -- better stay there.

DR. ROBERT CHAPIN: This is Steve Grant.

DR. STEPHEN GRANT: What would be -- Steve Grant. Right. You certainly want to catch the threshold of effect, but you want to be sure it's the real effect. You don't want a one-point curve, and then find out you missed the real effect because it was actually an order of

magnitude higher. So, you really do want to see more of the curve than just assume the first down point is the beginning of the induction or the effect.

DR. ROBERT CHAPIN: They've got two there, right? So, it's --

DR. STEPHEN GRANT: Infinitely more.

DR. DOUG WOLF: Can I respond to that? So, if it's not in between those two points, where is it?

DR. STEPHEN GRANT: No, no. My --

DR. DOUG WOLF: You can worry about it, but what you're seeing is a variability in the top.

DR. ROBERT CHAPIN: Okay. This is a discussion for a fermented beverage. Jim?

DR. JAMES BLANDO: Not to add more to the ferment; but I guess I too felt that, because the curve was very flat, I don't know that I agree that that's the only thing that a risk assessor would be interested in, is at what point do I see drop-off. I think, if you're looking at an assay where you want to have a

proof of concept, you want something that's sensitive enough to see a graded response.

Also, I think it's important to keep in mind, what I felt very unimpressed about, to be honest with you, was when you looked at the negative and positive controls. If I'm not mistaken, for, I think, the TEER results -- if I remember – I don't have it in front of me, but if I remember correctly, it was within the region for the negative controls, which made it even less convincing to me.

DR. ROBERT CHAPIN: The TEER for the positive control?

DR. JAMES BLANDO: For the experimental group versus the negative control, the difference, I think, that was observed was what was pretty much pretty close to what was observed in the negative control, if I remember correctly. But I, too, will say that -- if I don't remember correctly, then I apologize, but I remember not being particularly impressed by the data.

DR. ANNA LOWIT: So, just to make sure that we remember the historical context that

you heard from Alex this afternoon -- this is Anna Lowit again. I'm sorry. That the original experiments that Syngenta was working with was to look at the degree to which changing a formulation would change the response. And in those original experiments, they were using concentration curves across many orders of magnitude. And so, the strength of the response had already been demonstrated in the early experiments with those formulation evaluations.

So, to repeat that, when they were working towards deriving a point of departure, is really not necessary, because they had already evaluated those endpoints at those concentrations. So the more recent experiments were specifically designed for the purposes we're talking about of deriving a point of departure, for purposes of risk assessment.

And if the values are hovering within the background, that's actually not a horrible idea; because, as a risk assessor, what we think about when we do a benchmark dose is that we want the response level for our BMDL to be right at the edge of background.

So, if some of the experiments are hovering above and below where the controls are, that tells me we've actually hit the sweet spot where we're at the edge of background, but most of the time we can reliably measure it. So, that's actually the goal, and that tells me that we've achieved that.

DR. ROBERT CHAPIN: Very helpful. Okay. George?

DR. GEORGE CORCORAN: Thank you, Dr. Chapin. I just would like to add a perspective point of view for this committee versus Syngenta. I know Syngenta said we probably won't need to do histology on these in vitro cell samples. However, if you review all of the charge questions to us, we are going to be asked not only whether we believe this is an adequate system for risk assessment with chlorothalonil, but whether it's a secure, believable system that can be projected and generalized.

So, for that reason alone, I would say, if future studies are done, it will be very valuable to add histology on the in vitro.

DR. ROBERT CHAPIN: Okay. So, that's a useful thing -- to sort of answer his questions, that gets us into Thursday and Friday, I think.

DR. GEORGE CORCORAN: All right. I'll be quiet now. Thank you.

DR. ROBERT CHAPIN: Thank you. So, my question for the committee is, are there any other questions that we have for the people who generated or used the model, to help us understand?

DR. MARIE FORTIN: I mentioned earlier my impression that the endpoints that were chosen were not very sensitive. And I was wondering if either Epithelix or Charles River could provide information, with respect to, for example, TEER. Right? It's pretty much a yes, no, right? Because you lose your membrane integrity and then you lose that resistance. So, how many cells -- you know, in a percent maybe -- would have to die to get that class?

DR. SONG HUANG: Actually, for TEER, you don't need the cells to die actually. So just broken junctions, it's enough. So,

sometimes, when we have trouble with some of the batches of production, it's that these epithelia detached from the inserts to create a gap. Just detach a little bit.

DR. MARIE FORTIN: I'm not asking for TEER, specifically. I'm asking for all -- for cytotoxicity. It's argued that they all correlate, and that's the reason why they are employing the technique, because they say they're all basically providing the same readout. Those are, essentially, readouts of cytotoxicity, because that's how they model it. That's part of the AOP, right? So, how many cells die to get to that level where we can actually measure it?

DR. CLIVE ROPER: Okay. So, it's actually one of the most sensitive models that we've got. It's actually very -- picks out very quickly the endpoints. But I don't think anyone has measured how many cells that you're going to take. But they're quite sensitive, the tissues.

So, we do things like, you apply your material onto the tissue. That might do nothing. And if you see, at the end you see that the TEER is falling at the end of 24 hours, and

that's because we are actually doing physical things to those tissues. So, we are actually watching them, for example. That physical effect could actually damage those junctions and reduce the TEER, change the TEER.

But actually, they'll recover quite quickly. They also snot a lot. And I have to use that as being a bit of a colloquialism, but they produce a lot of mucin. So, again, we have to remove that mucin for some of these measurements. So, they are actually getting physically affected, but they do recover back again.

So, I don't think anyone's measured how many cells or what percentage of cells. That's why we use the other measurements. But what we are doing is we're looking at this, we're looking at a very, very easy measurement.

I think someone actually asked about how they're measured. The electricity is coming from the probe. You've got two probes. One in the top. One in the bottom. And it's a measurement of the electrical resistance across

that. It's a very easy method. You could even do that in animals.

DR. MARIE FORTIN: Like I said earlier, cell death is a very terminal endpoint for the cell, at least, right? At the organism level, no. So, adding an idea of the amount of cells that die, so a percent, right? Because we're making the assumption that that specific area, within that cell, that dose -- so, if we could get to, like, okay. That means that 20 percent of the cells are dying in that level, that would transfer, right, according to the model, to what's seen in the airways.

And then, the question in risk assessment becomes, is 20 percent cell death too big of an adverse effect?

DR. CLIVE ROPER: Can I just point this out? Monolayer integrity was determined by TEER. Okay? So, we've got other ways to measure toxicity in there. Okay? So, we are measuring slightly different things without measuring integrity. So, the integrity, you might not cause any cell death and see a big reduction in TEER. But once you get down to these

measurements, then we're measuring true cell death.

DR. MARIE FORTIN: Yes and no. So, if you use, for example, I mentioned the live/dead assay, right? So, that will look at something that's more sensitive. And you can use those facts to measure and calculate the number of cells.

Because, right now, it's all based on the assumption that the cell death that's occurring -- and oh, we had once on the variation change, and that's, you know, where, basically, we get our curves as being adverse; but we don't have any risk correlated to the number of cells or the specificity of the tissue and the percent of the cells within that tissue that are dying. Yet, that's what we're trying to do. So, that would be something to kind of work on in the future, in my opinion.

DR. CLIVE ROPER: I wanted to very quickly introduce a perspective on TEER that's an endpoint relevant for cell death, irritation, however we term this. So, I think if we look at OECD test guidelines, there are OECD test

guidelines in vitro, looking at irritation and corrosion, which routinely use TEER as an endpoint in that study. I think that that's one of the reasons we thought that TEER was an appropriate endpoint.

DR. MARIE FORTIN: But that's where I thought I'd be. There's a difference between saying yes, no, or even putting it in a GHS category, versus conducting a risk assessment and defining a value that's going to be the limit or basically a threshold with respect to workers' exposure. So, what I'm asking is a more refined approach, if we want to do it as part of a risk assessment.

DR. CLIVE ROPER: Going to this question again about it is almost -- it is very, very sensitive, the TEER. And when we're looking for our point of departure -- anytime you do a point of departure, you always do your most sensitive model. And that's actually -- bow. By the end of this, I'm going to have learned something.

In fact, one of the things that we've got is we've got the luxury that you don't

get in the animals. We've got lots of concentrations. You don't have lots of concentrations with your animals. We've got lots of luxury of lots of different endpoints. So, we're seeing things probably slightly earlier than in the animals because some of the times, in the animal, you're using just a really simple thing called death.

DR. MARIE FORTIN: But it's the same thing. We're using death in cells, right?

DR. CLIVE ROPER: We're not. For monolayer integrity, we're not. We're --

DR. MARIE FORTIN: Well, TEER is one effect, but the other ones aren't --

DR. CLIVE ROPER: The others are cell death.

DR. MARIE FORTIN: But that's written in the document. That's part of their hypothesis. That is the endpoint. So, if you're saying it's not the endpoint --

DR. CLIVE ROPER: And that's what we're measuring from LDH and --

DR. ROBERT CHAPIN: Okay. Dr. Grant.

DR. STEPHEN GRANT: Just a clarification. As I understand it, monolayer integrity means that as soon as you breach that one cell, one place, the electricity is going to find that open spot to go through. So, it's the first evidence of damage that separates the monolayer, right? It's not going to give you 20 percent. It's going to give you all or none.

DR. CLIVE ROPER: I was going to bring you the paper that was mentioned before. Someone mentioned the Sivars paper.

DR. MARIE FORTIN: Yeah. I've read it.

DR. CLIVE ROPER: Yeah. Sorry. Andy Dupont, can you please put the alternative on there.

What they did was very interesting. Because they took a library of their known --

DR. ANDY DUPONT: The Sivars paper?

DR. CLIVE ROPER: The Sivars paper is the one which was in the PDF. Yes. That one

there. And if we just go down a tiny bit. Stop. That's fine.

What they did is they went from the other direction. So, they said that we've got materials that have failed in preclinical, they failed in clinical, and they've gone to market. Can we pick up these failures early?

And what they actually identified was -- and I'm going to try to read it from here -- is predictability for respiratory toxicity were evaluated by cytotoxic barrier integrity, viability, blah, blah, blah, blah. Interestingly, it did show that the -- can't quite read it now. So, it basically says that a trans electrical resistance and cell viability by Resazurin predicted the in vivo most effectively. There you go.

DR. MARIE FORTIN: In the endpoints, they measured. If you add something else there, you could have something different.

DR. CLIVE ROPER: We could add loads of endpoints. We could add loads and loads of endpoints. You name them. We can add them. We can they test them. They might not be

relevant. There's lots of endpoints we can have. But it's a better one than just dead animal.

DR. ROBERT CHAPIN: Okay. Anna.

DR. ANNA LOWIT: So, Dr. Chapin, I kind of feel like we've crossed over from clarification to working some of the charge questions. So, there may be differences of opinion on the panel that we'll look forward to hearing when we do these charge questions, to make sure that the full breadth of opinions are represented when we do the charge questions.

But the one thing that I would add, as we sort of close out this piece of the session, is, if I put on my ICCVAM coacher hat, a common theme that we see, no matter what kind of endpoint we're talking about, is that people hold in vitro studies to a higher standard than the in vivo studies. And we're asking questions of the in vitro study that have never been pushed in the in vivo animal. Issues of validation, issues of the most sensitive endpoints, issues of sample size, a number of questions that have been raised.

If you actually understand the OECD guideline process, most animal studies have actually never been validated. The sample sizes in those studies have never been evaluated statistically. And the endpoints that are measured in those studies, generally, are those that are commonly done and can be easily done in CROs. They're not the most sensitive endpoints. They're not measuring mechanistic endpoints. Mechanistic studies were done specially outside of the OECD guideline process.

So, I want to make sure that when you all are evaluating the questions, that we keep that in context, that we don't ask of the in vitro studies more than we ask of the in vivo studies. And in fact, we'd want to go back to the comments that Monique and I made this morning of thinking about the animal as a gold standard. And is that really even the right question to ask?

That, given the distinct anatomical differences between a rat and the human, and the distinct dosimetry differences, and the small particles used in a guideline study

versus the much larger particles that humans are exposed to out in the field, what we're talking about here is not apples and oranges. It's more like watermelons and lemons. Trying to make these one-to-one comparisons is fought with a lot of uncertainty, and there's just a lot of challenges in making those comparisons.

We've tried. I promise. That was the first thing we did when Syngenta came to us on this. And we've put the side-by-side comparisons and struggled with, wow, these are different. But what does it mean? That's the question. What does it mean?

Human tissue is modeling humans. Human dosimetry modeling is modeling humans. When we know there's a distinct difference between the species, we have to make sure that we're modeling the right species. We're concerned about workers in the field exposed to chlorothalonil, as I think you would understand based on the potency of the compound.

So, I would just make sure that when you're looking at the questions that we're back to this reality -- sort of just a reality

check of weighing the uncertainties in the rat versus the challenges that we face in the new science. We ask questions of new science that we don't ask of old science. We hold new science to a higher standard, and that should not prevent us from moving forward.

I guess that's sort of the way I would end the presentations, that we want to make sure that, as we're thinking about bringing the new approaches, we're never going to know all the answers. I don't know all the answers using the rat in vivo study. As a risk assessor, I never know all the answers. That's inherent in regulatory science.

That's why we use extrapolation factors and uncertainty factors. That's why our exposure assessments use high-end assumptions. That's why you saw Syngenta today compounding conservative assumptions in the models that they're doing; that we never have all the answers, but that's why we push our estimates towards conservatism, to account for those uncertainties. It's inherent in the work that we do every day for every chemical.

So, I would just hope that all of you sort of bring that to the reality of, this is the situation that we face every day and that our goal is to move towards a more human-relevant approach where we understand the science. We're doing hypothesis-based testing, or we're doing relevant testing for the rat, for the species, and for the dosimetry.

DR. ROBERT CHAPIN: Right. That's, I think, a good re-grounding of our discussions and expectations, and might sort of help us think about separating the really-nice-to-haves from the what-we-got-to-have to make this work.

Let me see. So, I'm assuming that since we had -- I'm assuming that we're kind of done. We're well past 5:00. So, I'd like to thank our EPA colleagues for staying this long and allowing us to be on this issue some.

Let's see. We've had, I thought, a wonderful day. Tomorrow, the committee is not meeting, but I encourage the groups addressing each individual question to confer and do as much discussion of your question as you'd like to.

And then, we will start at 9:00 on Thursday with question one.

So, with that, unless there are any other issues from the committee? And I'd also like to thank the presenters. Thank you all for your time and patience with us here today. And I'll turn it back over to our DFO.

DR. SHAUNTA HILL-HAMMOND: Thank you, Dr. Chapin. I would like to thank the panel for your robust discussions and questions raised today. I would like to thank members of the public and panel, as well, for your participation. It's been a long day. Thank you all for staying with us. As noted by our chair, we will reconvene on Thursday, December 6th, at 9:00 a.m. in this meeting room. And with that, this meeting is now held in recess. Thank you.

[ADJOURNED FOR DAY 1]

DAY 2 - OPENING/INTRODUCTIONS

DR. SHAUNTA HILL-HAMMOND: Good morning. I would like to welcome everyone and thank everyone for participating in today's public meeting. My name is Shaunta Hill and I'm the Designated Federal Officer, or DFO, for the FIFRA SAP Review of EPA's Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study using a New Approach Methodology (NAM).

At this time I would like to reconvene the meeting of the FIFRA SAP. The FIFRA SAP is a Federal Advisory Committee that provides independent scientific peer review and advice to the agency, on pesticides and pesticide-related issues, regarding the impact of proposed regulatory actions on human health and the environment. The FIFRA SAP only provides advice and recommendations to the EPA. Decision making and implementation authority remain with the agency.

As a reminder, all meeting materials are available in the public docket

available on regulations.gov. The docket number and website are noted on the meeting agenda. With that, I would like to turn the meeting over to our meeting chair.

DR. ROBERT CHAPIN: Thank you, Shaunta, and good morning, everyone, and thank you for being here on time. My name is Bob Chapin. I drew the short straw, and I am the panel chair for this SAP. So now we're going to go around and introduce all the panel members, and I'll start. I'm Bob Chapin. I'm an independent consultant with reproductive toxicology, and we'll go this way this time.

DR. CLIFFORD WEISEL: My name is Clifford Weisel. I'm a professor at the Environmental and Occupational Health Science Institute at Rutgers, and I work in exposure science.

DR. RAYMOND YANG: I'm Ray Yang, retired professor from Colorado State University, consultant, and I'm a toxicologist.

DR. LISA SWEENEY: I'm Lisa Sweeney. I'm a risk assessment toxicologist for

UES, assigned to the U.S. Air Force School of Aerospace Medicine.

MS. KRISTIE SULLIVAN: I'm Kristie Sullivan, Physicians Committee for Responsible Medicine.

DR. NIKAETA SADEKAR: Nikaeta Sadekar, Human Health Scientist for Inhalation Toxicology with Research Institute for Fragrance Materials.

DR. EMILY REINKE: Emily Reinke, biologist and board-certified toxicologist with the U.S. Army Public Health Center and co-chair of the Interagency Coordinating Committee for the Validation of Alternative Methods.

DR. KATHRYN PAGE: Kathryn Page, public safety toxicologist with Clorox; also, board certified toxicologist, and my specialty is alternatives to animal testing.

DR. ROBERT MITKUS: Hi, I'm Bob Mitkus. I'm a toxicologist at BASF Corporation in Durham, North Carolina.

MS. ALLISON JENKINS: Allison Jenkins, regulatory toxicologist with the Texas Commission on Environmental Quality.

DR. JON HOTCHKISS: Jon Hotchkiss. I'm an inhalation toxicologist, and I work for The Dow Chemical Company.

DR. STEPHEN GRANT: Steve Grant. I'm a genetic toxicologist and geneticist at the AutoNation Cancer Institute at Nova Southeastern University.

DR. MARIE FORTIN: I'm Marie Fortin, Assistant Director of Toxicology at Jazz Pharmaceutical and also adjunct professor at Rutgers University. I do toxicology and risk assessment.

DR. JENNIFER CAVALLARI: Hi. My name is Jen Cavallari and I'm an associate professor. My expertise is in exposure assessment, and I'm at the University of Connecticut School of Medicine.

DR. HOLGER BEHRSING: I'm Holger Behrsing, principal scientist and head of the Respiratory Toxicology Program at the Institute for In Vitro Sciences.

DR. JAMES BLANDO: Jim Blando. I'm associate professor at Old Dominion University. I'm an industrial hygienist.

MR. ANDY DUPONT: Andy Dupont with the Office of Science Coordination Policy, federal designated official with EPA.

DR. GEORGE CORCORAN: George Corcoran, professor and chair of Pharmaceutical Sciences at Wayne State University. My areas of interest are liver entry, drugs and chemicals, bio transformation, and nutritional effects on safety.

DR. SONYA SOBRIAN: Good morning. I'm Sonya Sobrian. I'm at the Howard University College of Medicine. I'm a developmental neurotoxicologist.

DR. ROBERT CHAPIN: An illustrious group of scientists by any measure. Okay. Thanks again for being here. We've got a full agenda today. As you can see, we're trying to stuff the discussions for all the charge questions into today so that that will leave tomorrow for writing, while we're all still here, and that will really facilitate the completion of the writing assignments.

Let me do a couple housekeeping things before we get started. Please remember to

silence your phones. They can vibrate all they want, but vocal rings are distracting. We're going to have about 70 minutes for each question, so the discussions are going to go -- we're going to need to be fairly expeditious about this. I've been asked by our sound expert back there to try to make sure that we speak about five inches away from the microphone so that it transmits and can get out to the people who are listening to this on a webcast.

So, with that, I would like to help -- let me see. It occurred to me that there are lots of things that we could discuss about the proposed technologies, and not all of those discussions and suggestions and enthusiasms from the panel will be equally useful to the agency.

What we're here to do is to support the agency scientists who are interested in reducing this concept to practice. So I thought it would be useful to hear just a two or three-minute description from Anna Lowit, from the EPA, about what kinds of things would be most useful. So the question that I'd like to ask Dr. Lowit is what's the best and most useful sort of

feedback that we can give you, and what kind of answers will not be helpful for you?

DR. ANNA LOWIT: I'll try to do that in two minutes. So just sort of to back up for a second. What we're proposing along with Syngenta is new. It's very much new, but the NRC finalized their report on Toxicity Testing in the 21st Century over a decade ago. Many organizations, including many parts of the EPA and international partners, have been talking and working on advancing in vitro science, high throughput toxicology, computational approaches, to advance the science to more human-relevant, task-irrelevant approaches, and moving away from animal models that we know, in our heart of hearts, don't do a good job of predicting human health outcomes.

It's our view that, at least in the case of point of contact toxicants and inhalation, that the science is on the cusp of being ready for use in regulatory science. If we didn't think that, we wouldn't be here. We only bring topics to this panel that we know are challenging and hard and new and sometimes

controversial. So we're not expecting 100 percent consensus from this panel.

What we're more interested in is to hear all of your voices. We want to make sure that all of your voices and all of your opinions get captured on the microphone, so that not only the people in the room can hear that, but the people out on the webcast, but that all of your voices are also captured in the report. Because we'll take all of that information and look at the totality of it and look at how it intersects with our risk assessment process, where research is going, et cetera, and make our own difficult determinations on which areas to pursue and which to maybe not.

So it's most important to us that you all have a voice today. And that may mean some of you don't agree with each other, and that's perfectly fine. That is a healthy and natural part of the scientific process, and that's why we're here.

We have had reports in the past where we had a panel say, we agree with you, but the standard approach is not so good. But what

you're proposing has problems, but without tractable advice of what those challenges are and what we can do about them.

So, as you think about giving us your feedback today and recommendations to either the agency or to other stakeholders, what are those tractable things that can be done, not a 10 or 15-year research project?

We're not waiting another decade to implement Toxicity Testing in the 21st Century. We're doing it, and we're doing it now, because we're doing it in other areas. We have a lot of activities in this area going on. We want to make sure in the inhalation area that we're working appropriately as the science is there and is ready for prime time. That's why you've been invited here to give us that feedback.

So those are the things that would be most helpful, if that's helpful to what you're looking for. We do have two exposure experts on our team. I think there were a couple of questions that we needed to give a touch of clarification on. If you could give us a minute, I'll let Monique introduce our team, and they can

answer a couple questions I think had come up yesterday.

DR. MONIQUE PERRON: This is Monique Perron. Good morning. To my right is Cassie Wells (phonetic) and over to the left is Matt Crowley. Both of them are exposure assessors in the Health Effects Division. Primarily, we wanted to give a little bit of clarification regarding the activity level breathing rates because there was quite a bit of discussion yesterday. We just wanted to quickly touch upon that topic, and then we'll let you jump on in.

MATT CROWLEY: Hi everybody. Thanks for --

DR. ROBERT CHAPIN: Remember to identify yourself for the people online.

MATT CROWLEY: My name is Matt Crowley. My title is Biologist in the Health Effects Division of the Office of Pesticides Program, so I mainly deal with the exposure assessment and exposure modeling, not the toxicity side of things. I'm familiar with all of the monitoring data, like the actual field

monitoring data, that our division has used for the past 20 years or so, 30 years, in particular, the Agricultural Handler Exposure Task Force data that has been referenced in these documents.

So my focus here, I think the questions were on breathing rates. The particular scenario that's discussed for this SAP, this kind of case study, is applicators who are using tractors and driving vehicles to spray liquid pesticides or solutions. For that, we have a default breathing rate, and Syngenta used that in their modeling, of 8.3 liters per minute. And that is consistent with the value that is used in our risk assessment process.

The air concentrations that are monitored for those people doing that activity, spraying pesticide solutions with tractors, ground booms, that kind of thing, those air concentrations are then calculated inhaled amount based on that breathing rate of 8.3 liters per minute.

So to the extent that this methodology is extended to other scenarios, workers spraying with a backpack or pilots

spraying with airplanes or perhaps even exposure scenarios with not even applicators but bystanders or exposure scenarios with children, all of those scenarios carry with them our default, or standard, breathing rates that we assume for those scenarios.

For example, in this case, the tractor driver is assumed to breath at a rate of 8.3 liters per minute. For someone carrying a backpack, which is probably around 40 pounds, a full five-gallon plastic container carried on their back, that value we assume for that is 26.7 liters per minute, so just a higher value. And then there's an intermediate rate that we assume for other scenarios.

If there's any conversations on this panel or even amongst the team, we will for sure have to consider how breathing rate applies in this whole approach and making sure that we're continuing the same method and consistent with our risk assessment process and how we estimate inhalation exposure in this.

DR. ANNA LOWIT: I have one thing to add to that. Our exposure assessment

approaches that we use for all of our exposure assessment, occupational, residential, food, water, have been heavily vetted over the last 20 years after the passage of the Food Quality Protection Act in 1996. In fact, our occupational exposure assessments have been reviewed by SAPs several times over the years. Unlike a lot of other programs where exposure assessment is largely based on a lot of default approaches, our assessments are heavily data derived.

We have industry task force that develop, by monitoring studies of workers in the field, that then go into the approaches used by our assessors. We have many, many studies that are used to develop the algorithms used on a scenario by scenario basis. We're very advanced in this area.

Because of those advancements and the existing peer review, we have not brought to you the occupational exposure assessment that was done as part of the case study, and that adaptation of the scenario that they've done to other ones is a natural part of our process that

we do already. So we have not brought you a charge question on that, but we are keenly interested. There were some really good comments yesterday and good questions that came from the panel. We want to make sure that those are captured in the report.

Because, to be honest, we've been asking Syngenta a lot of those same questions ourselves over the last couple of years. So to have this group put those to paper would be excellent for us. Just to make sure that you understood why we hadn't asked you a question about that, is because those approaches have been substantially vetted over a long period of time and are heavily data derived. I guess that's all I would add, if Matt or Cassie had --

DR. ROBERT CHAPIN: So are you guys done? This is Bob Chapin. You're fine with sort of filling in the questions from Tuesday, the open things from Tuesday? Yup. Okay. All right.

DR. CLIFFORD WEISEL: I appreciate what you said about breathing rate. You said you have a lot of field data. The other big question

we had was about particle size from the systems. And I know when we look at spraying you get a lot of large particles, obviously we're focused on the small ones. Could you enlighten us any more on the particle size, particularly below 100 microns, that was used in this report or what you generally found? If not, we understand, but anything you can give on that is going to be helpful.

MATT CROWLEY: Sure. I can elaborate a little bit. This is Matt Crowley, again, from the Health Effects Division, Pesticide Office. Like Anna said, this is new, and the particle size piece of it would be new. The field data that's collected, the monitoring devices -- I think in Syngenta's presentation they showed a picture. It's like a cassette with a pump attached on somebody's collar. And that data does not include particle size information. Syngenta's approach, they did an experiment on --

DR. CLIFFORD WEISEL: I'm sorry. I understand Syngenta. I just wondered if you had field data that looks at particle size.

MATT CROWLEY: Not the field data for the individual workers, but there is information, otherwise, outside of the actual field monitoring data for another task force. For example, Spray Drift Task Force, they have monitors that are set up, and that has to do with how far particles will carry in the winds to off-target locations. So there is information about particle size, and I think Monique mentioned this the other day, that that's part of future conversations with Syngenta and other stakeholders to compile possible data that informs us on particle sizes, yes.

DR. CLIFFORD WEISEL: Okay. Thank you.

MATT CROWLEY: You're welcome.

DR. ROBERT CHAPIN: Okay. With that, let's go ahead and dive into the charge questions. Let's see. My understanding is that Dr. Perron will read the charge questions, and then we'll go to the lead and the associate discussants. Then, everybody else gets a chance to weigh in as you will. So, Dr. Perron?

CHARGE QUESTION 1

DR. MONIQUE PERRON: This is Monique Perron. I'm going to read the first charge question. It's nice and lengthy. 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, considered in the EPA's issue paper?

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 and causes cell death. Following repeated exposure, the repeated cell death results in a metaplastic response and differentiation of respiratory epithelium into stratified squamous epithelium.

DR. ROBERT CHAPIN: Okay. So we'll start off with the lead discussant for this, Dr. Grant.

DR. STEPHEN GRANT: Okay. This is Steve Grant, and, to some degree, we have had a couple of rounds of discussion. So we'll go through. I will pause for elaboration both from the rest of the panel and for some of my people to make sure that I have represented their opinions.

To begin with, the agency is to be commended for all its efforts in undertaking to advance the adoption of in vitro models, particularly those involved incorporating human cells to reduce the use of animals in protecting human health. The charge to comment on the biological understanding in this chlorothalonil -- that second L is the one that always gets me -- proposal was confounded by different interpretations of the charge. Prior to the meeting, many panel members felt that the charge was to understand the respiratory irritant effects of the agent.

At the meeting, it became more clear that the intent was to provide a model for the late unresolved metaplastic effects of the agent submitted into redosing/dosing in vivo study.

Finally, we want to take into account that we've been advised not to consider the existing animal testing system and the limited data obtained using this system as gold standards and not to hold the proposed new testing system to standards beyond those imposed or accepted for the existing test system. To some degree, however, these various charges are interdependent and sometimes at odds, so we'll try to address them all.

As to an understanding of the respiratory toxic effects of chlorothalonil, described as labored rapid breathing, gasping, wheezing, and rales, there is not sufficient data in the proposal to provide a reasonable biological understanding. All data provided demonstrate full respiratory effects, although this endpoint is not provided quantitatively. Although these data were pointedly cited in the

presentation, cellular damage to the respiratory -- I wrote this, and you must -- Holger, when you edited this, you screwed it all up.

Okay. So the in vivo data were not cited as precedent for cell death in the presentation. The damage in the respiratory system described in print as degeneration and/or necrosis, and expanded on in presentation as necrosis and ulceration, were noted in all treated animals in addition to the respiratory effects.

Since no sub-cytotoxic effects were documented, however, it was felt that an interpretation that airway epithelial cytotoxicity was intrinsic to the contact irritation and/or respiratory toxic effects was unjustified since all data was derived from a plateau of maximal effects on the induction curves of both endpoints.

There's no reason to discount the possibilities that sub-cytotoxic effects could induce the physiological reaction in the absence of overt cell death. Moreover, it was noted that other factors had been observed in nasal

irritation and respiratory toxicity, including but not limited to inflammation, olfactory effects, and sensory nerve effects. Inflammation was observed in the in vivo data but was dismissed as resolving with time. It must be noted that the existing animal data is not germane to the level of exposure required to initiate physiological effects.

Similarly, it was stated that olfactory effects could be discounted because of the modeled deposition profiles. This assumes that all effects are modulated only by the amount of contact, discounting the possibility that olfactory effects are much more sensitive and could be induced at levels that still are not associated with overt degeneration in other parts of the pathway.

Although unclear in the proposal, at the presentation it became clear that the proposed in vitro model was at least partly meant to satisfy a request for a 90-day chronic exposure study. Thus, instead of concentrating on establishing the threshold of acute effects that the panel generally felt was lacking in the

original data, the follow-up was more concerned with long-term effects. One again, all exposures in the two-week study induced both symptoms of respiratory toxicity and airway degeneration.

Squamous metaplasia of the larynx was the only effect that did not completely resolve after a further two-week recovery time; and this observation, therefore, became the focus of the follow-up studies, including the move to an in vitro system. Not least because 14 days is not 90 days and the suspicion that even this lingering effect would resolve if given a longer recovery, many on the panel were confused when the squamous metaplasia effect was given as the outcome of the adverse outcome pathway instead of contact irritation resulting in respiratory toxicity.

Referring to the previous paragraph, many on the panel felt that the initial step in this pathway, airway epithelial cytotoxicity, had not been shown to be intrinsic to the physiological processes. In the proposal presentation and later as a clarification, the proposers stated unequivocally that the only

biological effect of chlorothalonil was cytotoxicity and that there was no need to prove that it was true for its effects on the respiratory system. Some on the committee would prefer that this be proven rather than simply asserted as common knowledge.

Finally, despite great amounts of effort to distinguish areas of deposition in the CFP model, it appears that effects in different areas of the airway are invoked interchangeably in the proposal and that there is a general assertion that the model system is concurrently applicable to the whole pathway, rather than just the area provided by the donated tissue. For example, despite the fact that squamous transformation in the airway is a rather late event, clearly distinct from the onset of physiological symptoms, the fact that effects occurred at all doses in other areas, such as the larynx, is considered to mitigate that disconnect between generalized cell death in respiratory systems.

Thus, there's not general agreement that the contention that cytotoxicity

is the basis of the in vivo contact irritation and respiratory toxic effects of chlorothalonil have been established definitively enough to allow for translation to an in vitro assay. In general, there are two methods of justifying such a translation, as a mechanistic precursor effect or simply as a consistent and reliable biomarker. Since no data is available in the onset of systems in the in vivo model, neither of these conditions can be fulfilled.

This brings up a fundamental problem with the application. It attempts to both replace existing methodology with new methodology and to provide actionable data from that new methodology at the same time. We can't invoke the limited in vivo data as evidence for concentrating on a cell death endpoint without first ensuring that the in vivo data unequivocally supports such a translation and then showing that the in vitro data in some way reiterates the in vivo data.

This is not a case where we are trying to create new methodologies in a vacuum. Since there are existing methodologies, it's

important to understand the relative efficacy of a new system at determining or estimating human toxicity, in addition to factors such as throughput, money saved, and animals spared. It should be noted that there is a precedent for defining irritation as cell death in vitro, but that such data has not as yet been proposed for regulatory consideration.

I want to pause here because a number of people want to indicate that irritation has been used interchangeably with cell death in other related systems.

DR. HOLGER BEHRSING: So, yes, when it comes to other tissue models using assays such as the MTT assay, which quantitates the metabolic activity of tissues, is used successfully. For example, the OECD test guideline 492 for eye irritation, test guideline 439 is used for in vitro skin irritation. It's also used with corrosion, test guideline 431, in vitro skin corrosion assays. So that's used routinely when it comes to assessing products and the potential for irritation or corrosion.

DR. KATHRYN PAGE: I just want to add to that because the EPA and eye irritation method using the ocular tissue actually does also use MTT and also looks at cell death as an indicator of eye irritation.

DR. STEPHEN GRANT: Two issues with that. One is, if there are good bases for extrapolation of that system to inhalation. And the second is MTT as an endpoint.

DR. HOLGER BEHRSING: I know in the study that was conducted, (inaudible), which is different type of reaction. MTT has been -- and (inaudible) has been quite proven, historically, and there is a lot of basis there. But in my opinion, I think that there is some equivalence there between the two assays.

DR. EMILY REINKE: I would agree. There definitely is the old ones of that. Additionally, these OECD guidelines have undergone extensive validation in comparison to a large set of chemicals; and, again, whether the equivalency between respiratory and epithelial, or dermal is correct. I would say it probably is. The amount of data that has had to have been

collected by OECD. ICCVAM has also gone through extensive validations of these. So MTT is most certainly a good model for that and these other.

DR. STEPHEN GRANT: It's not an issue that MTT isn't good. It's an issue with the data that was being accepted as MTT, is the data that has presented, in this system, equivalent to that; so that we can take that acceptance and extrapolate it.

DR. EMILY REINKE: That actually begs the question, why was resazurin used over MTT. I'm looking at Clive. Instead of using MTT, why was resazurin used? I forgot to ask that on Wednesday.

DR. ROBERT CHAPIN: So, I've just been told that have additional input, I need to invite people. Dr. Roper, would you please come to the table and just clarify this? Thank you.

DR. CLIVE ROPER: Thank you. Clive Roper. I'm not wearing a clown suit, for all the people who are listening in there. It's just they're laughing at me running backwards and forwards. Sorry. I missed the question.

DR. EMILY REINKE: Sorry, Clive. Why, for the endpoints -- like I said, I forgot to ask this on Tuesday, I guess. Why did you use resazurin as the endpoint instead of MTT, because of the large amount of data with the MTT?

DR. CLIVE ROPER: Both assays can be used as very useful endpoints within this inhalation model, so we could easily have swapped them over. They're both destructive endpoints, so you have to choose either -- you've got three options. You either have to choose either MTT, which is actually a very simple assay. It's well known, as everybody has mentioned in here. You choose a very different assay, such as resazurin metabolism. Or you have to double your sample size, which is not really appropriate. So they're really both measuring a metabolic capability of that sample at the end of that experiment.

It doesn't matter if you're running it for 24 hours or a week. It's still just a metabolic competence, and it's actually a very simple and easy assay to run. So we could

have easily swapped them over, and then someone would have said why did you use resazurin.

DR. STEPHEN GRANT: The issue here was we're trying to say there are other irritation systems where cell death is an accepted substitute. But they use MTT. If we're going to transfer that precedents, it's a little bit harder when you're defining the same endpoint with different methods. So largely, it's not a question of -- it's another accounting that we have to take into account.

DR. CLIVE ROPER: I wouldn't see any difficulty at all just replacing it at all, just swapping them across. They're both measuring viability.

DR. GEORGE CORCORAN: From a metabolic point of view, I agree completely with Dr. Roper, that these are virtually identical assays. The same enzymes are involved. The same liabilities exist for the substrates. The same strengths exist for the substrates. So I believe this is a straight read through with almost no risk.

DR. STEPHEN GRANT: The bigger issue is whether dermal and optical irritation are directly translatable to the inhalation system. Anyone want to comment on that?

DR. JON HOTCHKISS: I've got a little different take on the resazurin assay. I agree that MTT and resazurin conversion, to raise the roof, are similar endpoints, but MTT is a single point assay that you can't go back from. You have one point in time, and you get one data point. And that's it. While with the resazurin assay, if the ultimate goal is to do repeated exposures and to monitor the health status of the cells during a long period of time, that's why we've chosen to use resazurin. So it measures the same endpoint, but you can repeat it. So you don't have to toss your cultures and increase the hand in order to be able to follow them over time.

DR. EMILY REINKE: I don't disagree. I just wanted to make sure more that you chose resazurin just because you did or that there was interference with MTT. That was all I was asking.

DR. NIKAETA SADEKAR: So to address the point, these OECD assays, or standardized or validated in skin models and eye --

DR. ROBERT CHAPIN: If you're going to look away from the microphone, at least be closer to it.

DR. NIKAETA SADEKAR: Sorry. Okay. So this is to address that these OECD test guidelines were standardized for skin model and eye corrosivity test. They used those respective tissues to test those. Therefore, cell death and using MTT, that makes sense in those models. But when you're talking about irritation in respiratory system, the respiratory epithelium is very different from those two model systems in vitro; and therefore, in physiological relevance, the irritation potential for these tissues is very different, comparing respiratory versus skin.

That's why I raised this point as to if you're comparing cell death as a point of irritation, in skin, I agree with those endpoints, with the way the corrosivity test is

done. But in respiratory, you would definitely get a signal before you see that cell death as a way of irritation in that epithelium.

However, if you were to model a representative of the vestibule in the nasal region, which the tissue there has resemblance to the dermal tissue, that would make sense to use the parallelism of the corrosivity test for the skin and eye for that particular representation. That's it.

DR. STEPHEN GRANT: I just want to say I think we're more in the view of there still exists the possibility of sub-cytotoxic effects, not that they're definite, because we're only interested in effects that are relevant to our endpoints. Okay. Actually, that's a very good introduction to the next section.

Another aspect of balancing the charges of evaluating the biological understanding of the proposal, both in the context of existing in vivo data and as freestanding information, is the question of duration. As responsive to acute issues from the first paragraph -- sorry, it's a written document

-- the submitted data do not provide the NOAEC and LOAEC data missing from the acute studies. So the in vitro data provide those missing parameters if the translation systems are accepted.

However, if this submission is also to be responsive to the request for a 90-day study, many in the committee express reservations that this can be done with a single acute study. The possibility of repeated dosing in the in vitro system has been discussed; and, clearly, the system does have a limited ability to provide such a capability -- although, we don't know what the in vitro equivalent of 90 days is, and that's something that we have to keep in mind -- but not in the context of cell death as a primary effect. Repeated exposures cannot have cumulative effects greater than cell death.

There was also concern on the part of the panel that cell death is no longer an appropriate endpoint in and of itself. In the presentation, much of the data involved tissue disorganization, presumably secondary to cell death, as the in vivo endpoint. One advantage of

the proposed in vitro model is it can reiterate such a three-dimensional effect.

However, it was felt that subjectively ranking histological effects, while visual, was not as quantitative as is possible with current technologies. It's also not clear whether decades of progress in defining mechanism of cell death have been incorporated into the assay system to ensure that the type of cell death observed in vivo was successfully reiterated in vitro. I'm done. Thank you.

DR. ROBERT CHAPIN: Thank you very much. Let me just survey the other panelists who were the associate discussants for this. Dr. Grant, if you could just tap the little button on your mic? Thank you. Survey the associate discussants and make sure we've captured all the things that you guys have to say. Now is the time to speak up.

DR. GEORGE CORCORAN: Dr. Grant did the yeoman's duty in collecting the input of those who are working on Charge Question 1, and he parsed the elements under consideration, I think, effectively in showing how the answers are

not clear cut in many circumstances. I think the biggest disagreement amongst this group of scientists was the value of cell death as being the indicator here for decision making and protecting human health.

There was a group within this charge question that feel it is, and some feel very strongly that it is, in spite of the different tissue types that have been discussed by Nikaeta. There's no question that this model can be further developed and can be explored as to whether it responds in a manner that you'd see in an in vivo study, such as reduce of cytokine, small molecule indicators, and physical disruption. That may indeed happen as this model moves forward.

So the question becomes at this stage in its natural history of development, can it be a productive tool for setting safe levels of human exposure. That is, in spite of the back and forth and the equivocation and all the things that could go wrong or might go wrong or possibly did go wrong, that's really what we're here to do today.

There's some amongst us in the Charge Question 1 who believe, based on -- and I know we were charged last night with not scrutinizing the in vivo data, but that's about really -- it will be the path forward in validating this MucilAir model, in my view, and bringing it to a point where there's enough confidence in it where it can relied upon for regulatory decisions.

As I judged the data in the rat inhalation studies and the values generated by those studies, my confidence level in the MucilAir model using cell death was increased because of the near concordance of values derived from the in vivo and in vitro studies. So despite the liabilities, the assumptions, the non-specification, at times, of the model in vitro versus in vivo studies, my belief is that this model -- first of all, it's essential for the agency moving forward in their charge.

I know, Steve, you began by commending the agency, but I think we all believe that this has to be done. And thank you for doing it, and we're here to help.

So I would, I guess, close my remarks on a note where the MucilAir model requires further scrutiny, careful development and refinement, I have, I want to say, some level of confidence that it will survive that journey and become a robust model in the future. I believe this is a valuable initial demonstration of its capacity.

DR. ROBERT CHAPIN: Thank you, Dr. Corcoran. Okay. Comments?

DR. NIKAETA SADEKAR: I would just like to add I completely agree with the entire Charge Question 1 discussions that have been going on here. But if you're looking at cell death for chronic effects, for chronic exposures, then I would be more comfortable to know that you're not classifying those effects or outcomes as irritation. Because irritation for respiratory has a very different meaning.

Irritation for skin, as seen from the tests, from the OECD validated and the available test guidelines, are applicable in that model. But for respiratory, it is far more sensitive. Therefore, the question for Charge

Question 1 is to review the AOP in terms of irritation, the biological understanding of irritation. That AOP does not address irritation. It addresses local effects in the respiratory system that leads to tissue remodeling due to chronic exposure effects. And it concurs very well with the in vivo exposures, and it is expected that you would definitely see those effects even in humans because the tissue is damaged and there is an effort on the part of the tissue to repair itself.

It is going to lead to that remodeling, whether it's fibrosis or squamous metaplasia. But those are local effects, and the irritation is before those cell deaths, overt cell death that is observed in this model. So I would be more comfortable if you could distinguish that these are long-term exposure effects instead of just irritation in respiratory.

DR. STEPHEN GRANT: Steve Grant. Again, however, the question becomes are we regulating on cell death, assuming that it is the most important endpoint. And where we're going

with that is, if we regulated on cell death, and we haven't eliminated or, to some degree, become comfortable with the idea that there aren't pre-cell-death situations, we don't want to feel that we have done a great job of setting limits, and yet they're not against the earliest effects, the irritation effects.

DR. ROBERT CHAPIN: I think Dr. Sobrian is the next person on the panel.

DR. SONYA SOBRIAN: I agree with all that's been said. I think my biggest reservation was looking at the effect of irritation in cell death and the fact that it was difficult to say how you use this model, how this model is going to be translated into a long-term system to look at 90-day toxicity.

DR. EMILY REINKE: This is Emily Reinke. Sorry. I'm trying to process. I agree with pretty much everything that has been said. I think the use of cell death as a marker for irritation is appropriate in that you need some marker in an in vitro system.

You could start looking at inflammation, but that has been messy, markers of

inflammation, in other models. It is not clean, and the fact that they used a three-pronged approach to look at irritation, so you're looking at the LDH, the TEER, and the resazurin, I think those are all good ways to kind of get the various different steps that you're going to look at initiation of irritation.

Overall, I think the points that have been made are appropriate, and my only other concern is why not an in vivo study? Other than that, I think it's good.

DR. HOLGER BEHRSING: I agree with the other panelists charged in looking at this question. Having worked with MucilAir for some time and reading all the literature out there regarding its use, it's quite a capable model. It has multiple cell types. It definitely better represents airway epithelium than any 2D model that I'm aware of. So the fact that it is competent when it comes to inflammatory responses. TEER is a fantastic endpoint. Certainly, the different ways one can measure cytotoxicity and/or loss of viability is key.

Another benefit of this type of a model is that you have different compartments. You have the apical surface, where you can do an airway-like exposure. In this case, it was using a physiological buffer, and that doesn't necessarily really reflect how inhalation may occur. Certainly, if one were to conduct repeat exposures, you may have confounding results with hypoxia, because of that buffer system that's on top of those cells that are going to be exposed to air.

Of course, you have the medium, where sampling was done to look at LDH release. I'm not sure if that was really the ideal way to go if you're looking for the most sensitive signal. They may well be in the apical compartment where the exposure occurred.

That being said, definitely the MucilAir model has a lot of potential, and applying it in a way that best reflects what may happen to human beings is really a good way to go. Thanks.

DR. STEPHEN GRANT: We allowed for time to reject, and then we've actually gone

around and made people talk. I'm going to try and tie it all up by being folksy. There's an old story about running into somebody on the street, searching diligently on the ground underneath the streetlamp. You say to them, "What happened?" "I dropped a quarter." You say, "Oh, I'll help you look, where did you drop it?" He said, "Over across the street." "Well, why are you looking here?" "Because the streetlamp is here." And to some degree, we have to be sure that the in vitro model isn't the streetlamp, and we're taking what we can get rather than what we need to have.

DR. ROBERT CHAPIN: Thank you, Dr. Grant. Any comments? Dr. Sullivan.

DR. KRISTIE SULLIVAN: Two brief comments. We were talking about, or some comments have been made about, cell death and whether it's upstream enough. I just wanted to point out that -- or whether we should be looking at further upstream effects. Cell death is already upstream of the effects that we currently look at and use to make a regulatory decision.

So I just want to keep in mind that we're already traveling upstream and using new endpoints to make these decisions, and that's important. The advantage of cell death as opposed to more upstream mechanistic effects is that you could consider it as a sort of converging key effect where it's capturing lots of different mechanisms.

The other thing I wanted to say is that the utility of AOP framework is that it provides this link between upstream and more apical effects and, potentially, shorter term versus longer term endpoints. So, with the right supportive set of evidence, it's possible to use a single exposure or a single endpoint to predict longer term endpoints. I do think there is biological plausibility within this pathway, this is sort of a known toxicological endpoint, and data was demonstrated to provide a link between some of these chemicals and some in vivo effects.

I think that more information could have been provided to support the pathway. We sort of got this long reference and a diagram

and there wasn't a lot of discussion about how that diagram was built from the evidence.

DR. ROBERT MITKUS: So two comments. I wasn't on this particular subcommittee, but I have two comments. So for me, the possible debate about sub-cytotoxicity and actual toxicity was clarified for me by Dr. Wolf on Tuesday when he basically stated that irritation, in this model, refers to cytotoxicity. So for me, there isn't really a debate. There's some hairsplitting, it seems to me, between what's going on at the subcellular level prior to cell death, and I don't think that's necessary for the agency's purposes. Cytotoxicity has been used as an endpoint from in vivo studies for modes of action for cancer studies for a long time. So cytotoxicity, as an endpoint in itself, is well known, and the agency is very familiar with it.

Beyond that, I would say, with regard to the AOP, which to me seems to be the meat and potatoes of Charge Question 1. The AOP, as outlined on page 19 of the agency's issue paper, is well supported by the in vivo studies.

Not just the four acute and repeat dose inhalation tox studies, but also by the studies conducted by the oral route, which support, in general, cytotoxicity as an initial key event from chlorothalonil exposure. It's not necessary to demonstrate evidence of every single key event. The major key events, yes.

So, in the case of chlorothalonil, the initial key event is necrotic injury to the respiratory epithelial cells, in vivo. That's been demonstrated. A few steps down, the squamous cell metaplasia has also been demonstrated in vivo. So to me, the AOP is well supported. The question then becomes does the in vitro model mimic or model well that initial key event. To me, that's really the thrust of Charge Question 2. For me, again, Charge Question 1, the AOP is well established. It's well supported by the in vivo data.

DR. STEPHEN GRANT: First of all, bringing up cancer and cytotoxicity, the absolute most important genotoxic effects are slightly sub-cytotoxic. The cell dies, you don't get cancer. The cell is damaged but survives, you're

in trouble. So sub-cytotoxic, you brought up the most important case where that's important.

DR. ROBERT MITKUS: Not to prolong the debate, but I would make a distinction between genotoxic events, which you just stated. So cytotoxicity, we're not talking about genotoxicity.

DR. STEPHEN GRANT: But as a reproductive geneticist to some degree, I usually teach that death, while a bad endpoint, is a good endpoint because you don't have the outcome to worry about. It resolves itself. So a cell takes itself out of the way, you don't have to worry about long-term effects.

DR. ROBERT MITKUS: So I'll just respond, and I won't go on. The agency is able to tease out differences between acting genotoxicants and non-genotoxicants and cytotoxicants, so I would say they're well familiar with that. I think, in this case, you're not dealing with a direct acting genotoxicant. You're talking about a cytotoxicant.

DR. STEPHEN GRANT: The other issue, and I thought that I brought it up well enough, but it doesn't seem to clarify. We had a lot of question about -- at least half of the panel didn't understand the AOP at all because they didn't understand why squamous cell metaplasia was the endpoint. As far as they were concerned, the endpoint should have been the physiological effects of contact irritation.

It took the presentation to be clear that, oh, this was really the response to the request for a long-term study and that you were looking for a long-term outcome. It still, however, is a late effect as opposed to an early effect. So, whereas it might be clear that cell death is associated with eventual squamous cell metaplasia, it's not clear that cell death is the initiating event in irritation.

DR. ROBERT MITKUS: So my response to that would be Syngenta has clearly delineated the AOP. If panel members would like more information, they really need to dig into the source to outcome approach document that Syngenta provided and also dig into the reference Rene, et

al., 2009 (phonetic), upon which the AOP is based.

DR. MARIE FORTIN: I'm Marie Fortin and the views are my own. Just a quick point, to your discussion, with respect to the AOP, but the AOP is not actually -- the first event is not cell death. The first event is reactive (inaudible) with degeneration and with cell damage. Cell death doesn't occur just by itself.

That being said, I think that the AOP that's being used in this context is appropriate. Because although it does not include all upstream events -- and Kristie alluded to that earlier -- converging AOPs is a concept where you have one type of molecular mechanism occurring going towards a key event, in that case cell death. And there's multiple pathways to get to cell death. And different irritants acting with different mechanism of action will lead to that same endpoint. And using that endpoint as our focus, is the right way to build this model.

The other thing that I want to mention has been discussed already, but I just want to voice my opinion. So, that being said, and agreeing that cell death is the right endpoint, the question of cytotoxic effects and in vitro exposure, I think it's one that needs to be addressed. My gut feeling from, is that those type of assays, I have the impression that if we repeated exposure for just a few days, we would see cell death at lower concentration. I think that needs to be addressed because we were trying to bridge that gap to the 90-day study.

The endpoints here, LDH and resazurin are fine based on their landing point, but those don't know -- we use that all the time. So, that's fine in and of itself, as long as it's done properly. And, you know, eventually a guidance document would provide how to do it right and so forth. So that's acceptable.

The one thing, though, that I felt was perhaps a gap is that we need to incorporate this into the physiology. And what it means in vivo, in humans, not in animals, it doesn't matter. We're trying to protect humans.

But we just need to benchmark that level of effect and that model. What does it mean when we bring it to nuance? I don't have the answer on how to do that, but we need to figure out how to do that. So that's what I wanted to say.

DR. JON HOTCHKISS: Overall, I think that the AOP is adequate to describe the model system, and the endpoints, and the cell system that was chosen is appropriate. You can nitpick about what is the best point of departure, whether it's a sublethal alteration before you get frank cell death. But that's something that can be worked on as the model develops.

For a direct acting point of contact toxicant, I think that this is a pretty good place to start. My only regret is that, because this is a real paradigm shift, that they didn't link the initial injury with the outcome. And this cell system is able to do that, whether it is a single acute exposure, but give it a recovery time, or post-exposure time to see how

the epithelium is remodeled. That's possible with this system.

The other issue is what a repeat exposure scenario will do to your estimated point of departure. Is that going to significantly change with what concentration you pick? Overall, I'm comfortable with the cell model, and AOP is a good start. It would just be nice to have a little more information to sort of fill this out. That's it.

DR. ROBERT CHAPIN: Other comments from other panelists? Okay. So let me go back to Dr. Perron and ask if you would like to ask any clarifying questions of the panel? Are you doing a little consultation there?

DR. MONIQUE PERRON: I guess two things, sort of linked. So we're definitely hearing lots of different opinions. We definitely want to make sure those are reflected in the report. I'm hearing a lot about the repeat dosing. Does that seem to be a consensus, though, that you think a repeat dose study would be needed to move forward?

DR. STEPHEN GRANT: I think the concern is that repeated dosing might lower the benchmark dose that would come out of the system.

DR. KRISTIE SULLIVAN: I think a lot of the discussion we had was that maybe not regularly in the future, but at least see what a seven-day exposure looks like, in this case just to kind of see what happens, see if you do have a concern. But that thinking to the future, we wouldn't want to say you would need to do in vitro 90-day to replace an in vivo 90-day. That's not the message I would want to give.

DR. LISA SWEENEY: I was not tasked with this question, but when I read the document, I thought, well, why not repeated exposure? Because it is a human system; and, in a real-life exposure, the recovery time between exposures is an issue in the outcome of acquiring long-term damage. An in vitro system, particularly a human in vitro system, that recapitulates that recovery period could be informative for repeat exposure effects.

DR. KATHRYN PAGE: Just adding to what Kristie said, I feel like I would at least

want to see evidence that repeat exposure wouldn't have an increased effect or decrease the point of departure. Then, also, reiterating what Jon said, I would like to see the recovery period also and what effect repeat dose has on that.

DR. ROBERT MITKUS: Just echoing the same sentiment, the in vitro model is a 24-hour exposure; so, in essence, an acute exposure. Let's say with the in vivo studies you didn't see any progression over time, or as we're seeing in vivo inhalation studies, you're seeing it's a very potent inhalational toxicant, so there's no NOAEC. So if there's a way to represent that, because it does appear that repeat exposures doesn't make things worse than acute.

DR. GEORGE CORCORAN: Just so we don't lose sight of the importance of pathology analysis and histopathology, in the summary comments for Charge Question 1, I think some comments were made about it maybe not being representative or difficult to quantify. Well, there's people who have their entire careers based on quantifying histopathology in a reliable

manner, a predictable manner, and a repeatable manner.

A very important part of a follow-on discussion for chronic exposure in the in vitro system would be the opportunity to do a broader analysis of the histopathological changes over time, which I think will greatly strengthen the contribution of this model for regulatory purposes and setting protective levels.

I want to make sure that that goes on the record of very great importance, even though there was very little time spent on it in the presentation to us on Tuesday. It wasn't a message that it wasn't important. It was a message that they had all this other ground to cover, and they wanted to focus on what was going to be presented to us. So I just wanted to clarify that point, at least from my point of view.

DR. STEPHEN GRANT: In the actual data given, the histopathological damage was on a scale of one to four, and there was some concordance with in vivo and in vitro. And I am not saying that there's lots that can be done

there, but there are stains and things like that that can be quantified. And you can actually show the same types of damage. There's a lot more that could be mined on.

DR. GEORGE CORCORAN: Particularly the metaplastic nature of the AOP in confirming that, in when it arrives, and whether it can be recapitulated.

DR. ROBERT CHAPIN: Okay. Getting back to Dr. Perron. That was the initial response of your first clarification. Any more clarifications?

DR. MONIQUE PERRON: No, I think we're good at this time. Thank you.

DR. MARIE FORTIN: The only thing I wanted to say that we do need to -- in setting up this just as far as we need to see and optimize -- if it was my lab, I would optimize what is the study duration that we need. That may be seven days. That may be ten. You have to look at the system, its stability over time. You know, all the controls addressed, then, if you dose them for 30 days. Maybe that's too much.

And understanding that, and then extrapolating. So, in the issue paper, it's a 24-hour study. And then it says that we don't need to account for study duration, and I'm not sure I agree with that. There's no safety factor applied for study duration in the calculation for the risk assessment.

I'm going to use an analogy. When sometimes we'll do a CSAF, a compound specific adjustment factor. And we'll leverage data that we have, usually PK, you know, to inform that difference between what we're doing for (inaudible).

I think here the gap we have, is we have an in vitro system. I think it's the right one for that type of endpoint. But where I see a gap is understanding how it relates to the human effect, and accounting for that repeated exposure.

I think if you're going to do a 24-hour exposure, then probably we need a safety factor to account for the possibility that longer exposure would result in a lower benchmark. After you have data that shows either way, the

way it's going, then, after that you can move forward.

DR. ROBERT CHAPIN: Okay. So have we exhausted all the possibilities for Question 1? Excellent. Thank you very much. Okay. So now, we'll go to Question 2. It appears as if by magic.

DR. ALLISON JENKINS: Could we have a break first?

DR. ROBERT CHAPIN: Sure. Let's have a break. So it's 10:10. Can we convene in ten minutes? All right. So we'll be back at 10:20.

[BREAK]

DR. ROBERT CHAPIN: We are resuming, and we will set the plow a little deeper this time with charge Question 2. Dr. Perron?

CHARGE QUESTION 2

DR. MONIQUE PERRON: This is Monique Perron. I'm going to read Question Number 2, also a bit lengthy.

Please comment on the strengths and limitations of using the in vitro test systems to evaluate a variety of membrane and cell damage endpoints (transepithelial electrical resistance, lactate dehydrogenase release, and resazurin metabolism) as markers of cellular response as described in MRID 50317702 and summarized in Section 2.2.4 of the EPA's issue paper. 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.

DR. ROBERT CHAPIN: Excellent. Thank you, and the lead discussant for this is Allison Jenkins.

MS. ALLISON JENKINS: Good morning. As in Question 1, we appreciate the U.S. EPA and Syngenta's working moving the science forward, and we appreciate the

opportunity to learn and comment on this approach presented using chlorothalonil as an example.

MucilAir, as an in vitro system, has several advantages in that it is a three-dimensional model involving human airway epithelial cells that allows direct exposure to chemicals at that air-liquid interface and mimics some functions of the human respiratory tract, including barrier function, mucus production, and cilia function.

The group's comments are focused around several areas of the studies that were reviewed and discussed in full FIFRA SAP meeting on Tuesday and include study design, including the method of application to the MucilAir system and donor tissue characteristics, in vitro endpoints selected in relevance to irritation, validation of reproducibility, and reporting details. The members do agree that this model is generally appropriate to evaluate the type of effect of concern: respiratory irritant, corrosive agent, or cytotoxic agent.

In terms of the study design, members of the group had concerns about relying

on a single 24-hour study design for replacement of a 90-day animal study. The study design as presented may not be sufficient to replace a 90-day animal study, even when the adverse outcome pathways suggest acute irritation, cytotoxicity as a critical adverse effect. If the model is used to replace a sub-chronic animal study, the group suggests repeated dosing to assess potential effects or repeated exposure. This study as presented only looked at acute effects with cell death as the endpoint.

The MucilAir model is viable for one year, according to information presented. If it is proven that repeated exposure over a specific duration does not change the outcome when compared to another duration, then the approach could be optimized for shorter study duration. For example, if data demonstrate that the same results are obtained following three months of dosing or one month of dosing, then it could be acceptable to conduct the study for shorter exposure duration.

Members of the group would like to see as a comparison application of material as an

aerosol, perhaps generated by an aerosol generator, in addition to the method of application outlined in the study.

One comment stated that with maximal deposition being modeled in the vestibule in the nasal region, considering particle sizes, the nasal epithelium needs to be represented for in vitro testing. The same goes for deep lung tissue, as the effects were observed despite lung deposition of the test chemical. This could be important when evaluating chronic exposure.

Members also noted that it appeared that chlorothalonil was not measured in media or tissue extracts at any point during the incubation period and had questions about the chemical stability, cell culture media, and biological matrices.

In regard to donor differences, the discussion on Tuesday clarified the MucilAir donor tissues and reasons for the five donors per group. However, members had questions about the absence of the presentation of the variability between the replicates per donor per dose. The study states that six replicates of this type

were used, but variation was not shown as error bars on the graphs or standard deviation in the tables, as the graphs shown during the presentation on Tuesday showed large variability.

The inclusion of cultures from multiple individuals is an important addition to this study and it would be helpful to present the range of baseline or control responses across individuals. If this assay is accepted and used, the requirements for historic controls would need to be developed. In addition, group members also suggest additional settings to confirm results in the nasal tissue model using tissue models from other regions. As stated in the study information and on Tuesday, only the nasal tissue model was available when the study was conducted.

Members lacked confidence in the discussion that the additional models would respond the same without data supporting that assertion. Further, during the discussion on Tuesday, it was discussed that the nasal tissue model cells are, or are usually, obtained from patients with nasal polyps, and there were questions in the group about those cells and

whether they might respond differently from cells from people without nasal polyps.

Some members commented on the lack of data on differences in donors in cell models that could impact responses or that could introduce additional uncertainty. At a minimum, comparative studies with several irritants should be conducted to demonstrate the comparable outcomes are observed between cells harvested from different regions. Comparative toxicity data with respect to irritant responses for different regions, using nasal, tracheal, and bronchial derived cells could substantiate the assertion and should be included in the study information.

Regarding endpoints and results, the TEER lactate hydrogenase release and resazurin metabolism are standard markers but crude markers of overt toxicity. Subtle changes may be occurring at the transcriptional and/or epigenetic level that are not measured nor assessed in this study but might result in an increased susceptibility to injury, especially upon repeated insult. The pivotal hypothesis is

that, by protecting for the initial cell damage caused by chlorothalonil exposure, effects that would be caused from repeated exposure would also be prevented.

However, since the markers are markers of overt toxicity, the current study design does not allow for an assessment for the potential sublethal effects that, upon repeated exposures, would lead to the same phenotype over time.

During the presentation on Tuesday, Syngenta presented information on TEER correlating well with other markers of cell injury or death. The group would recommend the addition of this information and any other information showing the other endpoints, for example, LDH and resazurin, and their correlation in other studies, to be included in the documents.

Group members commented on the need to include more of a metric assessment of exposure response, injury, adaptation, and that this MucilAir system could be a perfect system to assess a critical early key endpoint but weren't

sure whether there were enough data to prove that a single endpoint analysis is sufficient. Members commented that the dose response curve as presented in the study were mainly flat at most doses administered, and because a significant change only occurred in the highest two doses administered, may not produce a model that can accurately reflect the point of departure.

Members commented that it is important to have a full view of the response behavior by observing data across a range of responses, not just the last two data points as produced in this study.

Regarding study validation and reproducibility, members of this group were concerned about the lack of study validation or reproducibility presented in the study materials. There was no effort presented to repeat this study in different labs, or even in the same lab, or to use known controls from Syngenta's portfolio. Members would like to see evidence that this method is applicable to other irritants where NOAELs and LOAELs have been established in the literature, perhaps with human data.

On Tuesday's meeting, Syngenta stated that resazurin results from lower doses needed to be combined with the control to produce significant difference. These data should be included in future submittals. And that concludes our response.

DR. ROBERT CHAPIN: Wonderful. Thank you. Can we just sort of look around the room and query the associates we assigned, associate discussants for this question, and make sure that everybody is onboard and see if anybody else has anything to say? So Dr. Fortin?

DR. MARIE FORTIN: Marie Fortin and the views are my own. I have just a few things to add on. I sent them last night very late and didn't make it into the overall document, and most people on that team didn't get a chance to necessarily review it.

I stated earlier I think the model is conceptually the right model to answer that question. What I would like to propose or for you to consider is that, instead of using single donor, which leads to a greater variety, using

pools of donors I think would be more appropriate.

I also think that, from a replicate perspective, not just necessary to have six replicates, so six tissue replicates, but rather I think that you could have three replicates. You'll see when I say my reasoning on the MDL derivation why I'm saying that.

So three replicates, pools of donors. One of the things that also should be considered in moving this forward is assessing the technical requests for reproducibility. The issue is that, right now, the variability that is seen is dependent on the lab that did the study, the person that did the study, because within the lab you have variability. This will need to be addressed because, right now, the variability is what defined what is the response.

The BMR is based on the variability, so the greater your variability, the greater the threshold to observing that response. So I think it's important to focus on -- in having an assay that becomes robust, you need to

minimize the variability. So that's something that I wanted to point out.

With respect to the benchmark dose modeling, the approach that was taken was to model the dose response within a donor using the dose with the tissue replicates, but that's not consistent with how we would do it with animals. With animals, you would use the dose groups, meaning the different individuals in that group are pooled together for each dose.

So my understanding of the guidance is that it should be done basically -- so all the data, so the tissue replicates would be the endpoint for the donor, and the donors would be pooled together for those groups, and that would be the model.

From a modeling perspective, it would be less heavy. Because, obviously, if you're modeling every single donor individually, it takes more time than to do the mean and then model that. And then you took the geometric means of that. I think it should be reversed, the way it's done, and I'm not sure how it would impact the results. But I believe we should try

to align with the way it's done in the guidance document on different dose.

The other point I wanted to make is that we have two measurements, TEER LDH and resazurin. If we were looking at -- I don't like to make animal comparisons, but everybody understands them. So it's easy. To me, we're looking at three different endpoints. If we were looking at the kidney, the brain and the liver, we wouldn't do the mean of those. We would take the critical effect. We would take the lowest one. So I think I would expect, moving forward in the data on that, is just to take whichever is responding first. It might be different for different irritants.

The other point I wanted to make is with respect to the derivation of the point of departure, and I mentioned this a little bit earlier. Right now, this is based on the variability of the assay on that day, with that lab, with that operator. In my opinion, it should be anchored in physiology, and I've mentioned this before.

The right way to do that, I'm not sure. We talked about having morphometric measurements, content imaging, those are all ideas. But we should correlate that to a proportion of cell death. Because I think that's what we need in order to be able to do the risk assessment. Right now, the risk assessment ends up being based on the viability of that assay. If ideas from the assay (inaudible), you're going to get the different (inaudible). And I don't think that's adequate. So that's how I'm going to conclude for now.

DR. ROBERT CHAPIN: Other comments? I guess maybe we'll just go around the table. Dr. Sobrian, do you have anything to add?

DR. SONYA SOBRIAN: I agree with what our lead discussant has already said. I actually just made comments on the study design, the tissue samples and independent and dependent variables. All have been included in what's been said.

DR. ROBERT CHAPIN: Great. Thank you. Dr. Behrsing?

DR. HOLGER BEHRSING: I agree with the summary, and certainly that issue of repeat dosing keeps coming up. Certainly, a recommendation that I would have, if that is pursued, is that one does actually work the aerosol exposures. As I mentioned previously, the hypoxic effects of having that physiological buffer constantly on a topical surface would be confounding, and that also gives the opportunity to look at the particle sizes and match that up with what is obtained from that, from the spray nozzles that are used to apply the chlorothalonil. That's pretty much it.

I think the endpoints themselves, LDH, TEER and resazurin markers, those are good. As we discussed during Charge Question 1, the equivalence between the resazurin and MPT. That's a good thing, but certainly the MPT has that historical base to it, I think, that many researchers would find attractive. With that, I think that covers all of my comments.

DR. JAMES BLANDO: I agree pretty much with everything that was said. I think in the group, I was probably the one who was a

little more concerned about the shape of the dose response curve, and I admit that I wondered what the impact would be if the range of doses that were used showed a more significant trend and how that might impact the prediction of the BMDL for the POD. So that was something that I was wondering about, and probably a little more concerned about that than some other members of the panel.

DR. ROBERT CHAPIN: I'll just remind us that Dr. Lowit said that lots of negative doses is exactly what she was happy to see.

DR. JAMES BLANDO: Right, and I just disagree with that.

DR. ROBERT CHAPIN: Got it. Okay. All right. Let's see. Dr. Cavallari, anything to add?

DR. JENNIFER CAVALLARI: I agree with what's already been presented from members of the committee, and especially with what Marie has mentioned, that the most sensitive endpoint, rather than an average, should be considered. Thank you.

DR. ROBERT CHAPIN: Thank you. Dr. Fortin, anything else to add? No. Go ahead. Well, not yet. Dr. Sadekar, anything to add? Nope, nope, nope. Okay. Dr. Grant, over to you, and we'll open up to the panel for other comments.

DR. STEPHEN GRANT: Okay. As a geneticist, I would really like to know -- what I'd encourage you to do is look, whatever endpoints you're looking in the test, is establish a range of normal so you know whether you need to worry about interindividual differences. Largely in this study, there was very little indication of that. What I just don't want is for us to simply assume that there is or assume that there isn't.

It's one of those things that I think we need to study and find out whether there's significant interindividual differences. One of the issues, all of these -- and this is something I'm sensitive to. All of these donors were European, were they not? Yeah. Okay. Again, it's one of those questions which is we

have to make sure we're also modeling the population that we want our data to apply to.

DR. MARIE FORTIN: I actually would disagree with that, because we're accounting for the interindividual variability with the safety factor, so you do not need to model the populations. That's a flawed assumption that we can do that. You will need 100, 200, 2,000 samples to model the population. That's not the purpose of this assay. The purpose of the assay is to identify what's the hazard. That's why using pools is a fast way, more throughput way, to have something that's going to represent a population. I don't think you need to have more than five donors. I don't think that's the purpose.

DR. STEPHEN GRANT: Again, I think I said 10 or 20 on Tuesday, and what I'm uncomfortable with is that we just shouldn't pull that out of a hat. We should have some basis for, if we're going to use pooled samples, how many pooled samples should go into it.

One of the things we need to worry about is the interindividual variability is that

the group we're looking at is skewed to one side and the group that we're applying it to is skewed to the other. We want to not have two interindividual modulating factors.

DR. LISA SWEENEY: To follow off that particular comment, which wasn't originally why I raised my card, it seems like that's something that's a matter of characterizing the baseline assay, that there's no reason once you've done this assay on enough samples that you can't go back and see if there are demographic differences based on some pretty basic donor information. So that seems like a starting point that you would know in the assay is this different in people with different backgrounds.

If you can remove uncertainty in in vitro testing, instead of having to add a default uncertainty factor for interindividual variability, why not do it? It could be that it's too expensive to test it enough, and you're fine with the default uncertainty factor. Go ahead. But if the registrant is interested and paying to analyze the background database to justify why they don't need an uncertainty factor

because this assay is similar across different individual donors, why not?

And now for something completely different, it's probably not an issue for chlorothalonil, but it's also important to test your chemical in your in vitro system to see where it goes. I didn't see anything about the actual in vitro dose symmetry of the test countdown; and, as a particle, it's probably not going anywhere. But if this technique is going to be applied to other chemicals, you have to ask yourself where is the chemical going?

For my PhD work, I had issues with the chemical that I was studying being absorbed by plastic and tubing, and I was trying to pipe it from one place to another. So I had to do my in vitro work literally in vitro in glass so that it wouldn't be all absorbed by the compound and used expensive tubing in order to pipe it from one chamber to another. So, while not an issue probably with chlorothalonil, it should be part of the in vitro testing design going forward for other chemicals to consider the fate of the chemical in a test system without cells.

DR. ROBERT CHAPIN: Thank you. And then in the order in which they appeared, Dr. Sullivan?

DR. KRISTIE SULLIVAN: A couple of comments. I agree with Marie that we can't really represent all the populations of the world in an in vitro system, and I think what's really important to consider is the difference in response to the chemical. Is there a difference among populations for what we're concerned about, which is the toxic response?

For some chemicals and some effects where there may be genetic differences or differences in metabolism, that may be really important. And you may be able to model that or consider that in other ways. But I think when we're thinking about the endpoint that we're interested in, we need to think about will these different populations actually have a difference in toxic response. That's what should be kind of kept in mind.

I also wanted to point out, and maybe clarify from my early comments, that I think, according to the conventions of adverse

outcome pathway framework, it is possible to extrapolate from a single exposure endpoint to a repeated dose endpoint given enough supporting information. So I want to make sure that we consider that. And also that we're not proposing, or the agency is not proposing to replace a 90-day study with an in vitro study in a complete vacuum. There's a lot of other information about how the chemical already interacts with biological systems in vivo, and I think we need to keep in mind that we're using all of this weight of evidence and not just the results of one in vitro study.

DR. ROBERT CHAPIN: Dr. Page and then Fortin.

DR. KATHRYN PAGE: I'm also concerned with the variability that's seen in this assay. Specifically of interest is the resazurin where results from lower doses needed to be combined with the control in order to produce significant differences at the higher doses. It's my understanding that from Tuesday's discussion a direct comparison has been provided or will be provided to EPA to reassess this

endpoint. However, I do wonder, if this assay was repeated, whether the results would also still align.

This is important not only the protect the population but to make sure results are consistent across future registrations. I also think that a correlation of the in vitro effects with the pathology in vivo is important. Once we show this, if we see correlation, I don't necessarily think that we have to go a full 90-day assay in vitro all the time or do repeated histopathology every day. But I do think assay optimization will help derive the appropriate conditions in order to fulfill this particular data requirement for direct irritants.

I would also like to see a comparison of effect in other tissue types, like lung versus the nasal tissue seen here. I understand that this might not have been available at the time, but it is now. And I would have liked to see the corresponding point of departure and HEC with these results to determine what the most sensitive and relevant concentration of effect would be.

DR. ROBERT CHAPIN: Thank you very much. Dr. Fortin?

DR. MARIE FORTIN: I forgot to mention something earlier. Syngenta demonstrated that this model could be used to assess a different formula would produce cytotoxicity. In that case study, they used a formula to test. I think it would be in our best interest to test the active ingredient rather than the formula to avoid an active ingredient defense.

DR. ROBERT CHAPIN: Dr. Corcoran?

DR. GEORGE CORCORAN: Thank you, Dr. Chapin. If I'm correct in my assumption that things don't go on the record unless they're actually stated verbally during a discussion of the charge questions, at the risk of being repetitive of comments I may have made on Tuesday, I would just like to reiterate that the selection of the three endpoints in the MucilAir system are excellent choices in my view, with a couple caveats. One, that particularly the LDH assay be customized for the MucilAir system, which it was not in my view for the data presented.

Secondly, the dual use of resazurin to probe and evaluate two very different cellular capacities provides a liability of using -- if something is wrong with resazurin for one setting, it will be wrong for the other. So you're causing less confidence in two separate measurements, which should be probed with two different chemical entities. That's all I have.

DR. ROBERT CHAPIN: Great. Thank you. Cliff?

DR. CLIFFORD WEISEL: I'm going to start off saying I'm very impressed. This is somewhat outside my area. I'm very impressed with the MucilAir system and the discussions we've had. But this charge question asks for some limitations in how it's used.

One of the limitations that I'm seeing is this doesn't present the whole-body system and feedback loops to it. For the compound we're looking at now, that seems to be appropriate, because what I heard is that compound is very toxic, essentially kills it immediately. If you go into other contact

irritants, that may not be the case. And we have to make sure we justify using this system if those compounds may affect the system and some requirement.

Now, you talk about other in vitro systems, and you justify why you're using this one, and it certainly seems appropriate. But we have to make sure we reevaluate some of those other systems, such as the -- you know, they're all (inaudible) as they get better to see whether for other compounds they may be ones that you want to use. I just wanted to make sure that's in the record going forward.

DR. ROBERT MITKUS: Just briefly, two points. I just wanted to, I guess, congratulate the agency on looking at this particular model. It seems to me that it's a well-used model. It's used in Dr. Behrsing's lab there, with the smoking robot technology. It's been used by the tobacco industry along with MatTek EpiAirway. So the model, in addition to what's already been said, seems to be a strong and relevant model.

It models three sensitive endpoints: the TEER, the LDH, and the resazurin metabolism, which seem to me to be sensitive endpoints. Just the way the data were presented, the dose range of 200 milligrams per liter, I think because the preliminary data went up to 5,000 mgs per liter were not presented along with that, I think maybe for the committee it was a perceptual issue. They didn't see the top of the dose response curve. They really just saw two points going up at the high end of the dose response curve and so didn't fully appreciate the fact that it plateaus above that. So, it would have been nice to have combined both of those dose response curves together just to see the full dose response.

The other piece I would just add is that Syngenta and the agency's working together approach to use BMD was a strength that's relevant to this particular charge question. BMD analysis has been used by the agency for over a decade now, and I know it's becoming more and more common.

The only thing I would add is it would have been nice to have seen a BMD analysis of both the acute and repeat dose in vivo inhalation studies to see what -- if you would have obtained the MDL and where that would be. Not to validate the in vitro results against the in vivo, but because the agency scientists are going to naturally, because that's their current approach, is to use the in vivo rat data compared to an HEC and their look for the MDL. So that's from that perspective, not to validate. Thank you.

DR. ROBERT CHAPIN: Ray was next.

DR. RAYMOND YANG: I have a couple of points. First of all, I want to follow up on what Lisa said a while ago. She brought up a really important point, that is the plastic tubing. Myself, I've paid dearly with a chemical, hexachlorobenzene, in my research phase. This chemical attached to any and all plastics, so if you want to do quantitative analysis, a lot of it is on plastics. Also, this chemical has the ability of sublime, go directly from solid to vapor phase. So I totally agree

with Lisa's suggestion. I think Syngenta would do themselves a favor to check out the system with controls and try to see if your chemical somehow tied up with the system.

So the second point is related to the study. Personally, I think Syngenta has done a great job with this particular system and design and the studies for the purpose they are doing. And I want to echo what Anna said at the end of Tuesday. That is we are in academia. We are intellectualists and so on. We have intellectual curiosity. We tend to demand this, demand that, demand to know everything. But no system is perfect.

Therefore, no matter what you do with this system, you can study it to death, it will not become a human. So there's limitations. Therefore, after I said that Syngenta did a great job; nevertheless, since you asked questions about study design and message, I want to bring back the issue of repeated study. I totally endorse that. In fact, I want to go further. This is motivated by George's earlier comment about bringing pathology in and examining it.

Now, the chair, Bob, and I spend quite a bit of our prime life at NTP, so I'm thinking about the NTP protocol for animal studies and so on. 14-day study followed by 90-day study followed by two-year study, and these are not only acute, sub-acute, and sub-chronic study leading to a chronic study, but there's a dose setting regime in there. What I'm about to suggest to you for consideration is the study design incorporating the thinking of you go from acute to sub-acute to chronic to sub-chronic study. You have this dosage setting study. Take that into consideration in your repeated dose study.

Also, if you do see FD modeling, you have depositions and so on with different sizes of particles and so on. That quantitative information should be somehow incorporated into your study in terms of setting those as study.

So I'm not only suggesting you do repeated dose study but do a time cost study. For example, you do seven days, two weeks, 90 days, and see the progression of changes and so on, and probably incorporate recovery study.

These are all for what? To me, whenever you do an experiment, you've got to do it for a purpose. The purpose here is eventually invalidation process. Because right now you only have an eight-hour exposure scenario, one-day acute study.

Eventually, you're going to have to validate sub-chronic toxicity, chronic toxicity, maybe even carcinogenicity. Therefore, you need to have as much information as possible because you're a trailblazer. These are the issues that I think we are trying to help you and you need to consider. Thank you.

DR. ROBERT CHAPIN: Dr. Reinke?

DR. EMILY REINKE: I think what we're bumping into is two separate issues here. We have the issue of optimization of an approach. I'm not going to say validation because this is not. Validation is a whole other word with a lot of other connotations that I don't we really want to be talking about here.

So optimization of an approach, while also helping you make a decision on a registration. So we really need to, in some ways, separate

these out, and how do we best optimize the approach so that we can then help you make a decision on the registration?

I think what we need to think about is, yes, the general approach is appropriate. I have suggested, as many other people have, that maybe we need to be doing a repeat dose study with consideration of the fact that, as Holger said, leaving it on consistently could cause hypoxia. So maybe looking it as a repeated episodic dose, so it's only for a couple hours every day for a time, just to show that the repeat dose does not affect or does affect the outcome. Does that change the point of departure? And then also adding the potential for recovery.

But again, the optimization part is key. I concur on the selection of endpoints with the LDH, the TEER, and the resazurin. But as Holger had mentioned earlier, or in one of our conversations, maybe, again, optimizing whether LDH from the apical surface is more appropriate than LDH from the knee up.

Again, if you can show one way or the other that it doesn't matter, that's great. But there are some variabilities in here that we need to determine whether or not they do or do not matter, for this approach to be the best approach possible; in order to allow for a decision to be made.

As others had said, I would like to see whether or not the nasal, bronchial, and tracheal outcomes are different or if they're the same. Again, that would allow for optimization of approach, to say you only need to use the nasal if you're concerned about this area.

DR. ROBERT CHAPIN: Thank you. Jon, your card was up for a while. Close enough to the mic, please. Thank you.

DR. JON HOTCHKISS: I don't think anyone was reading my paper here, but they pretty much hit all my comments. So maybe I'm channeling all my thoughts around the room. I agree that the inclusion of multiple endpoints is really important, at least at this early stage, in order to get a full understanding of what the exposure response is to the test material.

Examination of the acute response in 24 hours is important, but so is recovery and the potential for repeat exposure. That would just be a suggestion as we move forward with this experimental design.

I also agree that it would be good to include a morphometric analysis of some of the endpoints associated with the tissues in terms of the injury response model. For instance, cell proliferation, looking at changes in the thickness of the distribution in types of cells that are present. That may not, in the long run, be required for every study; but as we gain confidence in this model, I think it's just really helpful to see that this system is recapitulating what we would expect to see in vivo.

I guess the only other thing is, as this model moves forward, what are we going to do about historic controls? How much data is needed as a new lab starts introducing this, and what's the requirement going to be? And what's the requirement for the specific controls for each experiment, not only a vehicle control, but

just an incubator control, just to allow for the aging of the cultures? They don't change all that much; but, again, to help build up confidence in the system, I think that's really important information to have as we move forward. That's it.

DR. ROBERT CHAPIN: Rob, your card was up. Do you still -- are you good? Okay. Holger?

DR. HOLGER BEHRSING: Two come to that, you know, added endpoints. And I know that George had mentioned the histology. In our summary, you know, we talked about transcriptional or epigenetic changes that we might want to measure. We need to be cautious that -- certainly, while we characterize the tissue, a lot of these endpoints are going to be very valuable, and we want to tease out those that are really the most important. Because ultimately, the way I envision these systems to work is we'll have a non-animal, human-relevant screening machine for all these different materials.

If we keep adding these other endpoints, that is going to greatly increase the cost and the time it takes to actually screen these materials. For example, if you want to do (inaudible), well, now you're going to have and (inaudible) type buffer there. You can't use that tissue for histology. You can't use it for other endpoints and so on and so forth.

So, we need to be mindful that when we do optimize and we do validate this model, that we select those that are the most appropriate; so that we actually have a practical, economically practical situation where we can rapidly move through these materials.

DR. ROBERT CHAPIN: Great. Ms. Sweeney? I'm sorry. Ms. Sullivan?

MS. KRISTIE SULLIVAN: I just wanted to make one additional comment that some of the things that we're asking for around the room and talking about, including potential differences between different regions of the upper respiratory tract, reproducibility of the method, whether plastic or other materials impact the results, I think a lot of these experiments

may already and do already exist. So I think it's important to point out that that existing evidence can be brought to bear. It's not that we need to do all of these experiments with this particular chemical.

DR. ROBERT CHAPIN: Great. Jon?

DR. JON HOTCHKISS: I forgot what I was going to say.

DR. ROBERT CHAPIN: Well, you're not going anywhere. Jim?

DR. JAMES BLANDO: I totally agree with all the discussion that everybody's had, especially about the repeat dosing. I guess the one reservation that I always feel when people talk about extrapolating from an acute study to a longer-term study, and all the discussion about the AOPs, is I do always worry about say there's a new chemical you're screening and there's an AOP pathway that you don't know exists.

For example, I think this case study is a good example of, if I understand what was presented on Tuesday, that the metaplasia would not be observed without longer-term repeat doses. If that is the case, that would be

example of, if you looked at the pathology, you might have an unexpected finding that maybe all the in silico and all the knowledge that you have about a chemical, you think you know how it's going to react. In fact, when you actually test it, it doesn't. I know we've had some compounds in the past that did not behave like the toxicologists really thought they would.

So that's the one concern that I always do have about when you're extrapolating. I understand the practical needs for some of the testing, but I do worry about, if you're trying to extrapolate too much, that you might miss things that were unexpected.

DR. ROBERT CHAPIN: Nature is somehow really good at surprising us, isn't it? Ray?

DR. RAYMOND YANG: I want to add one point. To emphasis, actually, what Anna said at the end of Tuesday and what I just said echoed her. That is any system's got flaws. In the modeling world, I teach PBPK modeling in my workshop. I always emphasis to the students this following statement by an imminent statistician,

George Box. "All models are wrong. Some are useful."

Now if you have a four-compartment, human PBPK model, that is an over-simplification of humans, and yet we don't have any problem of accepting the target dose derived from that for risk assessment and so forth. Now, this system I look at in that light. Thank you.

DR. ROBERT CHAPIN: The memory works?

DR. JON HOTCHKISS: Yeah. I had a breakthrough. I think for this system, the model that was chosen, MucilAir is a good choice, because that's driven by the regional dose symmetry. Even if you do a simple analysis with MPPV before you do a CFD determination, you're going to get an idea of where the principal area of contact is going to be. So that should be the driver for which model we use. If you've got something that's going to bang out Type 1 cells, then you need to use the alveolar model, and MucilAir's not going to be a really good system because it may not be sensitive to the effects of your toxicant.

The same thing with small conducting airways. They respond differently to the same toxicant, at least in our hands, so you just have to sort of be careful, not to just select the most sensitive system, but the one that's most appropriate for the test material that you're using.

DR. KATHRYN PAGE: I just want to clarify what I said previously and following on from what Jon said. I think what I was getting at was that it did appear that there was some particular matter getting into the lung. If we were to test both systems and then go through the calculations to determine if, say, the lung system was more sensitive, maybe you would get that effect triggered at a smaller dose. So comparing the HECs derived from both of those test systems, I feel, would be relevant, even though I'm not talking about the most sensitive result in the tissue itself.

DR. JON HOTCHKISS: I agree with you totally. If you do that, then you need to follow what the reasonable dose symmetry is and target the dose that you predict would be

relevant in the human. Then you could compare site specific sensitivities. So that should be the guiding direction.

DR. NIKAETA SADEKAR: So, on record, I agree with that.

DR. MARIE FORTIN: I also agree with that.

DR. ROBERT CHAPIN: All right. So I'm getting ready to come back to you guys and ask for clarifying questions.

While they're conferring, Dr. Jenkins, are you happy with stuff that's been going on? Does this fundamentally alter the stuff that you read earlier? I don't get the sense that it does.

DR. ALLISON JENKINS: I don't think so, maybe make some additions.

DR. ROBERT CHAPIN: Okay. Cool. I'll give them about five seconds, and then we'll go to our EPA colleagues and ask are there any questions that you want to ask the committee to clarify or comments?

DR. ANNA LOWIT: I don't think so. We heard a lot of really good comments and a lot

of good feedback. It's really excellent to hear so many sort of grounded, realistic suggestions that are tractable, and a couple comments that Kristie made I think are really important. As we think about the chlorothalonil case, it's a very data rich chemical. There's a lot of information on it.

So thinking about the system as fit for purpose in that context, and then the idea that there are thousands of other compounds out there for which it may be appropriate to moving away from the animal. So some of the dialogue that we're hearing may not be fit for purpose for chlorothalonil but may be directly fit for purpose for other kinds of things. So it's nice to hear that variety of feedback; but they may not all apply to chlorothalonil itself, per se.

DR. ROBERT CHAPIN: Okay. Success. Thank you all. That was a rich discussion. So we're at 11:15. We've been going a little longer than an hour for each question. My inclination would be to do Charge Question 3 before lunch, so my question to you all is do we

need a five-minute break before we dive into Question 3? Yes. Okay. Is five minutes going to be long enough? Yes. Okay. 11:20.

[BREAK]

CHARGE QUESTION 3

DR. ROBERT CHAPIN: This is Bob Chapin. First up is -- and we've got 3 on the screen. Dr. Perron, would you care to pose question 3 to the panel, please?

DR. MONIQUE PERRON: Hi, this is Monique Perron. Charge Question Number 3: 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 as discussed in the EPA's issue paper. 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 the 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.

DR. ROBERT CHAPIN: That's easy for you to say. The lead discussant for this is Dr. Lisa Sweeney.

DR. LISA SWEENEY: Lisa Sweeney here. Syngenta and the EPA Office of Pesticide Programs are proposing a new approach, or new approach methodology, for inhalation toxicology of a respiratory irritant, fungicide chlorothalonil. Their approach draws from the vision proposed by the National Research Council for toxicity testing in the 21st century.

Syngenta's pioneering approach is unusual in that they didn't wait for method approach to be validated or -- but I guess they're trying to optimize it, right, Emily? But they put together a suite of technologies that they felt could address specific questions regulators need answered for their Agency's risk assessment mandates. They believe their approach improves on traditional approaches -- conducting a 90-day rat study and extrapolating findings to human -- and their approach relies on in vitro experiments and simulations with greater human relevance than the traditional approach. Specifically in this charge question, we're asked to comment on strengths and limitations of the CFD model and the assumption of calculations made to counter polydisperse particles.

To summarize our findings, the panelists deemed that the use of the CFD model is an innovative approach to determining human airway exposure to chlorothalonil and the calculation performed to account for polydisperse particles are supported by information provided.

For the most part, the proposed process improves upon the current processes EPA would use for interpretation of in vivo data, with a consideration of the deposition of chlorothalonil particles in the human respiratory system to determine actual deposited doses to tissue.

Going forward, the panel would like to see a better justification for the chosen inputs and assumptions for the model provided upfront. Some of this information was provided in our Tuesday session. Basically, you should have given us more work to do upfront and given us more documents. I can't believe we're saying that. That additional justification and documentation would have provided answers to many of the questions that arose while reviewing the documents.

The panel also requests that EPA and/or Syngenta provide greater detail on eight topic areas that I'll list, and then we'll address each of those individually so that we don't wind up jumping around as individuals raise comments on them. But just to summarize:

One: Provide greater detail on and validation for the proposed particle size distribution, although we understand that there will be application-specific considerations down the line in future risk assessments;

Two: Consider the lung as the target organ of concern, in concert with exploration of the impact of oral, nasal, and/or mouth breathing;

Three: Determine the potential for additional upper respiratory tract deposition of chlorothalonil during exhalation;

Four: Move beyond an N of one for human upper respiratory tract geometry addressing CFD model parameter uncertainty in variability, and selecting parameter values appropriate to the relative and exposures scenarios such as level of effort;

Five: Address questions about the precision of the current upper respiratory tract of the CFD model;

Six: Address the potential for application of different or additional modeling

approaches to dosimetry calculations, such as MPPD or PBPK models;

Seven: Consider alternative dose metrics for the risk assessment point of departure;

Eight: Expand the use of the rat CFD model simulation findings to build confidence in the overall NAM approach.

Each of these concerns is discussed in greater detail. We'll start with the particle size distribution. A number of members of the panel had some difficulty following the proposal regarding the 35-micrometer MMAD particle size that's sort of the baseline, and then the 1.5 geometric standard deviation assumption. And also the CFD model assumed 20 degrees C in ambient humidity. It's unclear how this would affect the particle size distribution; and basically, it's an embedded assumption and there's sort of a lack of qualitative or quantitative description of the impact.

Some of the issues that was felt needed better documentation, including some of

the information on the laboratory experiments that were done, the fact that there's an assumption of no change in particle size due to humidity within the respiratory tract. And so I think we'll open it up to additional comments on the particle size distribution at this time. And I invite Cliff to go first, with the Chair's permission, since Cliff had the most comments on this.

DR. CLIFFORD WEISEL: Cliff Weisel. The CFD model and the way it was presented really is very dependent upon the particle size coming in; and that's separate from the changes that might go within it. If you look at how particles change in the environment, they are very dependent upon the relative humidity, how long they stay there, even the temperature.

The thing about spray, as I look more and more, the particle size distribution of spray is much larger than the inhalation. So you're looking at the tail end of what's going on.

Now, what was done in the laboratory, if I understood correctly, was you

had about 2.5 distance between where the spray was and the sampler. And one of the comments was, well, that's what you might be looking at for an applicator carrying a wand. But that's not what you're modeling, you're modeling a boom system, and the distance between the emission and the person is much larger there. So, you have a greater opportunity for changes in particle size than what you might see in the laboratory. And I think that's a critical thing, because that's your primary input into what's going on.

Even with that, I was trying to figure out how the calculation was made to get at that 35, and I'm still completely lost. It references a health-based particle size selective sampling and application note in TSI, and that really doesn't deal with the specific situation that you have. This is a very generic one and that's the only thing I can see.

In addition, there were two ways that the particle size was measured. One was as an injector. The other -- I forgot to ask about it because I missed it -- was an Oxford laser system, which actually is a full distribution in

real time. And the data from both of those were not presented.

And if you have data that gives you the real size distribution, why are you're using a calculation based on a very generic is a loss to me. As I say, that's critical as to how you move along.

And the secondary is, what was also mentioned, is once it gets to the lung, if you do have small sizes, there is growth. And there are CFD models of lungs that do incorporate it; so, that was not included on that. So, those are our major concerns that we have.

And I asked about the drift. There are plenty of drift models out there. Now, you may not want to go as far as drift, because that's much further -- your targets of the occupational individuals -- but it certainly becomes more and more important if you're looking at people surrounding this, and you're going to expanded past just the occupational exposure.

The other thing about the model, my understanding, again, of a boom system is you have more than one nozzle off and on in a boom

system. All your calculations are based on the amounts you got from a single nozzle. And so, that has to be looked at further to see what the real total amount is.

And then lastly, the issue of pressure. Pressure 40 PSI was used in the laboratory. I understand why that came about. But when the suggestion was that was related to an applicator, if you look at it as someone who's actually carrying something, the way those things work is you pressurize it, and then you start spraying. And when you do that, you're starting at a high pressure and you're going to a low pressure. I don't know how high they actually go when you're actually pumping, but you change that, you change both the amount and the particle size distribution coming out of it.

And then I don't know how well tied those boom systems are. You have different nozzles. Some nozzles you have a single pressure, some nozzles may be at a higher one, proximity. So, again, a sensitivity analysis to understand how those go, would be a very important thing. And I'll stop for the moment.

DR. ROBERT CHAPIN: Dr. Sweeney, are you pausing to let other associate discussants weigh in on this particle size?

DR. LISA SWEENEY: Particle size. Yes.

DR. ROBERT CHAPIN: Does anybody want to add anything to what she said, any of the associate discussions? Start pressing your buttons.

DR. EMILY REINKE: Emily Reinke. I concur.

DR. JON HOTCHKISS: Jon Hotchkiss. I agree that I had a hard time following the derivation of that 35-micron number. It may be right; like it kind of feels about right, but I just couldn't follow it. And they keep on harping on the really tight GSD, but that's going to impact of your estimates of regional deposition in the CFD model.

DR. ROBERT CHAPIN: Jim?

DR. JAMES BLANDO: I wasn't an associate discussant. I just have a comment.

DR. ROBERT CHAPIN: If you have a comment about this, now is a reasonable time to do it.

DR. JAMES BLANDO: I just want to make a comment that there are a couple other impactor types that are available. And it was unclear to me why you picked an impactor with the size cuts that it had, and my suggestion would be to pick an impactor that's closer to the size cuts that are relevant to your modeling.

Also, I just want to point out that a serious impactor, for example, is an impactor you can bring in a field and collect personal samples. And that would be, I think, really useful to have -- I don't want to say real data. But have data on actual operators, and those serious impactors are widely available.

The only technical complication you could have -- I know we dealt with this once in a lab -- is that if you do have an impactor that pulls a heavy vacuum, you could desiccate your particles as your pulling them through the impactor. So, that's just something for your aerosol scientists to consider, but I would

encourage you to use impactors that have size cuts that are more relevant to what you're trying to model.

DR. ROBERT CHAPIN: Dr. Yang?

DR. RAYMOND YANG: Ray Yang. Cliff's comments are very educational. Thank you. And that brings me back to what I said on Tuesday in terms of the spray is polydisperse, and yet the CFD modeling is monodisperse, meaning they use one particle size at a time to run the simulation. And just based on some common sense, seems to me when you have all these aerosol particles going into a narrow and winding space, they're going to have collisions. And some smaller particles are going to become bigger; and therefore, the simulation probably, really doesn't represent what the actual spraying and so on.

And I would urge the Syngenta folks and Rick Corley to get together, maybe do some further simulation using more than one size. Or maybe all those seven or eight sizes together and run your simulation to see if, in fact, the

impact and deposition, and so on, are still the same.

Those are some of the simplest things that one could do to really ask the question, "Am I having a good system?" Thank you.

DR. ROBERT CHAPIN: Dr. Cavallari?

DR. JENNIFER CAVALLARI: I just want to say that I agree with what was mentioned by many of my colleagues. I thought you did -- a good job was done in choosing the spray application versus mixing and loading and choosing that fine spray. But looking at other factors that may influence particle exposure, like the pressure, is also important. I would have liked to see justification for that.

And another factor that I think is important to consider is when we look at biological endpoints, we look at the most sensitive markers within that. Should we be considering that for exposure inputs, and should we be considering the 75th percentile of exposure in that same way? So, when we look at this

exposure data, thinking about whether the mean is most important when we get this data. Thank you.

DR. ROBERT CHAPIN: Dr. Reinke?

DR. EMILY REINKE: I just wanted to respond to what Ray said about the particle size distribution. I think the way that it was modeled with the individual particle sizes, and then combined to the percent distribution was accurate and adequate. I don't think it necessarily needed to be one CFD model with a polydisperse exposure versus six CFD models with percent distribution. I honestly think that that was okay.

DR. ROBERT CHAPIN: Rob?

DR. ROBERT MITKUS: Just two points I wanted to make. One is just kind of echoing what Cliff had said about using a kind of a theoretical distribution. I think I understand maybe why you guys wanted to do that; maybe to generalize this for other compounds that use that density function. But it seems to me that since you were running the OVS against the RespiCon sampler head to head, it seemed to me, based on Dr. Flack's presentation, that you actually did

have actual particle size distribution from the RespiCon that they could have used in the model. So, I would just echo that.

The other thing is sometimes, whether it's PBPK models or PSD models, like those are CFD models, there's always a perception that this is a boutique model. This is very fit for purpose, maybe overly fit for purpose and maybe can't be extrapolated to other situations and scenarios.

My recommendation to maybe to overcome that perception that might exist for the Agency, and at the same time advantage the modeling science that the Agency is using, is to use kind of an approach that’s in between. So, currently, you guys are using RDDR for when you're making your HEC calculations, which was referenced back almost 25 years ago in the Agency's RFC methodology. You could use the MPPD software, multiple path particle dosimetry software, by Applied Research Associates of New Mexico, which, in my opinion, would be a step up from the current RDDR software. And at the same time, it doesn't -- you're not wading into

territory where you have to validate, or explain, or check differential equations for every model that is submitted to you by every company for every particular formulation in exposure scenario.

So, the MPPD model, from my having used it, it has a lot of the same benefits of the CFD model that was proposed and described by Dr. Hinderliter. It's free, it's publicly available, it's very transparent -- unlike the RDDR software -- and it's widely used.

I know Dr. Lowit asked for some tractable specific recommendations. I think one that could be used, not just for this particular situation, but could be applied with an HED more widely, is to investigate that MPPD software. And maybe that could be a step forward from the current HEC calculation approach to offer that.

DR. ROBERT CHAPIN: Before I call on Dr. Hotchkiss, I'll just remind us that we've got eight sort of paragraphs that we're working through on Dr. Sweeney's thing. So, if we fully explore each one of these things, lunch may be late. Dr. Hotchkiss, your sign is up.

DR. JON HOTCHKISS: I'd like to agree with Rob's comment about the CFD model and other applications for its use. Not everyone who may want to use this approach will have the computational horsepower to run a CFD model; some are lucky, some are not.

One of my comments was even though the MPPD model is less precise in terms of regional deposition, and how closely you can dissect what the regional dose is, it is pretty simple to use. And there are well-established regional surface areas, or humans, or rodents, or whatever you wanted to do.

And it would just be interesting, and maybe this has already been done, if there was a comparison between the more precise CFD estimate of dose per unit area relative to a more average method using MPPD. I don't know. That would just be an interesting exercise. It wouldn't take all that much time, and it would just tell you one way or another whether or not a simpler approach might be more applicable across the range where this model's going to be used.

DR. ROBERT CHAPIN: Two more comments. Dr. Page?

DR. KATHRYN PAGE: Maybe this is my lack of understanding here, but it was my understanding that, and I think it was said on Tuesday that the CFD modeling is based on the particle size and can, in fact, be extrapolated to other compounds. Therefore, maybe the Agency would consider the development of a databased set of values using the CFD model. If it is felt that the MPPD model is not precise enough for their application, they could use that reference set. Just something for consideration.

DR. ROBERT CHAPIN: Dr. Fortin?

DR. MARIE FORTIN: I just want to include, I think, that if it's feasible to employ a simpler model to identify the region, that's going to be the target of the highest exposure. This way, it would enable -- it would be easier for more companies to adopt this approach, easier for the Agency to review. And I think a lot of faith is put into this CFD model. I think there are probably ways to appreciate the limitation of

the MPPD model, and account for that in other manners.

DR. ROBERT CHAPIN: And the last word goes to Dr. Weisel.

DR. CLIFFORD WEISEL: Just some very specific recommendations. Dr. Blando suggested you use field impactors. I don't even think you have to do that. We understand -- since these are very dilute particles we know the density. You can actually use some real time scanning systems to get the particle counts across a very wide range of systems and calculate the deposition.

The other comment that was about whether you need polydisperse versus monodisperse in the CFD model, in this case, I don't think you do. Because you're starting with fairly large particles already, and that's not what we're worried about.

Where you do need it, is when you assign the small particle size range, and you're looking at changes of particle size of increases in the lung, and not including that would be a potential problem if you're assign the small,

because that would very much change the deposition. Whereas with this size, everything would be coming out of the top, if the change is larger, it's not being so important, but some of the small ones are.

DR. ROBERT CHAPIN: Back to Dr. Sweeney.

DR. LISA SWEENEY: That will cut down some of the discussion on number 6 since we've already talked a little bit about that. Just to follow up a little bit, is that basically we're modeling the water droplets. So, to the extent that other spray systems have, again, water droplets, the estimate would be applicable to other chemical applications. But depending on how much the density of the particle that's sitting in that droplet changes. The overall density could change the simulations even for something with water.

But moving on to number 2. The consideration of the lung as a potential human toxicity concern in oronasal breathing was an area that a number of members of the panel had comments on. Significant concern about the CFD

approach as implemented in current case study is it neglected to address a significant potential target organ of the lung. Lung is identified as the target organ even in an obligate nose breather, the rat; albeit the testing was done with smaller particle sizes and droplet sizes in the rat, than might be a present in some of the applications that would be of concern for human use of chlorothalonil.

The predictions in the Corley model and, also, MPPD simulations that were provided by Syngenta indicated that smaller particles in the inhalable range do pass through the trachea deeper into the lung. While human fractional lung deposition is highly dependent on particle size, and it may be lower than what's delivered to the upper respiratory tract -- again, depending on particle size -- the larynx dose is not zero, and the lung is not zero. So, it needs to be carried through a little bit further.

A CFD model with proper assumptions provides a valid approach for calculating cumulative deposition, and the

specific application described here has some assumptions, which the panel recommends should have better documentation overall. The CFD model assumed a breathing rate for a sedentary adult male who was a nose breather. Individuals spraying chlorothalonil are likely to breathe at a higher rate for at least part of the time than the assumed sedentary breathing rate since applicators exert themselves and carry appointment.

The higher breathing rate discussed in a later point on the parameter assumptions would increase the mass of aerosols inhaled and increase the linear velocity of the air through the respiratory tract and could cause more air to penetrate deeper into the lungs. Higher breathing rates are also associated with the shift from an individual being a nose breather to a mouth breather. These conditions could change the deposition pattern.

Inclusion of oronasal breathing of the model to ascertain its effect on compound deposition should be considered. The panel suggests using a CFD model that can examine the

deposition for both mouth and nose breathers and recommends the sensitivity analysis for breathing rate be conducted. The panel would like to see the source to outcome approach extended to computational modeling of lung deposition in humans during mouth breathing as a worst-case scenario for delivery to the lung, and possibly to human exposures with 100 percent nasal breathing, and with mouth breathing augmenting nasal breathing.

Habitual oronasal breathing is not unusual, and a 1981 study showed that habitual oronasal breathing occurred in four out of thirty subjects, and that switching from nasal to oronasal breathing at higher ventilation rates is the norm and occurred in 20 out of 30 subjects in the study.

So, while it may be that these elements did not add greater understanding to the approach, and may not be of concern in future cases, for a first application, it is recommended that this be considered for the chlorothalonil case study.

DR. ROBERT CHAPIN: Comments from the associate discussants; things to enrich this summary? Anybody else on the panel? Lunch just got closer.

DR. LISA SWEENEY: Number 3: Consideration of further upper respiratory tract deposition during exhalation. The CFD modeling of the upper respiratory tract assumes no deposition during exhalation of the compound, but no specific evidence was provided in support of this assumption.

Inclusion of exhalation in oronasal breathing to ascertain its effecting compound deposition should be considered, and particles that are deposited during inhalation can be assumed to be stuck. They're probably not going to come off during exhalation, but the regional deposition of entrained particles in the exhaled breath may lead to a different deposition pattern, or just increase the tissue dose.

The modeling of lung deposition, which was recommended, could support or challenge the validity of the assumption that there was significant deposition of chlorothalonil occurs

in the upper airway exhalation. In a sense, if it all deposits in the lung, yes, you've proved that you aren't getting more from exhalation but oops, now you have a dose in the lung that you have to consider. So, that's sort of a "can't win" scenario in a sense.

We recommend that the exhalation be considered, especially with the additional detail of understanding deposition in the lungs. So you have to know how much is coming out and could be further deposited in the upper respiratory tract, especially in the larynx, which has been identified as the target tissue.

DR. ROBERT CHAPIN: Enrichment by the associate discussants. Anybody else?

DR. LISA SWEENEY: The general ideas of variability and uncertainty are unavoidable when we deal with populations, as is the case in risk assessment. More transparency on the sources of parameter values, and the scenarios they are intended to represent, would also be desirable.

Inclusion of sensitivity analyses of the upper airway CFD model would have greatly

enhanced the understanding of the uncertainty and potential variability of CFD modeling outcomes for use in risk assessment. The model geometry is based on an end of one individual, described in Kabilan et al., 2016. Current submission does not place this geometry in any context to indicate whether this individual is likely to be a representative of the population.

There's no detail provided in the submission to support the assertion that the CFD modeling is applicable across individuals. And EPA stated that it was within the range of other simulations but didn't really quantify what "in the range" means.

Sensitivity analyses would identify key model parameters that could focus the assessment of the representativeness of the CFD model, and the panel recommends that such analyses be undertaken.

For example, in the present report by Corley, et al., 2018, the nasal breathing model is based on a 35-year-old healthy male. But in two earlier publications, from the same group, they had CFD models for an 84-year-old

female, who hopefully won't be out doing agricultural spraying, and an 18-year-old male volunteer.

The question is whether the CFD simulations would have been different if the dosimetry, based on these individuals, had been run instead. This question seems particularly important since in their original 2012 paper they noted that using a single volunteer was a significant limitation of their approach.

So, the panel recommends that simulations with these additional upper respiratory tract geometries be conducted as a first step toward understanding interindividual pharmacokinetic irritability for chlorothalonil deposition.

Panel also encourages EPA and Syngenta to consider the possibility of a Bayesian approach or Monte Carlo approaches to the extent the data are available to allow these types of modeling exercises, which are more computationally intense. It still could be useful, but at least starting out by exploring multiple geometries would be a good start.

The EPA gave some additional detail this morning about the breathing frequency and inhalation rate for the CFD model. It was noted by the panel that the CFD model assumes 20 breaths per minute and 7.4 liters per minute. And that differs from the rate for the HEC calculation, which was 8.3 liters per minute, and 12.7 breaths per minute.

A sensitivity analysis would let us know sort of what is rate limiting in terms of deposition. Is it more important the total mass that's delivered and the concentration times the number of liters per minute, or is it the number the breaths? Because both of those factors are different in the two models. So, if you don't know which is rate limiting, you don't know which is the appropriate way to adjust in developing an HEC that's specific to a different breathing rate; breathing rate in terms of minute volume or breathing rate in terms of breath per minute.

It was noted by the panel that driving a tractor might be a light activity rather than a sedentary activity. So, the rate of 7.4 that was used in the modeling might not be

representative of the higher level of activity of someone driving with a tractor.

And Dr. Hinderliter did relay the finding that breathing frequency results in higher deposition rates, but not a change in distribution. Question is, how much higher is this breathing frequency? Because it's one thing when you perturb parameters by ten percent; it's another when you start tripling them, such as could be the case for a high exertion scenario. So, additional detail of what has already been done would be helpful.

We also had a question from a panelist that wondered just to what extent are the CFD model parameters driven by differences in age and sex, because we really haven't explored that at all. If we knew which parameters were sensitive, then we'd say, oh, well, we know that that is something that changes with age or based on gender. So, a sensitivity analysis would let us know which questions are the ones to really pursue in detail.

I think that's it for sort of the variability uncertainty and specific parameter

values on the CFD model. So, time for panel input.

DR. CLIFFORD WEISEL: I want to reemphasize a couple of things. One is, you mentioned sensitivity analysis a few times here. Actually it's something that should be done across everything that's being presented to us, because what you're proposing is does this methodology work? And at the very beginning, when you're doing a new methodological system, particularly modeling is a key that should be done.

I also want to back up and congratulate EPA. CFD modeling is something we're starting to understand because we can now do it with a computer capability. I'm glad to see that you're taking the forefront on that, but it's critical that you use the right ones in that area.

The other thing that there was talk about is variability. As you mentioned, there wouldn't be a likely 84-year-old woman. I'm not sure that's not true. You actually have a lot of field day around that population that's

involved. And it's not just the person that's driving the tractor. You often have other people walking by doing other things in a field at the same time. And, often, in some of these things, it is a family operation.

So, I think you should go back and look at the data you have on who's really involved and use that as your input into here, not only at the most healthy, but look along that distribution of who's involved, what they're doing, and the level of exercise.

So, if you have someone on the tractor at one rate and you have someone that may be a couple of meters away doing something else that's a little more energetic, they're going to get the exposure as well. And, so, you should probably take a look at your patterns around each activity.

DR. ROBERT CHAPIN: Dr. Yang.

DR. RAYMOND YANG: I just wanted to add a little bit to what Lisa presented. In the PBPK modeling world, a very active area, which was advanced by Frederic Bois, was to use a Bayesian approach. And to adopt Bayesian

approach, you have to have a very high computational power; and therefore, Markov chain Monte Carlo simulation incorporated into this assessment to address the issue of uncertainty and variability of the parameters that you use for modeling.

Now, I have never done any CFD modeling, but any modeling is going to be involving parameters. If you have parameter which is -- has a very wide distribution, you are probably not going to have a very good job done. And since EPA is actively involved in this, I want to specifically mention the latest revision of methylene chloride -- or dichloromethane risk assessment very, very nicely utilized what EPA calls probabilistic PBPK modeling, which is really the Bayesian approach incorporated with Markov chain Monte Carlo simulation.

So, I would strongly urge the possibility of looking into the possible use of this type of technology -- it's already in your shop -- to address the issue of variability and uncertainty in CFD modeling. Thank you.

DR. ROBERT CHAPIN: Dr. Sullivan.

MS. KRISTIE SULLIVAN: Thank you. Kristie Sullivan. I just want to maybe add on to what Lisa and Cliff had said about this idea of an N of one and needing to consider other respiratory anatomies. It may be as a supply to other chemicals, such as detailed analysis may not be necessary; but as we start off, we want to consider some of these variables and make sure they don't have an impact.

DR. ROBERT CHAPIN: Anybody else for this particular issue of heterogeneity of the modeling? Back to Dr. Sweeney.

DR. LISA SWEENEY: Next issue is one that actually didn't really come up in the presentations on Tuesday. Maybe in part, because Rick Corley wasn't here to present on the CFD model, but it's not clear to the reviewers that the CFD model mesh is sufficiently fine to accurately estimate those to specific hotspots. Regional doses are presented as distributions -- that is percentiles -- in a fairly limited way. We were given the -- not records -- mean or median in the 75th percentile, and then the max, as opposed to real gradations. And they state

that the 75th percentiles are stable, but the higher percentiles could not be.

At least one reviewer said that stability might vary with the number of mesh segments for a given region. So, it might be that the 75th percentile is reliable for one region, but not for another. And if it's not based on the region side of the number, elements, or facets for each region, why is that not the case?

And panel member found that the 75th percentile doses that were reported were approximately linear with the airborne concentration with a strong correlation coefficient or squared of .991. But the deviation between that linear estimate and the lowest concentration for the trend line was 19 percent. So, is that precise enough?

And there were not similar calculations provided for the humans. So, it's hard to know just exactly how precise the human model estimate is because we didn't see predictions for a range of concentrations.

So, lack of that kind of detail makes it hard to be confident about the mesh information and the stability of the dosimetry of calculations, in particular, the 75th percentile.

DR. ROBERT CHAPIN: Enrichments from anybody on the panel? Back to you, Dr. Sweeney.

DR. LISA SWEENEY: We already talked a little bit about alternative deposition modeling options and possible expansions of the modeling approach. EPA and Syngenta appeared to have determined that CFD modeling of the upper airways best suited their purposes. But other modeling options have been suggested by one or more member of the panel, who have already revealed themselves by commenting on question 1 in this regard.

While CFD modeling has potential to drive better cite-specific doses in terms of mass -- compared to the MPPD model, the MPPD model has the advantage of being freely available and widely used with reproducible simulations. So, to the extent that those regional doses

produced by the CFD model can be compared to the MPPD model, it might be nice.

Now, whether that would really confirm the model or suggest that there's a problem with MPPD having such a gross reporting, well, that would be something that we could debate if we had the data. But we don't have that in front of us yet. So, it's possible that there could be some insights gained as to when the CFD modeling versus MPPD modeling is fit for purpose.

It was also noted by the panel that the CFD model did not include a clearance mechanism and was not run for repeated exposure scenarios. Now, to the extent that the pharmacokinetic parameters are not altered by repeated exposures -- such as changes in breathing rate, or any changes to the airway structure -- it wouldn't matter, but it should at least be considered and made explicit that they don't think that's a concern; and therefore, that a single breath simulation would be adequate to count for repeated exposure.

As Ray noted, PBPK modeling can be used to consider systemic exposure as well. In the case of this risk assessment that is focused on a portal of entry effect, it could be that PBPK modeling does not enhance the risk assessment effort. However, in general, it would be helpful for both the Agency and the registrants to sort of explain the rationale for the choice of the level of detail of the modeling chosen, whether it's CFD, MPPD, or PBPK, to understand why a particular strategy was pursued.

DR. ROBERT CHAPIN: Any additions or enrichments from the panel? Jim.

DR. JAMES BLANDO: This may have already been stated, but did the model include mouth breathing? I remember there was some -- because I'm wondering if you have an activity that's strenuous, I wonder if that's something that should be considered, depending on the specific scenario that you're looking at, because I imagine deposition pattern would be different.

DR. LISA SWEENEY: The short answer is no. For one thing, it doesn't include the lower respiratory tract, but you could even

sort of simulate that by subtracting that from the airflow that goes into the nose. So, there is consideration for how having mouth breathing, instead of all nasal breathing, would have an impact on the dosimetry.

DR. JON HOTCHKISS: In an effort to be totally transparent, in terms of the capabilities of the model and how you're deriving regional dose, do you foresee the EPA will define its best model? What I'm worried about is that there will be multiple models being run by eight people who are coming to you. And then, surely, you'll select your own model too. So, I'm just wondering is there going to be a common methodology that you perceive, or is it going to be up to the registrants?

DR. ROBERT CHAPIN: We want to make recommendations, not ask questions. So, now's the time to make a recommendation.

DR. JON HOTCHKISS: I would recommend in the commonality across laboratories and registrants, that there be some thought giving to a common model, whether it's -- there are a couple of different ways to run CFD's, and

if you can just pick one. That would be my recommendation.

DR. ROBERT CHAPIN: Thank you. Other comments or enrichments?

DR. CLIFFORD WEISEL: Just to follow up what Jon was just saying, and I sort of said this. I put this idea into the next charge question. Since you've developed a new methodology and have a lot of inputs into using the models, and everything like that, putting together a decision tree basis that looks at all the inputs so you can decide what parameters should be included. May not have one model that works for everything, because some models are more complex to run than others. So, nose only models take less time and energy and inputs than one that combines it, including the relative -- again, as well as the confidence.

But you can have a series of models, and if you have a decision tree that will help you point to what you should be using, what are some of the criteria deciding when default works and when doesn't; and this is, again, goes back to sensitivity analysis. As you get more

and more experience, then it becomes easier. That might be one approach you can use to help with that.

DR. ROBERT CHAPIN: Back to Dr. Sweeney.

DR. LISA SWEENEY: Here we are, winding down a little bit. Next issue is the selection of the dose metric. And it was noted that there are localized regions with higher deposition in the CFD modeling. And this contrasts to the way the MucilAir system is tested, in that you have a consistent interface. So, a question of if you have that sort of variability within the respiratory tract, and yet a constant concentration in the test system.

So, the direct applicability is perhaps called into question a little bit. There's a question of whether, again, the 75th percentile is the appropriate dose to be using in the risk assessment.

DR. ROBERT CHAPIN: Additions from the panel? Dr. Sweeney.

DR. LISA SWEENEY: The last one was on making use of the rat data. While the NAM

approach emphasizes human-relevant simulation in silica methods and in vitro testing, the parallelogram approach still has merit, especially when it can be applied using existing rat in vivo data.

And as I noted yesterday, the predicted 75th percentile dose in rat transitional epithelium is not that much lower than the doses in the larynx. And, yet, we didn't hear anything about whether transitional epithelium was also the cytotoxicity in the rat.

Now, whether that's because it happened, and it just wasn't brought to our attention, or the level of information on the in vivo studies did not detail that. It would be helpful to know that. And the greater concordance that can be observed in the rat dosimetry versus the in vivo severity correlation, the greater confidence one can have in applying the same strategies to -- that they will be predictive of human in vivo effects.

To a certain extent, we do have previous human use data with this compound. So, maybe we'd already have seen it by now if this is

an issue. It was noted that this chemical has a history of safe use, and that's reassuring; but with a new chemical, it might be a little more of a concern to be worried about whether we're predicting the right endpoints. So, to the extent the EPA and/or Syngenta can maximize insights that can be gained from past rat studies, that helps us move forward possibly in being comfortable applying these methodologies in testing in the future where we might lack that data.

And that wraps it up for the issues that the panel members that were assigned this question brought to my attention. So, I suppose first we want to see if anyone has a comment specifically on the use of the rat data; and then, after that, opening up to other topics related to this charge.

DR. ROBERT CHAPIN: Perfect. So, use of the rat data, anyone? Jon.

DR. JON HOTCHKISS: The parallelogram approach has a lot of merit in making us feel better about, say a rat in vitro model matching up with the rat in vivo. But

you're still going to be comparing then rat in vitro to human in vitro. And I would not want us to get too hung up if those don't match up directly, because that's sort of the whole point.

We're not trying to mimic the rat in vivo exposure. We're trying to get a better estimate of what's going to happen in humans. So, it's nice to make those comparisons, but we shouldn't be shocked or dismiss the human in vitro system if they're not alike. And that's just a comment.

DR. ROBERT CHAPIN: The good news is this is not their first rodeo. Dr. Sullivan.

MS. KRISTIE SULLIVAN: Just to emphasize what Jon just said. There are other cases where the parallelogram approach is sort of being -- trying to be used to assess an in vitro method. And, in fact, there are methodological differences between the rat in vitro and the rat in vivo that make it difficult to make these comparisons. So, just to add to your question.

DR. ROBERT CHAPIN: Other comments about charge question 3? You guys are rocking this.

DR. LISA SWEENEY: Or very hungry.

DR. ROBERT CHAPIN: Or very hungry. Okay. Before we break for lunch, I think what we'd like to -- I'm foreseeing that we won't need to stay here all day tomorrow and work on this. We're making great progress today, and specifically, because you guys have put in so much time in getting your comments back to our lead discussants and allow them to fold stuff in.

So, what I'd like to do, with your concurrence, is plan on using the rest of the afternoon to work on charge questions 4, and then the monster of number 5. And then basically, go home tomorrow. And that will leave tonight for people, for the leads, to do their final tweaking, and solicit things back and forth from everyone while we're still here in the same place. Is that okay for people?

DR. JAMES BLANDO: Thank you. I just had a quick question. So, from now forward, after we discuss this within, we can reach out to everybody on the panel, not just the subcommittee for tonight as we edit? The final tweak, so to

speak, we can email or reach out to everybody now that's like public, so to speak?

DR. SHAUNTA HILL-HAMMOND: All of the comments that you need to receive from the panel overall should be addressed now. So, in your email communications, you should still limit that to your subgroup to make sure that you've captured all the points.

DR. ROBERT CHAPIN: If that's suitable for everybody -- let me, before we -- I'm sorry I missed one. Missed a concept, and that is to get clarifying questions from you guys. Do our EPA friends want to ask any questions of the panel for clarification for charge question 3?

DR. MONIQUE PERRON: We really appreciate the many different aspects of this one. We know there was a lot that went into this. And as I mentioned on Tuesday, we are working through that particle size distribution question with Syngenta, as well as people from other stakeholders as well.

And ultimately, the idea is that we would have particle size distributions that

would represent the different scenarios appropriately. Whether that's one that would do all operators, or whether that means ground boom's going to be different than air blast. We're still working through that, and we appreciate that you're picking up on some of the same questions that we're trying to work through.

And then, also, just that the idea is that also with the modeling being basically a water droplet, that it would be independent of a chemical; so that if somebody comes in with a ground boom for another chemical, they wouldn't have to do any actual modeling, because we already have that information done for one before it. So, the hope is that we can generalize this in some way so that all of that work doesn't need to be done every single time.

But keeping that in mind with your recommendations would be really helpful to make sure that that aspect is also considered when providing your input.

DR. ANNA LOWIT: It's really good to hear a lot of conversation about the MPPD and Dr. Weisel's comments about coming up with almost

a tiering framework. And I hope to hear more about that in question 5. Because as we thought about going past chlorothalonil -- to in the PMN space or to a new compound, where you don't have a lot of information, how do you make those choices about -- you know, CFD shouldn't be the first choice. What are those incremental steps that get you from a traditional default to a full-blown CFD sort of approach?

We've had a lot of registrants come to us requesting us to use the MPPD, and it's good to hear this panel sort of confirm those conversations. And we're looking forward to those comments on finding that space where the different models have their utility and are fit for different purposes.

Understanding that unlike the IRIS program that has the luxury of time often, the pesticide office and the toxics office are statutorily required to make certain deadlines. We don't have the luxury to do the full-blown Bayesian kind of statistics on every assessment.

In an average year, this program does over 100 risk assessments. We have to use

our resources appropriately to put resources where they're needed. Keeping that in mind as we think about sort of tiered framework for moving away from the animal studies, think beyond just these data-rich examples.

DR. ROBERT CHAPIN: Jon.

DR. JON HOTCHKISS: I agree that you can make a generic case for a water droplet or whatever of various sizes to finding the regional deposition, but there may be a difference in how the active ingredient is distributed within that water droplet.

So, in this case, the assumption was that it's an insoluble particle that's just sort of floating around inside the water droplet. So, the water droplet of a certain size defines where it's going to be deposited. But if you're a cell there, it's going to look a lot different to you because most of it's going to be water. But if you happen to be the cell that gets that solid particle deposited on it, your regional dose is going to be much different than if the material was uniformly distributed throughout that water droplet.

I'm not arguing that you shouldn't use the generic case. It's just that that may be an additional complication, or kind of a surprise element when you're looking at a specific active ingredient.

DR. ROBERT CHAPIN: I love the rich irony of Dr. Hotchkiss reminding the EPA that life is complicated. So, let's take an hour for lunch. Be back here at -- we're going to try to start at 1:25. Are we good over there? Let's try to be back here at 1:25, and we'll round down to 1:30 if we must. Thank you all. We'll see you in an hour.

[LUNCH BREAK]

DR. ROBERT CHAPIN: Excellent. Thank you. Let's see. For the people on the phone, I'm Bob Chapin, the chair of the committee. Let me just remind everybody that we want to be within five inches of the microphone so that the people online can hear us. So here we are. We'll do Charge Question 4 and then we'll take a break. And then we'll all gird our

loins for the heavy lifting, Charge Question 5. But first, we get to do 4, and that brings us to Dr. Cavallari, the lead discussant for Charge Question 4. How are you doing getting your stuff up on the --

DR. MONIQUE PERRON: Dr. Chapin, we have to read the question.

DR. ROBERT CHAPIN: Oh, I'm sorry. That's right. I apologize. Thank you.

CHARGE QUESTION 4

DR. MONIQUE PERRON: Hi. This is Monique Perron. I'm going to read question 4 into the record. Please comment on the calculation of the human equivalent concentrations. 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.

DR. ROBERT CHAPIN: Dr. Cavallari?

DR. JENNIFER CAVALLARI: Thank you. This is Jen Cavallari. As mentioned in the other charge questions, we appreciate the agency and Syngenta's willingness to consider these new technologies and approach. We have the benefit today, for question 4, of following all the rich discussions that have already occurred with respect to a dosimetry, the CFD model as well as the in vitro point of departure evaluation. Since these numbers are used in the HEC calculation, we just want to stress how imperative it is to incorporate the suggestions, of course, that they do into the HEC calculation.

With respect to the HEC calculation, members of the group agree that all the data elements are present to calculate the HEC by using data from both the dose symmetry modeling in conjunction with the in vitro POD results.

As discussed in detail, in the evaluation of the CFD results, we'd like to see how different model parameters effect the HEC results. Thus, sensitivity analyses, of course, are suggested. However, some of the members

expressed a little confusion over the equation used to calculate the HEC, as well as some of the values used in the calculations.

First, I'm going to cover the evaluation of the calculation as we presented, and then I'd like to turn it over to my colleague, Cliff, to kind of discuss some of the other thoughts on uncertainty factors.

The first step of the calculation was moving from the monodisperse to the polydisperse, and the calculation of the cumulative site-specific depositions per breath. To calculate the total site-specific deposition per breath is, we believe, an appropriate first step; and the method used seemed appropriate.

First, the adjustable inhalable fraction was determined. And as mentioned in the evaluation of the CFD, there are some questions with the assumptions of the 35 micrometer MMAD, as well as its standard deviations. As EPA has already mentioned, you and Syngenta, along with others, are kind of working together to refine that, and we appreciate that.

So, rather than reiterate some of the points that have already been discussed, I will just stress the importance of using a relevant particle size distribution and standard deviation. And also, should the agency accept the mathematically derived human-relevant particles PSD, comparison should be made against the sampling data, and sensitivity analyses should explore alternate MMADs as well as GSDs.

In order to determine cumulative deposition, the data on the discrete particle sizes in a single breath were then incorporated using the CFD model. An evaluation of the CFD was already addressed, as I mentioned; but additional considerations or emphasis of the following should be considered.

We really like the use of the 75th percentile for the discrete particle size. We thought that was a good choice. And as noted above, the choice of the particle aerosol diameters in the CFD analysis should be informed by the sampling results.

The second step of the HEC determination, is the calculation of site-

specific total deposition, which we, again, found very reasonable. While the method used to calculate this seemed appropriate, we offer the following considerations with respect to the breathing rate. So we felt that the breathing rate should better reflect the exposure scenario, where exertions required during tractor or backpack application of the product in an active breathing rate may be more appropriate.

For example, in the CFD model, a deposited mass, per breath, was calculated with 7.4 liters per minute and 20 breaths per minute. So then in the HEC calculation, the number of breaths per minute is decreased to 12.7 per minute. So the adjustment factor would then be 12.7 divided by 20 or .635.

However, this scenario is supposed to represent a minute volume of 8.3 liters per minute, which would be an adjustment factor of 8.3 divided by 7.4, or 1.12. So it's critical to know what's the rate limiting factor in the CFD model, the number of breaths or the amount of air taken in. We found it appropriate that the

region with the highest deposition values were used in moving forward with the calculations.

So, the final step of the HEC determination is the calculation of site-specific HECs. So there was some confusion about the relevance in the final step of multiplying by an aerosol concentration of one milligram per liter. So we believed that the assumption came from the fact that a milligram per liter aerosol was used in the CFD results and presented in Table 2.23.1 in the agency report. But we believe that additional clarity around this calculation is justified.

So I think that was all I had with respect to the calculation of the HEC. However, I'd like, with the chair's permission, to turn it over to Cliff.

DR. CLIFFORD WEISEL: This is Cliff Weisel. Let me just get my notes here. When I looked at the HEC, I'm not a risk assessor, so I went back and tried to find out what that really entailed. According to what I could see in the EPA June 2008 document, TSC for non-cancer REL -- and this is an appendix there

that says, estimated human equivalent concentration is used in the US EPA default approaches to adjust the dose in animal inhalation experiments to dose that human will receive in the same air concentration. And this is done using uncertainty factors for interspecies toxicokinetic differences. It goes on a little bit more on that about what the other ones are.

What's being proposed here is a paradigm shift away from animals to human cell cultures, such as the model we see now, the 3D model and others. So, that doesn't quite fit into the definition I just read, because that's specific to in vivo animal studies.

Now, what I sort of saw in the documents I had, was they're saying, since we're using human cells, we don't need an adjustment. That may be true for this case, but I don't think that's an appropriate response. If we go back to what we talked about earlier about that parallelogram, and whether the parallelogram is the right geometry or not, essentially, one side is the human in vivo, and that's what we're

trying to get to. And the other three sides are information that we're gathering, and we can measure, trying to appropriate. I think each of them has to be considered as to where the uncertainty may be going from one spot to another.

What I'm sort of suggesting is that, really, what you should do is get an in vitro to an in vivo HEC; and call it something different than just HEC. Because you really have to look at that and see whether there are uncertainties that need to be addressed. Now, the uncertainty may be one, and maybe you can make that claim for this case it is. But I think that should be your starting point, not saying since we're using human, and in the past, we only used these species, we don't have to do it now. I think you really do.

That's sort of the crux of where I'm coming from. I think it just has to be developed; figure out what the concerns need to be in doing that. And we talked a lot about them before. I think that's an area we can discuss in much more detail. The mathematical models

consider even physiology. They consider the differences between in vitro and living organisms, the feedback mechanisms -- all these things may or may not be put into these models; or they may have some default values, and we only have a range to consider.

What was pointed out to me, in this case, that maybe since it's a very toxic agent contact, that you don't have a lot of extraneous things that are going on. But that's really for the toxicologists to argue, rather than myself, as to whether the uncertainty factor of one is correct. But just going and making the blanket assumption that since we're using human cells it would go that way is, I think, incorrect.

DR. KATHRYN PAGE: This is Kathryn Page. The study presents acute findings for (inaudible). We've already covered that, and we've covered that it doesn't reflect repeat dose. Therefore, the exposure duration that was suggested by Syngenta, that reduction, the duration should remain at ten, in my perspective.

However, the interspecies uncertainty factor seems over-restrictive for a direct acting irritant. So, the EU, NAS, and EPA all align on an uncertainty factor of three in the literature for direct-acting irritants. So, I just wanted to point out that that would make the uncertainty factor 30, without accounting for any additional considerations, the database adjustment or anything for the in vitro system to whole systems.

The other point I wanted to make was on the benchmark dose. So the method used to derive at benchmark dose was chosen individually, based on the results from each endpoint. That seems inappropriate to me. There is evidence from other studies on this model to support methods chosen.

And TEER used relative deviation from the response of the control group. That, as a standard EPA analysis, is chosen ahead of the results. It seems logical when you read through the issue paper. However, the other two endpoints didn't do that. LDH used a point at which the response reaches a specific volume.

Now, again, that is a method the EPA uses, but it seemed arbitrary, and added later to clarify that an effect happened, rather than before.

Same with the resazurin results from lower doses where, again, as I pointed out before, lower doses were combined with the control, and then results from the two highest doses were used to compare relative deviation from the combined groups. Again, this seems strange to me. And maybe the wrong doses or not enough controls were selected for this endpoint. Or maybe the endpoint isn't appropriate, or both.

DR. ROBERT MITKUS: I just want to make a few comments. For me, I thought, overall, the framework approach, the three steps that were taken to calculate or estimate the HEC made sense. I thought they were rational, I thought they were cogent. We may quibble over exactly how that's done, or the uncertainties at each step along the way, but for me, overall, I thought it was rational and cogent.

Just a small quibble with regard to the BMDL calculation. I think the BMDL that was chosen, Syngenta did an analysis and then

probably Dr. Visioni did his BMD analysis. And then he chose the untransformed data. I'm sorry. The BMD and BMDL values using the transform data were lower, and therefore considered protective. Although, the untransformed data had lower AICs, and therefore it'd be more reasonable to choose those.

I would just caution, you know, the agency of arbitrarily choosing a lower endpoint because it is, quote/unquote, more protective. To me, it makes more sense to use what makes the most sense when you're choosing the best model among adequately fitted models. For that, it'd be emphasis on the AIC.

I can probably, maybe, address Dr. Weisel's comments a little bit. He's correct when he quotes from that particular agency guidance, but HED isn't actually using that particular approach in its calculation of HECs. It's taking an airborne animal concentration, adjusting for the duration of exposure, and then using a site-specific deposition in a ratio between rats and humans to estimate the HEC. So that's actually what's being done.

I can understand why certain members of the panel may not know that. They're not familiar with that particular approach that OPP is using. I think that approach is what we're trying to move away from.

An HEC was not calculated using the agency standard approach, based on the in vivo animal data. I did it using the RDD software last night, and it does give a very low HEC. The question is -- and I think this is why you're trying to move into this other direction. When you have local toxicity effects, the RDD value is always lower, much lower, than the systemic RDDR value. So usually, for local lung toxicity, you're going to get a much lower HEC for local effects than you would for systemic effects.

The advantage, or the benefit, of this particular model is you're actually using human cells. That's where the NRC is moving us to. It makes sense the HEC calculation, performed by Syngenta, is not going to match up with the calculation performed historically by the agency.

At the same time, Agency scientists are going to use that as their benchmark, just because they're familiar with it. That's what they know. That's what they've been using. I think internal comparison within HED -- I would say use the RDDR software to calculate an HEC, as you have been historically, and then compare it with the HEC that was estimated from this current model, and then kind of see where they line up; just to give your staff more comfort with where you're going.

Last but not least, again, as I mentioned, the three-step approach of the HEC calculation makes sense. You're ultimately going from a concentration, you're trying to estimate a local dose, basically, so milligram per square centimeter.

Now, the in vitro model involved a 24-hour exposure. You've taken steps along the way. You're comparing that to an eight-hour applicator scenario. My suggestion would be to probably adjust your BMDL for the eight-hour exposure. Because the BMDL is based on a 24-hour

exposure in vitro; you're trying to estimate an eight-hour exposure in real life.

So I would adjust that. And then, using it as an acute HEC, it makes sense. I wouldn't use it for repeat dose exposure; but based on the calculations, which to me makes sense, I think it's a good estimate of an acute HEC.

DR. ROBERT CHAPIN: Since he was responding to Cliff, can we get Cliff to just weigh in?

DR. CLIFFORD WEISEL: I just want to get your advice because this is not what I do consistently. If I understood you right, you're saying that the HEC that's normally calculated is not what's was essentially done here.

And this might lead to confusion. You think it would make more sense to have it called something else, such as an in vivo equivalent concentration? And therefore, there'd be two pathways, depending on which people go, and take some of the confusion out? That's sort of what my point was.

DR. ROBERT MITKUS: I understand what you're saying, Cliff. Yeah. Sure. Calling one an HEC in vitro and the other the HEC in vivo, or HEC standard, or HEC sub-historical, something like that makes sense.

DR. CLIFFORD WEISEL: Maybe just calling it -- if you take away calling it in vivo, you call it concentration. And so you're taking out the -- take out the -- so, I'm putting this, obviously, as what we'll put out -- and EPA would have to make the decision as to what it is, but maybe having something so it's clearer, because you really are producing a new way of doing things. And if you try to keep it the same terminology, I find that people will go about -- when you get to my age, you remember what you used to do, and you keep going if it has the same name. And so, if there's a new name, I have to think a little harder.

DR. ROBERT CHAPIN: We can leave the details to them, because no matter what specific we decide, they'll be wrong in that specific context. Dr. Fortin?

DR. MARIE FORTIN: This goes a bit to Rob's point and Cliff's point. When I was trying to evaluate the value of this approach, I come here on the HEC that was derived as part of this case study, and the one that was based -- part of the kind of registration back, and there was also (inaudible) review. Based on a (inaudible) LOAEL in rats, at which overt toxicity was observed.

The one derived, using the in vitro approach is 37 times higher. So, for me, it doesn't mean that the approach is not adequate. It means that we perhaps have not fully captured the relationship between how we do it and how we extrapolate what it should be.

DR. ROBERT MITKUS: Sorry. Maybe some perspective. I thought about the same issue, Dr. Fortin. I think maybe one thing to keep in mind is that the in vivo rat studies, the animals were exposed to a 54.7 percent AI concentration. And the HEC is basically for a concentration about tenfold lower than that. The estimate is for 4.9 mgs per liter, I believe. So that may -- I'm sorry?

DR. JENNIFER CAVALLARI: It's 4.9 percent.

DR. ROBERT MITKUS: I'm sorry. Thank you. 4.9 percent in the diluted end use product versus 54.7 percent. Thank you. Of the AI and the in vivo inhalation study. So, that may account for some of the difference, that wide margin.

DR. MARIE FORTIN: But the air concentration was still adjusted. The HEC that was calculated, based on the in vivo effect, was based on the air concentration. That was the LOAEL. And that was 0.002 mg per -- I think. Or was it 0.003?

DR. ROBERT MITKUS: Right. What I'm just saying, is if that exact experiment were repeated using a 4.9 percent chlorothalonil exposure, you'd probably have a higher LOAEC because the diluted product is dilute tenfold.

DR. MARIE FORTIN: Right. But we're looking at the air concentration milligram per liter, right? So it doesn't matter what -- you're diluting it in air.

DR. ROBERT MITKUS: No. I guess if you're diluting it in air -- if you're diluting a tenfold diluted formulation in air, then you would expect a higher concentration in air to cause the same effects as you're seeing at the 54.7 percent.

DR. ROBERT CHAPIN: My suggestion is maybe this be an offline conversation and get this sort of straightened out until both of you are thinking the same way, whatever that is. Are there other parts of your comments?

DR. MARIE FORTIN: Yeah. More comments, but maybe he'll have the same argument. The other thing I did, is I looked at the reference dose that was derived for chronic exposure, the other oral route would give me the critical effect. And again -- actually, it's funny how the numbers lined up. So if you used the RfD and use a 70 kg bodyweight, and if you use the HEC that was derived using this approach, and a 10 cubic meter breathing volume, and apply the safety factor of ten. Because, you know, I want to compare apples to apples. I also get the 37-fold difference between the two.

Again, I was trying to wrap my head around, we're using these in vitro approaches and we're landing higher. What I'm thinking is that we need to -- in our review of this approach, we need to make sure that that extrapolation actually passed that test where I would have expected that we (inaudible). So, if I found like 3-fold difference, I would have been kind of okay, that's close enough. But we're talking more about 37-fold, and that's concerning to me. Because we're going to use this for future risk assessment. That's the comments I had on this.

DR. ROBERT CHAPIN: Okay. Other comments from the panel on question 4? Sorry. Go ahead.

DR. KATHRYN PAGE: I just had a clarification point for the HEC. So the HEC is a human equivalent concentration. It doesn't matter where the data's actually come from, whether it's from animals or from in vitro. Whatever transformation that happens, you're trying to get to the concentration that's relevant for the human.

So I would disagree with calling this a different word or a different acronym. Because at the end of the day, the data point that we want to get, regardless of where you get it from, is still the human equivalent concentration.

DR. JAMES BLANDO: I just had more of a comment for EPA. One of the things that I noticed in this discussion, not just here, but from trying to find materials online about HEC -- and I know I pulled a document, I think, that was from 1994. And then listening to, Rob and Cliff, you guys talking about how the HED doesn't do it the way that's in that 2000 and whatever document.

I suspect that I might not be the only person on the committee that had trouble following and felt a little confused about how this is done; combined with the fact that I've really had a lot of trouble finding clarity through EPA documents.

A suggestion I might make for EPA, is to consider maybe putting together a really clear, concise, succinct document about HECs and

how they're computed; especially, for people like myself who might be consumers and users of the risk assessment but might not be doing it as a daily task in my job. So that might be a suggestion I might make for EPA. I think that might be very helpful for a lot of folks.

DR. MARIE FORTIN: To second James' point, I think if it was thoughtful to have a bit more transparency in the equation. By that, because the model, to me, it's very cryptic. I'm not the modeler. I make friends with the people who know how to model, and I asked them questions.

When I was trying to think about how we do the same type of assessment in other cases. For example, for a hair product, we use the surface area of the scalp, more or less. So, understanding that we want to protect the region that's most exposed, I was wondering if we could use the BMDL with the corrections I suggested earlier. The surface area, the fraction that's deposited there, and then the breathing rate, rather than the deposited dose to the area.

Because that number is hard to know -- because it's really based on the model.

And although it would be the model outputs that are used to do the same equation, it would be more transparent. We talked earlier about using MPPD. We can get those values from MPPD. We can have the surface area that would be kind of standardized. And then I could, basically, take my in vitro values, take those value MPPD and do it. That's just a suggestion.

DR. KRISTIE SULLIVAN: I just wanted to make a point about adjustment factors. I think that the use of human cells does mean that you mirror an interspecies adjustment factor. There may be some cases where in vitro to in vivo extrapolation means that you need to add an adjustment factor; but there are data driven ways to conduct and IV/IV. I consider it sort of this modeling approach that was used, one of those ways to do that.

DR. ROBERT CHAPIN: Other comments from the panel? All right. We're going to come back to you guys and ask if you have any

clarifying questions, or comments, to ask us to make sure that our thoughts are clear.

DR. MONIQUE PERRON: This is Monique Perron. I'll start and then Anna can add on. I guess I'm hearing a lot of the comparisons of the HECs. I would just caution that comparison because don't forget that you have the CFD model that is modeling larger particle sizes. And that gets incorporated for HEC in this approach. Whereas for the rat, that's not happening.

So it's taking more externally because less is being deposited; if you think about it that way. So the HEC should be higher, because of the human-relevant particle sizes that are being incorporated. It's not just a simple apples to apples comparison, again.

So that's a lot of the difficulty here in all these comparisons that people keep trying to make, is that it's not apples to apples. So, keep in mind those differences.

DR. ANNA LOWIT: Just to add a little bit to that. Also, keep in mind the level of refinement of the two different approaches.

The RfC method and the RDDR are designed to be conservative default approaches. Default approaches, by their nature, are conservative and less data derived. The computational for dynamic modeling is the far extreme of that. So, in the realm of oral risk assessment, the default would be dividing by ten or possibly do a three-quarter bodyweight scaling.

The equal to the CFD would be a PBPK model, where you're actually modeling the systemic absorption and distribution at the target dose.

So, in this case, as we think about those comparisons, if the RDDR -- if the traditional RfC and what we're calculating with the new approach were the same, I would actually be worried. Because it would tell me that we were gaining no levels of refinement in accuracy in our assessment.

DR. ROBERT MITKUS: Thanks, both of you, for your clarifications. That's a good point you made, Dr. Perron, about taking into account the particle size. I hadn't really thought about that during my reanalysis.

At the same time, the one good thing about the RDDR software, if you have it, is you can put in the MMAD for your particle cell. Let's say you had -- again, defaulting to the rat study. You had two rat studies were the MMAD is three and one and 35 microns; and the other with a GSD estimated for both. I guess in theory you could compare those HEC calculations. Thanks for reminding me of that.

DR. ROBERT CHAPIN: Okay. That brings us to the end of Question 4. Let's take a break. Come back at quarter after. And I've got two minutes of or one minute of. Come back at quarter after and we'll dive into Charge Question 5. Period. Anything from our DFO? No. Okay. We are adjourned for 15 minutes. I'm sorry. Recessed.

[BREAK]

DR. ROBERT CHAPIN: We're back from recess. We're newly energized. Dr. Perron, would you read question 5 into the record, please, ma'am?

CHARGE QUESTION 5

DR. MONIQUE PERRON: This is Monique Perron. Question Number 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 chlorothalonil and other contact irritants, as well as its potential to be used for other chemicals that cause portal of entry effects in the respiratory tract.

DR. ROBERT CHAPIN: Such a simple question. Dr. Blando, the one taking a deep breath.

DR. JAMES BLANDO: Sure. Okay. I was in charge of coordinating the response from the subcommittee on their thoughts about this particular question. We sort of framed this question as developed more generalizable comments, which is what we think you guys wanted, sort of thinking about chlorothalonil as sort of a case study example. That's sort of how we tried to approach answering this. There were

lots of comments that were received, and I tried to distill it down into overall themes. And we had six different themes that we came up with.

Some of these may be redundant from what's already been discussed. And I apologize. If I start repeating something, just let me know and I'll stop; because some of this reflects some of the questions that we've already had. What I thought I'd do is I'll just read what I wrote, and then people can jump in.

This does reflect about midnight last night. I did try to update it during the day today, but I didn't do a very good job. So I know that some of our committee members have some disagreements with things I'm about to say. Just jump in. But it was my best attempt to try to synthesize this together. I'm just going to read what I wrote. And I will admit, for the subcommittee members, I did plagiarize some of the things you guys wrote to me and just copied them in. So I apologize for that. Okay.

In vitro testing has great promise and offers many potential benefits, such as reduced reliance on in vivo animal testing and

reduced burden on animal welfare; potentially avoiding the pitfalls of animal to human extrapolation, and faster screening throughput for chemical safety evaluations.

The proposed approach is a step forward in the use of human modeling and tissues for assessment of the inhalation toxicology of certain chemicals. The use of the criteria developed by OCSPP for the evaluation of NAMs, or new approach methodologies, is extremely helpful as outlined in Appendix B.

These include decision context, biologic relevance, reference chemical set justification, reliability within the context of use, transparency, description of uncertainty, access by third parties, and independent scientific review. EPA's discussion of whether the approach meets the criteria for its intended use is, for the most part, persuasive. Additional information would help to increase confidence.

The MucilAir system has been used in over 100 publications starting in 2008. Although not all these are relevant to the

current question, some may provide additional supporting information to increase the comfort of applying this approach to other chemicals.

The overall approach to utilize a human in vitro model of local lung toxicity, to refine the human health risk assessment for chlorothalonil, serves as an instructive example. It is an example of an in vitro to in vivo extrapolation, and the agency should be commended for entertaining this approach. One strength of this approach is that it seeks to identify and utilize a relevant human in vitro model for the endpoint of concern, local lung toxicity. The model is not designed to and cannot evaluate systemic toxicity.

Another strength of the overall approach is that it proposes a model novel toxicology approach to the current risk assessment for chlorothalonil, for which a NOAEC has not been attain.

A third strength is the demonstration of how modeling, for the particle size distribution to estimate site-specific

deposition in the relevant target organ, can be utilized.

Additional strengths include use of human tissues and human respiratory anatomy, the ability to use many doses in replicates, the tissue model is well established, and the literature widely used. The CFD demonstration modeling and ten dose experimental design allows for a quantitative risk assessment using an in vitro approach.

Derivation of the BMD standard deviation followed accepted EPA guidance; ability to discern upstream toxic endpoints and provide mechanistic understanding; retention of intraspecies uncertainty factor; potential for toxicity investigation using tissues from sensitive subpopulations. There's potential to do that. Cytotoxicity as a measure, allows the capturing of several possible mechanisms leading to cell death.

EPA should continue to explore and carefully consider the utilization of in vitro models. In vitro methods should be evaluated to ensure they protect the health and welfare of the

public and the environment. So that was theme number one. I suspect -- did anybody have any comments about theme number one? Otherwise, I can move on. I think that's the least controversial. Sure. Kristie?

DR. KRISTIE SULLIVAN: I just want to add a caveat to the statement that it cannot be used to evaluate systemic toxicity. I would say the evidence that we've seen here, it's not being proposed that way. I would hate to have that be a statement of the future for all cases.

DR. KATHRYN PAGE: I just want to add to that. For this case, systemic toxicity is covered by the oral toxicity studies. Oh, Kathryn Page. Sorry. In this case, it wasn't an issue because the oral toxicity study covered the systemic toxicity.

But I do want to stress that this would need to be determined to be the case, or not, for future applications, and it be considered when this is use in the future; especially for chemicals that don't have any information associated.

DR. JAMES BLANDO: Okay. Going to theme number two. In vitro testing methods have their own set of limitations and will not necessarily resolve all the uncertainties that exist with currently accepted in vivo studies. While likely to be potentially very helpful, it is not likely a magic bullet that will fully resolve the common uncertainties and risk assessment. It is also important to recognize, at the outset, that some of the deficiencies of the specific in vitro approach, that the panel identified, are also deficiencies of the current in vivo approach.

So, to expand on that, the specific subpoints were: intraspecies variability still exists with in vitro studies and, in fact, maybe higher when using donors who are not inbred as is often done with many animal tests. It was noted in this proof of concept model evaluated for chlorothalonil, that only five donors were used, who were all Caucasian, with female donors being relatively close in age.

Despite this relative similarity among the donors, there was still variability in

the results, and this variability could be much higher, should a wider or more representative donor population be used. This is particularly important because this can result in a much less precise estimate of the BMR against the BMD and POD. In vitro testing should attempt to utilize donors that are representative of the appropriate target population, but it is crucially important that health protective and conservative methods are utilized when estimating the BMR and BMD.

A tertiary point was, for example, in this chlorothalonil case study, a significant limitation of the submitted work resulted from the use of means, geometric means, or standard deviations that result in a less protective estimates of risk. And I know some folks disagree with that, I'm just reading what was submitted to me.

Some of the members of the subcommittee felt that this was not appropriate. It would also be helpful, in the future, to know if different cell culture brands could also be used to perform in vitro studies, giving some options for users of this technology.

I still have additional points within that theme. Then, I'll continue to go on, unless folks want to jump in. I'll just continue and just jump in.

DR. ROBERT MITKUS: Sorry. Just briefly. With regard to the comment about using standard deviations and that's not protective enough.

My only comment was that, I think, these are standard measures of variability that we see in toxicology studies. It was also my impression that for some measures Syngenta proposed using the geometric standard deviation, not just for particle size but for other measures, to capture that variability. For me, it was adequate.

Perhaps, the next step you'd want to do, probabilistic, to incorporate measures of variability and uncertainty across parameters, especially as you're doing your HEC calculation. But that's an open question.

DR. ROBERT CHAPIN: Let me just remind the committee that, for the record, we

need to precede our comments by our name. This is Bob Chapin, or post-script it.

DR. HOLGER BEHRSING: I fully agree. There need to be options when it comes to different vendors. Commercially available tissues are out there. For the airway tissue, I'm not aware of too many commercially available types or manufacturers thereof. For some of the other -- for example, skin, the reconstructive modeling, you're going to have more options.

That being said, the manufacturers of these tissues are going to have their proprietary recipes, and their media that they use to expand and mature the tissues. I don't know if that's really going to play a role in ultimately validating the model.

There are many different laboratories that actually create the tissues themselves. One laboratory, in particular, that I had the pleasure of visiting was that of Scott Randell at the University of North Carolina. But he's been doing this for 20 years and has published the recipes and the approach that they take. So that does make it a lot easier for

laboratories that do want to create these tissues to do so.

Everything that I understand about it, is that all the conditions can be very tightly controlled. So, even if you do have multiple manufacturers of the tissues, the quality of the tissues may not be the same and they may behave differently. Again, when it comes to having multiple options, that's great, but you also want to have similar results.

DR. EMILY REINKE: This is Emily Reinke. Holger, just to kind of expand upon that, that would be a place where EPA could step in with some sort of performance criteria around each of the models; to say, you know, you need to show with a package of 16 chemicals that it behaves the way that we expect it to behave; in order to show that your model is applicable within the larger domain.

DR. CLIFFORD WEISEL: This is Cliff Weisel. Just to follow up on one of the things that's being alluded to here, about only having five cell lines. One recommendation that I thought would be worthwhile, we sort of touched

this earlier, is to have developed some baseline responses across cells to understand both the variability within the system; and then look across different ages and genders, the two genders, and ethnicities, and potentially health status. So, you have a sense as to what type of variability might exist. And that would help push the whole area forward.

DR. ROBERT CHAPIN: Anybody else?

DR. JON HOTCHKISS: I wouldn't want to exclude other in-house cell systems, just offhandedly. But one thing that you do get with using the commercial sources, is they spend a lot of time upfront validating the system. And they essentially come to you with a verification that they meet all the standard criteria from lot to lot and batch to batch. That is just one way of reducing the variability between laboratories. The downside is that they're not cheap. But that reflects all the work that's gone into make certain that they're consistent. They're not contaminated, they have no mycoplasma, and they're really the cells that you think they are.

DR. KATHRYN PAGE: I just want to add to that. A lot of effort was put in with the development of the skin irritation OECD test guideline, where a similar thing was done. So there is precedent for doing this where you have different performance criteria with different brands of the 3D models. I think if a similar approach was taken against a performance criterion, this could be overcome.

DR. JAMES BLANDO: So the next sort of subpoint, within that theme, was the specific choice of cells used in the culture for in vitro methods must be carefully considered and should be representative of the target organs for toxic chemical exposures. Critical parameters, such as sensitivity and cellular response, should be similar and representative of the populations or ecosystems exposed, if this was an eco-tox application.

In this particular case study with chlorothalonil, the study utilized cells that were harvested from the nasal passages. It was unclear if this harvest location produced in vitro cultures that would respond in a similar

way, and with similar sensitivity to other locations in the lung, that could be exposed to a test chemical.

It is very important that the cells used in the in vitro cultures are representative of the cells that would receive a dose, in the population under consideration, for a specific chemical or another risk assessment.

I'll just continue going on. So the next subpoint within the them was, in vitro testing protocols are still subject to the challenge of choosing appropriate adverse endpoints for consideration.

Based on some of the discussions we've had previously, several of our subcommittee members felt that the endpoints of the TEER, the LDH, and -- I can't pronounce it -- the resazurin. However you pronounce that. Were very crude markers of cell damage, and therefore did not detect important steps in the pathologic process.

For example, a better understanding of the specific correlation of these crude measures with cell death, might

better facilitate a more accurate interpretation of the meaning of the study results. So, there was some debate about what is the endpoint, especially if we have chemicals that have more complicated modes of action.

While it's important that the endpoint be sensitive, measurable and represent an underlying pathologic response, it should also be physiologically relevant. Variability in the measured response for an adverse endpoint should also be considered, and the impact this variability will have on both the detection limit and interpretation should also be considered.

If highly variable responses are used, the most protective values should be used, not necessarily average values. Effects of inactive or inert ingredients should also be considered, but it is still important to have an assessment of the pure active ingredient because of the numerable combination of mixtures that can be produced for products reaching the market.

As such, it may not be practical to test all mixtures, or even predict which mixtures or formulations may be produced to meet

consumer demand. Therefore, assessments of the pure active ingredient are still valuable and useful.

So, theme number three, moving on to another theme is, estimates of exposure for relevant scenarios in the corresponding target cellular dose are critically important when using in vitro assays for safety evaluations of chemicals. If the exposure (inaudible) the cellular dose is not estimated properly, the results of the in vitro assay may not be applicable or even result in errors when characterizing the risk.

It is crucial that the human equivalent concentration be computed correctly and accurately. This has kind of already been discussed, so I'm just going to skip over this. But we also, for Question Number 5, the subcommittee, we also had a lot of discussion about the clarity of the HEC calculation. I had some difficulty understanding how that was done and its relevance. I think a lot of that has already been discussed.

There was some discussion -- this probably was already mentioned. It was discussed, at length, about the particle size distributions assumed in the study.

There was concern that -- and we already discussed that. Some of the operational parameters of the nozzles could greatly impact the particle size distribution, and many of the other things that we've already discussed as it related to the computational fluid dynamics model. All of this has sort of been discussed. There was concern about a lack of clarity on the HEC. Okay. So I'm just going to skip them.

Chemicals with different physiochemical properties should be carefully considered. Important parameters such as volatility in the form of the chemical, as present in the environment, must be carefully considered. In this chlorothalonil case study, there was considerable discussion about its volatility and how the chemical was applied, and in what form, whether it was dissolved, emulsified, volatile, et cetera.

The physiochemical properties of the chemical in the form, through which it exists, greatly impacts the appropriate method in which the chemical is applied to the in vitro culture, because the application of the chemical to the in vitro culture may significantly impact the results and responses seen.

For example, chemicals that are more volatile may behave very differently. For example, if they're applied to an open culture plate, they might even be lost as they volatilize from the plate. Okay. This is going a lot faster than I expected.

So in theme number four, it was not clear that the format of the in vitro 24-hour assay was representative, of sub-chronic exposures, where you have repeated doses and potential recovery and re-exposure of the cells in vitro.

The subpoint for this theme was, it was clear from the data that the length of time in the cellular metaplasia, without recovery, would be highly dependent in the total length of time of the toxicity test in the case

study example. I think we had discussion about this, but I'll just read it. It does not appear that a 24-hour test is long enough to ensure that any evaluation of these longer-term exposures would necessarily be elucidated by this test. I think we had discussion, but I don't know if anybody wants to comment. Go ahead. Yes.

DR. KRISTIE SULLIVAN: Maybe this is just a clarifying question. If we had discussion about this earlier, but it was under a different question, is that still okay in terms of putting it into the final record.

DR. ROBERT CHAPIN: You can totally bring it up, again, if we need to. We may not need to beat is as much as we beat it before, but simply reminding us that this is still an issue, if you want.

DR. KRISTIE SULLIVAN: I would just say that it's possible to use shorter term endpoints to predict longer term effects.

DR. LISA SWEENEY: My comment relates to the exposure duration and whether or not that needs to be adjusted for in the HEC. We're talking about an irritant endpoint.

I actually followed some of my own advice and went back and looked at the acute in vivo rat data; to see the differences between the two, four, and six-hour exposures and the incidence and severity of the inflammation effects. I wouldn't necessarily say that a six-hour exposure is three times as toxic as a two-hour exposure, looking at some of the incidence and severity information that was in Slide 13 of the Syngenta presentation.

For example, with the males that are exposed to the middle concentration, so you're not at the highest concentration, so you're not necessarily maxing it out. And the epithelial necrosis and ulceration, the incidence is the same, three out of five animals for two, four, and six hours. And the severity scores go from 1.8 to 2.

Looks to me like you don't really need a time adjustment on two hours versus six hours. Which is not to say that you don't need an adjustment for one day to 14 days. So that suggests, to me, that you need to think about your time adjustment that you've proposed in the

HEC; whether or not you need that sort of duration adjustment, just based on the acute effects in vivo.

DR. KATHRYN PAGE: Building on that a little bit, I think more generally looking ahead with use of this for the chemical component. I would like to see a few other irritants with known direct-acting irritation effects. And to see if this really does need to be a repeat dose long term assay, or if it wants to be short term. And if it does want to be repeat, how long for? I think we talked about this a little bit earlier, but I just wanted to reiterate that I think that is important to find out.

DR. STEPHEN GRANT: I want to weigh in. Simply longer duration of a cytotoxic dose is going to be cytotoxic. Period. What has convinced me that longer term doses -- and we need to look at the model -- is the idea of repeated doses with recovery times in between. So that we might see whether or not sub-cytotoxic levels become cytotoxic with time.

DR. JAMES BLANDO: Sorry. I was trying to capture that.

DR. ROBERT CHAPIN: Take your time. Capture it.

DR. JAMES BLANDO: If I don't, I'll forget.

DR. ROBERT CHAPIN: We'll be here. Plot amongst yourselves.

DR. JAMES BLANDO: So theme number five was any in vitro test should be validated for the expected modes of action of the chemical being evaluated for safety.

The subpoints in this were: starting out with a proof of concept evaluation for in vitro studies, is helpful to initially test chemicals based on their expected mode of action, with initial chemicals being those that have extensive and well-understood toxicity. This will likely help further understand validation studies, and likely help the risk assessor understand the limitation of any in vitro study used.

Standardization or harmonization of testing protocols will likely be very helpful

to end users, especially those with a global footprint. Information supporting the reproducibility of the MucilAir system, and other similar systems, are also needed and should be considered when proposing use of these systems. Assessment of the validity of the model approach, for future uses, need not include prospective trials comparing in vitro results to in vivo results with dozens of chemicals. Comparisons to current in vivo models and model results may not be fruitful.

Relevance could be supported with an adverse outcome pathway, and other information, and the assessment of the reliability of the test system. Some comparative data was already provided using the system to assess some inhaled pharmaceuticals and other chemicals. Reliance on an AOP can support the use of upstream effects, like cell death in this case, to make regulatory decisions and avoid in vivo testing.

The idea is that once the AOP has provided biological relevance for the upstream effect, and the test system addressing that

endpoint is considered reliable, then other chemicals that have the same effect may cause the same applicable endpoint.

While a fully endorsed AOP is not necessarily needed, detailed explanation about how the AOP was constructed, and how the endpoints were selected to fit into the AOP, would be useful in order to support application to other chemicals with similar modes of action.

DR. KRISTIE SULLIVAN: At the beginning, when you said an in vitro test should be validated for expected modes of action, I think I would not want to imply that every potential mode of action needs to have a separate validation study. Maybe something better to say, would be a test should reflect the expected modes of action.

DR. EMILY REINKE: This is Emily Reinke. Sorry. I'm gathering my thoughts. Yes, I would agree with what Kristie said, that you do not need to validate every single endpoint. You need to validate the key events that you're seeing happen within an AOP. And any methodology that addresses those key events within and meets

performance criteria as specified for that key event, would be applicable as a good methodology, if that makes sense.

DR. JAMES BLANDO: Okay. Theme number six: I guess I saved this one for last. I'm just going to read it. An in vitro test should be externally validated or at least initially be compared to other conventional methods to assess validity. It is clear that animal studies have limitations, and some argue that, in fact, they may not be the gold standard they are so often thought to be. However, there has to be a method to evaluate the performance and predictive ability of any new test method under consideration. Careful thought should be given as to how this can be done.

For example, one can ask that if a comparison of the results of your in vitro test method, to results from chemicals with already existing animal to human data and well-known hazards exists, this can serve as some assurance that the in vitro test predicts risks accurately. Performance of in vitro test methods should be periodically reassessed, as new information

becomes available, to determine if they continue to provide accurate risk estimates.

That's it. Those were the six themes. People also provided -- Dr. Yang, in particular, provide me some -- I haven't had a chance to look at them and incorporate them yet. But I attempted to try to incorporate all comments, and I know other folks had some other comments. And feel free to --

DR. ROBERT CHAPIN: We'll just work down this row. Kristie?

DR. KRISTIE SULLIVAN: Hopefully, I can go back to one of the other themes.

DR. JAMES BLANDO: Go ahead. Yeah.

DR. KRISTIE SULLIVAN: At one point, we said something like, it's important that the cells in the cultures represent the populations of cells that will receive a dose. I think we want to have the concept of functionality in here. I guess, in this case, we're talking about different regions of the respiratory tract. So if there are functional

differences between different regions, then, yes, that should be represented and modeled.

But I don't think that we need to -- if there aren't functional differences, then we shouldn't have to model every single section, I guess, is what I'm trying to say.

DR. STEPHEN GRANT: On that same point. I thought there was some discussion early on that different points in the airway had different pre-existing squamous cell contributions. So the issue would be that if you get the cells from different places, do they reiterate that in vitro. And are the cells from one are more or less susceptible to the effect? Just something that you have to keep in mind, even when you're taking cells from the same donor.

DR. EMILY REINKE: Jim, can you reread the first sentence from that last point?

DR. JAMES BLANDO: Sure. I will repeat it. I thought I could sneak it through there. That was my attempt to sneak that under the rug. Okay. Read theme number six again. An in vitro test should be externally validated or

at least initially be compared to other conventional methods to assess validity.

DR. EMILY REINKE: I am trying to figure out how I want to rebut that. As has been stated, numerous times in this meeting, the traditional methods, the animal methods, have never been validated. They have decades of use. But it's only been as we have better mechanistic understanding of how each different system functions, that we can actually see where the animal models that are traditionally being used have been failing.

So, I would hesitate to say that we need to be comparing our new in vitro methods directly against the animal methods, for which we already know they fail. And this is where validation becomes a very -- I'm choosing my words very carefully here. Validation becomes a very baggage filled word. There are a lot of thoughts and feelings around the word validation and what it actually means, and how you can fulfill that.

Again, this is where I would say we need to have performance-based criteria around

a methodology of how you know -- and this is maybe another panel has to come together to determine that. What criteria do you need to meet to show that a model is doing what it should be doing? And you can use the animal data to inform that. But I would say that comparing it to animal data may not be the best way to do it, where we know the animal data is failing.

DR. ROBERT CHAPIN: I'll try to do this in the order in which I hope these things appeared. Kathryn?

DR. KATHRYN PAGE: Okay. I have three points. Some of this was reiterated earlier today, but I just want to say it again.

The data generated in the CFD can be used for the chemical assessment to similar properties, for example, density. But again, I just want to clarify that restriction should be placed on the scope of bridging these data, just like we do for any of the bridging and waving of data requirements would be. The future applications where sensory irritation will be a concern, for example, if we noticed the cold symptoms seen in vivo sometimes. And alternative

paired approaches that assess these additional endpoints, should also be considered for future approaches, evaluating new chemistries. Jon can comment if you want more information on those.

If this alternative approach is correct, it does mean that the gold standard in vivo model is vastly over predictive, and unnecessarily overprotective for this endpoint. It could mean the potential for a large adjustment of other direct-acting irritants that are currently on the market.

Since the EPA's main goal is to protect the public, we do need to make sure the rationale behind the approach is sound so we can be confident that we're still protective. That goes without saying.

The numbers seen here are vastly different from the in vivo and the in vitro derived approaches. It's important to consider. If we're confident that these data support a more realistic approach, whilst also protecting the population, are we now to assume that the animal model is not a relevant system to look at these direct acting irritants? And that this type of

alternative should not only be suggested, to avoid minimal testing, but encouraged as the right approach to be more humanistic?

DR. CLIFF WEISEL: I had mentioned in Charge Question 3, something about developing a checkoff list for an evaluation. This is probably where it should be, because this really encompasses everything that we're trying to do as a full risk assessment.

I would like to say I can give you guidance on how to develop that. I don't think I can within the time period that we're here. But some very generic systems should be, you have a whole series of equations, which we're doing equations more now, and you have some experimental work. So you take a look at the inputs that you have for the equations and find out what are the key parameters that govern -- if you've done a sensitivity analysis, you'll find which are the most important. And that's how you might start developing the criteria you want all along there. That would be one of the main suggestions.

Among this room, if anybody could think of things they work with, maybe I'll try and think of some in the exposure area of what I wanted to provide. I think that would be helpful to our colleagues in the EPA.

The other thing is, to go back to one of the comments that Jon had made about the advantages of a commercial lab setting these up as opposed to individual labs. Now, EPA is very good about putting out something called a QAP, quality assurance protocols. And any time you put in a proposal, we have to do that.

And that might be your starting point for this as well. Put out the quality control, quality assurances, that need to be put in for any cell developed lines. The test standardizations, what they have to meet to be considered usable.

So, that would be a starting point that -- presumably, the commercial labs would take this and say, great, I'll work on it, and make sure I meet it. But even those that are not commercial, like myself, will complain and mumble under our breath. But we know if we want

funding, we'll have to do it. That might be a way to get people up to at least a minimum standard that you think is acceptable.

DR. HOLGER BEHRSING: I wanted to touch on the comment about cells derived from different regions. As long as we obtain the functional characteristics, that would be a good way to assess potential effects in the regions. What I don't know is whether or not those different culture media, that are being used to develop those tissues, are the same based on the different cell types. For example, once you do those isolations, it's possible that they may actually change from their original phenotype a little bit, based on that same culture media that's used across tissues that are being developed.

One of the reasons I bring that up, is because, in this case, I think healthy donor tissue was used. But there's many circumstances when tissues such as MucilAir are selected because you can actually obtain diseased tissue. And then the question is, well, you differentiated these for a period of many weeks.

Those cells from that smoker aren't smoking any more, and do they really still contain the smoker phenotype? I've kind of heard arguments both ways, but there's also a concern. I just want to raise that point.

DR. JON HOTCHKISS: This was not a charge question that I was assigned, so this is just a stream of conscious discussion of points that I thought about when --

DR. ROBERT CHAPIN: We've only got three hours. So, just rein it in just a little bit for us.

DR. JON HOTCHKISS: It's a small notebook. In terms of the approach taken, I thought in this case it was a well-reasoned approach that they used, so I fully support it. It's appropriate. It's an appropriate 3D model to assess the direct toxicity. The use of CFD modelling to determine regional dose symmetry is, I think, a really strong point.

The acute cytotoxicity that was used to identify the point of departure, in this case for a direct-acting toxicant, I think, is appropriate. And we can quibble about whether

TEER can be a more subtle indicator of sublethal injury. But that'll come out over time; and it'll be different for different materials.

The only caveat is that repeat exposure and/or acute exposure and recovery was missing here. That's still sort of a gap that I see. So that would be really nice to have that approach.

The strength of the approach is the use of the correct in vitro model based on the dosimetry that they solve. And it generally is likely to be appropriate for any direct acting toxicant. So you just have to look to see where the dose is going to be. Dose is dose for these directing-acting things. And you live or die, depending on what you're exposed to.

As far as the limitations, in this case -- and I think it's just because of a rich history of this material, it jumps over hazard. So, for new materials, which is something that I'm mostly interested in, there has to be some way of getting that hazard data in there. So I don't know if that means you just always start with the active ingredient, with a pure -- only

do a dose response. And so, that gives you some sort of an estimate of where you are in the exposure response continuum. For instance, that's going to be needed to set OELs for use of the materials.

It would be nice to have some way of addressing the potential for sensory irritation. So if you look at the OELs that are out there, over 60 percent of them are based on sensory irritation, as opposed to frank toxicity. How we incorporate that into these developing models is somewhat of a challenge. Whether we can use cheminformatics or modelling reactivity with the family of trip receptors, that are responsible for that, that work is ongoing, and we'll see in a year or so.

We need to include some way of assessing what the mode of action is. And that will help define what the appropriate AOP is; which in turn will help drive the selection of the appropriate cell model. So is it respiratory toxicant? Is it metabolic poison? What is it? Then, you can use a fit for purpose in vitro

exposure model. So that's just a refinement that I see coming down the line.

We talked about setting up a hierarchical -- tiered approach. I'll use the acronym IOTA (phonetic). For us, our IOTA includes a whole series of steps that we use for any new material, which starts off with cheminformatics to look at the chemical. What are the structural alerts? What's the potential mode of action, and what toxicity classification is it likely to fall in?

In the big picture of things, we're not real worried about threes and fours; but you really don't want to miss ones, twos, and the tweeners there. So that is a really good first place to start. That's your first step, and then the regional dosimetry can help identify what the target site's going to be. And then that drives your selection.

For materials that you have an estimate of what the exposure concentration people are likely going to be exposed to, I think that's where the CFD modeling can really help in defining your exposure response profile. Because

you're not just guessing what the exposure concentration should be for the dose to the tissue. You could predict what it should be, based on a human exposure and use that as your starting point and then go both ways.

So, it's a little more efficient and kind of gets you to the answer a little bit quicker. Overall, it's a really powerful model. It should be really good for testing formulations, once you know what the profile of the activities. That in itself can really reduce a number of acute exposures that need to be done for formulations. That's about it.

DR. ROBERT CHAPIN: I think I had Rob down next.

DR. ROBERT MITKUS: Jon covered a lot of the topics I was going to propose.

DR. ROBERT CHAPIN: Kristie was next.

DR. KRISTIE SULLIVAN: Yeah. I take the easy way out and say I agree with what Jon just said. I also had a couple of comments about this case study being a good demonstration

of the work you put together in IOTA. I think what you said there makes sense.

I wanted to come back and thank Emily for highlighting theme six. If you listened to what James had said, a lot of our comments actually didn't say that we needed an extensive validation compared to conventional methods. So, I think that's sort of demonstrative of our on-going discussions and working through our opinions. So, I would agree with what you've said there, and then I had one more -- nope. No, I didn't. Sorry. Thanks.

DR. KATHRYN PAGE: Just following on a little bit, again, from what Jon said. Again, love the let's use IOTA rather than other words to explain this. But I would really like to see -- and I'm sure the EPA is planning on this, but I'm just going to state it anyway -- really like to see an updated guidance document with some framework or decision tree to help guide registrants through supporting rationale to select one model over another for different scenarios.

DR. STEPHEN GRANT: Again, we wrestled with this idea of should we be looking at the animal data, and then the in vitro data as filling in the gaps; or whether we're making a complete break. One of the things, I think, a published secondary data analysis, is that let's not throw away that huge amount of data that we have.

One of the things that I felt was lacking, in this presentation, was the referral to previous studies with other chemicals. There were a few references to it, but I don't think the best use of that data was made to justify assumptions made in the current studies. I really can't emphasis enough, that that data is there and existing. For whatever it's worth, it should be mined and it should be used to the degree that it's useful.

DR. ROBERT CHAPIN: Thank you. Holger, did you? Nope. Marie?

DR. JAMES BLANDO: I was going to say something really controversial, so I'll get you later than. Do you want me to --? I guess with regard to theme number six, I'll admit I

think our subcommittee had lots of different opinions about it.

When I think about the validity question, which seems to be a bad word, I guess the difficulty that I have is it sounds like, to me, there's a sense of, okay, the animal models aren't that good. And that you almost just have to accept, on face value, that we're going to do these in vitro tests, and we have nothing to compare them to, so therefore you just have to accept that.

I know that's not what you're saying. But because we understand the biologic mechanisms, therefore, we have to have faith in that. And I think that we should. But I would also just kind of give you a different experience.

So I've been involved with a lot of cases where the toxicologist told us that the risk assessment is fine, and that there is no adverse pathway. And yet we have somebody in, say, for example, an emergency department, who the poison control center is now calling us up and saying, how could this person be sick?

I'll give you an example, when we dealt with the bromopropane. I remember with our index patient, in that case, we got a call from our poison control center and the information that we had initially was, well, bromopropane, it's different. There was a lot of lack of clarity about how could this be? How could you have a patient in the emergency department that's poisoned from this particular chemical?

So I guess I just worry about -- I don't know how to word it, but I worry about lacking a full appreciation that sometimes, when you do these tests or you do these screens, you might not actually know all the details you would want to know about a chemical. And the problem is that I worry about missing things.

Of course, being the guy who's the industrial hygienist; you go out in the field, you're the one who sees the people who are getting sick, and you think, well, how could people be getting sick? Because everything says 100 bpm level is an acceptable OEL. And this is an acceptable exposure standard.

So that's why, for me -- I know, for me, validation doesn't seem to be as much of a dirty word, to me. Just because I've always just been concerned about, what do we do about the things that we don't know about yet? Because it's always bad, from an epidemiologic standpoint, when you're looking at people that have now become cases.

And you think, geez, we never knew that people that grind wood for a living could end up with nasal cancer, depending on the wood. I remember the days we thought, oh, wood dust is just nothing but a nuisance. Until somewhere -- I guess that's the not particularly refined way of saying it.

But that's just a thing that does concern me a little bit about -- I just want to always recognize that, whenever we do these tests, risk assessment is a tool, and that there is the opportunity for those tools to be wrong and need to be revised.

I understand what you guys are saying. I totally understand that you can't really validate these things. But I just would

hate to have that feeling of, we approved this in vitro test and it's the end all, be all. If somebody's sick out in the field, well, the in vitro test says that they're alive.

I can't tell you how many times, in industrial hygienics, I've been in facilities where people complain about being sick, and I've had people say they were not exposed above the OEL, it's all in their head. They can't possibly be sick because the threshold's 100 bpm and their exposure was 80 bpm, so they can't possibly be sick. It's all in their head.

I apologize for the lack of refinement in the way I'm describing it, but that's just something that I worry about when you think about risk assessment. I don't ever want to forget that there are things that we might not know. There might be adverse pathways that nobody ever thought actual existed with a particular chemical. And I wouldn't want people to say, well, no, that can't be because the test says this.

Maybe I'm stating the obvious. I don't know. That's what I was trying to kind of

get at with theme number six. But, obviously, I didn't really word it properly. I'm just trying to get at that.

DR. ROBERT CHAPIN: That's what some of the back and forth between you and the associate discussants could beat about to try to help sort of solidify that.

DR. MARIE FORTIN: There was a lot of discussion about validation, for lack of a better term. But I see it as method validation the way we see it in the lab. I don't see it as a comparison.

And I do not believe that in this we need to conduct this by comparison with animal studies. But my computer falls asleep. Sorry about that.

I think that we're trying to pave the way forward with a new approach. And what I would like to -- for all of us -- and I think that's what Jim is getting to -- is it needs to be health protective. At the end of the day, we need to be able to protect the people that are in the field.

I think that what I feel this proposal is missing, is the quantitative relationship between the value that's in the model and what happens in the lungs. I'm not sure how we get to that quantitative relationship.

I know down in North Carolina, you guys have the human exposure chambers, so that could be an option. But I'm not sure that going through the animal with the parallelogram is the way to do it. But we need to understand what that value that we derive, using this approach, what it means in the human body. And basically incur it from human physiology.

Instead of doing human exposure study, I think we can probably use what's already known. And I know there's a host of challenge for you guys to use human data. But I think it would be your due diligence to do that. And compare with -- basically, there's a vast number of other irritants that are known. And for which we know that when you go into that -- it doesn't matter what the industry, but you go into that

plant, or that camp, and it's an irritant, you feel it.

So we have measurable levels that make people feel irritated. And we need to be able to backtrack to how that model is predicting that and have that quantitative relationship. Because right now it's qualitative.

And that's the drawback of the AOP framework. So it's qualitative relationship. We need the quantitative relationships. That's my opinion.

DR. KRISTIE SULLIVAN: I think I would agree with both of you. Certainly, I think sort of the occupational and the environmental public health perspective is extremely important, in this regard, in the consideration for follow up monitoring. And consideration of what actually happens in the field, and to people, is important.

I wanted to just respond to a little bit of what you said about missing things. I think we are missing things already, whether it's because we don't have a specific model, that we're using to test for it, or whether we didn't

have time to assess every single chemical, in every mixture, for every endpoint. I certainly do not want to miss things with an in vitro approach; but we need to recognize that we're already missing things, or might be, and probably are with the in vivo paradigm.

I think what Stephen said about using the in vivo data is right to the extent that it's useful, it's a weight of evidence approach, right?

And finally, to come back, I just wanted to point out, again, this idea of criteria for assessing the liability and relevance of methods. Lots of thought has gone into this. This case used a set of criteria that were in OPPTS strategic plan for implementing new methods. So, I think taking another look at that, and seeing if that seems appropriate and relevant, is a good idea.

DR. RAYMOND YANG: Let me start out by saying when the chair opens this for general discussion, I will talk more -- isn't this about validation? Okay. But I can't help to jump in right now to echo some of the comments

earlier defending animal toxicity testing, or the utility of that.

As I've said over and over again, and Anna also put it very elegantly, Tuesday, any system has flaw and limitations and so on. Therefore, animal toxicity testing, likewise, has a limitation. But to consider that as failed, I just can't accept it. Because I have more grey hair and am older than you, I could philosophize, okay?

That original toxicity testing program from NCI, is grown out of the chemo therapeutic program, and has saved a lot of lives. Because a lot of the cancer patients go to NCI hospital as a last resort. There's no other way they want to use experimental drug, to hopefully have a miracle bullet. And those drugs don't go through today's drug pharma developmental process. They do quick and dirty studies in animals and it goes into patients. And if you don't know what you're doing, you kill people. You save a lot of lives.

And also, the present day PBPK modeling was grown out of that project, because

toxicity differences and so on. Two chemical engineers, Bob Dedrick and Kim Bischoff, developed PBPK modeling to study pharmacokinetics and so on, differences and so on and, therefore, the advancement to today.

When I was a graduate student doing research and so on, people laughed at people chromatography, because now we got HPLC and GC and so on. But I always remind them paper from chromatography won someone a Nobel Prize.

There's a tendency -- the younger people today want to poopoo the older testing methods. Your methodologies may not necessarily be better.

DR. ROBERT CHAPIN: So, we want to focus this on the recommendations for the agency, okay?

DR. RAYMOND YANG: No. I just want to jump in and make this clear. There are utilities, and otherwise, IRIS wouldn't exist. Maybe some of these negative feelings influence the (inaudible) to kill the IRIS program.

DR. LISA SWEENEY: A little bit more on validation versus other terms described.

I tend not to use the validation terms, and I think more in terms of things that build confidence in weight of evidence.

For example, in an IRIS-derived value, you'll have a description of high confidence, medium confidence, low confidence. Perhaps something like that could be at least crudely applied to in vitro systems. When I see the way things are going in terms of things like systematic review and study quality, and those sorts of evaluations, they are doing that for in vivo studies, and epi studies, and stuff like that.

They're having a little more trouble figuring out how to apply that to in vitro and mechanistic studies. So, I see kind of a synergy between the concerns here, for developing NAMs and the same sorts of data that other EPA programs are dealing with; in terms of how you understand what makes a good study; and that that helps sort of drive the people that do this testing to meet certain standards on how they do things and how they share their data.

So I'm not sure if there are other internal agency lessons learned that can be applied to understanding how good the components of the NAM methodology are and bring that forward into either the current risk assessment or future risk assessments, which obviously this is evolving. It's definitely not a set procedure.

DR. JON HOTCHKISS: I wouldn't suggest throwing away all the in vivo data. Because where that really comes in handy is in building your cheminformatics database. What is really needed, is a broad representation of both animal and human exposures, through various roots of exposure. Apparently, the most important thing that we see, with the model that is being developed in our lab, is that what's critically important is not just to know these things are toxic so your structural alerts pop up; but what really makes the cheminformatics assessment powerful, is when you can see what doesn't trigger that response.

So, you have to have both positives and negatives in order to make a deterministic decision on what the potential

activity of the material, and what the mode of action is. Otherwise, if all you had are negative things, your world view is really skewed. So these systems would just pick up structural alerts and have nothing to compare it to. So you tend to get pretty poor data.

I know this wasn't addressed in this submission, but that initial cheminformatic approach to identify potential toxicities and mode of action, I think, is important in a development of these in vitro systems.

What's important, also, is to understand the absorption in metabolism and potential systemic exposure through different routes of exposure. So, we happen to use one program, but there are many expert learning systems out there that can predict what the blood levels are going to be, both after an acute, and then with repeat exposure.

That'll sort of help guide whether or not it's going to be important to -- what tissue you're going to look at, and whether there's going to be a real impact in terms of repeated exposures. So if you have something

that goes in, gets metabolized, then you start at zero again the next day, an acute exposure is probably as good as anything.

I know it doesn't align directly with this in vitro model, but I think it's a critical component, and like an integrated approach to moving away from animal exposures.

DR. EMILY REINKE: I feel like I should probably clarify something. And, Jon, you make some very good points. When I'm thinking of validation, I'm thinking of the definition of validation as it stands internationally right now, which is a very baggage-filled definition.

I do not disagree with validation, and I'm not saying don't use the animal data. What I'm saying, is that we are cautious about using the animal data as our standard by which to compare a new methodology. The animal data has informed a very large portion of our mechanistic understanding of pretty much everything. So, without that animal data, we wouldn't be where we are.

So, we need to use the animal data. We need to use the animal data in a weight

of evidence approach. My caution is in using the animal data as the standard by which we judge a new methodology, that is not animal based. I think that's really what I was trying to say.

DR. ROBERT CHAPIN: George, your placard was up for a while. Do you want to make a comment?

DR. GEORGE CORCORAN: I was searching for a slide that I once saw presented by Thomas Hartung, who's well-known to many of you in this room. It was stunningly simple. It was three domains: human, animal, and in vitro. And he showed the concordance between any pair of those circles, and it was never above 0.6.

So we are, in some ways, attaching our future to high-quality in vitro systems, based on human tissues; and it is totally logical, and I think the correct thing to do today. What I think Thomas might do -- and I don't want to put spots on his figure. But what I would now add as a fourth domain, is computation and artificial intelligence. I know he of the strong belief that computation and artificial intelligence is already outperforming

in vivo animal studies. And will soon outperform virtually all sources of data verification.

I take, Lisa, your point on validation. But validation doesn't necessarily mean only pre-existing in vivo studies. It is the weight of evidence concept that somebody mentioned earlier -- I gathered you were driving at it, and I wholeheartedly endorse that.

But, thinking back to what my friend Thomas Hartung taught me, in that one lecture, is not overprescribing the importance of any one of those circles, and embracing all four of them now. I guess I would leave it there.

DR. STEPHEN GRANT: I'm still trying to decide what to say. I have worked in computational toxicology, in predicting cancer for the most part. And one of the issues that I have, is that prediction is never good as measurement. I'm a big advocate of functional tests.

As a geneticist, I'm often brought data, microarray data, stiff data, and asked to predict the phenotype of a cell, or a person, or a thing like that. And I try not to do it

because you're far more likely to be wrong. Because for whatever amount you know, you know there's more that you don't know.

I'm choosing amongst stories to tell. I'm from Florida, and last year we had a hurricane. And they have AI created spaghetti models of where the hurricane's going to go. I live in Ft. Lauderdale, so when the hurricane was first coming, it was coming up my coast, so we got all worried. And then there was a model that said, oh, it's going up the other coast. Oh, we're okay. But, let's go up to Orlando just to be sure. It ran over Orlando. Okay?

The meteorologist would say the variability in those models was ridiculously small. All of them were right. Except the difference is being hit by the hurricane or being missed by the hurricane. We need to acknowledge that that difference is significant.

DR. JAMES BLANDO: I also just want to add perspective of a user of a risk assessment as opposed to a performer of a risk assessment. I would just add -- I guess maybe it's really self-evident, but I would just add

that whether you use in vivo or in vitro animal testing, whatever is done for the risk assessment, the user of a risk assessment, someone like me, it still is always important to have a clear understanding of what the assumptions are in any risk assessment.

One of the first things we oftentimes do is, you know, you assume, okay, I have a 35 micrometer MMAD, because the nozzle is operated this way. Then you go out in the fields and you find the guys have 1000 PSI on their nozzle, and they're generating droplets of completely different particle size distribution. So, whatever decisions are done, it still -- the obvious fact, that everybody knows, is those assumptions for the users of a risk assessment are really crucial for us to continue to easily digest and discern; even if we are not biologists or biology types that can understand this.

Because for us, it's in the application of what does this mean when I go out in the field and I see people that are exposed to these particular chemicals? And that's not going

to change whether you're using in vitro or in vivo to understand those assumptions.

DR. ROBERT CHAPIN: I'm seeing no other name placards up. I think I'll take this moment to weigh in on something that I heard in your number two, Jim. This is Bob Chapin.

There was some comment about the measures of the LDH, resazurin and TEER were not tightly linked to cell death. My understanding of the literature is significantly different. And I was under the impression that there's a significant correlation of those things, the cell death. And maybe the take home message for the agency would be that they want to clearly state, or clearly refer to, the literature that supports the use of the endpoint that they've chosen, as a good reporter for the effect they're trying to find.

So they just want to support and defend, if you will; or reference the literature that supports that these are the appropriate endpoints to choose for what they're trying to refine. We can go over the wording later on.

DR. KATHRYN PAGE: Just a point in clarification; that I think that where we're intending to go with Jim's original response was more of if this has been what was happening in this model. And this is a good reflect of what's happening in the 3D model. And that variation, in that, has been assessed and addressed.

Syngenta presented some slides looking -- or somebody presented some slides showing that TEER correlates nicely with the effect. I think that the point was just to -- and we've actually addressed this as one of the earlier questions, too. But just making sure that the other endpoints that we're going to look at, for this type of assay, has also been assessed in this way. Just as part of the validation, and a (inaudible) validation approach.

DR. ROBERT CHAPIN: All right. So we've got Ray. I'll come back to you guys. I'm looking around. This is Bob Chapin. I'm looking around the committee one more time to make sure that -- Dr. Yang.

DR. RAYMOND YANG: I'm sorry. A question. Have we actually gone through the whole committee discussion of this particular question? Or we have just finished the associate folks in the group?

DR. ROBERT CHAPIN: We can formally open it for collective committee discussion if we need to do that. I was sort of thinking that everybody was kind of piling in. I was kind of thinking that we were done with that. But if there's more to say, please enlighten us.

DR. RAYMOND YANG: I'm going strong.

DR. ROBERT CHAPIN: Let me get some coffee.

DR. RAYMOND YANG: I promise I won't take too much of your time. I need to bring up my writeup. Let me explain first. Originally, this particular writeup was in question 3 as a sort of big picture discussion. But as time goes, I feel more and more its right place is in question 5. So this afternoon, just before reconvening, I gave James my writeup on

this because I don't want to give EPA's internet too much trouble. So, he just distributed this.

This is the writeup on the discussion I made first thing Tuesday morning. I was the first one to raise issue after Monique's presentation. And in it, I did some recommendation that for a new approach like this, the most critical thing is validation, validation, validation. And I put them in quotation marks. I hoped putting them in quotation marks will make Emily feel a little better.

I'm thinking in the discussion we just had, multiple people used the term of validation. So, we all understand what this word is. We're dealing with semantics. So, I don't have any problem. If validation is too offensive to some of you, we can use reliability index, or quality index, or something like that. I think the EPA and Syngenta has to go through this process, because eventually they're going to use this for regulatory purposes and so on.

Therefore, I have some new stuff. What I said on Tuesday morning, it's in the

record. I want to put the rest of them in the record. And it's just a paragraph. I'll read it to you. This some questions raised.

DR. ROBERT CHAPIN: Make sure you're speaking -- when you get over there, you're not speaking into the microphone. Thank you.

DR. RAYMOND YANG: Yes, sir. So for the present proposed NAM, N-A-M, approach, what is validation? What comprises an appropriate validation of any approach? How many chemicals is enough to show that it works? What are we validating against? These are some of the questions in our group, question 3 group, raised. I'm going to give you my initial thought on this. After this, you can jump on me. We'll have argument or debate and so on.

At the outset, it is important to set the boundary and state the assumptions/understanding in this validation process. The boundary, or what is validation, and what are we validating against, is the final comparison of risk assessment values between the proposed NAM and those from IRIS database on the

set of chemicals preferable respiratory irritant. That's what I propose. In that sense, whether the IRIS values were derived from human epidemiology studies or animal studies are inconsequential.

If the magnitude of differences between the two approaches is consistently and relatively small, let's say within a factor of two to five -- now, this is to be determined by scientific community -- then the NAM may be considered an adequate replacement of the conventional approach.

Of course, in the present case, the goal was to replace an inhalation sub-chronic study. Thus, the final risk assessment values would be for sub-chronic toxicities. In other cases, comparisons might be made by using NAM sub-chronic toxicity value, i.e. RfC, coupled with uncertainty factors to estimate values for chronic toxicity or even carcinogenicity for comparison. Much the same way as EPA has a chemical with very little information, but they have to do risk assessment.

As to how many chemicals in such a testing set are to be considered adequate? Of course, the more chemicals undergoing such a validation process the better. However, the Charles River's -- I think it's Dr. Roper's presentation -- test set of 15 chemicals presented that the meeting could very well serve as a starting point. As time goes, similar information will become available for more and more chemicals. This is precisely the essence of Bayesian approach.

For the validation process to work, the following assumption/understanding must be clear.

Number one: we understand that no approach for human risk assessment is perfect; and therefore, there are limitations in any of the available approaches. For instance, many consider human epidemiological study results are the ultimate answers, but there are genetic polymorphisms issues.

In the case of dichloromethane, that is methylene chloride, if we use lung adenoma and carcinoma as an endpoint, a key

enzyme, Glutathione S-Transferase Theta 1, is absent in about 70 percent of the Asian population. In such a population, one would expect to see a bimodal risk distribution with a large portion of the population at the zero-risk level. This is published by El Masley (phonetic) et al, 1999.

Further, Sweeney, et al. -- in case you're wondering, this is our Sweeney. These are Sweeney 2004, reported evidence of bimodal distribution and transformation enzyme for dichloromethane, cytochrome P450 2E1 in humans.

Two: we assume that IRIS risk assessment is the gold standard of the world or hope the best we've got. Even though there are scientific critiques toward the accuracy and reliability of such a gold standard. This is what we are validating against.

Three: our goal is to develop in vitro and in silico systems, which could help EPA do risk assessment much more quickly and efficiently. If it works, who cares if it is not a perfect and it is not human? After all, we

just discussed above that we are all different. Then I say, in the modeling world, George Box talks about all models are wrong, some are useful.

Also, I used the example this morning of four compartment PBPK model for human. If we can accept derivation of internal doses from that for risk assessment purpose, even cancer a risk assessment, why can't we accept something less than perfect? Along that line, I want to say, do we understand everything about cancer? Far from it. Yet, we're doing cancer risk assessment.

So I conclude by saying, if it works, whatever. Even a crystal ball. Other than intellectual curiosity, do we need to know every step of the way how it works? When you do your word processing, you don't know every line of code behind those. You use it. It's a tool. Okay. Thank you.

DR. ROBERT CHAPIN: Thank you, Dr. Yang. Next up is Emily.

DR. EMILY REINKE: This is Emily Reinke. I feel like I need to defend myself a

little bit here. I don't hate validation. Validation is extremely important. I think what we need to do is we need to figure out what validation -- and Ray did say this in some points. We need to figure out what we're validating. And having the specific -- you know, specificity, sensitivity and variability. So, we have to really rethink how validation is occurring, and what we mean by validation. That's what I'm trying to say.

The paradigm around the word validation right now is very different then, I think, what we want to try and do. I would also caution against saying that human epi studies are the end all be all because they are extremely messy. There are lots of confounders, and you usually don't have exact exposure data except for in mass exposure events. And how many of those do we actually have in human history? We are trying to be health protective, but I would caution against using epi data.

DR. KATHRYN PAGE: I just want to reiterate a statement I made earlier. We know with our case study that the values that we're

getting from the HEC, when you compare in vitro and in vivo, are vastly different. But I would argue that doesn't necessarily mean this approach is wrong. You know, the in vitro approach is wrong because, you know, it is providing a value that is very different.

It could even suggest that our gold standards, the animal tests, are necessarily overprotective. And an important point to consider -- again, I made this earlier -- is that if we are confident that these data support a more realistic approach, the in vitro data, whilst also protecting the population, then we may want to assume that the animal model is no longer relevant. That doesn't mean get rid of the data. We're using the existing data in both humans and animals, as well as your MOE, to establish your confidence in the new approach.

We may find that the animal model isn't thought of as relevant when we're looking at direct-acting irritant, which is what we're specifically talking about today. And this type of alternative maybe shouldn't be suggested to be used to avoid animal testing but encourages the

right approach to take because it is more realistic and more humanistic.

DR. ROBERT CHAPIN: Dr. Lowit, would you contribute to the conversation please?

DR. ANNA LOWIT: Thank you for recognizing me, Dr. Chapin, and Kristie for helping. I want to pick up on something Emily said two or three, maybe four, times in the last couple of days. And just maybe give a little bit of context and try to channel my good friend and colleague, Warren Casey, who wishes he was here; because I've been getting texts from him all day wanting to know what's going on.

If you don't know Warren, he's the Director of the National Center -- the NTP Center for Alternative Test Methods. And Warren is really one of the world's leading authorities on how to determine whether or not an assay is fit for purpose, and the confidence building exercises to make them ready for regulatory use. I think what you all are calling validation.

In the international context, the word validation comes with it a lot of baggage. What we mean by that is, at the OECD level,

there's a guideline called GD 34, that has historically defined what the word validation means, in terms of the alternative test methods space for what we call the VAMS, ICCVAM, ECVAM, KoCVAM, JaCVAM, and then their Canadian equivalent.

Organizations that conduct three-ring trials, around the world, and have validation management groups. And these activities have led to the existing OECD guidelines. Quite honestly, to do a validation, according to OECD GD34, takes years and millions of dollars. And what we're actually finding is that those actually don't led to fit for purpose assays that can actually be used by regulatory agencies. We continue to have to work with them to establish their fit for purpose.

At the ICCVAM level, over the last year or so, Warren has really spearheaded this idea that we move away from OECD GD34, and create a new paradigm for evaluating fit for purpose and making assays -- what he calls building confidence. So, the activities that go around building confidence.

If Warren was here, the first thing he would say is that words matter. If you've ever heard Warren give a presentation on this, he always starts with, "word matter." In this case, the word validation, in the context of alternative test methods, has a very distinct meaning.

So, every time that it will appear in the report, under the word "validation" there will be people around the world who read that as, the MucilAir system can't be used until it has gone through a GD34 three-ring trial that takes who knows how long and how many millions of dollars.

I don't think that's what you all are meaning by the word validation. I think when you all are using the word "validation," I've actually started making nots, and I think I found like five different meanings. Everything from optimization, to confidence building, and sort of some things in between there. Verification, I think, in some cases.

I would beg you, for lack of a better term, to be very careful of this word

"validation" because I don't think that's what you mean. I think you are meaning something is valid for use, or it's fit for purpose, or we are confident that it's useful in this purpose. Because every time you write the word "validation" into the report, you put us deeper into a hole of when we can use that, because of this international connotation.

As we think about the comments, and your written comments, and what goes into the report, words matter. And I would beg you to choose them wisely.

DR. EMILY REINKE: Thank you, Dr. Lowit, for filling in some of the things I was having a hard time saying. This is why I have been saying validation. So, I concur with what you say.

DR. ROBERT CHAPIN: Let me just get a clarification from Dr. Yang. When you gave your hit one, hit two, hit three on validation, did you mean certification that the test is fit for purpose and sort of a confidence building exercise that it was really reporting what we thought it was?

DR. RAYMOND YANG: Exactly. I don't care about using the word validation. I think given what Anna was saying, it's well taken. We don't want you to get into trouble. I think as long as all of the scientist are here --

DR. ROBERT CHAPIN: Could you define, for us, what you meant by using other words? Sort of crystalize what that meaning really is, and then we'll stop for validation?

DR. RAYMOND YANG: You have a new approach, which hopefully will replace an old approach. But the final decision point is whether or not human risk assessment would work in both cases. What I mean by validation, is that this new process will have evidence presented to the scientific community that it works just as well, or very close to it, as the old approach.

DR. ROBERT CHAPIN: Excellent. Thank you. I think Kristie's up next. While Kristie is gathering her thoughts, let me just confirm that nobody around the table is invoking a series of ring trials when we use the word validation. Is that right?

DR. JAMES BLANDO: I'm glad you pointed that out. I had no idea about the baggage behind it. When I think of validation, I think of like NIOSH sampling methods, and that's the way they use those terms. So I had no idea it had that connotation.

For me, what validation mean, or what I mean to communicate when I say the word validation, is I can be confident that when I go out in the field, and guys and gals are using this product, that I can use the risk assessment as a tool to help me make a recommendation I can feel comfortable with.

DR. STEPHEN GRANT: I would simply say, since we want to take away the baggage of validation, that we need another word; but those are the processes that precede application. And in the old days, the validation was considered to be definitive, and then you applied. But nowadays we know that it's a loop, and you feedback, and you go back to it.

But we need something that says, what are the criteria that now say this is ready for application? Perhaps on a speculative basis,

but it's gone through some preliminary tests, and screenings, so that it's now ready for field testing, whatever that means.

DR. KRISTIE SULLIVAN: Okay. I think that -- jumping off of what Steve just said -- that building confidence is a process. It is not all or nothing. It's not yesterday we didn't have, and now today we do.

I think this is part of that process. I think that the way that the agency has approached the use of a case study is very well thought out, in terms of this is going to be the way that we're going to build confidence. It's going to be seen how NAMs can be applied in certain cases and seeing where else that those methods apply. And continue to build that confidence.

The case study approach is showing to be very powerful, internationally, in terms of building harmonization and confidence in how new approaches can be applied. So, I think that a big part of this process is going to be case studies. I would just want to really emphasis that, which I have.

I also think we want to look at the context of use of the method. I don't agree that IRIS risk assessments are the gold standard for this application. We're talking about a very specific case. Maybe we're also talking about expanding into other similar chemicals with similar modes of action. But I just don't want to transmit the recommendation that IRIS risk assessments are the comparator for all in vitro or in silico approaches.

Because Emily told me to, I will say, again, that there are criteria that EPA has outlined in some of its guidance, related to the strategic plan under the new TSCA that are, I think, very relevant here.

DR. ROBERT CHAPIN: Thank you very much. Steve, you were up next if you still want to say. And then after Steve was Marie.

DR. MARIE FORTIN: I have two points. One is more of a process. I understand your concern with respect to using that word, and then being tied to that OECD validation process. That's very cumbersome. However, I also understand Jim's point of what validation means.

When I think about method of validation and HPLC validation, it's all sort of things. And we're talking about method, and it needs to be validated. I don't think there's another word, in the English language, that allows to communicate that idea.

But I would like to propose something in order to be able to write what we're trying to say. And if we're going to put that in our report, we need to have consensus on that. What if we said, in our introduction or something like that, that when we employ that word, we are not making the assumption or requiring you to work under that guidance. What if that was there?

DR. ROBERT CHAPIN: Okay. So, we're not asking questions of EPA. At this point, we're making recommendations. Then, you can ask the panel.

DR. MARIE FORTIN: So, I'll ask the panel. Are we all comfortable with saying that we are not tying EPA to validating under the OECD process?

DR. ROBERT CHAPIN: I think that makes a lot of sense. And we'll just work in a working definition of validation in the introduction or someplace in the report.

DR. MARIE FORTIN: I just want to be pragmatic about this thing.

DR. KATHRYN PAGE: I think that's a great idea, Marie. I would, however, say that if we are going to use that word, validation, that we need to define it, or use a different word. Like saying confidence, qualification, optimize.

DR. RAYMOND YANG: Jim, what I will do is I will totally avoid using the term validation. Because I totally appreciate what Anna was saying. There are paranoid scientists out there. They get a fit when they see a word like that and automatically channel their fury toward EPA. And I don't want you to get them in trouble.

I will use something like confidence or reliability or whatever. Whatever you guys want to do, if you want to use

validation, and you want to define it, fine with me.

DR. LISA SWEENEY: Getting back to the point of whether the new approaches would be quote/unquote, "as good as" or "better" than previous approaches is pretty hard to quantify how good any safety or risk assessment process is. It's not quite like testing widgets. It's not even like -- with, for example, an FDA drug approval, you can say if you have too many adverse reactions, then, gee, maybe their process didn't work well. Because they have reporting systems and things like that.

I think with safety, especially something like environmental risk assessment is even harder to identify what the effects are. Maybe occupational. You have your OSHA reporting and things like that. But for an environmental general-population human health risk assessment, it's going to be pretty hard to say how good our current system works. We like to think that we're out there protecting public health, but it's really pretty hard to quantify.

I think that the idea of the statement that we're building confidence by serving on this panel, and giving our input, and doing the best to help them make this new process as good as it can be, I think we are helping to build confidence. But I'm not sure that we can really come up with metrics that are going to allow us to compare, before and after, which risk assessment processes were better or equally good.

DR. ROBERT CHAPIN: Kristie.

DR. KRISTIE SULLIVAN: I just wanted to make a suggestion to use the term reliability and relevance to refer to the validation process.

DR. CLIFFORD WEISEL: I worked with Amalah (phonetic) who said he never validated anything, he always evaluated it. And maybe that term might be -- I don't like the word optimization because optimization means something very different than this. I also want to agree with Lisa that we don't know, in the environmental system, whether we've done things to be helpful or not. Usually, a lot of the time we find out, if it's not, it's very unfortunate.

That said, I still think epidemiological studies and case studies help us understand it, so I don't want to put them off to the side. All you can do is do the best you can.

The one thing that you should be doing is, after you put this data in -- and this is often not done -- is evaluate and go look at the communities and see whether you really are protective.

It's often not done the way it should be. You put in your risk assessment, you do your risk management and then you walk away. Really, after risk management, you should have a new risk assessment in the field.

DR. MARIE FORTIN: This goes a lot different than the validation discussion, and says a point that I wanted to make, because I don't want it to be forgotten. Considering that irritation is really the effect that's addressed, and if we protect for irritation, we're protecting for the other more severe effects. Considering that irritation is an indigent effect, I think that bridging to human, from in

vitro, is actually very realistic, unlike many other endpoints.

That can be done by a variety of approaches. You can have agricultural workers, and you have personal samplers, you know, questionnaires. You can have human studies with volunteers, or you can use epidemiological data.

But I think that it's important, before putting this forward, that we understand the relationship, and the quantitative relationship of that value that we derive to the human health effect of interest. I wanted to make that very clear.

The other point, going back to that discussion with the term that we're kind of being asked not to use, I do risk assessment. And one of the things we look at is study reliability. One of the things you use for that, is look at -- well, did they use a validated -- I might say. That's one of the things we look at.

If we open the door to that, I have concern that -- I think it needs to undergo validation because that is the term that's used with respect to how you make sure that your

method is protective for what you're trying to measure. I'm not saying it needs to undergo that specific process, but I don't know that there are other words to convey that idea.

DR. ROBERT CHAPIN: Let me just clarify whether or not when you use the word validation, do you invoke the OECD ring trial stuff?

DR. MARIE FORTIN: No, I don't.

DR. ROBERT CHAPIN: Thank you. George.

DR. GEORGE CORCORAN: I'm trying to simplify everything I've heard over the last several hours, particularly around validation. I, as a simple thinking man, would be very satisfied if the agency would consider a standard for a NAM, as simply certifying that informs the hazard identification and risk assessment performed by the agency.

It informs you. It doesn't have to be better, worse, bigger, smaller, cheaper, faster, but it informs the process. And if it meets that standard, by my way of thinking, it would advance admission of EPA.

DR. STEPHEN GRANT: Thank you, George. So, validation means assertion of the truth. Veritas is the truth, verification means the same thing. And if it doesn't have the baggage of, well, what are you verifying -- because, again, validation is a comparison to previously existing -- we may have to define something else. And it may be we have to do this because in vitro tests are not new in vivo tests. It seems silly. We've been sitting here -- most scientists aren't also humanities majors, so maybe we need to get a different panel to figure out what's going on here.

But the bottom line is, whatever it is, we want it done. And EPA is in a unique position to say, in vitro test, or a test that is fundamentally different from the existing gold standards, have to have these criteria before we consider applying them.

And that application is, by definition, an evaluation. The only reason I don't like evaluation, is it's a process. It's not an endpoint. Evaluation is ongoing and cyclical. Every time you have a piece of new

data, you reevaluate the whole, or at least I hope so.

DR. ROBERT MITKUS: I just wanted to revisit what Dr. Fortin said and what Dr. Corcoran said. I think they both made some really good points. If you put yourself in the perspective of an agency reviewers -- feel free to pipe in if you'd like to, since you do it more actively now. But they do look for guideline studies and base decision making -- they put more weight on guideline studies. If there is no guideline, then that's raised a question.

At the same time, for things like cancer mode of action studies, in vitro studies are submitted for those, to inform the hazard ID and to inform the mode of action without guideline studies. So as long as they're conducted scientifically reasonably well, then they can be used.

So I'm just wondering if now is a good time to broach the subject of a tiered approach that Dr. Lowit had brought up earlier. When she communicated to us that -- for this portion of the discussion that it seems to me

that she's looking for recommendations for a tiered approach; specifically, for this in vitro method, as applied to chlorothalonil. I'm just wondering if now is a good time to discuss that.

DR. ROBERT CHAPIN: I have no earthly idea.

DR. ROBERT MITKUS: Sorry. I didn't mean to put you on the spot. It seemed to me that a lot of the discussion that's taken place all day today, there seems to be a consensus that the current 24-hour exposure of the in vitro model should not be used for repeat dose risk assessment. I could be mishearing that, but that's kind of what I'm hearing.

So if that's the case, then it seems to me that the 24-hour exposure of the in vitro model wouldn't be particularly relevant, to the data call in, for a repeat dose inhalation study. If that's the case, then the question is what is the model and the results conducted with chlorothalonil good for?

Personally, I think, with some tweaking, it is relevant for an acute exposure scenario, an acute risk assessment. I guess I

would offer that as maybe a thought starter, to launch into maybe what are some tiers that this data can be used for. Jon had mentioned earlier cheminformatics as an early step, QSAR, the in silico approaches. Here we have an in vitro approach, with data, and then we have an in vivo exhaust.

I wonder how others feel, or what they're thinking about. How this particular assay, and the results that we have with chlorothalonil, could fit into a tiered approach?

DR. ROBERT CHAPIN: I noticed that Dr. Page stuck up her placard very shortly after Rob started speaking. So, let me go ahead and see if she's got something to contribute to this.

DR. KATHRYN PAGE: One of the points that was just brought up about definitely the repeat dose in vitro study is needed, I don't necessarily think -- that wasn't the way that I wanted my opinion to be perceived. It was more of that hasn't been evaluated to be required or not. And there's a couple of other points that also haven't been evaluated to have an effect or not.

To me, that evaluation step, plus a consensus, goes to that confidence; and goes to that confidence or valuation, however you want to say it.

It's the addition of the extra evidence that is required. Not saying the approach that's been done is wrong; I'm just saying that I think we need a little bit more evidence to show that the approach is right.

DR. LISA SWEENEY: I agree with Kathryn that I also said that I thought that a repeat study in vitro would be better. But I definitely do not want that to be construed as to say that it's necessary.

As a person who does risk assessment, you do the best you can with what you have. And depending on how good you think what you have is, that effects the MOE that you're comfortable with, or the uncertainty factors that you apply.

Syngenta indicated that they did have some data on recovery that -- I believe they said it was incomplete, the 24-hour. Well, depending on how far it is from complete recovery

of these cells, that might affect what sort of a MOE you're looking for, or the uncertainty factor that you're going to apply, still using that you already have, the single dosing data.

DR. RAYMOND YANG: I just thought about something I want to recommend to Monique, since you are the lead scientist on this initiative. That is, you have very good resource, that's Rusty Thomas, and his National Center for Computation of Toxicology.

I would strongly urge you to sit down with him, and some key people, to talk about this whole thing. Because I understand he is looking into a lot of these issues that we talked about; about the reliability of the animal toxicity testing, the IRIS analysis and so on. I think you would probably gain a lot of insight if you work with him. Thank you.

DR. KRISTIE SULLIVAN: I wanted to jump off of what Kathryn and Lisa said. What I heard was that the consensus was that an advantage of this method is that it could be used for repeat dosing and that we thought some work should be done to see whether repeat dosing of

the cells, or a single dosing recovery period, had an impact on the risk assessment. Not that going forward in the future you would always need a repeat dosing in vitro study, necessarily.

DR. ROBERT MITKUS: I would like to force the issue if I could, or revisit this tiered approach. As I understand it, we've already been through a few tiers. We have a lot of in vivo inhalation tox data. Those studies are not showing a NOAEC. It seems to me that if the traditional agency uncertainty factors are applied using those studies, the risk assessments fail.

And if I understand it correctly, from Dr. Wolf, the PPE that would be required to make those risk assessments pass is just completely prohibitive from a business perspective. The workers, they're not going to purchase the compound and the formulations if a requirement for wearing tie-back suits in 110-degree heat goes along with it.

So, we've worked through that tier. So, the next tier is to try to refine the risk assessment by looking at this in vitro

model. So the question -- I think that rather than kicking it to Rusty Thomas, I think the panel has been tasked with providing a recommendation as to what the next steps should be for the risk assessment.

DR. ROBERT CHAPIN: That's not my interpretation. My interpretation is we're supposed to answer these questions. And then my proposal would be, after we hear from Dr. Lowit, and if she agrees, and we all feel like that would be a useful thing to do and the EPA feels like that, then we could tackle that. But I think our charge was pretty well laid out here. I just sort of did a deer in the headlights thing there for a minute. Sorry about that.

DR. ROBERT MITKUS: Thanks, Bob. Thanks for the clarification.

DR. ROBERT CHAPIN: Dr. Lowit, would you please contribute to the discussion?

DR. ANNA LOWIT: I'm actually glad you just said that because when Rob spoke a couple minutes ago, before a few others, he said something about what I had said, and I wanted to make sure it was clarified.

In our mind, Charge Question 5 has been answered, and we don't need any additional information. We've gotten a lot of great information; not only specific to chlorothalonil, but other things around computation, and bioinformatics, and other things that we can take and look at the big picture. So we're not asking for more than has already been provided.

DR. ROBERT CHAPIN: That's very helpful. Thank you. Jim, your name was up. Did you want to say anything?

DR. JAMES BLANDO: I don't know if this is relevant or not, and I think this is sort of implied. But irrespective of whatever policy decision or whatever decision is made as a result of the input, I would just say that I still think that, even if the decision is made that the in vitro studies are the way that people want to go, that EPA still should have the ability to reserve the right; that if their scientists decide that they want something different or more, they should still have the ability to request it.

DR. MARIE FORTIN: I would like to ask the panel, and my colleagues, if they have a suggestion for another word?

DR. ROBERT CHAPIN: I think my approach would be, let's do that offline. Because that will at least allow me to go consult more learned resources than what I carry around with me. And I'm thinking that there will be more value in doing that offline.

DR. MARIE FORTIN: Okay. So what I would like to state, on the record, is that if there's another word that conveys that idea that I want to convey, that's proposed, I would be happy to use that word.

DR. ROBERT CHAPIN: Thank you. So I'm not seeing any more nametags that are up. We just heard from Dr. Lowit that -- I think the polite equivalent of, "that's enough, that's enough." Let me just confirm with Dr. Blando that you've got more than enough stuff here to take and refold into the soufflé that you're folding in for question 5.

I think we're done discussing these charge questions. We're not done with our

work. Certainly, I'm done for today, and I know that everybody else who's actually been doing the heavy lifting here, you are, too.

Before I congratulate you, let me turn to Shaunta and find out what the status is. My understanding was everybody's going to be here tonight, not here in this room, but here in town tonight. We have some additional things on the agenda to address. So let me, I guess, give this over to you.

DR. SHAUNTA HILL-HAMMOND: Thank you. We have now reached the point in our agenda where we will address clarifying public comments. We did receive one public question by email that was sent directly to the FIFRA SAP staff. That question has been shared with the panel, as well as the appropriate EPA staff representatives and will be loaded to the docket for the public record. I will read the question, and we will look to members of the EPA to address the question. The question reads, "Why is dosing done in milligrams per liter? How is it converted back to concentration in the air particles or droplets?" Thank you. EPA?

DR. MONIQUE PERRON: This is Monique Perron. The answer to this question can be found on Page 21 of the issue paper, where the dose in milligrams per liter was converted to milligrams per centimeter squared, using the internal diameter of the MucilAir insert, as well as the volume that was applied.

DR. SHANTA HILL-HAMMOND: Thank you. At this time, our chair is now available to provide a recap of the discussions that we had today, and then we will talk about what happens later.

DR. ROBERT CHAPIN: This was the okay, dog, in front of me, go over that way. All right. I think congratulations and thank you. We covered a tremendous amount of ground today; and I think we did so with significant productivity. We stayed, bless you, focused on the questions that were asked of us, by and large. It's my interpretation that the lead discussants have an awful long way towards having a final written summary of the comments and responses from each of the associate discussants. For that, you should all feel justly proud.

Let me see. This is only my second time around this track, so I'm looking significantly at Shaunta. I'm going to sort of take small steps and you can jerk on the leash when I get it wrong, okay?

My understanding is that what we'll do is we won't go home tonight, which is to say our travel reservations are in the process of being -- we will not meet as a committee tomorrow.

My intent would be to give us tonight as a time when the leads can get in touch with the associates, and anybody else on the committee, and go back and forth and do clarification things. And I'll go home and consult -- I'll go back to my room and consult various dictionaries about alternatives and propose things for the V word that must not be said.

Let me see. We'll still have tonight to beaver away on this. But then, basically, as soon as the sun comes up tomorrow, it's my intent that we could start to wing our way home. How does that strike you? Is that a

doable thing? That's fine by you. I love it. Okay. Final thank yous. Steve?

DR. STEPHEN GRANT: What is the final thing, what I've distributed and signed off?

DR. ROBERT CHAPIN: It goes to her.

DR. STEPHEN GRANT: She's saying it goes to you.

DR. ROBERT CHAPIN: That's right. She points at me and I point at her. It goes primarily to her with a copy to me. And then what will happen is the SAP staff will -- if you can turn your thing. Thank you.

DR. STEPHEN GRANT: I don't know that I have your email.

DR. ROBERT CHAPIN: I can change that.

DR. SHAUNTA HILL-HAMMOND: I'll just preface this that the final details of the report we will cover in an administrative meeting following the closing of this public meeting.

DR. JON HOTCHKISS: Just a point of clarification. As far as travel arrangement, we'll just stick with what we had?

DR. SHANTA HILL-HAMMOND: Hold that question.

DR. JON HOTCHKISS: Imagine I didn't say it.

DR. ROBERT CHAPIN: I should close this meeting, I assume. Thank you all. You've done a great job. I really appreciate it. Are there any clarifying questions from the EPA that -- do you dare ask a clarifying question?

DR. MONIQUE PERRON: I just want to thank all of you for all of your time. And we really do appreciate all of the back and forth discussions. This public discourse is really important to us in the transparency of our process and making sure that we're utilizing the best available science to make our human health risk assessment decisions.

I would also, once again, just make sure that all opinions are being reflected, even those who are outside of the subgroups. Make sure that all of those opinions are captured

in the report. We really do utilize those reports as a totality, and we want to be able to make sure that we understand where there was consensus and when there was not.

DR. ROBERT CHAPIN: Let me, speaking for the panel, thank the public commenters and particularly Syngenta for doing the heavy initial lift on making this work. Dr. Lowit?

DR. ANNA LOWIT: I was going to reiterate your comment about the public commenters and those on the web who've been listening very intently. And a big shout out to the SAP staff. It's a huge amount of work to put these meetings together, and it doesn't end for them in a couple of hours, putting the report together, helping all of you with your travel. So, we appreciate all of them. Happy travels getting home, all of you.

DR. ROBERT CHAPIN: With that, I'll close the public portion of this -- I'll say thank you. Thank you.

DR. SHAUNTA HILL-HAMMOND: All right, everyone. Once again, my name is Shaunta

Hammond, I'm DFO for this FIFRA SAP meeting. On behalf of the FIFRA SAP staff, I would like to thank the members of the public, as well as the members of this panel, for your participation this week, and your very robust discussions.

As our chair has mentioned, we have completed all of the discussions and deliberations on our charge questions. This will close the public portion of this meeting. I do ask that all panel members join me in the breakout room for an administrative meeting, following the closure of this meeting. With that, we are officially adjourned. Thank you.

[WHEREAS THE MEETING WAS ADJOURNED]