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Welcome and Opening Remarks

Moderator: Sarah Rogstad, PhD Senior Scientific Advisor, OPQR, OPQ, CDER, FDA

Presenters: Darby Kozak, PhD Deputy Director, OGD, CDER, FDA
Michael Kopcha, PhD Director, OPQ, CDER, FDA
Robert Lionberger, PhD Director, ORS, OGD, CDER, FDA

Session 1: Nitrosamine Drug Substance-Related Impurities (NDSRIs)

Co-Moderators:
Sruthi King, PhD Deputy Division Director, DPTR, OSCE, OGD, CDER, FDA
Dongmei Lu, PhD Policy Lead, DRG, OPPQ, OPQ, CDER, FDA

- Public Comment Presentations on NDSRIs
- ***N-Nitrosamine SAR Modeling of Potency – Current Status and Future Needs***
  Kevin P. Cross, PhD VP, Regulatory Science, PI, FDA Research Collaborations, Instem
- ***N-Nitrosamine Drug Impurity Research at FDA/NCTR: Assessing the Mutagenicity of N-Nitrosamines and NDSRIs***
  Xilin Li, PhD Visiting Scientist, DGMT, NCTR, FDA
- ***NDMA and Beyond: A Biased Kinetic Model to Assess Nitrosation Risk in Solid Drug Products***
  Ian W. Ashworth Principal Scientist, Chemical Development, AstraZeneca, UK
- ***CMC Considerations and Bridging Bioequivalence Studies of Reformulated Products Impacted by Nitrosamines***
  Martin Ehlert, PhD Vice-president, API R&D, Apotex Inc.
- ***Physiologically Based Pharmacokinetic Absorption Modeling to Support BCS Based Waiver of In Vivo BE Studies***
  Fang Wu, PhD Senior Pharmacologist, DQMM, ORS, OGD, CDER, FDA
- ***Nitrosamine Impurities: Beyond a Compendial Standard - Learnings from USP's Nitrosamines Exchange Community***
  Naiffer Romero, MSc, MPH Principal Scientist, Scientific Affairs - US Pharmacopeia

Panel Discussion

In addition to moderators and presenters listed above:

Public Panelists:

Tausif Ahmed MS, PhD	VP & Head, Biopharmaceutics & Bioequivalence, GCM, Dr. Reddy's Laboratories Ltd.
Daniel Snider, PhD	Head, Global Quality Systems IT Quality/Technical Quality, Viatris

FDA Panelists:

Robert Dorsam, PhD	Director, DPTR, OSCE, OGD, CDER, FDA
Naomi Kruhlak, PhD	Scientific Lead, DARS, OCP, OTS, CDER, FDA
Bing V. Li, PhD	Associate Director for Science, OB, OGD, CDER, FDA
Bhagwant Rege, PhD	Division Director, DPQA VI, OPQA I, OPQ, CDER, FDA
Diaa Shakleya, PhD	Senior Research Scientist, DPQR, OPQR, OPQ, CDER, FDA
Matthew D. Vera, PhD	Supervisory Chemist, DPQA II, OPQA I, OPQ, CDER, FDA

Sarah Rogstad (FDA): Good morning, everyone, and good afternoon. And good evening to some of you online. I think we're going to get started. It's about almost 3 minutes after the hour. So welcome everyone to the FY 24 generic drug science and research initiatives public workshop. I am Dr. Sarah Rogstad. I'm a senior scientific advisor in the Office of Pharmaceutical Quality Research here at FDA.
And I'm really happy to be moderating this initial session with some high level overview presentations.
So we're going to start out with a joint directors message from both Dr. Darby Kozak, the deputy director of the Office of generic drugs, and Dr. Mike Kopcha, the office director for the Office of Pharmaceutical Quality. I think let's just jump right in because we have a really packed day to get into but I'm really excited to hear about all of these research initiatives for generic drugs.

Darby Kozak (FDA): Thanks, Dr. Rogstad.
So I'm thrilled to welcome everyone to the 12th Annual Generic drug science and research initiatives public workshop.
So we've held this workshop annually since the generic drug user fee amendments in 2012. And it's the cornerstone of our GDUFA science and research program.
The conversations we're going to have here today, as well as the feedback you give us in this workshop shape and direct drug science and research program by helping us to establish the priorities for the coming fiscal year.
GDUFA science and research program is an essential component of FDA's mission to protect and promote public health.
GDUFA science and research ensures that FDA's thinking remains current and the FDA's evidence-based regulatory decision-making is informed by the most up to date scientific and technical insights.
The science and research conducted through the GDUFA research program continually advances FDA's ability to ensure the American public has access to safe, effective and high-quality generic drugs.
So I actually know firsthand just how important this workshop is to FDA's ability to facilitate the development assessment and ultimately the American public's access to high quality drug medications.
Before I became the OGD deputy director early this year I actually worked in OGD's Office of Research and Standards. One of the offices you'll be hearing from today that oversees the GDUFA research program and is helping out in hosting today's event.
I first became aware of the GDUFA Science and research program in 2013, when I participated actually as a public attendant and commentator such as you guys in the audience today. And it was my unique understanding of this unique scientific program, this collaboration with the

American public, as well as that overarching commitment to improve the public health that really drove me to join the FDA. And specifically, OGD, and this GDUFA research program.
So opening today's event is actually a real full circle moment for me. I'm really inspired today as I was then by the impact of the GDUFA research program. And I'm also honored to be part of it today in this capacity.
The GDUFA science and research program creates enormous value by proactively identifying potential scientific challenges in the development and assessment of generic drug products. The research program helps address and establish increasingly efficient approaches industry can use to develop generic drug products. It also ensures that our FDA reviewer teams are familiar with the latest scientific technology and knowledge and the methods for assessing drug applications.
For patients and consumers, what's more important is that this translates into earlier access to high quality, safe and effective generic drugs.
Today and tomorrow's workshop allows us to hear directly from you about the upcoming challenges and research needs.
It is motivating to see the inroads we are making together to better understand, develop, and communicate approaches to resolve scientific challenges as they arise.
In addition to the public sessions, I'm very eager to hear more about the continued challenges in assessing and controlling things like nitrosamines, the development of complex device drug combination products, and where the use of artificial intelligence can facilitate generic drug development as well as assessment.
We take the feedback we hear from you today and from the open research initiatives dockets that's on our FDA website as well as the regulations.gov to establish the research priorities for next fiscal year. This feedback also helps FDA strategically design research projects and studies to address the knowledge gaps and product development challenges.
You'll hear more about this later this morning from Dr. Robert Lionberger, the director of the Office of Research and Standards.
Since its establishment the GDUFA Science and Research Program has funded more than 100 research projects within FDA and with leading academic industrial researchers. The scientific foundation gained from our GDUFA research program outcomes enables FDA to provide prospective generic applicants with timely technical advice.
This advice helps them prepare their submissions in a timely manner more compatible with the most current scientific insights and regulatory expectations.
For example, outcomes from the GDUFA research allows FDA to provide potential applicants on whether a proposed product development approach presented to FDA in a pre-ANDA product development meeting is likely to be suitable. Last fiscal year FDA facilitated 71 of these product development meetings with the goal of assisting applicants' development programs and encouraging submission of high-quality drug applications. And this can ultimately improve application assessment efficiency.
Similarly, FDA communicates its recommendations through the publication of new and revised product specific guidances and general guidances for industry. The key outcome from our generic drug program is the development of these product specific guidances and its implications.
These guidances help prospective applicants understand FDA's expectations to focus their product development and mitigate potential risks.
You can find product specific guidances for more than 2,000 products on our website. And last year FDA issued over 244 new and revised product specific guidances, of those 174 were for complex products.
In addition, FDA issued more than 80 new and revised product specific guidances for topical products based on the GDUFA research program. These PSGs make it more efficient and

feasible for prospective applicants to apply formulations for topical products that matches its reference standard product.
The recommendations in many of these PSGs that we posted would not have been possible without GDUFA science and research.
What's that mean? That means your input matters. And this is what we're really looking forward to here today as experts in the field of drug development. You are in a unique position to advise FDA on the specific types of research that could address some of the scientific challenges that we face. We're looking forward to hearing your thoughts during this workshop, and as well as in addition to the public docket, a link to that docket, the FDA-2024-N-0119 is on our website and announcement for this public workshop.
In addition, as part of FDA's commitment to expanding its collaboration and communication, we also communicate and work closely with the GDUFA Funded Center for research on complex generics also known as the CRCG. It's a unique forum for the FDA generic industry and other subject matter experts to engage in scientific discussions on challenging issues. In addition, FDA and the center for research on complex generics helps communicate some of the GDUFA research outcomes that you'll be hearing about and also setting priorities for today, and then in ways that can help directly guide generic industry and develop the most challenging studies.
For example, a couple of weeks ago the FDA and the center for research on complex generics co-hosted the considerations of potential regulatory applications for a model master filing workshop.
In this workshop we sought to illustrate how modern model master files could improve the assessment efficiency when modeling and simulation is used in a generic drug development program.
In October 2024, we'll co-host a workshop on scientific and regulatory considerations for the assessment of immunogenicity risk for generic peptide and oligonucleotide drug products, something of very considerable interest and scientific research need. And also in December we'll be co-hosting a workshop on the navigation of transition to low global warming product propellants and products. I hope some of you will also be able to join us for those events.
For people attending in person today a complete listing of those upcoming workshops will be available as a handout, and for our virtual attendees we're posting this information and links into the chat box.
These workshops are tremendous opportunities to learn and understand how GDUFA research outcomes can help you develop generic products as well as opportunities to engage with and provide feedback to FDA and other subject matter experts in that field.
So in concluding my remarks, I'd like to thank all of our presenters and panelists for providing this scientific input. And for all of your attendees for their support and feedback for this important research program. I'd also like to thank the volunteers that are working hard to make sure that everything is running seamlessly here in the room as well as online.
On behalf of the extraordinary team who organized this workshop, both within Office of Generic Drugs and the Office of Pharmaceutical Quality, I'd also like to thank all of you for your engagement over the next 2 days of this public workshop.
We are deeply grateful to all of our collaborators within FDA and at institutions around the world, and to many throughout the global generic industry for the success of the Science and research program.
This success would also not be possible, if not for our extraordinary collaboration with the Office of Pharmaceutical Quality headed by Dr. Mike Kopcha.
In addition to supporting the GDUFA Science and research program, the office of pharmaceutical quality contributes to the assessment of nearly every type of human drug marketing application, this includes new drug applications, generic drug applications and biological license applications as well as establishing the quality standards for over-the-counter drug products and facilities.

With that I'd like to hand over to Dr. Mike Kopcha, director of OPQ, to tell you a little bit more about the shared efforts in GDUFA science and research program.

Michael Kopcha (FDA): Thank you, Dr. Kozak, and welcome everyone. We appreciate your engagement today with FDA as we identify the most pressing research priorities related to generic drugs in this next fiscal year.
I believe, as does my office, that everyone deserves confidence in their next dose of medicine, and it is pharmaceutical quality that assures the availability, safety, and efficacy of every dose that patients and consumers take.
The office of pharmaceutical quality, or what we call OPQ, mission at the FDA is to assure that quality medicines are available to the American public.
As part of this mission within CDER, both OPQ and the office of generic Drugs, or OGD, lead many of the GDUFA funded research projects which involves coordination and collaboration across all of FDA.
These collaborations involve a number of offices as well, and I want to highlight at least some of those offices which include the office of translational sciences within CDER, FDA Center for Devices and Radiological health, FDA's National Center for Toxicological Research and FDA's office of regulatory affairs.
We also work closely with research collaborators within institutions around the world. So it's a global effort.
You here today are one of the most important collaborators, though, and this annual workshop plays a very essential role to ensure that we maintain our focus on the most current scientific challenges and evolving research priorities within the industry.
This continual advancement and emerging issues and pharmaceutical science and manufacturing provide ongoing challenges and opportunities for generic drug product development.
GDUFA funded research improves the efficiency with which generic drugs can be developed as well as assessed.
This benefits public health by making it feasible for manufacturers to develop certain complex drugs which reduces the risk of drug shortages, facilitates competition within the industry, and enhances patient access which is the most important piece to help make safe, effective as well as high quality drug products widely available.
The GDUFA science and research program continuously improves efficiencies through scientifically supported and evidence-based quality and bioequivalence standards. The emphasis here being evidence based.
Such quality standards can create more opportunities for competition.
GDUFA science and research program also creates opportunities for competition by providing scientific and regulatory clarity to all prospective ANDA applicants who are eligible for pre-ANDA product development.
Each year GDUFA funded research improves patient access to generic products that were practically unfeasible to develop as recently as only a few years ago.
One way the research achieves this is through the development of advanced methods to characterize product quality as well as that product's overall performance.
These methods facilitate practical and efficient approaches to develop generic products, including complex products. Those methods also help FDA assess the bioequivalency and quality of complex generic products once the ANDAs which utilize these advanced methodologies are submitted to the agency.
For example, in July of 2023, FDA approved the first generic Naltrexone extended-release injectable suspension which treats alcohol dependence as well as prevents relapse of opioid dependence.

The first generic approval of this product is a notable achievement. Why? Because of the product's scientific challenges to develop, manufacture and demonstrate bioequivalence.
This generic product uses a long acting, injectable, biodegradable polymer microsphere technology called PLA.
This formulation confers the product with a sustained effect that only necessitates patients to be dosed once a month.
FDA approved the first PLA product more than 30 years ago, and the complexity of developing and manufacturing PLA products is so great that there have been relatively few PLA products approved ever since.
This product is both the first Naltrexone extended-release injectable suspension generic product to be approved as well as the first generic PLA product to be approved.
This product's approval is notable not only because of how scientifically challenging it was to develop and manufacture, but also because this product treats 2 illnesses, alcohol dependence, and also prevents relapse to opioid dependence, 2 major public health issues affecting millions of individuals within the United States.
FDA and our collaborators' ongoing GDUFA funded research on PLGA products during the last decade has systematically advanced scientific insights and developed new tools to support an efficient demonstration of bioequivalence and pharmaceutical equivalence for complex generic drug products such as this one.
The research focused on developing analytical methods that would ultimately facilitate the reverse engineering, the characterization, and then ultimately the selection of the suitable polymers, which was a critical first step for generic drug product development and quality for this product.
GDUFA funded research also focused on developing suitable in vitro drug release testing methods.
These methods ultimately elucidated how a drug is released from such a formulation and how different manufacturing processes and polymer characteristics can influence that product's drug release.
The GDUFA funded research on polymer and formulation characterization helped to establish a viable scientific approach and regulatory pathway for generic PLGA based products.
It directly supported generic drug product development and prepared FDA to assess PLGA based products when submitted in ANDAs.
The approval of this complex drug product exemplifies how we can achieve with effective coordination between FDA and the generic drug industry to bring these products to market. As Darby mentioned earlier, the GDUFA science and research program fosters early engagement between FDA and industry to help identify specific priority areas for GDUFA research.
Science and research then facilitate continual engagement potentially through pre-ANDA meetings during product development, to discuss how insights from GDUFA research can be leveraged.
Following ANDA submission, science and research continue to support productive technical discussions and meetings between FDA and the ANDA Applicant on these scientific matters.
Our collaborative engagements to advance the GDUFA science and research program have been exceptionally effective at addressing scientific challenges for complex drug product development as well as the assessment of those applications. The scientific advances it has fostered have continuously helped to ensure that the generic drug industry has the most efficient and modern tools and approaches to overcome barriers to generic drug product development.
The GDUFA science and research program also assures that FDA is able to use the best scientific insights when implementing evidence based regulatory standards to promote development of these generic products.

This will enable American patients as well as consumers to benefit from having multiple suppliers for their medications, which not only builds redundancy into the supply chain, but helps to mitigate the risk of drug shortages, but it also enhances the availability of affordable drug products to patients.
Now this all begins with the work we'll be doing together during the next 2 days.
We will identify and clarify the current and emerging challenges for generic drug product development and assessment to prioritize the research that's needed to address these challenges.
Our next speaker, Dr. Robert Lionberger, will provide an overview of the current portfolio research across the 8 scientific areas that are GDUFA science and research priority initiatives under GDUFA III.
This will provide a background for research that is already underway to address known challenges and also to set and establish the foundation for discussing additional research that FDA should prioritize for next year as well as the years ahead. So please join me in welcoming Dr. Robert Lionberger, director of the Office of Research and Standards in the office of Generic Drugs.

Robert Lionberger (FDA): Thanks, Mike, and thanks, Darby, for your introduction and your time. I think you might have learned from Darby's talk that this is not only a science and research priority event, but it's also a recruitment event. My office is very sad to lose Darby to his new position. But that means we're looking for a replacement. You can go to the 21st century cures page and see the vacancy there and I encourage you to think about that.
So with that, it's my great pleasure today to give you an overview of the entire research portfolio. Hopefully, this will give you a sense of the things that we're currently doing and will help set the stage for the discussion today where we focus on a few areas. But again, we're always open to suggestions within these portfolio areas.
So what do I mean by a portfolio view? This is a higher level than the project view. So I'm not going to talk about each individual research project that we have. We probably have about a hundred of them across OGD and OPQ that are active right now. But this is a little bit higher level. And this is the way we organize the research priorities. So you can go look at our current research priorities. We also use this to organize our science and research report. And so that'll be available soon.
And in the Science and research report that really goes down and lists all the projects that are active. It gives highlights and details, it gives references to all the publications in the last year, all the product specific guidances that were linked into research activity. So we expect that will be available within the next few weeks. That's really the details and the follow up to this talk.
Okay, so what does our research portfolio look like? And, as was mentioned by Mike, we divide our portfolio into 8 different areas.
And these are impurities, complex active ingredients, bioequivalence for complex routes of delivery, bioequivalence for complex dosage forms, bioequivalence for oral and parenteral generics, drug device combination products, quantitative medicine, artificial intelligence and machine learning.
And so again, what we generally try to do is on each 5 years of the GDUFA cycle, we try at the beginning of that, and we did this a few years ago, leading into GDUFA 3 to try and redefine what the broad portfolio will look like for the next 5 years. So we want to have the portfolio level trying to keep that stable.
And each year at this workshop we dig into a few of these areas. So today we'll have sessions on impurities on what we call predictive tools. You can also think of this as quantitative medicine linking into the activities of CDER's recently announced center of excellence in quantitative medicine which is driven by a lot of the creative initiatives that we've developed in the generic

program. And then, as well as we'll have a session tomorrow on drug device combination products. In previous years we covered other parts of the portfolio in depth.
But again, we're always going to be open to within those portfolio areas. Every year we have to make a decision about what projects we initiate, what projects are our highest priority, where we invest resources in. So across the portfolio each year, we're always open to listening for within those different areas, what are specific things that are currently challenging to the generic industry. So we can adapt at the project level much faster.
And at this year's workshop we've tried to compared to previous workshops mark out much more time for the public comment period. So you'll hear a lot more open public comments here. We try to encourage people who are interested in potential research collaborations to participate in those public comment periods. We want people to make those comments publicly. So our panelists, who will be from both FDA, who have to help set up the portfolio, but also the generic industry who provide valuable input from those public comments to really say to us, are those public comments addressing issues that you in the generic industry also see? So we're looking for some validation from our panelists around the public comments. That's very helpful with us. We hope this will be a very interactive day from all the different participants.
And as well today, we're always listening for input into which product specific guidances are the highest priorities. So again, we have commitments under GDUFA 3 around the availability of product-specific guidances for newly approved complex products.
But again, that leaves out all the complex products that were approved before the start of GDUFA 3. So we still have prioritization to do within our science and regulatory efforts on those guidances that are not covered by those goal dates.
And so if you're familiar with our PSG program, we have a forecast list. We just posted an update last week. We have some improvements to the forecast list. Now you can go to the forecast list and see what's changed recently on the forecast list to make it clear what's new or what forecasted dates have changed.
But we're most interested in this workshop on product specific guidances that are not on the forecast list. If it's on the forecast list, that means we're actively working toward it. We have a target goal date. We have research and regulatory evaluations coming together toward that. But if something's not on there and should be on there, this is the place where this is one place where we'd like to hear about that. You can also send your PSG request into the generic drug mailbox as well through the controlled correspondence process as well. So there's other venues to indicate interest in product specific guidances, both in a public way and in a private communication way. So we're always listening for that to help direct our science and research programs to be the most effective and make the most impact on generic drug development.
So again, what I'm going to do for the rest of my talk is try to give you an overview of the entire research portfolio. Both the things we're going to talk about today in more detail, and the other sections where we will be listening for comments as well. And I hope this will stimulate discussion. And we also want to help you focus on things that we're not doing. So if there's activity that's really underway right now, it's good for us to hear like that's on point. That's solving the problem. But we're really looking also for are there things that are missing in our portfolio? Are there emerging issues that will be great value. And I'll point out some of the examples in the past where we've learned a lot from this workshop that have informed this portfolio, and informed some of the very significant regulatory outcomes that we've achieved.
And so the first aspect of our portfolio, and so I've tried to shorten the titles of these down to one word, if possible. So if you look at our priority list I have a lot more words in there, but trying to get to the essence of it, and this really first covers impurities. The goal in this area is tools that efficiently evaluate and mitigate the risk of potentially harmful impurities in generic products.
And I think everybody knows that the most important aspect of this are the nitrosamine related compounds, and we'll have a whole session on that coming up in the next few minutes.

But I also want to point out that before the nitrosamines became the giant issue that everyone was thinking about, people came to this workshop and said this is an emerging issue. It's going to be bigger than the first few products that are affected by this. So if you're listening carefully at this workshop, then you could be aware of what that is. I think, from us as a research science and research perspective at FDA have the ability to be thinking about that and preparing for that, so it can be very valuable. This is an example where at this workshop if you're looking for what the emerging problems and challenges will be, you can hear them here. And this is a really good example of that.

But some of our key accomplishments in this area you'll hear about more is really some work in our FDA labs really demonstrated that antioxidants can reduce some of these impurities. And so there are formulation reformulations that you can make that reduce the formation of the nitrosamine related compounds. I think this is a very valuable finding and demonstration from our colleagues in the FDA labs.

But once you realize this happens, you say, well, wait a second. If I want to actually reformulate my products to reduce nitrosamines, which is a valuable goal. That's what we want. We really want to reduce the toxicities of these impurities, you want to have ways to reduce them.

One of the focuses of the research activity has been on identifying ways that we can make these potential reformulations more efficient. And we look specifically at antioxidants that have been known to reduce nitrosamines to try to identify any kind of biopharmaceutic risk for those products. And this has really informed our thinking on the type of data that would be used in reformulations of nitrosamine products, and I encourage you to discuss those with FDA through the appropriate channels as you come up.

And just for each of these areas I want to give this overview of some of the continuing projects that are active in here. We have some work that's been completed on the biopharmaceutics, understanding the effect of antioxidants on drug absorption. This really helps evaluate the biopharmaceutic risk, and we have a bunch of internal projects mainly lab based, but also with our center for toxicology research. You'll hear about that as well in the session on methods for evaluating the toxicity of individual nitrosamines and developing the pharmacokinetic infrastructure for that. I think realizing that the whole of the generic industry really is not designed for a system where later in the product life cycle, you identify new impurities, the generic system works best when the new impurities are evaluated through the new drug review process that characterized at the time of the new drug approvals. And there's a baseline understanding of the safety of those impurities. There's qualification through ICH thresholds. And so late emerging impurities of this whole class really challenge the generic industry. We do understand that, and really trying to develop an appropriate infrastructure to move this area forward. And it's really based on scientific tools through the center for toxicology research and some of the formulation science aspects of it as well. But we realize this is a fundamental, difficult challenge for the generic industry. And we want to try and develop appropriate solutions in this area.

Second element of our portfolio is what are called complex active ingredients.

And so these are cases where it's not a simple, small molecule. It's something a little bit more complicated. Goal in this area is to really be able to characterize those complex active ingredients especially, and their impurities that are formed.

And really the biggest challenge we see in this area is managing the immunogenicity risk for these complex active ingredients. Again, generally, you think that clinical and safety risk should be the same between the brand and generic products, because the active ingredients are the same when the sameness of the active ingredients is more complicated and the impurity profiles are more complicated. There's the potential for safety risks that are associated with those potential differences. And we want to have appropriate tools for us to characterize those differences, but also to evaluate any immunogenicity risks that may be there in an efficient and effective way.

And in this area, really 2 product categories of focus. One are Peptides.
Again, only about 10% of the Peptide products have generic competition. But we're really seeing a surge in ANDA submissions for Peptides. I think if you look at the market and the realities of the world, you will understand why there's a whole new class of products, huge markets that basically will have a huge public health impact if they're widely available accessible versions of those products. And so we expect to see huge numbers of ANDA submissions for Peptide products in the future. And we want to have an appropriate, efficient way to evaluate, to characterize those generic versions and evaluate any types of immunogenicity risks.
This is going to be a key public health impact over the next 5 years. Again, another category which is the focus of our research, are oligonucleotide-based drugs.
You see several of these approved each year. Some maybe for orphan indications. But it's a new technology class. There's lots of challenges to characterizing these products. Lots of you have many potential at every base, at every base position. There's a possibility of a substitution to figure out how many different molecules you could have. You get to some huge number. So lots of appropriate analytical methods for characterizing the oligonucleotides, understanding the risks of these products, especially for products that have maybe smaller clinical programs as well is a challenge going forward. Again, no generics approved in this space yet. But again, an area where we see lots of activity, there's going to be a whole class of products for which you'll want to develop generic versions. And we want to build up and develop the scientific foundations for this area.
And you see lots of activity flowing out of the research program, new product specific guidances for Peptides and oligonucleotides. Later this year we'll have a workshop focused on immunogenicity with the center for complex generics. We know that's been a challenge for implementing some of the study designs in that area.
So again, a key area. And if you look at some of our projects, we have several external collaborations. Again, a lot of internal work, especially on the characterization work with our OPQ lab colleagues for the Peptides and the oligonucleotides. That's been very important to building that foundation. If you don't know what's there, then you can't do anything else.
Third part of our category is what I call complex dosage forms and formulations, and I wanted to distinguish this from the other parts of the complex products is that this part of the portfolio is focused on efficient bioequivalence approaches for systemically acting complex dosage forms rather than locally acting products. We'll talk about them separately.
But one of the key areas of focus in this area has been the long acting injectables and implants. And you heard Mike Kopcha talk about the recent approval of the first PLA-based product. Again, that was based on years and years of research collaborations to identify what the key attributes are, what type of analytical methods can develop, and how similar do polymers have to be to provide the same effect. But again, lots of challenges for the long acting injectables and implants remain. There's fundamental challenges about how to do the in vivo studies, especially ones for drugs that are not safe enough to use in healthy subjects, really have a lot of quantitative medicine focused on efficient study designs.
Another key category here, which you see challenges in generic drug approval are some of the more complex injectables, liposomes and iron colloid products. It's been many years since we've approved generic versions of the iron colloid products. So we're interested in a lot of focus, especially in our characterization based approaches as to what's the best way to identify the challenges in those products. There's also citizen petitions on some of the naming issues related to those products that make even more challenging. So again, complex science, significant regulatory environment. Those are the 2 areas. Again, mentioning the Naltrexone products is one of the key accomplishments in this area.
And again this area has a much bigger research portfolio. Here we have really on the PLA products, a wide range of some of the key collaborators and the academic experts in this area

have worked with us to build the scientific knowledge moving toward the foundation for PLA-based generics.
The next part of our portfolio is what I call the complex routes of delivery, and this is again with a goal focused on efficient bioequivalence approaches for locally acting complex dosage forms distinguished again from the systemically acting products in the previous section. And here again, this is probably the largest area of our portfolio in terms of the number of projects, because there's a lot of different areas where there's interest in generic competition and lacking one. So I've lined out 5 of the areas here that the routes of delivery, which have been very challenging for the generic industry.
Again, the most important one to us is the inhalation area.
There are still very few MDI and DPI generic products available.
And we've been working very diligently through the research program and through updating our product-specific guidances to make sure that it's absolutely clear, through our product-specific guidances that there are alternatives to the FEV1 based clinical bioequivalence studies for these products.
And I think this will be a key foundation for efficient access to these products. This is a longstanding goal of the program. And again, very challenging to implement these and develop these products. So we're really working on developing both the analytical and the computational foundations that'll help make the development of these products more efficient. But you can expect to see again guidance updates coming. If you look at our guidance agenda in this area.
Another aspect of the inhalation products. And this is something again, if you follow this workshop, you would be at the leading edge of this. Several years ago people came to this workshop and said, one of the things you have to pay attention to is this transition to environmentally friendly propellants for the metered dose inhalers. Again, there's a desired transition from the current propellants will be phased out over 10 to 15 year period. This is going to be a challenge to reformulating these products. Again, the reset of the whole areas. People also pointed out that the last time there was environmentally driven change to propellants, it caused a lack of generic competition for about 15 to 20 years. And that's not something we want to happen this time. We really want to be prepared for that through a scientific and regulatory foundation, and it was really helpful to us to hear that being brought to this meeting very early, before it was on a lot of the radar before it was on the radar of people within FDA before even we started seeing new drug applications that wanted to do this. And so this area has evolved. We've been listening to this. We have research activities in this area. We have a workshop coming up with the center for complex generics on this specific topic later. But again, we want to be proactive on this. We don't want to be in a situation where this transition to environmentally friendly propellants leads to an interruption in generic competition.
Other important areas that have complex routes of delivery are the topical areas. There are many, many generic products in this category, this is an area where we've been very successful at really resetting the field and moving toward characterization based approaches. Last year there were 20 topical products approved via characterization methods. Again, many more than we've ever approved, based on clinical endpoint type studies in a single year. So very successful program and topical products. When we look at that space, 2 priorities moving forward, one is understanding that if we restrict some of these characterization based approaches to Q1 Q2 formulations that may leave out competition. So we're looking to make sure that our scientific foundations can be expanded to formulations that might have small differences and have good methods to characterize those differences and predict their clinical or safety impacts. And again, some of the challenges we see, as we see more people moving into these characterization based approaches. Sometimes we see challenges in implementing the new in vitro methods. There's less infrastructure available for them. There's less CROs available that are familiar with FDA's guidances around that. Compared to a more established field like, say, bioanalytical methods, for in vivo PK studies. There's a whole infrastructure and

understanding around how to do different types of studies for emerging and novel technology approaches. There's more, we see more challenges in the submissions of these. So we see lots of submissions. But then, as the FDA reviewers dig into the applications, they find problems with the in vitro studies, understanding how the in vitro studies need to be conducted like their bioequivalence studies, as well as some of the scientific issues in implementing them consistently across the laboratory. So one focus of our research activity here and through some of the upcoming workshops that we'll have, as well as making sure that we have efficient paths to implement the new analytical methods that support these in vitro characterization approaches so they can be as reliable and efficient as the PK studies that we normally do for bioequivalence in generic drug products, and that just saying, Oh, do this type of study. And there's a common understanding around that. And that's, I think one thing we've learned from this workshop over the many years is as these new technologies are established and set up and presented in the product specific guidances, there can still be challenges in implementing them that may slow access toward generic products. So that's again, a growing focus of our research program to make sure that these things can be translated through the guidances into the application review.
Again, 3 other areas, ophthalmic and otic products. Again, big successes in here, due to research projects due to research programs, we now have access to generic versions of the ophthalmic emulsions, such as cyclosporine and difluprednate, very much based on a lot of the characterization work there. So again, an area where the in vitro approaches have been established used to approve very complex products. Again, some of the things that are in our portfolio for this area now are a focus on what type of data is needed to support changes again for ophthalmic products. You're allowed to have differences in preservatives. But again, that's sometimes challenging or concerning about the therapeutic substitution. So we're looking at ways to even just implement our current regulations on excipient recipients. What type of studies are needed to support those?
And again, another category where we've been seeing more NDA submissions are sort of longer acting, ophthalmic implants.
So these are longer sustained release dosage forms for the ophthalmic routes. So this leverages some of the work we've done on the long acting injectables. But we see this is an area where there's no generic versions of these longer acting ophthalmic products available. So we see that's the focus for research activities.
2 other areas where we have research activities are the nasal products. Again, we've recently, in the last year, really updated a lot of the nasal spray product specific guidances to show to remove some of the PK studies that we think now are unnecessary. Now that we have appropriate in vitro and computational methods that have mapped out the key critical attributes of those aspects again for GI acting products. Again, we've been improving products in a long staying. So an area that's a little bit more established with pathways for generic products.
And I mentioned the topical and nasal products of some of the areas here. But here again, I said, this is probably the biggest area of our portfolio. I need probably one page for each of the areas here for nasal and inhalation products we have set of grants and again, contracts focused on the access to the inhalation products. The topical products have been supported by a range of products really making building out the infrastructure. For here again a large number of products in the topical space. And we want to have robust methods that work across that full space.
And again, a slightly smaller portfolio for the ophthalmic and otic products.
And the GI acting products again, make us again a smaller part of our portfolio. So again, that's this area. Again, traditionally been the area with the most significant barriers to generic competition. When you can't use a PK study as part of the bioequivalence approach because the product is delivered directly to the site of action.
So moving on to other parts of portfolio, and this is again another one that we'll be talking about later in this workshop is the drug device combination products.

And here our focus is on methods to evaluate differences in the drug constituent parts compared to the reference listed drug. So really, on the user interfaces, we see the challenges. And we had a recent CRCG workshop earlier this year on this topic, heard a lot about the challenges and also about the opportunities in this area.
And again, that's really the focus is understanding the human factors study for these products really understand, also reading carefully after his guidances and understanding that when you see a difference in the user interface, it doesn't mean that you have to go directly to human factors studies that there's alternative methods to evaluate user interface differences. That's part of the research portfolio to really help identify that make this development of generic products that have differences in the user interface that aren't clinically significant, more efficient.
And again as well transdermal systems also fall in this area. We have work there, to continue efficient approaches for transdermal drug delivery as well. We have a collaboration with the CRCG and industry on more efficient methods for transdermal adhesions. And one of the impacts of this area is beginning to see is in the product specific guidance for any product that has a device constituent part that you'll begin to see device advice added to the product specific guidance is really pointing out is this a drug device combination? What's the drug device constituent part? What types of characteristics you need to consider during development. Hopefully, this will help people focus on not missing those aspects and provide a basis for more generic competition. But again, this is an area where we have a research profile. Here, we recently also have the ability to conduct human factors studies through a contract with the CRCG, so we are also in the process working with the CRCG to develop some places where we will be able to generate some new data that may help address some of these challenging interface issues in a sort of product, independent way that'll make future product development more efficient. So we have lots of interest in this area within a session tomorrow to hear input, on what are some of the key challenges? What are specific? And I think, really looking there for what are specific device interface challenges where you think more data would help set the risk benefit balance between generic allowing generic competition in this area.
Again, our research portfolio also covers oral and parenteral generics. We want to make sure that people understand, complex products get a lot of the attention. But most of the products that provide access to generic products are the oral and parenteral products. And it's really important to have efficient bioequivalence approaches for those products access. This is where the access issues, drug shortage issues come in.
And so we're always interested in aspects of how we can make that fundamental part of our regulatory portfolio more efficient. And this is again another example, where there's been huge impacts from this meeting in about 2018. We had a focus session in this workshop on do we need fed bioequivalence studies? And that's fundamental. And I don't think at that point people thought that 6 years later we'd be on the verge of a globally harmonized approach that really reduces the default expectation for fed bioequivalence studies, not just in the US FDA, but also in a global, harmonized way. So it's been a huge success. It's been driven by science based research on this data collection modeling and simulation analysis looking for risk factors. This is all fed into a global harmonization initiative through M13, which is led by FDA, but participants from the generic industry as well. Again, a huge accomplishment. We look forward to actually the finalization and implementation of M13 later this year, again, the process is working through. But again, huge impact in the development of the full space of generic products hopefully making it much more efficient going forward.
Again, a second area focused in this area is looking at the strength waivers for modified release products. Again, waivers are in quotes because we read our guidances. It's not really a waiver. It's some other way of demonstrating bioequivalence. But it's really for the other strengths of the modified release products. We had a recent workshop on that with PQRI few months ago, really understanding that we can make the development of to make our guidance much more clear guidance on modified release products much clearer, and to put the development of a

consistent bioavailability across the strength as a fundamental focus of our biopharmaceutical and pharmaceutical science understanding. So we have a portfolio of research activities in this area. Again, a lot of internal activities in this area supporting methods to make our bioequivalence determinations across the vast majority of our products more efficient.
So again, not as trendy as some of the other areas, but again, the fundamental importance having an efficient and effective regulatory program.
Again, I mentioned, we have some also crosscutting areas. So one of these biggest crosscutting areas is what I call here quantitative medicine. This is modeling and simulation tools to help generic drug development. And these are approaches that can be used across lots of different product categories.
And some of the things we focus on here in the generic program that create the most value to the generic industry and increase access to safe and effective generic products are the PBPK models for the local routes of delivery. So that's a big part of our research portfolio. We've tried to develop and build the modeling foundation available in each of those 5 different areas to make generic drug development more efficient.
Especially when PK studies aren't the gold standard you want to have something else that you can look at to try to understand what changes in my product are affecting local delivery. And we've been very successful over the program at building this foundation, but also making this foundation available in commercial software products that you can purchase in development programs. So there's access to these outcomes. Again, I mentioned in long acting injectables, model integrated evidence approaches for more efficient bioequivalence studies and linking into the oral products done a lot of modeling and simulation work in support of waivers for bioequivalence, either in the example of nitrosamines, expanding BCS waivers supporting the M13 changes to the fed bioequivalence studies.
Again, we currently have a meeting pilot program for the model integrated evidence running again. That's a key aspect of integrating these new scientific approaches into the review process. So you have an opportunity to have a special focused meeting on a model development with complete focus on that modeling approach that integrates into the pre-ANDA meeting program so hopefully, this will be successful. As people try to move these novel technologies into application review. You have additional opportunities for interaction and feedback from FDA.
And so again, here we have a lot of projects in quantitative medicine that I've mentioned in other sections. We also have a few, a small portfolio of research projects that are focused on method developments that cut across that. So we have a portfolio of 20 to 30 research projects mentioned in other places that use quantitative medicine. But we also have some specific ones really developing new approaches that cut across these areas.
Final part of our portfolio is we call AI machine learning problem. And again, this is part of our research portfolio. I mean, now, it's the super trendy thing. But if you come to this workshop 5 or 6 years ago you would have been hearing us talk about AI and how to use it in generic drug development, how FDA looks at it.

And again, you can stay ahead of the trend. We've been working on this again before it's taken off. Certainly it's dominant now people have realized across all of society how important these methods will be, how transformative they'll be and how significant they'll be again. The key for FDA is, how can we use these methods to improve the efficiency and consistency of our scientific assessments and advice?
That's the focus of our research program. And we're doing things like natural language processing to understand drug labels and other FDA data, these help us develop product specific guidances faster, more consistently. We're also looking at on the methods question, what are the best ways to use AI to help develop other types of quantitative methods and quantitative medicine models. So we have several research projects using the AI to develop and

fit a PBPK model or QCP quantitative clinical pharmacology model to data. We have some open source tools that have been available. So you can develop models faster for other things.
But again, just thinking about it, there's also just fundamental opportunities to make our review processes better as we learn the best way to use. And I think the research focus is what's the best way to use AI ML large language model tools on the type of data that FDA encounters in a way that protects access to the information. And this means that probably FDA has to do some type of training and analysis inside our data firewall. This can't be you can't just take one of the current products and say, Well, tell me about this drug application.
I think, from FDA leveraging the reviews that we've done in the past, and the type of data that we've seen. There's lots of opportunities to have something that's better than that, but also has to be protected in that. So we're looking so we have to have internally the understanding of what's the best way to do that in a computational and efficient way.
And I think that as we look about as we look into this area. So we but we think that there's a lot of opportunities in which this will make our generic drug review more efficient. I know the industry always talks about it's about consistency. But as you develop these tools, you'll be having tools that can help reviewers ensure that their review is consistent with other FDA reviews and identify places where there might be differences. And I think you'll be as we develop these tools and implement them over the next 5 years. I think you'll see a lot of ways. It just transforms how people do their jobs in a more effective and efficient way. But this is an area of your research. And you begin to see some of this in our generic drugs assessment, where we have really data, driven data flows for products. And we're trying to use these type of tools to extract information and make the review process more efficient already. And so we're building an infrastructure internally to do this, and where we can put as we develop new tools really rapidly insert them into our research program.
And we have several research collaborations and internal activities in this area as well.
So summary, really look forward to your input as we refine and focus our research portfolio to accelerate access to safe and generic products. We really welcome the input on what specific things and areas we can be doing that can make the generic drug development faster. We've tried to provide lots of opportunities for lots of different perspectives to provide their input into these questions over the next 2 days, and I really look forward to hearing them.

And with that I believe we'll be taking a 15 minute break, and then we'll be coming back for our session focus on nitrosamines. So thank you all for attending online in person and stay tuned in about 15 minutes as we come back.

Sruthi King (FDA): Good morning. My name is Sruthi King. I'm the Deputy Division Director for the Division of Pharmacology and Toxicology in the Office of Safety and Clinical Evaluation within the Office of Generic Drugs.
Welcome to session one, which is focused on nitrosamine impurities in drug products. My co-moderator, Dr. Dongmei Lu, and I are looking forward to today's discussion.
I just wanted to acknowledge the efforts that went into putting this session together. Organizers from OGD, OPQ, and also three individuals who are helping make this session a success: Elise Bailey, Dr. Juan Crespo Barretto, and Commander Steve McMillan, who really are behind the scenes in helping this session be a success. Thanks to our speakers and panelists and our public commentators who are coming to participate today to provide this feedback.
We're going to be hearing about current work that's being done in this area from a safety and quality perspective, also from a bioequivalence perspective, to address the challenges that have been posed by nitrosamine impurities in drug products. Our faculty and panelists will be discussing risk assessment strategies, advancements that have been made to date in setting acceptable intake limits, safety testing approaches, mitigation strategies, and modeling

approaches for evaluating bioequivalence. We'll also hear about the scientific and regulatory challenges that have been shared via the nitrosamine exchange.
Through this discussion and feedback, FDA will identify what research should be prioritized to better address this challenge that's been posed by nitrosamine impurities. This research is funded by GDUFA so that we can better serve the generic drug program.
Just a reminder that the intent of this session is really to focus on the scientific and research needs rather than discussing regulatory policy or any application-specific issues. So with that, I'm going to welcome my co-moderator to get us started off with the public comments. Thank you.
Dongmei Lu (FDA): Thank you, Sruthi. Hi! Good morning, everyone. My name is Dongmei Lu, and as a really brief self-introduction, I'm a policy lead in the Office of Policy for Pharmaceutical Quality, and I'm really honored to have this opportunity to be a moderator with Dr. King for this session.
For our public comment session, there are six public commenters going to present: four in person and two are going to present virtually. I just want to indicate that related to the questions, due to the time limitation, the questions will only be limited from the panelists and the session faculty, and the maximum one to two questions, just for clarification purposes. If the audience has any questions, please hold until the panel discussion.

So let's start the public comment session, and we'll start with the first one: Dr. Connie Chen from the Health and Environmental Sciences Institute of HESI, and she's a senior scientific program manager. Please welcome Dr. Chen.
Connie Chen (HESI): Thank you again for this opportunity to share some comments and for some opportunities to leverage public-private partnerships to address nitrosamine safety liabilities. As introduced, my name is Connie Chen. I'm with HESI. For those that are not familiar with HESI, HESI is the Health and Environmental Sciences Institute. We are a nonprofit scientific organization based in Washington, DC, but operating internationally for now 35 years. We provide a platform basically for collaboration between the public and private sector to work on scientific programs that wouldn't otherwise be able to be achieved if a single sector worked individually on their own. The HESI model is really about bridging research to translation for improved safety, innovation, and human health and environmental health.
We have four main focus areas, including that focus on accurate and efficient chemical risk assessment, safe and effective medicines (obviously a focus of today), environmental quality and sustainability, and food safety. We do this by basically pooling expertise and resources across our partner organizations, which number over about 300 organizations right now across 18 different scientific committees, each of which has a different technical focus area. The focus of the comments today is really the nitrosamine research program, which is housed under our genetic toxicology technical committee.
The nitrosamine research program really has only been in existence for about two years, and within that short amount of time, it's become kind of the central convening point for the collective prioritization, execution, resourcing, initiation, and analysis of nitrosamine safety-related studies. We have pooled industry and FDA grant funding, we have expertise and data and participation from pharmaceuticals, generics, contract research organizations from folks from the chemical, food, academic, and government sectors. We do have global participation from those different sectors, including from the regulatory and government agencies.
We have robust participation from colleagues at FDA CDER in the Office of New Drugs and NCTR. The nitrosamine research program really is focused on shared data and methods developments and initiating novel experimental studies to build best practices.
We've got four main focus areas. What launched the NRP was really the Ames optimization. It's a 16-member laboratory ring trial focusing on Ames, putting together an Ames protocol

predictive for the carcinogenic potential of nitrosamines, and this specifically received specific funding from FDA to support some of this work.
We have a new working group focused on identifying and verifying in vitro assays with alternative cell models. Currently, they're focused on the HepRG cell models, but with headlines to additional cell models as well or cell lines as well. We have a number of different programs focused on developing in vivo strategies to verify the Ames data within the frame of ICH M7. More recently, and you'll probably hear from one of the future speakers in the session about a newer working group focused on refining, extending the CPCA using QSAR and QM for improved predictive performance.

HESI was also co-organized and co-sponsored this research roadmap workshop last May with FDA focused on the hazard and risk assessment of nitrosamine impurities in drugs. This really was a global stakeholder exchange on ongoing research in the public and private sector. Through the two-day workshop, a number of different research needs and gaps were identified in need of funding and research. The nitrosamine research program work that was ongoing was presented as a specific input point during the workshop, and the output from this has informed new work that is going on within our program. I would encourage the panel, if they're not familiar with the research perspective that was submitted, to familiarize because it does list a number of research areas that are in need of addressing, not just through HESI, but externally as well.
I spent a majority of my time focusing on HESI and the NRP because it relates to specific opportunities for GDUFA funding and specifically for some staff in the Office of Generic Drugs. As I mentioned, we do have broad participation from a number of different regulatory agencies, and FDA specifically within the Office of New Drugs and NCTR. But I don't believe we have any participation from any staff at the Office of Generic Drugs. So this is an opportunity for that staff to weigh in and provide their input and help shape some of that public-private research. Specifically, the GDUFA funding could also go towards supporting some of the ongoing work streams to help progress and advance the depth, breadth, and speed of the work. All the work that is performed through HESI is performed largely by in-kind contributions. So additional funding could help support that work.
Specific financial support can also go to support new work that is not currently being funded or planned by the NRP. I've listed a couple here that were identified through that workshop.
One very specific area that I've been hearing is an area of need is that related to the NDSRI in vivo carcinogenicity studies. Within the NRP program, the in vivo working group that I've mentioned has some ongoing work looking at mutagenicity and carcinogenicity studies for robust NDSRIs. But an area that appears to be lacking is some studies looking at negative carcinogenicity studies, and we know that those studies are very costly and not really being prioritized or funded either through the NRP or through industry currently. So that's an area that really is ripe for additional funds. We know the two-year rodent bioassay is very expensive. So alternatives to that can include the six-month TgrasH2 transgenic assay, which could provide some quick answers.
I believe I'm almost right at time. So I wasn't able to get into a lot of details of a lot of the working groups within HESI. But I provided QR codes throughout the presentation. So I encourage the panel and others to look into that and the information available. And again, familiarize yourself with the output from the workshop last year. And with that, I'm happy to answer any questions.

Moderator: Thank you, Dr. Chen. Are there any questions from panelists to Dr. Chen?
Alright, thank you.
Next is Dr. Pallavi Silva, and Dr. Silva is from Advanced Manufacturing Technology at USP, and she's the technical director there.

Pallavi Silva (Advanced Manufacturing Technology at USP): Alright. Thank you. Thank you for the opportunity, and on behalf of USP and the team, we are grateful for the opportunity. Today what we would like to share is some of the research areas around emerging analytical technologies and advanced manufacturing technologies (AMT), how doing research around these topics could support the generic industry. I'll first talk about setting some priorities around AMT specific to generics, and then I'll delve into how those areas could benefit the nitrosamine research.

To begin with, setting a priority to facilitate the AMT adoption specific to the generic drug production, and why and how that would be beneficial. I mean, there are FDA-approved drug products that are produced using AMT that has shown using AMT, you can increase the efficiency in manufacturing and at the same time decrease the unit production cost. And if you think about that, that will eventually help for generic drug products to tackle the drug shortage products and eventually decrease the generic drug cost for our patients.

Well, when it comes to the AMT adoption, we have seen the rate of adoption is significantly lower for generics compared to the branded. At USP, we had several engagements and opportunities to work with our stakeholders, and what we learn is the barriers to AMT adoption, the challenge. There are unique challenges when it comes to generics.

One of the main pain points is lack of use cases to assess the benefits over the original investment. So this has to do with the complex and costly landscape of developing new methods, validating them, and integrating it to existing infrastructure, because with generics you are dealing with high turnover of products, a large portfolio with variable demands. So that means we need a tailored approach when it comes to generics to support the adoption of AMT. The solutions could be multifaceted. But when we think from a scientific standpoint, we need a concerted effort as a community. We need to work together. We need private and public partnerships. We need a strategic approach investment in technologies and innovation through our funding opportunities to support the R&D tailored specific to generics to explore how certain AMT technologies could benefit. So this could be continuous manufacturing or using specific type of process analytical technologies (PATs), right? So these could be for different to having that of funding support to explore certain PATs specific to a drug product category. This is how we can support the community.

And, like mentioned, the major barrier is the lack of use cases. Having a funded approach supporting collaborations, we would be as a community, we would be able to share business cases with the community and at the same time what we are learning through this process doing specific research around generics, we would be able to inform developing technical guidelines and best practices. And now there'll be to accelerate the adoption of AMT.

And now I would like to speak a little bit more about one particular type of AMT that would specifically be advantageous for nitrosamine research: process analytical technologies. This is a bigger field, like a larger field, it could encompass emerging analytical technologies and existing ones, like spectroscopy and other current analytical technologies.

So we are all aware, because this is a nitrosamine session, that there has been tremendous progress when it comes to understanding the basic pathways for nitrosamine formation. The current limitations of the analytical technologies that we are using limits us getting a comprehensive understanding or a full picture of the mechanisms behind different pathways. Some of those limitations are the matrix effect and the nature of the measurements. These are indirect. You are not doing it in situ. So this will limit getting a more accurate understanding of the origin of nitrosamine formation. So this is where we could leverage process analytical techniques and new emerging technologies. What we need is technologies that can enable in situ testing in a real or near real time and doing it in a high throughput manner.

And we can also learn from other industries like food industry and clinical diagnosis. They have matched with technologies that can detect impurities in complex matrices, even down to single

molecule detection. So for us, having doing prioritizing research, fundamental and applied research to understand how we can repurpose these technologies and develop and refine them and apply them to nitrosamine research would be beneficial to get a comprehensive understanding of a molecular mechanistic mechanism behind nitrosamine formation. And one of those technology phases is spectroscopy, optical spectroscopy.

In pharmaceutical manufacturing space in general, there has been a lot done with spectroscopy, optical spectroscopy, sensors, and pH. Having those in the toolkit now, we would be able, as a community, to expand the risk assessment moving from molecular structure-focused approach to manufacturing process-focused factors as well. How different the impact of different manufacturing conditions, how the environment would affect. Having that kind of developing that kind of framework will help to reduce the extensive testing of nitrosamine and eventually come up with a more risk-based approach, guidelines, decision trees, and strategies specific to a drug product category.

And so with that, I would like to conclude the presentation emphasizing how investing research and supported by funding mechanisms to explore utility and usage of emerging analytical technologies, process analytical technologies, and different advanced manufacturing technologies could help different aspects of generic drug product manufacturing and specifically in the nitrosamine space. Thank you.

Moderator: Thank you, Dr. Silva, and are there any questions from our panelists?

Nope, thank you very much.

Next public commenter is Dr. Amar Chittiboyina, and he is assistant director from National Center for Natural Product Research in the School of Pharmacy at University of Mississippi. Please!

Amar Chittiboyina (National Center for Natural Product Research in the School of Pharmacy at University of Mississippi): Good morning, everyone. You can hear me well, right? Thank you very much, Dr. Samuel and all the team members for giving me this opportunity. My name is Amar Chittiboyina. I'm coming from University of Mississippi, and I'd like to comment about the right now existing testing technologies or testing methodologies out there for detecting nitrosamines in pharmaceutical APIs or maybe in a complex mixture.

You know, again, just wanted to give you a brief overview. All of you know very well about this. Nitrosamines - what I wanted to tell you is close to 300 nitrosamines are known, and more than 90% of them are actually reported to be carcinogenic in various animal models. As of 2021, I know it's very old, more than five nitrosamines are actually discovered in certain medications. When it comes to formation, it's sometimes intentional, sometimes it can be very unintentional, due to various conditions, whether it's chemical or biological processes involved. And so sometimes environmental factors can also influence how this nitrosamine could be actually formed within your matrix.

The important thing after this is that many of them are actually known in animal models to be mutagenic, also reported for the carcinogenesis. The underlying information is that these nitrosamines actually degrade to form a DNA alkylating agent. As a result of that, you can actually modify the DNA. Example is diazomethane from NDMA. This is very well established. Well, those more than 1,400 product lots are actually recalled because of some kind of nitrosamine impurities existing. And this is not only just to certain class of drugs. There are many, many class of drugs, many therapeutic areas. And, in fact, FDA issued a final guidance on acceptable intake limit for the nitrosamine drug substance-related impurities.

So how do I know that? What is the best way to detect within my complex mixture? You know, in fact, the best thing is to do is remediate your processes, or you can actually mitigate some of those processes. For example, you know, nitrosamines in water, you can actually do the preliminary destruction method, or where you can do some kind of filtration as a physical removal, or you try to modify your existing methodologies so that you know you can actually prevent formation of such intermediate.

How do I know them? What level they exist? You know, there are several methods out there. Most of them are actually isolated chromatographic methods. Whether you use a gas chromatography or a liquid chromatography, you can actually reach to, you know, nanogram per liter in our echo solutions. Often these involved extraction. You take up large volumes of solutions, and then you extract tediously, and then you, once you concentrate them, you actually go through the chromatographic separation, and the separation will give you components. You can detect them. Then you can characterize them. What is actually the underlying nitrosamine impurity is.
These are the classical reagents. Often people use it the way do you? So, for example, I showed you NDMA, you break down nitrogen-nitrogen bond, and then resulting amine, you can actually quench with a fluorescent agent, so that you can improve the detection, for example, is a dansyl dimethyl amine, either, or a phenyl adduct. If you have NDMA in your drug product. Other option is that a Griess reagent is very well known, very well established. It's a highly colorimetric test, very sensitive. But here, what they do is you actually do sulfonamide. If you have a nitrosamine in the mixture, it'll goes to diazonium, diazinium, iron that actually are coupled with naphthylene to give you azo dye.

There are new technologies around there now, coming up where we're talking about a carbon nanotube-based sensors. Those sensors can be utilized for detecting a nitric oxide.
What are the problem in this? So you know that the idea? I was actually not idea I was telling you initially about a carcinogenesis right? The carcinogenic is actually coming from alkylating agent. That's very, very important. Example is a methane from MDMA. So you know, the diazomethane.
You know, a nitrosamine can be metabolically activated or thermally activated to give you this nitro. So the diazo intermediate and this intermediate can actually alkylate DNA backbone. Then you actually have a modified DNA that's the central mechanism for the carcinogenesis.
But what we are doing here, you know that you actually break down nitrosamine into two parts, one as an amine. Other one is a nitric oxide. So you have a method to detect nitric oxide using Griess reagent. You have a method to detect the amines using dansyl chloride. But the whole idea how carcinogenesis is all based on alkylating agent. Are we actually, are we actually detecting or analyzing the alkylating agent? Or we are actually completely missing that boat here.
So the current methodologies out there, we are actually looking only the amine and nitric oxide, but not anymore on the diazo alkylating, the alkyl ammonium diazomethane intermediate.
So there are, you know, the I was talking about analytical technologies out there. As long as you know what's in your mixture. If it is volatile, you can actually use a gas chromatography. But if it is non-volatile, you can actually use a liquid chromatography. As long as you know that component you're interested, you're looking at. It's very straightforward using all of these technologies.
What about if I don't know what kind of impurity I have, especially that nitrosamine impurity. So there are sophisticated technologies out there which can be utilized in a high throughput manner where you do intramolecular dye digestion. And as a result, you actually have a fluorescence off from two fluorescents on there are different chemical probes out there talking about. You know, these probes can be utilized for detecting in your process.
One example I can tell you about. Coumarin-based one. This is a fluorescent probe you can actually, people actually demonstrated the tobacco-derived specific nitrosamines using this technology.
What I was actually talking about is instead of breaking down into two pieces into an amine or nitric oxide. Is there any way one could actually develop a methodology in place where you actually keep intact of the molecule so that you can have a fluorescence off or fluorescent on.

And then you can actually characterize a whole nitrosamine fully characterized instead of indirectly testing those amine or a nitric oxide.
So there are a lot of developments on photosensitive technologies, modifications. Then people can use chemical probes using these alkylating agents with that. Thank you.
Moderator: Thank you very much. And are there any questions to Dr. Chittiboyina?
Alright, then, thank you very much.
And the next public presenter is Dr. Eric Munson. Dr. Munson is a professor from Purdue University.

Eric Munson (Purdue University): Thank you for the opportunity to talk today about some of our work that we're looking at to actually prevent nitrosamine formation. So I'll begin by just once again reminding everyone here who is probably very familiar with the fact that nitrosamines have been recalled or drugs containing nitrosamines have been recalled for various reasons. And so the presence of NDMA.
So one of the questions that we're trying to ask is, why did why do these nitrosamines form. And so one of the things that we were really interested in is looking at a paper that came out in 2020 that looked at different nitrosamine lots. And this came from this is a paper from GSK, and what they did is that they tested in 2019 different lots of ranitidine hydrochloride that been manufactured. And so you can see sort of in the red and the pink and the green three different sources and manufacturers associated with the production of the ranitidine hydrochloride. And so one of the things you can see is that some of them had very little for example, it's reported in the GSK lots had very little, others kind of were all over the place, and so one of the questions is, why is it all over the place?
So one of the things that they did is that they looked into supplier 2 that actually made ranitidine using two different processes one process was very bad. The other process was quite good. And so, and once again. Also, the ranitidine that GSK produced was also very good. So if you look at the degradation rates associated with these, the process one material had a very high degradation rate. The process two material had a very low degradation rate as well as the GSK material. And so one of the questions arises is okay. Well, what's the difference between process one and process two. Is there any way that we can sort of detect what those differences may be. In fact, the answer to that is, there is.
And in fact, the only difference in the ironically, is the fact that manufacturing processes for process one process two basically were identical except for the final salt formation or the crystallization step. So the bottom line is that the difference between the materials were identical except for how they were crystallized. If you crystallized it a certain way, you get very fast degradation. If you crystallize it a different way. You get very slow degradation. And so somehow, during that crystallization process, there must be something that actually causes the material to become much more reactive.
So we actually have some experience in this, and what I wanted just to talk about some of our experience in trying to understand these processes. So when GSK was looking at it. They basically tested all of them. Okay. And they really couldn't see any differences except for some slight differences in TGA, which is thermal gravimetric analysis and some differences in morphology. But there wasn't anything that they could actually test that would result in something that they could specifically quantify and say, this is a difference between material produced using process one versus process two, and so what we want to do is to see is there a way that we can tell why these materials have such different reactivities? And can we actually identify methods that may be able to differentiate between the two of them.

So I do a lot of work in characterization of materials in the solid state. And so one of the things that we have found through many years of research, including research with the FDA, is that the primary source of degradation in these crystalline materials is something called crystal defects.

And you can produce these crystal defects by any number of ways. One of the most common ways is actually physical transformation. So, for example, milling or grinding or compression can result in the production of these crystal defects. Another way that you can produce these crystal defects is crystallization. So when you crystallize from different environments, you actually get different properties associated with that particular material. And so one of the things that we have also shown is the fact that materials that contain more crystal defects, have faster degradation rates than materials that have fewer defects, and so the more defects that you have in these crystal materials, the faster they will degrade. And one of the things that we've been doing, and I am an expert in solid-state NMR spectroscopy. But one of the things that we have found is that you can actually measure materials in the solid state and look at these. The number of crystal defects are present in both drug substances and in drug products. And so I really want to thank the speaker from USP for basically motivating what we're talking about here. What we need to do is get that mechanistic understanding of why nitrosamines are formed particularly, not just in drug substances, but also in drug products, and also have the possibility for being able to understand when are they going to be formed and actually predict whether or not they're likely to be formed in a drug substance or in a drug product before you put them on stability. And so that's one of the things that we're trying to do.

So just very briefly. You can see, for example, we correlate this with surface area. But basically, as you process material, you can see that there's a higher degradation rate of the ground material when it's just stored for four days at 60°C. So once again, that grinding process results in a higher production of nitrosamines.

Okay from the predictive aspects of it. This is where the solid-state NMR data comes in on the left. We've actually looked at aspirin degradation. On the right was a project that was funded about 15 years ago by the FDA. Looking at gabapentin degradation, we were actually able to correlate the amount of gabapentin degradation in both drug substance and drug tablets with the NMR relaxation times. And so with that, I'd like to answer any questions that you might have.

Moderator: Thank you very much, Dr. Munson, and are there any questions from panelists? Dr. Munson? I just want to confirm, based on your discussion. So your proposed research priority is to explore the API. The impact of API crystal defects on the nitrosamine formation in solid state.

Eric Munson: Yes, both the crystallization as well as the processing aspects, because processing could also produce crystal defects, and both of those can result in faster degradation rates.

Moderator: Thank you very much. Another question, if I may please go ahead. So I understand that ranitidine is from the paper that you cited is a little idiosyncratic, in that it's believed that it could sort of nitrosate itself. Right? So do you think this effect of crystal defects is applicable to other APIs? Or do you think it's something that's related to that sort of peculiar behavior.

Eric Munson: No, it's related to basically to everything. So, for example, if you were to have a material where you had processed, it produced more of these crystal defects, for example, at the source of the reaction was due to the presence of nitrites. You're exposing more of that drug substance to those nitrites that can then have a faster degradation. Right? So like a surface area. So yeah, actually, exactly. In fact, that's what this plot is here is surface area. Okay? Thank you.

Moderator: Thank you very much, Dr. Munson. And additionally, we still have two virtual presenters for the public comments. There are Dr. Rafael Nudelman from. He is a senior director, impurity expert in Teva Israel, and the second virtual public commenter is Dr. Marco Trampus, and he is a scientist in early developments from Sandoz in Slovenia.

And for the questions, all the questions for the virtual public commenters. And please hold until the panel discussion.

Rafael Nudelman (Teva Israel): So my name is Rafi Nudelman from Teva pharmaceuticals, and I will be presenting a short public comment on the topic of confirming correlation between mutagenicity and carcinogenicity potencies, and NDSRIs.
As a disclaimer. The opinions expressed in this presentation are mine, and do not necessarily represent Teva pharmaceuticals.
NDSRIs do not have carcinogenicity data, and most probably will not have such in the future. Approximately 25% of NDSRIs as presented in the Lhasa complex nitrosamine data sharing initiative are positive in in vitro mutagenicity assays, and thus are likely to be positive also in vivo. But the question is how can positive in vivo mutagenicity data be used to set limits for NDSRIs.
Currently, according to the ICH M7, question and answer 7.2. It is clear that in vivo studies for mutagenicity cannot be used to set compound specific impurity limits, however, they can be used as part of a weight of evidence on a case by case basis. And the reason for this is that in vivo gene mutation assays alone are not validated to directly assess cancer risk, because the endpoint is mutation and not carcinogenicity.
So where does it take us to? With nitrosamines, positive in vivo mutagenicity data essentially has no use. And the possible consequence of that is that NDSRIs that are positive in the enhanced Ames test will essentially not be tested in vivo, because they'll most probably be positive in vivo, and therefore there's no use of that in vivo data. And all this may lead to serious market shortages.
Again, people follow up working group within the HESI, GTPC. MGRA working group just recently presented in the HESI's annual meeting just back in April. Goals for this sub working group, and the goals generally state that in vivo mutation data for seven model nitrosamines with robust carcinogenicity data would be tested to confirm the TGR assay as a surrogate for carcinogenicity, for known nitrosamines.
Furthermore, this data will provide information and comparators for assessment of relative potencies, namely, assessing mutation frequency relative to the TD50. Concurrently, they will also collect tissues for error-corrected NGS to show consistency with TGR for the endpoint and provide data to support a more amenable methodology.
And several companies got together, and each one tested a different comparator or exemplar nitrosamine in the TGR studies, either in MutaMouse or in Big Blue assays, and the data was contributed to the HESI GTPC. And here we see the results, that of the data that has been contributed for six exemplar nitrosamines, namely, NDMA and NDEA, and NDEA was done both in MutaMouse and in the Big Blue assay, nitrosomorpholine, nitrosopyrrolidine, nitrosopyrrolidine and nitrosodiethylamine.
And plotting the data coming out from the in vivo study namely, after a benchmark dose analysis and derivation of the good confidence interval the BMDL and BMD L50s were compared to two sets of TD50s. Either the most sensitive TD50s coming from literature or from data that's posted in the Lhasa data, carcinogenicity database or the health authorities TD50s that were used to derive the published acceptable intakes for each one of these comparators.
And if we plot the BMD L50 versus different TD50s. We get essentially two different plots. Here we see the blue plot which is using the most sensitive TD50s against the BMD L50, and the orange plot which is using the health authority's TD50s also against that same BMD L50. And what we see here is a very, very nice correlation between the in vivo mutagenicity and carcinogenicity with very high r square values.
When using the actually most sensitive TD50 that we in the industry do believe, is the more accurate TD50 to use. We get a very, very high correlation with an R square of 0.94 this correlation is quite sufficient in order to essentially conclude that there is a very strong potency correlation between the in vivo mutagenicity data coming from these exemplar nitrosamines and their respective TD50, namely, carcinogenicity potency.

The following showing this strong correlation between the TGR data and the carcinogenicity data and enabling a potency comparison for these exemplar small nitrosamines, further analysis is planned to be done with the tissues coming from the in vivo studies using error correction NGS, which would hopefully additionally add to the validation of this analysis, and our proposal to FDA is to even further confirm this methodology by taking exemplar NDSRIs that have tested positive in in vivo mutagenicity assays and test them in transgenic carcinogenicity studies with the hope to show this same correlation between the potencies and overall the goal would be to show a very strong correlation between in vivo mutagenicity and carcinogenicity, and to enable the use of positive in vivo mutagenicity in setting acceptable intakes for NDSRIs. Thank you. And if we have any questions we can always follow up for following this meeting.

Marco Trampus (Lek Pharmaceuticals, Sandoz): Hello! To all the attendees, both in person and virtually. My name is Marco Trampus, and I come from Slovenia. I work as a scientist in Lek Pharmaceuticals, which is part of Sandoz, and I am very honored to be able to present you our research proposal today on the topic of nitrosamines. The proposal is entitled Individual Studies on metabolic stability of NDSRIs and properties of reaction metabolites, and it was prepared, together with my colleagues in Nagorno yacht and Zinca.

As you know, it supports a higher acceptable intake limit for nitrosamine drug substance related impurities than the one it is calculated by the CPCA approach. The agency requests either compound specific data, such as mutagenicity assessment via enhanced Ames test, or read across approach based on a surrogate with robust carcinogenicity data.

Now it is stated that additional safety data may be required by the agency to support this higher acceptable intake, meaning that the negative enhanced Ames test read across approach, are not considered sufficient evidence.

Look at the mechanism for the mutagenicity of nitrosamines. We know that nitrosamines are metabolically activated by drug metabolizing enzymes such as cytochrome P450. This metabolism usually takes place on the alpha carbon and via a series of reactions. Reactive metabolites, such as diazonium cations or carbocations are formed. This species may then further react with DNA, alkylating it on the guanine.

The reactivity of this metabolites is known to be well dependent on the structure of their substituents, meaning that the initial structure of the nitrosamine matters greatly when it comes to deal with the mutagenic potential.

Last year Agency published the results of a comprehensive investigation. It was aiming to evaluate the risk of a model NDSRI and to identify optimized testing conditions for its individual safety assessment. The model NDSRI was starting, using various experimental systems, which is different bacterial strains, and also different human cell lines, either with or without S9 pre incubation to evaluate mutagenicity, genotoxicity, and to identify specific human enzymes involved bioactivation.

However, the study did not address at all any kind of chemical analysis of the metabolically activated NDSRI to the extent of metabolism and any kind of identity or metabolites. The amount, but was not analyzed at all. Furthermore, the stability or the reactivity of this metabolites were also not addressed in the study.

We would like to propose to the agency to prioritize research and provide guidance on in vitro studies, on the metabolic stability of NDSRIs on the generation of reactive metabolites. And the reactivity of this metabolites. Specifically, what type of experimental systems need to be used to evaluate whether alpha hydroxylation, metabolism takes place or not with the absence of detection of these potentially pathogenic metabolites seen as valid evidence, justify higher acceptable intakes. And what would be the required analytical limit of detection also with a quantitative comparison of generational reactive metabolites between NDSRIs and a surrogate complement, the reader crossed approach to justify higher acceptable intake limits.

Furthermore, the agency could also provide some guidance on understanding the stability of the reactive metabolites and the reaction kinetics with DNA, as already mentioned in larger

molecules due to difference in electronic and steric effects. may have very different reactivity compared to well evaluated small nitrosamines. These metabolites may be more stable or less stable, meaning, of course, that mutagenicity risk in this case could differ considerably.
Thank you for your attention. I'm open to any questions, and looking forward to the discussion.
Alright, that's all for our public comments, information, and thank you so much for our the information from our public commenters, and we really appreciate the sharing of your thoughts on the research priorities with us. And now I turn it over to Sruthi.

Moderator: Thank you. We're going to begin our presentations from our speakers. Our first speaker is Dr. Kevin Cross. He's the Vice President of Regulatory Science at Instem, where he's the principal investigator of FDA and Instem research collaborations. I just want to remind everyone that detailed bios are available on the meeting web page. So, Dr. Cross.
Kevin Cross (Instem): Thank you, Dr. King and Dr. Lu, for inviting me to present today.
So I'm going to talk a little bit about N-nitrosamine SAR modeling of carcinogenic potency. This will review some of the comments that have already been presented, particularly by our last presenter, Dr. Trampus, concerning SAR potency or carcinogenic potency of nitrosamines, why they're potent and how we can leverage structure activity relationships to understand and quantify that potency.
So N-nitrosamine impurities in drugs is well known problem. And it's really been discussed, I think, already quite at length here. Important to note, this is an ongoing problem. But it started back in 2018. So it has been going on for quite a while.
So acceptable intake limits for nitrosamines have also been discussed here. Really, there have been now several methods that people can use to assess acceptable intake limits for nitrosamines. I'm not going to focus on the experimental ones. But today here, I'm going to focus on the structure activity relationship considerations that can be used in deriving acceptable intake limits for N-nitrosamines. And these are basically two items. Originally, the read across of known robust carcinogenic data for nitrosamines was used and had been proposed being used for driving acceptable intake limits. In 2023, in August health authorities issued a document called the Carcinogenic Potency Categorization Approach or CPCA, which allowed the derivation of a set of acceptable intake limits for nitrosamines based on structure alone.
So I'm here to point out that there are several different methods for assessing nitrosamine risk, including the enhanced Ames test which has been touched upon to read across, which has been mentioned in vivo testing which has been mentioned. But I'm gonna focus here on the CPCA, which is a structure, activity, relationship approach to establishing AI categories, not specific TD50 values or, highly precise AI values, but categorically approach to determine whether you have a high or low potency nitrosamine. And again, it's really a quick approach. That has been developed by FDA and other health, Canada and EMA, others. That uses simply a two dimensional non stereochemical structure as input. So there's no animal testing or in vitro testing involved. So why do we want to use SAR approach to assess N-nitrosamines? Well, as been mentioned here, the adequate data for many NDSRIs, the large drug, like substance, related impurities are not available.
Also, historically, smaller molecule nitrosamines have been primarily studied, and the relationship and doing structured activity relationships and read across from those is not been very adequate, because adequate analogs from many nitrosamines are not available and read across. Arguments themselves are hard to justify to regulators. Lastly, as been mentioned in vivo mutagenicity testing or carcinogenicity. Testing is very costly, very time consuming. And there's been a real effort to reduce animal testing and use of animals and carcinogenicity testing. So we're expecting that we're gonna see a very limited production of carcinogenicity two-year assay data in the future.

So what can a SAR approach provide to us? Well, we could look at this to provide gap filling where there is no data available for carcinogenicity studies or enhanced Ames studies. But we can look at the structural features of known compounds with known data, and read across from those from a substructural standpoint to assess a structure, activity, relationship. So these are identification of the structural features and physical chemical properties that may increase or decrease potency, either mutagenicity or carcinogenicity.
Okay, as was mentioned earlier. This is an overview here now of the principal mechanism for nitrosamines that was mentioned. Potent nitrosamines are those that are result of metabolic activation. And this includes the activation drive a pointer here. Let's see, how does this work? Okay, that points to this screen? All right.
Alright. So if we look at the far left there, one of the dependencies for the metabolism is having available hydrogens for abstraction. That result in a carcinogenous result in metabolism. Hydroxylation at that particular form point. So alpha carbon hydroxylation is the main source for metabolic activation and potency of nitrosamines, particularly high potency nitrosamines. But it should also be noted that two other, at least two other things play important roles after the hydroxylation step itself. The diazonium ion is formed, and this may or may not be stable, as was mentioned by the last speaker. A very stable. Ion doesn't react with DNA. A very unstable ion is solvated away. So there's a sweet spot which most of the or higher levels of potency can occur. Also, there are issues. You know how the DNA adduct is formed where it is formed in terms of how potent the actual mutagenicity will be, or if it will be mutagenic, and there are cause, of course, DNA repair mechanisms as well.
So the things to remember are that what really causes carcinogenic potency around nitrosamines is the extent of metabolic activation, stability of the formed diazonium. Ion. The ability of it to form DNA alkylations and stability of the DNA of adducts, and then any repair of that. So these events, of course, are dependent not only on the chemical structural features around the nitrosamine moiety, but also the physical chemical properties of nitrosamines.
So as I mentioned in August last year, the CPCA category potency categorization approach was developed. To roughly categorize nitrosamines into five different categories corresponding to four different AI limits, not going to go through a great deal of information on this. This is available on the website, and there's a paper that is on the web available from health authorities, which describes us in considerable detail.
But it should be noted that there are, you know, a wide range of potencies that are predicted from 26 and a half nanograms per day or 18, if you're EMA up to 1,500 nanograms per day, and the last two categories are the same.
So what are the issues going forward when we're talking about? How to really assess nitrosamines going forward. Where does this approach help us? Where are there some limitations.
So what we should notice is that if you have features which denote carcinogenicity, you can have a compound that gets put into a high potency category. If you have features which adequately deactivate or reduce metabolic activation, you end up with compounds that are in the low potency categories 4 and 5. However, there, if you don't know much about the compound in terms of the, or as if the model doesn't know much about the compound in terms of its features. Whether they're activating or deactivating, they can still end up in a fairly high potency default category.
So from a industry sponsor standpoint, there are, you know, two issues. And there's and they're associated with each end of this category. One, some of the compounds that are some of the drugs that they're predicting NDSRIs for fall into a very high potency category. But there's really not a lot of information supporting the type of chemistry that's involved in that. And on the other end, for the low potency category. There's a question about whether those compounds should be limited at 1,500 nanograms per day, or if the value should go even higher, the 1,500 is there corresponding to the TTC level in the ICH M7 guidance.

So those are our two initial considerations for ongoing research.
But to delve a little bit closer. And to the CPCA approach, it's basically a consideration. We're looking at the availability of the hydrogens, as I mentioned, around the nitroso group. But then, if there are any deactivating features chemical features immediately adjacent to that moiety. Or if there are any activating features that could actually increase the metabolic activation. And then these are scored. But basically looked up to see if what the if those features exist. And and then there's a score associated with each feature those are. Then, added simplistically, they're all added up together to come up with a potency score which then ends up with an overall category. There's actually an additional algorithm that is run in a prior before the score is actually calculated. That's shown here. We won't go into detail on that. But that's really just assessing how many hydrogens may be available for abstraction. And actually, if you even need to re run the program at all.
So the CPCA approach has a number of algorithm features. I've listed them here. It's not really necessary to go into them. But it's important to say that most of the features are involved in identifying a reduction of metabolism by electron withdrawing groups. There's feature which is in present which increases are known to increase the metabolism and the potency. There is also feature there, that one feature that's related to the solubility. That's the presence of the carboxylic acid anywhere in the compound. And there is one feature there currently that addresses increased ion stability which would result in higher potency in this assessment.
So if you, if you, this is an example of this. You could the dere, the various software vendors who support computational chemistry, computational toxicology, like my company and others. Have. Now, you know, I made this calculations available through commercial access. And you, part of that is, you actually see how the different deactivating features will affect the overall potency, calculation how the score is derived. Here again, this is pretty hard to read, but basically, you see a list of features that are highlighted on a particular compound, and their score associated with them, and at the bottom they're just added up to come up with an overall score which isn't translated into an AI limit.
Okay, so considerations for the future. Where you know, where? Where should we go with this. There. The list of deactivated features which we have is a good start, but it's not by any means comprehensive. I can say this because I wrote the paper which a lot of that was drawn on, and I can tell you it's not as comprehensive as it could be. And it could be expanded into include similar deactivating features. Additionally, we can look at chemical features that may stabilize the diazonium Ion or the carbenium ion, and maybe activating, or maybe inactivating, as our last speaker actually mentioned. So there's work to be done there on making the predictions more granular or more or and more accurate also. There's certain domain that every good model has, and we want to make sure that we are able to include in the calculation, the presence of other common drug features that are present in NDSRIs. This included things like ketones, alcohols, microceptors, and the benzylic features.
There's more a lot more of this description, of course, again available on the forthcoming paper paper and proof as well as the website. So there's also work to be done on assessing the ability of bulky carbonyl ions to form DNA adducts. And whether those adducts after they're formed, if they readily degrade or how they may be repaired.
So one approach to trying to address these knowledge gaps has been for us to address, using quantum mechanical methods, and as opposed to experimental methods, so that we might further our knowledge and improve the process in a speeding exit and a speedy and cost, effective fashion. So here.
Okay, here, we're talking about designing in silico experiments. We have, we want to ground those experiments with compounds that have experimental data that we can validate, that the that the QM. Calculations for things like bond association and energetics, kinetics of forming the extent of metabolic activation for and also for identifying the stability of the forthcoming carbenium ions. We want to be able to validate that we want to be able to validate the current

rules. That's our rules that are used in the CPCA. To make sure we understand how they're being used as well. But also, since it's in silico, it gives us a chance to explore a lot of areas quickly that we may not be able to do from a experimental standpoint. And when we learn from one experiment, we can continue to another experiment right away. We can pivot based on the current knowledge that we've learned from our last experiment very quickly, whereas in a experimental setting that's very difficult to do.
So we're looking at providing performing. And this work. And this was work that has been done in the HESI nitrosamine team. That Dr. Chen mentioned earlier today. And we're looking at doing more work in validating CPCA. Refining and providing additional structure, features as well as considering physical chemical properties for improved and accuracy of predictions and expanding the domain. Applicability to predict additional nitrosamine classes, because there are some other compounds other than N-nitrosamines which are in the ICH M7 cohort, that are concerned which are not covered by the current. CPCA algorithm.
Okay, so there's an opportunity here for GDUFA, the generic drug team at FDA to become more involved in the HESI team studying nitrosamines, and we have already participation from the health authorities in this as well as a substantial number of industry and collaborative groups. So here's, for example, is a list of 26 private industry participants, 10 government regulators and 7 academics and NGOs.
So with that, I will stop and thank you for your attention.
Moderator: Thank you, Dr. Cross. We're now going to welcome Dr. Xilin Li. He is a toxicologist at the National Center for Toxicology at FDA, and he will be speaking about assessment of mutagenicity, of nitrosamines and NDSRIs. Dr. Li.
Xilin Li (FDA): Thank you for the introduction. Dr. King. Today. I'm very happy to be here to give you some updates on FDA NCTR's research project, assessing the mutagenicity of nitrosamines in, particularly on NDSRIs.
So today. My talk will cover three aspects. First, is our major effort optimizing the Ames test to detect the mutagenicity of nitrosamines. Second are in vitro mammalian cell and follow-up studies to further characterize the region of nitrosamines in human cells. And third, I'll briefly talk about our collaboration with HESI, GTPC.
So in the past three years. One of our major efforts at NCTR is to optimize Ames test for nitrosamines. The reason why we're doing this is because historically conducting the Ames test has produced inconsistent results. We think, the standard Ames test may not be sensitive enough to detect the mutagenicity of nitrosamines. Another issue is that very little is known about the mutagenicity of NDSRIs in the Ames test. Considering the structural diversity of these compounds, we really don't know how standard Ames test can perform on this particular nitrosamines. Therefore we think there's a need for enhanced version of the Ames test that can detect the mutagenic nitrosamines to the greatest possible sensitivity that will increase FDA's confidence in the test findings.
You know, there are a lot of parameters you can play with in the Ames test. But based on our previous experiments in the in the literature search. We developed a strategy to test the most promising protocol choices on a series of nitrosamines, including NDSRIs for the tester strains. We didn't modify them. They are pretty standard. They're obviously guideline type of five tester strains. I think we put our major efforts in the metabolic activation in a standard Ames test. There used to be 10% rat liver S9. But we we incorporated hamster liver S9, and we boosted up the concentration from 10% to 30%. So we have a total of five metabolic activation conditions for each nitrosamine for the pre-incubation time. We included 30 min and 60 min to compare. And for the solvents we always use water as a priority, followed by acetone, methanol and DMSO will be our last choice.
So our initial trial. Involved the testing of 12 small molecule nitrosamines and 17 NDSRIs with different chemical structures. This testing strategy will give us a total of 50 test combinations for each nitrosamine.

I'll briefly give you the summary of the results. First of all, a total of 18. Out of the 29 nitrosamines were tested positive. This include 8 out of the 17 NDSRIs. And we further break down the 50 test conditions into their metabolic metabolic activation conditions into five columns. In this heat map, you can tell that in this heat map, the basically the darker the color is. The mutagenicity of nitrosamines tested out in more tester strains and pre-incubation times. You can see, for example, that only two black colors in the heat map. They indicate this compound, NDSRI of ranitidine. Here is tested positive in all five test restraints in both pre incubation times. And these nitrosamines were arranged by their molecular weight from small to large, and you can tell that the the several of this largest nitrosamines NDSRIs. They all tested negative in the enhanced Ames test, which indicate that the the molecular weight of these compounds may affect their mutagenicity in the in the Ames, test

Several general conclusions or observations. Out of this study pre incubations with hamster S9 were generally more effective than the rat and pre incubations with 30% S9 were generally more effective than 10%. And if you use 30% hamster S9, you can detect out all 18 of the positives. But if you use 10% you will lose two of them. And these are NDIPA and NDBA, two dialkoxy nitrosamines. I just want to point out that. NDIPA is a weak roading constituent here. And if you use only rat liver S9 as as indicating the standard assay. You're gonna lose additional one which is nitrosarcosine.

We further look at the performance of different Ames tester strains and several observations. Here are TA1535, and WP2 uvrA are the most used for tester strains to detect the region as if nitrosamines and known nitrosamines we're uniquely mutagenic in TA100 TA98 or TA1537, which means, if you combine the TA1535 and WP2 uvrA you can detect out all of the positive, nitrosamines. And NDSRI of ranitidine and NDSRI of sarcosine here were uniquely positive in the WP2 uvrA. And we in terms of the pre incubation time. We compare the 30 min versus 60 min, and there's no major differences between there.

And in addition to the Ames test, we also conducted a bunch of individual mammalian cell-based follow-up studies. So to further characterize the mutagenicity of nitrosamines and NDSRIs in human cells. So the two cell system we used here are human lymphoblastoid TK6 cells and HepRG cells, and we see them as complimentary with each other for the TK6. We. These are pretty pretty standard genotoxicity in testing cell lines, and we genetically modified them to to indulge us to express one of the human CYPs. And we conducted standard genotoxic acid and mutagenicity assays on them. And for the HepRG cells they are more like human primary hepatocytes. They express both phase one, and then phase two human enzymes endogenously

One of the major questions we would ask is, how does the mutagenicity results generated from enhanced Ames test compared to the ones generated from the human cells. So, using the TK6 cells, we were able to compare that. As you can tell, that for these 12 NDSRIs, which have pretty diverse structure, features the mutagenicity generated from the enhanced Ames test is highly consistent with those generated from the genetically modified mammalian cells. This really supports the use of of enhanced Ames test for hazard identification on these NDSRIs and using the genetically engineered TK6 cells, we were able to identify the specific human CYPs. That account for the bioactivation of each one of these positive NDSRIs. From this study it looks like the the CYP2C19 and 2B6 appear to be very important for their bioactivation. This is actually different from the small nitrosamines where CYP2E1 appears to be the most important one.

So for the HepRG cells. It's much more difficult to measure the mutagenicity because they don't divide that much, and there's no standard. FDA compliant the standard assays to measure the mutagenicity to overcome this hurdle. I think my colleague, Jian and Carol. They. They adopted this error correct, NGS methods to sort of measuring the mutagenic frequency in these cells at the whole genome level. So basically, they use this 2D and 3D cell cultures. And they treat them with NDMA as a model compound, and they collected genomic DNA for error corrected NGS.

So the particular method we're using is called PacBio high fidelity reads, I'm not going to go to the details of this. But this is our preliminary study basically shows NDMA may induce the dose dependent increase in the mutation frequency in this HepRG cells. In both 2D and 3D cultures. Currently, we are optimizing the test protocol and hopefully increase the dynamic range and sensitivity of this particular methods for NDSRIs.
And last, I'll briefly talk about a collaboration with HESI. I think Kevin and Connie have already presented this slide. In particular, we're mainly involved in the first two goals of this program, which is Ames optimization and in vitro assay strategies.
So the HESI set of the nitrosamines tested in the Ames test is quite different than the ones we tested at NCTR. Where we mainly focus on the NDSRIs. But the HESI compounds address this from a different angle, because all the 32 nitrosamines chosen have previous carcinogenicity data. And you can tell there are 11 negatives and 21 positives for the carcinogenicity, and they distributed quite well into different CPCA categories. I think we are assigned to the six HESI compounds, and to our surprise, two out of the six compounds are actually direct, acting mutagens, which are not that common for small nitrosamines
So some general observations or conclusions. All of this preliminary collaborative studies that enhanced Ames protocol is really high, really sensitive for predicting the carcinogenic nitrosamines. I think the sensitivity is about 95%, which is really good for a single assay. And again, hamster S9 was more sensitive than rat and 30%. Hamster, liver S9 improved the sensitivity and 30% hamster S9 did not significantly decrease the specificity. The overall accuracy of the assay is around 77%. This is likely because of the low specificity. There are many reasons for that. One reason I want to bring up is the false positive compounds that are direct acting, which means they does not require the metabolic activation to exert the mutagenicity in in bacteria, and they are not carcinogenic in animal studies. So we really need to focus on these compounds and see why they're giving a false positive results
In terms of the in vitro mammalian cell studies. FDA NCTR is sort of taking the lead on this, particularly my colleague Carol, is in the steering committee of this subgroup. The overall goal of this of this group is to identify alternative in vitro approaches that is, follow-up assays to the Ames test findings. Ideally, the assay results should be informative for in vivo study design and for potency ranking in the weight of evidence. We're currently focused on HepRG cells like Connie just mentioned. And we're working on protocol harmonization and endpoint development, particularly for error corrected NGS.
And to a very small degree we're involved in the in vivo part. I think industry is taking the lead on the in vivo transgenic rodent assays testing particularly, we are only providing expertise in the error corrected NGS methods as an alternative to transgenic assays for NDSRIs.
In terms of the future directions. For the enhanced Ames test, although the sensitivity is is very high at this point that we're satisfied with this, we ask the question whether the enhanced Ames test can be further enhanced by changing the pH. I think people in here all know that the nitrosamines NDSRIs are formed under acidic conditions. We're testing whether the lowering the pH may help, you know, increase the assay sensitivity. Furthermore, and this is particularly for those ones that are very weak in the enhanced Ames test and all those, and for those which are equivocal in the enhanced Ames test.
And second, we were categorizing the mutagenicity of NDSRIs in the human cells using error corrected NGS. This is particularly for HepRG cells. Think this will provide some mutagenic information on their mutagenicity. And third we're doing in vivo transgenic, transgenic rodent gene mutation, assays particularly on the lacZ genes in-house. These are Big Blue rats studies. And we're using a selected NDSRIs. Of course, we also want to compare the in vivo mutagenicity results between the traditional transgenic rodent assays and error, corrected NGS methods at last. We always get to ask this question whether we should do any carcinogenicity studies. What I can tell you is that it's under discussion right now. We're really hoping to have carcinogenic endpoints to sort of verify what we already generated in the Ames test, in vitro

studies and in vivo mutagenesis, study, and sort of want to build up this connection between mutagenicity and carcinogenicity.
Just want to, thanks to all the contributors to this project, particularly Bob Heflich is our NCTR. Leader in this, and Aisar and Tim. They are the CDER leaders of this project. Thank you.
I'm going to start by thanking the organizers for the invitation to participate in this meeting. I would say. Oh, okay. Thank you, Dr. Li.

Moderator: Our next speaker is Dr. Ian Ashworth. He's a principal scientist in chemical development at AstraZeneca. He is going to be delivering his presentation virtually. Dr. Ashworth, whenever you're ready. Thank you.

Ian W. Ashworth (AstraZeneca): Thank you for the invitation to speak to everyone today. I'm going to share with you the biased kinetic model that we've developed and used to support nitrosamine risk assessments in relation to solid drug products. Let's see if that works. That's better. You've got me now showing a picture.
Can I have the next slide, please? Back one.
From a drug product perspective, the common presence of amines and nitrite means that amine nitrosation has the greatest relevance in terms of routes of forming nitrosamines within the context of a drug product. So that will be my focus.
I'm going to give a bit of background on nitrosating agents and amine nitrosation, touch briefly upon the utility of nitrosation experiments to assess complex structures because you don't always form nitrosamines when you nitrosate things. I'm going to then move on to the solution phase kinetic model that we originally developed to support drug substance nitrosamine risk assessments before moving on to consider how does nitrosation occur in drug products? And therefore how might we set about understanding it, rationalizing it and possibly predicting it? And finally, I'll discuss the biased kinetic model that we have developed.
Final point is, yes, synthetically, there are ways of making nitrosamines that don't start from an amine. Actually, from a drug product context, they're relatively low relevance because you're not likely to have those species present. Next slide, please.
In the presence of nitrite from the excipients, there is potential for nitrous acid to be present, which means you can have the potential for nitrous acid-based nitrosating agents under the right conditions, which is relatively low pH. At high pH, formaldehyde can catalyze nitrosation by nitrite. This is a pathway that has been validated in solution, but it hasn't been validated as a real pathway within the context of a pharmaceutical product.
Present if you expose secondary amines to atmospheric NOx, you have the potential to get nitrosation because NOx contains traces of N_2O_4 and N_2O_3, which have been shown to be perfectly competent nitrosating agents.
This is likely within drug products that some of the nitrite that we detect analytically when we analyze for nitrite is actually present in the form of alkali nitrites. At low pH, contact and acidic conditions, they behave just as nitrous acid-based nitrosating agents. They degrade to give you nitrous acid. At high pH, there is a direct pathway for an alkali nitrite to react with amine. But it's a really inefficient pathway, and it has really extreme geometrical constraints that make it unlikely to be a high yielding pathway within the context of a solid formulation.
Now, one thing that can be said when you convert nitrous acid into N_2O_3, so you dimerize it, protonate it to generate nitrosonium cation, or react it with a non-basic nucleophile in the presence of acids to generate nitrosyl halides such as nitrosyl chloride, the one shown—all of those reactions between the free-base form of the amine and the nitrosating agent show extremely high rate constants occurring. Well, they show diffusion-limiting behavior which has been taken as indicative of reactions occurring when encounter occurs. That's in contrast to the reaction for the alkali nitrite talked about some minutes ago. Next slide, please.

Right, so most APIs are structurally complex. They have the potential to form a range of products upon nitrosation. And so nitrosation experiments provide a route to determine if an amine or nitrosatable to form a nitrosamine, including actually identifying is my amine what I will describe as an activated tertiary amine. Because the majority of tertiary amines nitrosate slowly because they first have to dealkylate to make a secondary amine, which then nitrosates. There is a small number of molecules which, when you nitrosate them directly, eliminate a nitrosamine from the nitrosammonium, and you make—and can we go back, please?

And that gives you a different risk, and a nitrosation experiment will tell you that because you'll get a similar reactivity to the reactivity you would expect from a secondary amine. And that's what you're trying to find.

Dialkyl anilines fall somewhere in between the two. And they're worth doing the experiment on, because occasionally they nitrosate on the ring rather than nitrosating. And if you do get multiple products from your nitrosation experiment with doing the ones which are described in the reference at the foot of the screen, you can actually follow up with experiments using sub-stoichiometric nitrous acid, because that will give you a picture about what will my product be when I have trace nitrite. If you're still getting two things? Well, yes, you have two problems. But if you get only one thing when you're down with trace nitrite, then that's saying that's really where I need to focus from the point of view of my risk. Next slide, please.

Now, nitrous acid-based nitrosation chemistry has been thoroughly studied in published literature, and that work provides a basis for understanding and actually prediction. So reactions between the free base form, secondary amines and nitrous acid-based nitrosating agents show features which are consistent with them being diffusion encounter-controlled processes. They're insensitive to the structure and the basicity of the amine. They show very low activation energies.

And that means that actually a general model for dialkyl amines is possible because they all show pretty much the same reactivity. What differs is how much of the amine is in the reactive form as a function of pH, so you do have pH dependence. So, my little schematic—pKa of your amine still matters.

To complete the model, yes, you need pH dependence, speciation of nitrous acid as well. And you actually need to calculate those as a function of the pH for wherever you're trying to simulate, because it's the actual concentration of nitrous acid that's present in the rate laws over these arrows, and it's the free base amine concentration, not the total amounts that are present. So you'll have a pH-dependent fraction term.

And then what the model consists of: you've got the kinetic observed rate law. You've got a rate constant, and you've got an equilibrium constant for the formation of nitrosating agent in the case of N_2O_3 and nitrosyl chloride. Data for nitrosyl bromide is also available. There's just a limiting rate constant used in the case of protonated nitrous acid. That's very rarely a pathway, but it is occasionally at very, very low nitrous acid concentrations. But for a given scenario, calculate the initial rates through all of those pathways and add them together to give you a total initial rate.

If you just multiply that by time, in the simplest sense, you have a simple zero-order model which will over-predict because it's not making an allowance for reagent consumption. But you can implement that in Excel without any specialist software. If you want to do a full simulation that takes account of the decrease in reagent concentrations, then you need some form of kinetic package which is capable of integrating differential equations to run the model.

The model will should over-predict over reasonable time scales because it makes no allowance for the one thing that we do know about the behavior of nitrous acid in solution, and that is its decomposition, whereupon it makes nitric oxide which is lost from the system.

Next slide, please.

And this is a brief "does it work?" So this is data from some of the studies carried out within the IQ consortium, using 4-phenylpiperidine, which is an amine where there was—and when we did

this study—no data in terms of its nitrosation. We did relatively simple experiments. This is part of the development for the analytical method, because we use it as a model compound. But we basically incubated the amine in the presence of nitrite at a number of different pHs for a finite period of time—24 hours, 25 degrees C—and analyzed for how much of the nitrosamine was present at the end of that time.

The dots are what we observed, and the line is what the model based on the publication on the preceding slide will give you, allowing for the pKa for phenylpiperidine. And the model is doing what it's designed to do, which is over-predict.

We did a similar thing with diethylamine, and there we observed 2.3% conversion to nitrosodiethylamine versus the complete reaction you would have expected. And that's because this is actually a very concentrated solution of nitrous acid. We're quite hot, but in nitrous acid terms, and decomposition is a significant factor. But it's not doing too badly.

Good job. I was just about ready for that jump. No, I'm fine with the one it moved on to, thank you. I'll have next slide, please. I do apologize for the slide jumping. They appear to be using the timings which shouldn't have been on it.

Now, thinking about a solid drug product. How does nitrite present in our excipients meet an amine present in drug substance, either as the drug substance itself, or as an impurity in the drug substance, and undergo a multi-component reaction? Because to some extent you've got to turn that nitrite into a nitrosating agent which is going to involve some form of acid-base chemistry. It's then going to have to find another component to make a nitrosating agent, and then that's going to have to meet the amine, which may, if it's a salt, have to ionize first to get to the state it needs to be in.

And at this point we've reached the conclusion it can't at a surface contact between two particles. That just isn't a logical thing that could happen. But we know the reaction does happen. So how? And have the next build, please.

Our hypothesis: it doesn't occur at particle contact. It's a solution-based process because you need a medium to allow it to occur. And it's occurring within the saturated solution layer present within the product which bridges the particles, enables the components to come together. And now there's precedence for this model in terms of studies of pH-sensitive degradation in pharmaceutical products. And it seems like this is a reasonable method for describing nitrosation as it occurs within the context of a drug product.

And I'll come to the bottom reference again on the next slide. The bottom reference relates to the actual data. Next slide, please.

Sorry, slide after. How do you apply such a model? Well, you have some things that you should know, because if you started thinking about your drug product, and you're trying to think about the risk you have that you're going to make nitrosamine in the drug product. Hopefully, one of the first things you'll have done is find out well, just how much nitrite is the daily dose going to contain? And how much amine? And when I consume the limiting one of those two components, am I going to make enough of a nitrosamine to be in excess of the acceptable intake? Because in some cases you don't, depending on the product, thinking here with things like inhaled products where the doses are extremely low.

So you're probably going to start with a total nitrosation calculation. So you should know how much nitrite you've got and how much amine you've got. And from the context of trying to simulate nitrosation within that saturated solution layer, what you need to know is how much of them is available, and that's an unknown.

And so pragmatically, we took the conservative approach, which is to say, well, if we assume all the nitrite that we believe to be present is available, and that all of the impurity amine that's present is available, we can't be wrong in the sense that it's going to be making us predict less that could be there for the drug substance.

You can make an estimation based on its saturated solubility. Going to one of the earlier speakers this morning, how you've processed the substance does matter there, because, of

course, if you've generated a lot of defects, or what some of my colleagues would term as amorphous material while you've done that processing, you've got an enhanced solubility situation. So that may or may not impact you, but you can start from a saturated solubility model for the drug substance.

Next build, please.

So one of the unknown parameters we have is, how much moisture do we have in there? What's the volume? And normally, for a product, you'll have some idea about how much moisture you have in the product from the analysis you've conducted on stability. You also may be able to put that together from moisture isotherms for the product to tell you, well, at a given humidity, how much moisture will that product have absorbed? And you can model that versus pack as well if you're using the appropriate predictions. But you get to water content for the formulation. The most conservative thing to do is if you have multiple water contents is use the lowest, because that will give you the highest reactant concentrations and therefore the highest predicted rate. Next build, please.

The final thing that you really need to put this together is you need to know what the pH of that saturated solution layer within the product is because pH is a really important parameter when it comes down to predicting rates of nitrosation. Now you can measure the pH of a slurry of the relevant blend of materials for the tablet in a low volume of water. If you do that with several low volumes of water, and extrapolate back to zero, it will give you an estimate of what that intrinsic pH is, with the contribution from any trace acidic or basic components from the excipients and the contribution from the drug substance.

And that really is probably about the best thing you can get to as a number. Failing that, if your drug substance is reasonably soluble, then that saturated solution layer is going to contain a reasonable amount of the drug substance, and its buffering capacity is likely to overwhelm everything else unless you've deliberately modified—added a pH modifying component to the formulation.

There are ways you might approach it, but then that becomes a little more complex. And actually, that measurement may be the best thing to do again. I'll have the final build, please.

So what we try to do here is choose initial conditions which are set up to maximize what we'll get as a predicted rate of nitrosamine formation. This model is designed to use as part of a risk assessment. It's designed to be wrong. We're biasing it. So if we get a conclusion from it that says we don't have a risk, we can be confident that we do not have a risk.

Next slide, please.

And now does this work? So this is coming back to some of the IQ consortium's studies on nitrosation within model drug products using 4-phenylpiperidine hydrochloride as a model drug substance. Two different tablet formulations: a high load, 10% drug load, and a low drug load intended to mimic an amine impurity more than anything else. Formulation, native nitrite levels in the excipients. So depending on which of the two formulations we had, 0.55 or 0.6 ppm, mostly from MCC.

6% free water in the tablet based on moisture isotherm, and actually, for phenylpiperidine is reasonably soluble. So a pH of 5 from pH of a saturated solution, the drug substance and the tablets to be prepared by—in the case of the high load—wet processing as well. So we have that as an additional factor. I have the build, please.

Next slide, please. Thank you. So now, what we have is data. And you should be able to see that the top graph—that's the high drug load. We have two sets of points: unfilled points, which is the dry process formulation, and red points which is wet process formulation. That says something about what your processing does to making the nitrite from the excipients potentially available. The red line is what the model actually predicts with that formulation. So the model is over-predicting significantly how much we would expect to see. But we did get to a reasonably significant level of nitrosation. That said, we got to just under 40% of the maximum level of

nitrosamine we could have expected to make if we consumed all the nitrite present in the excipients, which is the limiting reactant.
When we look at low drug load, you see the effect of concentration on slowing the model prediction, and you see the effect of concentration on slowing the amount you actually form. So the real data is, yeah, we formed hardly anything in that low load product, which says something about how conservative those availability assumptions I've had to make in getting to a model are.
But we're over-predicting. Now, unfortunately, this was accelerated data. So it's not ideal for saying what will happen when we consider something over a shelf life. And I have the next slide, please.
So what I now have is application to a beta blocker. And this is one where our initial looking at it from a modeling perspective said we have a risk that the product is going to contain significant levels of a nitrosamine. And so we proceeded to test.
When we got the testing data back from the retained samples, we got three years' worth of data, and compared that to model predictions. What we found is, yes, we have a product which is forming nitrosamine over the shelf life. And it's forming over the shelf life—it's going up over time. But the model again is over-predicting, which is what it's meant to do. But we're less than the acceptable intake of 1,500 nanograms per day. So the model's done what it's designed to do.
We also applied the model to look at the formation of dinitrosodiisopropylamine due to the presence of trace diisopropylamine as a potential drug substance impurity. And in that case the model predictions came to a conclusion that the maximum daily dose would contain less than one nanogram, which is clearly less than 10% of the acceptable intake for NDIPA. And 10% of the acceptable intake is where we'd set our safe threshold for the model to conclude no risk. So in that case, yeah, we concluded no risk and didn't test for NDIPA.
Next slide, please.
I'll say thank you to the colleagues I've worked with on this and helped me get there, and also my collaborators from the IQ consortium, who challenged and provided much intellectual stimulation to get us on the journey to the model that I've shared with you today. And the final slide should have the question I have for your consideration. So I've talked about something where we built a model, and that's been based on very limited data to enable us to set a conservative availability assumption. So we've been incredibly conservative for trace impurities. That's probably too conservative. But the data isn't there to say, yeah, what can you apply as an availability factor?
The agencies should be seeing a large amount of data from companies who have tested. And so FDA possibly have the opportunity to evaluate that data versus the model to enable realistic factors for availability of reactants to be set with a view to actually companies testing for the nitrosamines they need to test for and not for ones which are not a risk.
And the other challenge is NOx can lead to nitrosamine formation. There are limited studies ongoing to probe that risk. But it's an area of developing science and unknown risks. And what happens in commercial formulation equipment? Because there's one publication that says just certainly you can form NDMA from presumably DMA reacting with NOx during processing of a metformin product, and from an industry perspective understanding how to manage our equipment would be a good thing. Thank you.

Moderator: Thank you, Dr. Ashworth. Now, I'm going to welcome Dr. Martin Ehlert, Vice President of API R&D at Apotex. He's going to be speaking to us about CMC considerations and bridging bioequivalence studies of reformulated products.
Martin Ehlert (Apotex): Good morning, everyone. Thank you to the organizing committee for giving me the opportunity to speak at the workshop today. I'm very excited to do so. I think we've heard some excellent presentations, both on QSAR and on the in vitro and in vivo studies

of the potential potency of nitrosamines of varying types. I'm going to take a little time to delve into some practical considerations here. And with that..
Hence there we are. Oh, this is interesting. What's this? Is there a way to manually advance the slides? Oh, sorry. That's mine. Thank you.
Okay, so just a disclaimer. The thoughts I expressed today are mine alone and not those of my employer.
So I'm going to cover two aspects. I'm going to consider some CMC considerations about nitrosamines method performance and nitrosation precursors in drug products which Ian just alluded to a little earlier. And secondly, I'll go on to bridging bioequivalence considerations.
We are there. Okay? So last August the FDA issued a major update with the final guidance on NDSRIs. Kevin spoke to it earlier and gave you a walkthrough of QSAR. So I won't go over that here. But I do want to take this opportunity to thank the FDA and the other international regulatory partners, who must have worked very hard and very long on developing the QSAR. It's made it much more efficient and certain for marketing authorization holders to develop their risk assessments to do what they need to do, design drugs that are safe for patients. So I do recognize what went into that. Of course we've heard some thoughts about how it will continue to evolve in the future, but it really was an important milestone last year, and very uniformly adopted by major regulatory agencies throughout the world.
So now get on to the analysis of NDSRIs themselves particularly in drug products. Essentially, the standard today, although you can use GC-MS, is LC-MS, because that allows you to do both volatile and non-volatile nitrosamine determination with very sensitive spectrometric detectors like triple quads and orbitrap detectors. And this is really allowing us to get down into the parts per billion sensitivity range.
On the other hand, the QSAR is now resulting in AIs of up to 1,500 nanograms per day. I remember just a few years ago, when the default was going to be 26.5 nanograms per day, and I think many of us in the room struggled with that at the time.
In low maximum daily dose drugs, this can result in—at least in the nitrosamines world—high NDSRI specifications. For example, a 1 milligram per day drug with an NDSRI AI of 1,500 nanograms would result in a specification of 1,500 ppm.
So what challenge can occur with this? Well, it's connected with the previously issued guidance, the original guidance "Control of Nitrosamine Impurities in Human Drugs." That guidance requires that all nitrosamine analytical methods have to have an LOQ of not more than 0.03 ppm. This was especially relevant at the time when the guidance was issued, because it was based on the sartan products, of which some have relatively high MDDs. And that was the early days of nitrosamines, and it made sense to have that level of sensitivity, to have an LOQ that low in the analytical methods since regulated limits were at that level or lower.
However, now, with the advent of the QSAR and with low maximum daily dose drugs, achieving a linear response in an analytical method that spans from 0.03 ppm to 1,500 ppm is challenging or impossible, to say the least. That's a five order of magnitude range that one method would have to span.
So that proves especially—I have a lot of sympathy for my analytical colleagues at my company—a great challenge when developing and validating methods for drugs in those ranges. And, in fact, I've had discussions with them about the various workarounds they've had to do. Another factor that enters in: you generally don't have one linear range, you have to apply multiple dilutions in testing products. This does add to the complexity of testing and release of products.
Furthermore, in very low maximum daily dose drugs, there's a high proportion of excipients. It can make recovery difficult, and you can struggle with accuracy at these very low LOQs.
So although I'm breaking a bit of a rule and recommending a policy change—I will get to research recommendations—I would suggest that the FDA look at considering revising that part

of the original guidance, and perhaps adopt something like having a default LOQ at 10% of the specification, as is done, for example, by the EMA and Health Canada.

Moving on. Okay, so this is the second part on sort of the analytical chemistry part: analysis of nitrites in excipients. So current state of the art analytical methodology, it's mostly chromatography based, and depending on the methods used, there's parts per billion sensitivity. I've taken an excerpt from a table here, maybe a little difficult to read on the screen, but of various methods being used for nitrite detection. And I pointed out there, you know, MCC being studied. There are also some tissues and other types of samples. But we're definitely down in the parts per billion range for detecting nitrites in materials.

The challenge associated with this—and doesn't offer an opportunity for research, which I'll get to in a moment—is that in effect, all of these analytical methods convert the nitrosating species to either nitrite itself in the analysis, or to an organic derivative like that. In other words, it's not necessarily the original species. Ian earlier presented, and I've recapped them here, the various species that can act in one mechanism or another towards nitrosation.

The other thing that these analytical methods do is they measure the average level of nitrite in the entire excipient, not the micro spatial distribution. Again, Ian mentioned to you that it's unlikely that you're getting nitrosation between an amine and nitrosating agents at point contact between an excipient and an API, for example. So we don't know how those are distributed. And so the same excipient from two different manufacturers may have, in fact, different micro spatial distributions in them. So that's another challenge associated with this.

So the other thing it can lead to is a request, as you know now, in doing nitrosamine risk assessment and putting control strategies in for drug products. If an applicant is using nitrite limiting specifications on one or more excipients, there's a question of how do they derive a functionally useful specification for that excipient, for that nitrite. There can be requests to do what's often done in impurity purge, and, for example, drug master files and APIs to do spike and purge studies. But from the previous slide I just showed, the question is, what species do you have in your excipients? How do we know they all react at the same rate, and that they're all equally available? So I would caution that the use of spiking studies to determine appropriate nitrite specifications for excipients is one that may be a bit fraught and may mislead in a way to achieve those. So here's where a research opportunity comes about.

It's either to design and conduct studies on widely used excipients to speciate nitrosating agents in materials from different manufacturers. Or, if that's not possible, to do that chemically is quite challenging. Again, we're talking in the parts per billion to single digit parts per million range. If that ends up being difficult to do—you can't tell without trying at first—to develop potentially a protocol that can be standardized to differentiate nitrosating kinetics on excipients or the nitrosation potential. There was a discussion amongst IQ members about a year ago on this possibility, so that one would have a way of, say, rank ordering a given excipient, like a lactose from one vendor to another in terms of its potential to nitrosate a vulnerable amine in one's product.

So this has come up earlier by Ian and mentioned elsewhere: challenges with NOx. So I'm showing an excerpt from a paper sort of showing the NOx cycle in the atmosphere. Concentrations can range from hundreds of parts per trillion in rural areas to tens of parts per billion in urban areas and have a diurnal variation. For example, I'm showing the nitrous acid cycle there. It's highest at night. There's photo degradation during the day.

I can say from my own experience we have had a case of a secondary amine API, where the NDSRI content increased from about 50 parts per billion to a hundred parts per billion in only 4 hours exposure to the ambient atmosphere in the laboratory. So not only is that presenting potential problems with drug product manufacturing, it presents a challenge with even measuring in the API in the first place, and in this case the QSAR-based specification is in the order of a hundred ppb for this particular product. So you can understand why that poses a difficulty for drug product manufacturing and testing.

I'll recap something presented at the CRCG Conference last June by Sandoz researchers, and what you're seeing here are particularly in the tables below. If you read through them, you'll see that aerial exposure again to ambient NOx levels which were being monitored where the samples were being exposed in some excipients—particularly, you know, croscarmellose is an example there. Starch as well. We see very rapid increases in apparent nitrite levels again, and only on the 4 to 24 hour timescale. So I had given you the example on an API. But we can see here the equivalent of nitrite absorption from the atmosphere is quite a dramatic increase with relatively short exposure.

And finally, in reference, this is the paper by researchers at Towa, in Japan, where they—a lot of points here. But thankfully, they're color coded. The same metformin drug product is manufactured in two different sites for Towa: one in Osaka and one in Yamagata. Exactly same process, same API, same excipients from the same sources and same drug product process. It turns out what they demonstrated is the level of NDMA that forms in the drug product during manufacture is dependent on the location it's made in with all else being held constant. The Yamagata site had lower levels of NOx in the ambient atmosphere—light blue triangles—and then correspondingly lower NOx, lower NDMA in the metformin product in the blue circles below, and then the magenta and red correspond to the other site. So here's a direct example where atmospheric—the only difference is the atmosphere, and it results in a different quality of product.

Okay, going on now to the second topic to be covered: bridging bioequivalence studies. I won't cover all the ways those of us in the generic industry—you know with, especially with NDSRIs coming to the fore. Many of us with large portfolios have literally hundreds of products to reformulate. FDA has established timelines for doing that. That's submitting changes. Step 3 in August 2025.

Now, there are different ways to do that, and I'm not going to cover them all here. But one of the ways that seems to be working, if not in all products, but in many of them is the addition of nitrosamine inhibitors. So in other words, you think of as antioxidants? So and FDA itself has done work in this area, as I know.

So the challenge with that, though, is according to SUPAC, that's a SUPAC level 3 change. That's a prior approval submission. That means for those of us with large portfolios, there will be literally hundreds of prior approval submissions coming in. And, you know, overloading the system, challenging the applicants. It's a challenge for everyone. So can we consider potentially some different ways to treat post approval changes on drug products when the intent purpose is solely to control for a nitrosamine that was discovered in the product.

Well, an important consideration—now this is more in the space of my bioequivalence colleagues rather than mine. But the two important considerations—some work has already been done on that—is, depending on the BCS class of the drug, how is permeability affected by putting an additive, an antioxidant type additive into a modified drug formulation to control the corresponding nitrosamine? So the two things to—two areas to have been concerned with: one is with passive diffusion, and I've summarized here both presentations from CRCG last fall and from a recent publication. I think it might be out now. Jim Polli's here, an author on that actually a number of you and Don make where a different cell model was used to determine permeation, the permeability, passive permeability. This is all in BCS class 3 drugs. The findings in both bodies of research were that tested antioxidants had no impact on permeability themselves, at least for these BCS class 3 drugs.

Similarly presented at last year's GDUFA Conference, there's the question of what about active transport? So again, research funded through this initiative tested a large number of antioxidants on three different active transport models. And similarly, again, the study finding was that tested antioxidants had no impact on active transporters.

So overall, this is positive in this sense, if one is going to entertain the consideration of a lower category of filing for changes post approval.

So one thing we'd like to have considered is that using this information, PBPK modeling and the biopharmaceutics risk assessment process, we could go on a case by case basis using things—and Fang will be speaking about this shortly—things like parameter sensitivity analysis, and other information, of course, coupled with in vitro testing, discriminative dissolution studies, etc., to basically consider the notion of filing in the lower category these reformulations for this purpose to help with that. This is a research-based workshop for today. We would propose that funding be directed towards expanding the scope of permeability research, for example, and why limited to class 3? Why not go to BCS class 4 actives and to check—do some cross check across model cell lines to make sure that this finding, the findings have been presented so far, are robust, and apply across a greater variety of products.

Again, this—these biowaiver approaches, and I've got the BCS classes here—are meant for immediate release products. However, another proposal to consider is that this way of reasoning and justification for changes could be considered for modified release products as well. There's, as I said, there's a very large body of products that do need reformulation, and that will be—I'm sure you're receiving files already, and will continue in the coming years. So these are some thoughts for where funding research can be directed. And with that I thank you for your time.

Moderator: Thank you. Next up is Dr. Fang Wu from OGD ORS. She's going to be speaking on PBPK modeling to support BCS based waiver of in vivo BE studies. Thank you, Dr. King.

Fang Wu (FDA): So today I'm going to talk about using PBPK absorption modeling to support BCS based waiver of in vivo BE studies.

Here is my disclaimer. This presentation reflects the views of my own, and should not be construed to FDA's views or policies.

So first, I would like to talk about the background, including the approaches to control the levels of nitrosamine impurities. Then I will introduce about some research highlights and including the in vitro testing of the impact of antioxidant on the permeation and including two case examples of PBPK absorption modeling to support the expanding the waiver for BCS class 3 drug products.

So, as you already know, nitrosamines are formed by interaction between a secondary amine or tertiary amine and the nitrosating ion. One approach in controlling the levels of these nitrosamine impurities is using antioxidant and/or use pH modifiers in the formulations when reformulating the drug products.

So, according to the recent updates on possible mitigation strategies by FDA and the possible NDSRI mitigation strategies include addition of antioxidants, such as ascorbic acid, or alpha tocopherol to formulations, or also include the incorporation of pH modifiers, such as sodium carbonate, that can modify the micro environment to neutral or basic pH to reduce the nitrosamine level and also include the other mitigative strategies.

So here comes the research questions: so adding the antioxidant, do these antioxidant or pH modifier impact bioavailability or bioequivalence of generic products? And can studies other than in vivo BE studies support these reformulated products?

So I would like to introduce some past FDA research to address these questions. FDA actually funded a few series of external research, and also conducted internal research to support addressing these questions, and we assume, first assume that antioxidant does not impact the dissolution or solubility, or it can be measured, then the risk is associated with impact of this antioxidant on drug permeability because of BCS class one, class 2. They are high, permeable drugs. So those are low risk products.

So we are not including these in FDA research. So we conducted and supported studies on a set of BCS class 3 drugs which has low permeability and also using the idea also to see whether the antioxidant will impact the permeation of these BCS class 3 drug products.

As just now mentioned by Martin, in last year we presented this in CRCG workshop about a couple of the research. One is using IDES system to investigate the effect of antioxidant on the

intestinal permeation of BCS class 3 drugs by our pharma, and the other one is the effects of antioxidant on the intestinal drug transporters, and also we internal research. We presented the PBPK to evaluate the impact of excipients on the BE of BCS class 3 drug products.
So here I would like to give you a brief recap of the this IDES study, and that this individual dissolution absorption system was used to measure the rate of permeation of the model drugs, including acyclovir, atenolol, and ranitidine, and those are pre dissolved. And then we measured these permeability of drug products in the absence and the presence of four antioxidants, and each added three high medium and low concentrations, and also we use a parallel control, with no antioxidant included.
These were presented last year by Chris Bowden in the June CRCG workshop.
So here is a representative figure of showing that the permeation of acyclovir in the presence and in the absence and the presence of a different concentration of alpha tocopherol antioxidant. So apparently permeability were calculated, based on the slope of these profiles of these accumulative concentration in the receiver versus time profile.
And then the as shown in the right table, we compared to the control without the antioxidant added, we can see that with the addition of high medium and low concentration of the antioxidant, alpha tocopherol, we don't see much or little or no effect on the apparent permeability.
So here, this actually summarized the research results of the all of the four tested antioxidants on the permeability of the four BCS class 3 drug substances. So we can we summarize that there's little or no effect on permeation of these acyclovir, atenolol and ranitidine, these four BCS class 3 drug substances in the in the presence of four antioxidants listed here.
So, but we don't know whether this—we only tested four antioxidant. If there's other antioxidants, and there are some changes on the permeability, what would be the impact of this permeability change on the absorption or bioequivalence of this BCS class 3 drug product
So here comes the research question, can we use modeling PBPK modeling to predict the impact of this antioxidant on BE by incorporating this tested eventual permeability change data.
So here, I just give you a quick like example of using PBPK acyclovir modeling to evaluate the impact of excipients on virtual BE as showing in this figure with the increase, apparent permeability change incorporated in the PBPK modeling. When I see the BE parameter C max AUC ratio between test and reference with the increase apparent change the ratio, also increase.
And using virtual BE simulations we conducted, we can see that 60% increase of apparent permeability will result in the non BE scenario.
So in summary that the individual testing system provided relevant information, such as the in vitro permeability changes by the excipients, and our acyclovir PBPK modeling and virtual BE simulations. We use to assess the potential impact of these excipient, mediated permeation change on the BE of acyclovir IR product.
And we this suggested that more than 60%, or double even double the permeability could cause the BE failure, for acyclovir immediately released tablets. However, I would like to mention this is a case by case evaluation. The sensitivity of a permeability change on the impact of a BE would be dependent on the drug substance properties could be different.
Another example I just want to mention here is which is based on the research recent SBA research project. And we know that BCS based waiver would require rapid, a very rapid dissolution for BCS class 3 waiver.
However, for not all— of the atenolol tablets the RLD does not display very rapidly in HCl solution. We use the PBPK modeling and the established safe space, actually upper bound and lower bound that they can be bioequivalent also is bioequivalent to the RLD. Using these simulations, we can support that over 85% dissolution, even within 60 min could be bioequivalent to the RLD.

So this can be used to support expanding the BCS class waiver to now very rapidly dissolved drug. Besides, class class class 3, drug product.
So then, with that, what are the next research topics? And we we propose some of the potential topics for consideration: what type of formulation changes could be used to to reduce the nitrosamine formation and in addition to the antioxidant and pH modifiers, and also what scientific data are most useful to support alternatives to in vivo BE studies for nitrosamine reducing reformulations. This could include the in vitro testing could include modeling. Yeah, these could be considered for our next research topics.
With that I would like to thank all of the people and for their support, and from the ORS, OPQ, and also from a contractor. Chris Bowden is in vitro testing. Thank you.
Moderator: Thank you very much. Our last speaker is Dr. Naiffer Romero from the USP. He's principal scientist of scientific affairs. Naiffer Romero.
Naiffer Romero (USP): Thank you. Good morning, everybody. Good afternoon. It's truly a pleasure to be here and present to you about this initiative. The USP started probably the beginning of all this journey of nitrosamines. My name is Naiffer Romero. I'm a principal scientist in USP, and we probably all know USP about standards.
But today I'm not talking about standards, and that's exciting for me. Before we go there, it's really important to really highlight what's our role as an organization? We are in the business of creating public standards that really safeguard the quality of medicines. But that doesn't happen in a vacuum.
We really need collaboration. And again, the slide really highlights the pillars of that creation of that standard. It's essential that each of those pieces are present for us to be able to create standards. We cannot do that in a vacuum in the basement of the building in Rockville. So again, we need that collaboration with regulators, with the industry, the purpose of that standard and the need for the use the application of that standard. That is a very complex process, you may think. Hey, there's an idea. And you know, couple of weeks later, we have a standard. It's a very complicated idea that involves a lot of people involved from maturing that idea all the way input, from industry, input from regulators, input from stakeholders as we progress through that journey until we finally materialize and create a standard. But the standard is not always the answer to help mitigate problems that face industry many, many times.
So in order to do that, we have to think out of the box. And again, what I would like to spend the rest minutes of my presentation is to really highlight of this particular initiative that we created, or we started for the nitrosamines. As you probably know this timeline here describes a little bit of the nightmare that became for each of us nitrosamines from the very beginning, from that very first report of nitrosamines back in 2018 from the regulatory point of view. Again, we saw the first official guidance back in the middle of 2020. So during those first two years there was a lot of uncertainty. We know this is something that industry was facing. We didn't really know where to go, how to go about it. What was going to be the regulators' expectations on each of the findings that were happening pretty much on a monthly basis.
So with this in mind, we start thinking, well, what can we do to really help accelerate sharing knowledge, sharing information, and really ease the journey or facilitate the journey that industry was facing at that time.
So with that the whole idea of creating a knowledge hub really came about. I'm highlighting few things there that it was really the hypothesis of this initiative. We wanted to take use of the power of online community. Again, this is happening. Remind you, during COVID times, opportunities to have this kind of encounter was out of a question. We really needed to find a way to accelerate that scientific knowledge and scientific sharing. We have seen, and we have heard, an incredible amount of knowledge and best practice that has been generated in the past four years, where we—that doesn't serve anybody. If it's just sitting in a journal or on any document. So we really need to find ways to spread that word, spread that knowledge and land into practical approaches, and how we apply that. So that was one of the purpose of this

initiative to create a sort of a sense and community. Again, every scientist dealing with nitrosamines, we didn't want it to feel alone, right? There was a whole community of scientists out there dealing with the same problems, trying to answer the same question. So can we bring a set of community to that also democratization and inclusion of that knowledge? We saw a lot of these discussions happening in close rooms in close organizations, so could we have a venue for us to really accelerate and share all that information and all that but best practice that was being generated as this progressed.

So that's how then the idea turned into the topic of nitrosamines, and we decided to then go ahead and launch what we call the Nitrosamines Exchange.

I'm going to focus here on the right side of the slide. That is, really it was a big journey for everybody to convince that we were trying to do was not related to a compendial space. And that's why the title of my presentation said beyond the compendial space. We didn't want people to feel that anything that they contribute or any share was suddenly going to be making into a new standard a new requirement. That was not the case. So again, lot of education, our stakeholders to open their eyes and help them understand what is it that we were trying to do with this knowledge hub.

So that's how we launched the Nitrosamines Exchange back in 2021 with that idea, as I have explained to this point. Today, we have close to 5,000 members in that community with really a global footprint and with capacity to translate the language of the platform into 22 languages that has incredibly facilitate exchanges really global.

This highlights for you a little bit of the footprint that we have. I'd like to say that we pretty much have a good representation, global representation in the community. And again, every day we see more and more folks coming together, exchange information, exchange knowledge, and really learning from each other. So the next rest of the presentation, I'm going to highlight few of those examples that really illustrate that sense of collaboration. When all these started methods, everybody. I'm an analytical chemist. So of course, I feel sympathized with my analytical chemist, and Martin alluded to the challenges that analytical methods meant for all the folks out there.

So we saw initiatives from the regulators putting in their website methods that were developed to facilitate the testing and accelerate the developing of methods for testing all this? Not just an FDA, but other agencies. So we said, okay, why don't we also put our grain of sand here to help? And we develop as part of the Nitrosamines Exchange a section call analytical hub, Nitrosamines analytical hub.

And the idea is to grow this as a sort of repository of analytical methods that company organizations can access to help accelerate developing their own methods. Again, this doesn't substitute any compliance or compendial methods is really a place for you to have a sort of a jump start point for your analytical development.

Again, as we embark on this analytical sharing of methods, we start seeing now peers coming together to help each other. And again, I'm showing one example of that where somebody's trying to develop a method. And several folks in the community coming to try to help and try to help troubleshoot the challenges that that developer was being put to that the community is really helping us also identify trends on the common issues, the common challenges that industry is facing. And again, I'm highlighting few of them. Some of those has been touched today, one of those being the NOx, as the next headache, as a lot of like to call it in this space. Also a lot of discussions, you know, about refinement on some of the models that are used in terms of other type of dosage forms. And again, some other examples that I'm putting there. For, you see, every time any regulation it's updated, everybody, the entire community turned into its own to try to understand the impact of that regulation and the practical terms. So it's a really insightful conversations that start happening every time a new revision take place of any guidance. Not just here in the US. But really globally.

Another example of folks coming together and developing tools to help the community itself. So this you see, couple of gentlemen from the community, one from Japan, scientists from Japan and the other one from Brazil, coming together to develop a simple Excel based calculator tool. As soon as that QSAR framework was published again. This has also helped everybody to kind of understand how to do the calculations, how to apply into their into their companies.
So let me now share a little bit of the voices of the community. And again, how we see the all these evolving in this space. Again, I'm not going to stop and talk about this. There's definitely a whole evolution of the safety discussions. How do we assess? But there's definitely opportunity to develop that additional understanding, a lot of really good science. But again, how do you land that science into practical approaches, into something that is practical, tangible for organizations to easily apply. So I think there's an opportunity here to sort of like, digest that and make it into easy ways to share that information.
That public information and knowledge is, it's essential. And again, I think we can help accelerate all that through the community and other efforts that we have. And again, standardization and adoption of this is critical. Again, how do we accelerate the adoptions of a lot of these new kinds of alternative ways to assess the safety of these compounds.
We have seen some of this evolution right again, couple of examples there going from the default limit of 26.5 to now having a QSAR framework of going from the Ames test to now having an enhanced Ames test that's starting to be a lot more acceptance on it, based on all the research again and contributions of the agency as well. So I think there's a really need out there expressed by the community about these increase awareness and understanding of using all this prediction and all this model again, to land it into a very practical approach. How can people apply these approaches?
I think there's a big thirst out there for the adoption of all these in silico tools that has been mentioned as well, but that doesn't happen on its own. There's a lot of education, collaboration, and experience sharing that will need to happen for to accomplish that wide adoption.
Risk assessment again. Everybody here that had to deal with nitrosamines had to undergo risk assessment. However, we still see this an area, an opportunity to continue, grow and continue evolving. We haven't seen risk assessments evolving probably as much as they should. We continue to see risk assessment based only on a declaration letter or any checklist.
And definitely, we need to evolve from that. So that's an area where I definitely see us coming together to share best practice and really anchor on best practice and sharing knowledge of what good organizations are doing there. How do we bring that into a practical toolkit?
On the analytical? There's a lot of challenges to be faced. We still need a lot of robust methods. Every nitrosamine, every NDSRI requires its personal method.
Another highlight that we continue to see through the community is the challenge of having reliable materials to do the testing. This continue to be reported in the community as a big concern, especially for the analytical lab standardized approaches, and again, that support to peer, to peer is essential to again accelerate all this understanding. The future challenges. I think the already mentioned by several speaker NOx. I think that that's the next thing that we really need to understand. How is the really gonna affect everybody. The role of the compendium again, as a as a USP, it's important for us to understand and again get feedback on what's the role of the compendium into all these? Do we need to develop tools that can help facilitate, mitigate control this impurities? Do we need to create additional standards that can help and steer all this discussion again. We're all open years to do that.
Another thing that again was alluded by my previous the previous speaker is the scavengers. We have heard endless about the use and the benefit, the benefits of scavengers in a whole mitigation strategy. But there's also room of getting that knowledge and sharing that knowledge on early development. So we're starting to also see organizations now incorporating scavenger screening into their early formulation development. So again, how do we take some of those lessons learned how some of those knowledge and create a sort of a roadmap to do and

support early development. To prevent nitrosamines down the road, and again, hand with hand with what was presented before is, how do we bridge that scavenger, solution or mitigation strategy. To now real data or real evidence that could really support that decision in the terms of permeability and absorption, and the usable either, like the dissolution testing with a permeability cell, perhaps that could also help understand some of these mechanisms as we start to apply as scavengers.
So that's my presentation. Thank you so much for your time and again I'll invite everybody to join the community. Thank you very much.
Moderator: And since our session is running late a little bit, and our panel discussion will stop at 12:15, so hopefully you can stay for a little bit for the panel discussion a little bit late for the panel discussion and also for the audience in the room. If you have any questions and there is a floater mic, and for your convenience to answer questions.
Next, I'm going to introduce our panelists in addition to our speakers, and they are Dr. Tausif Ahmed. Dr. Ahmed is the Vice President and head in biopharmaceutics and bioequivalence in Dr. Reddy's lab.
And Dr. Daniel Snyder. Dr. Snyder is the head and a global quality for a global quality system. IT quality and a technical quality in Viatris pharmaceutical.
Dr. Robert Dorsam is the division director of Pharmacology and Toxicology review in OGD.
Dr. Naomi Kruhlak is the scientific lead in DARS, OCP, OTS in CDER FDA.
And Dr. Bing Li is the associate director for sciences in office of bioequivalence in OGD CDER, FDA.
Dr. Bhagwant Rege is the division director for biopharmaceutics in CDER OPQ one.
And Dr. Diaa Shakleya is a senior research scientist in the office of pharmaceutical quality research.
And the last one is Dr. Matt Vera. It's a supervisory chemist in DPQA 2 in the office of pharmaceutical quality assessment I in CDER FDA.
Moderator: Thank you. Dongmei. I'm going to start off the panel discussion with some questions for our CMC experts on the panel. We've heard several approaches being described to inhibit nitrosamine formation. Could you please comment on what are additional approaches, approaches that should be considered, and what combinations of approaches should be used or considered to mitigate formation of nitrosamines.

Matt Vera (FDA): Yeah, I assume that's kind of aimed in my direction. I would say that the recognition, particularly with antioxidants that that just using one isn't guaranteed to be a solution. There's a lot of case by case development work. I think, that would go into an antioxidant effort in a formulation. Dr. Shakleya might be able to expand on that a little bit, because he's certainly one of our experts here internally on how antioxidants can work in addressing that issue. Not sure if Diaa has anything to add.
Diaa Shakleya (FDA): Thank you, Matt, so actually the work that we did here at FDA was very interesting. We took some three antioxidants I think you have seen the publications on pioglitazone, and we screened them at different concentrations. It was interesting actually to see the effectiveness, even at low concentrations, the ascorbic acid. It could prevent the nitrosopioglitazone from forming, even after six months, stability at accelerated conditions.
We did another molecule, which is metformin. And we saw a little bit different results. So antioxidants may fit in one drug, but it may not fit in the another molecule. So, early of development, using those antioxidant. I think it's essential to see the effectiveness even under accelerated conditions. Thank you.
Martin Ehlert: Martin. Go ahead. Sure. Another approach that's relatively simple, that certainly has a to a degree mitigates formation is especially for oral solid dose drugs, is keeping them dryer, controlling water content in the drug. If it's not affecting dissolution, performance or bioequivalence seems to help in a lot of case to slow the rates of nitrosation and that's

consistent. I can see Ian, shaking his head. It's consistent with the model for the solid drugs. So there's another thing. Use use of desiccants, more vapor impermeable packaging, etc. But I want to echo, what Diaa had said that certainly in our own experience, and why I spoke to it earlier. Antioxidants are effective in many cases. But ascorbic acid works beautifully in case one doesn't work at all in case two. So you've got to try a variety of them, and it will depend on the drug. Thanks.

Moderator: Thank you. Related to that question are, you know, once there are reformulated drug products. What are some critical considerations to reestablish bioequivalence. And would you consider it that these approaches would be different, depending on the type of reformulation approach that you've used?

Bing Li (FDA): Yeah, maybe I can start. As I sit here, listen to today's presentation. I sort of, you know. Put this our efforts. Our whole efforts on this nitrosamine issue into three buckets. Okay, one is what are the acceptable level of the nitrosamines in the drug product? That bucket one. The second bucket is, you know, we have a whole lot of effort. Study the mechanism of nitrosamine formation, the analytical method to quantify the nitrosamine, and all of that is pointing to an end purpose of reformulate your product to make this product with a product, with an acceptable level of nitrosamines.

And then, after that that comes to the third bucket, which is subsequent to having an acceptable level of nitrosamine product. How do you reform? How do you put your product onto the market? That comes the bioequivalence, which is the focus of the area that I'm in. So I'd like to share few points and few comments of my thoughts. First of all, when you think about your bucket two, which is, you know, making your product into a good product. Okay, you have to think about your bucket three. Which is what would be the maximum, the optimal method. Okay, that you want to use to reformulate your product, to improve your to change your manufacturing process and etc. You know, when you think about that, what would be the optimized strategy? Meanwhile, you need to think to reduce the bioequivalence data that support this change. Okay, we talked about the existing bioequivalence guidances that we have. Okay, three guidances, SUPAC, change solid dosage form and other dosage forms. BCS, which is talking about waiver and API changes. Okay? So you can utilize these three existing guidance. The scientific principle underlying these three guidances to think about ways beyond what is three guidances give to you? Okay? In other words, FDA is also open for alternative approach, which is more streamlined, you know, as few speakers touch upon already more streamlined, more simplified methods to demonstrate. Your products to be bioequivalent.

Okay, then, what is the factors that you should consider to develop these approaches? Okay? First of all, the API property of your drug. Okay, is your the. For example, BCS classification of your product, whether your product is an NTI drug product and etc. Second, the your product performance. Okay. First, it is API, second, is your drug product performance? Are you having a product that demonstrate the same dissolution profile? Right? Do you have a product that still have maintained the same specification of your product. And etc. And third would be what would be the most streamlined approach that you use to reformulate your product. Okay, to minimize the bioequivalence data that supports your product. Okay? And the fourth one would be, you know, when you add antioxidant or other excipients to your product, to reformulate the product? Are you considering, you know, the inactive ingredient level of those additional excipients being lower than those have already demonstrated the safety. So those are few key points that you know you should consider when you reformulate your product and mitigate the nitrosamine level in the nitrosamine risk in your in your product. Think about alternative. But bottom line is okay. Any changes that you're thinking of should think of, you know. You know whether your API you'll change your API permeability, whether you change the absorption of the drug product. So that's few thoughts that I have. Thank you.

Moderator: Thank you, Dr. Li. I saw Dr. Ahmed's hand was raised. Just want to give him a chance, as he's one of our virtual panelists.

Tausif Ahmed: Yeah, thanks a lot. I hope I'm audible. Yeah, am I audible? Can you confirm, yeah, okay. I think just to take forward what last person has given the feedback, I think from a industry perspective, what we see. It's a big burden. And Martin also alluded to that. For the reformulated products. We have to repeat the these studies, and I think, we need to establish a framework where we could do again, case by case evaluation. But some of the aspects are, can we look into a multimedia dissolution comparison between the reformulated product and the the reference product. Can we do a biopharmaceutics risk assessment to mitigate the risk from the bio perspective. Can we look from a take guidance from the SUPAC level? And obviously what Fang referred is from a PBPK approach. So I think this is all different approaches which we can try to avoid, or where we could mitigate repeating the BE studies. And since this topic is on the the research initiatives, I think some many speakers presented the the article published by FDA and James Polli. I think that is one area which we could do further, more research where we could assess the impact of the excipients, or come up with a database where these excipients up till this level will not have an impact on the permeability of a particular class of drugs. That database, or coming up with more data in this aspect will really be helpful. From my industry perspective, I think this will really reduce the burden on the generic industry by avoiding repeating the studies. These these are some of the comments which I wanted to make. Thank you for giving me the opportunity.

Moderator: Thank you. Any additional comments from our panelists.
Fang Wu (FDA): As Dr. Ahmed mentioned, modeling can be considered as alternative approach to support the waiving. For example, I gave the example of supporting BCS class 3 based bio waivers, and I think this can be through sensitivity, analysis, and also the virtual BE simulation to see what could be the risk of a failure of BE when there is a reformulation. And of course these need to be supported by in vitro testing data, and, for example, the permeability, testing and sometimes in vitro dissolution testing as well. So those all of the approaches can be used to support the waiving or reduce the in vivo BE studies. Yeah, these knowledge gap can be also filled by the research project. Thank you.
Bhagwant Rege (FDA): Yeah. I think Bing and Fang have already touched upon this BCS can form the foundation for what bridging approaches should be considered for approved products that are facing this issue. Obviously, BCS class one and class 3 drug products are low risk products because they're the basically class one especially, there is no dissolution rate limit or permeability limit versus BCS class 3 products. If it is shown that the addition of these quenchers can does not affect permeability, that's again becomes a lower situation. So whether we can have scientific bridging based on in vitro approaches that certainly can be considered. Obviously, we are not getting into policy issues here. But I also want to make a point about extent of reformulation. I mean, if you're just adding small amount of antioxidant or pH modifier, it does become a smaller change versus if you have to do extensive reformulation. So the approaches for bridging may be different. In those situations.
And another point I do want to make is I believe. Martin, you presented some information from Japan, where, depending on the site you had different level of nitrosamines. So at least going forward. I hope generic industry is already screening molecules for potential to form nitrosamines. And just as you do polymorph screening or salt screening? I hope there is additional preformulation involved in terms of screening of addition of either antioxidant or pH modifier that might be needed, because you might think you don't need it. But if if depends on site and atmospheric content of nitrosating agents, then you may want to be a little bit more proactive. So to avoid bridging in future.
Moderator: Thank you. Any comments from our panelists here, go ahead.
Daniel Snider (Viatris) : So one research opportunity might be to take a look at historical information from other companies. Change from all companies, changes to kind of have a refresh of the information included in SUPAC. Given the number of products that are potentially

impacted by needing to be updated, I think it's going to become exceedingly important for both industry and the regulator to be able to have as much clarity in in SUPAC so that products, that that there's a simple solution can go through a simplified regulatory process and the and leave the review resources for products that are more simple. And I would think there's probably decades of information that says that there's the kind of information there's information that says these sorts of changes may lead to very little impact on the performance of the drug. Think? One example is is in order to decrease the potential for nitrosamines, you basically just cut the tablet weight in half and reduce the amount of reactants in the form of excipients. These kinds of pragmatic solutions, I think, are how we get through this this topic.
Thank you.
Moderator: Okay? So we've heard about CMC, strategies and BE strategies. Mitigation is certainly I think. Very important, and then reformulating and establishing BE when that is not feasible or still remains to be a challenge. We do have an option to conduct, risk assessment by performing safety evaluation. And you know this is a strategy that we have seen being used to understand the risk and also establish limits.
And so I'd like to call on our safety colleagues on the panel to discuss. You know, we heard about use of modeling and quantitative quantum mechanics. I wondered if you could talk about developing and implementation of these models similar to how you know we have. ICH M7 guidance where some of these models are appropriate for use are considered acceptable.
So what resources or additional research would be necessary to ensure that we can leverage some of the modeling approaches for safety aspects, safety, assessment of nitrosamines, and what would be necessary to ensure that these models are standardized for use with nitrosamines.
Naomi Kruhlak (FDA): Go ahead, Naomi. Thank you. So I can certainly start by commenting on this topic. You know, I think it's recognized that when the QSAR was rolled out it's deliberately intended to be conservative. There may be some opportunities for perhaps dialing back some of the limits that you initially get based on the QSAR. Which, in cases where we don't have enough information are going to kind of default to the more conservative limit by then supplementing that with additional experimental testing data that's being generated. And there's a lot of data that's being generated across industry, and also at FDA and by other groups.
I think, as Kevin has alluded to in in his presentation. There are certainly ways that we can start to use that data to get a better handle on certain areas of chemical space where perhaps we don't fully understand the deactivating features that may be present, and we can then incorporate that into future iterations of the QSAR. And I I think it's important to remember that QSAR is a starting point.
And in the future it's likely to be updated with additional knowledge. I think there are challenges associated with access to that data. It's something that you know, we even as regulators, although we see some data coming to the agency, we certainly don't see all of the data that is being mentioned when we go to these scientific conferences.
And so if there's a way for that information to be made available more broadly, that allows the modelers to really be able to take advantage of it. But then, I think you know, as Kevin mentioned, too, in his presentation. There are also opportunities to use these methods like quantum mechanical modeling, where, rather than consider using that as an alternative approach, we can use it as a complementary approach to really refine and better understand certain structural features, to then implement them back into the QSAR, so that in the future we'll have a more comprehensive and hopefully a more refined model.
The other thing I just wanted to mention was that it's also important to remember the QSAR is, is, is modeling carcinogenic potency rather than Ames mutagenicity. Although we do see Ames mutagenicity as a surrogate for carcinogenic activity. It's not a perfect surrogate and so there may be opportunities down the line to start to build out our repertoire of models so that we could maybe have a QSAR model of the enhanced Ames assay that would also be able to be used in

combination with the QSAR. Again as part of a battery of tools. So I think these are all valid directions for research, that you know, I would like to see us going in.
Robert Dorsam (FDA): I would just add, one thing there. And follow up on that quantum mechanical modeling. Excuse me, we're really talking about very coarse modeling. We're not. I mean, traditionally, you know, computational chemistry has been a very precise, very laborious activity. But in this context, where we're looking at it to understand structure, activity relationships, and in the context of a broader feature analysis. So we're really not interested in, you know, highly precise, accurate predictions or calculations we're really interested in. You know, what can we? What can we learn from these calculations? And can we establish a high, medium, low sort of assessment, you know, like the categories in the QSAR. And also the goal is not to come up with a regulatory guideline that says everybody has to do quantum mechanical modeling. Because that, you know. Is a very specific skill set. Take some resources, compute resources and parameters and doing it. There's still a lot of work to be done there. But really the goal there is to fall back, then, on what we learn from that to enhance the structure, activity relationship rules so that we can quickly still quickly assess. Carcinogenic, carcinogenic potency and mutagenicity. Using extensions to the QSAR. For example.
Moderator: Thank you and just picking up on that some of the points that were made we heard in in our public comment period about perhaps leveraging some carcinogenicity studies for candidate molecules. I wanted to get a sense of you know it is a resource. Intensive effort. However, is there a utility in pursuing carcinogenicity studies, and what criteria could be used in selecting compounds for testing.
Robert Dorsam: I would just chime in on that. We went through the process of enhanced Ames testing and the EMA contract that I've been involved in as well and as well as the HESI ring trial work and compound selectivity is probably one of the most important but contentious areas. You're really looking to fill a data or knowledge gap. And so you'd want to be very careful about you know what you actually selected, and give it the due diligence. And forethought before you went ahead. And anything is time consuming as a and as expensive as a two year carcinogenicity study. You would really want to know that that is the absolute compound that was of interest to you.
Naomi Kruhlak: Yeah, I would agree. I mean, I think it's very challenging to come up with, and in this case it would be a very small pool of structures that we would want to invest in. Just given the time and the resources. Would that would be needed? Yeah, I think it would be. I think it would be difficult to come up with the criteria that you would need in order to in order to select those. So that's not a that's not a simple task.

In fact, I would suggest taking the those resources you might consider for that and putting them into TGR studies or duplex sequencing studies with the goal, as I was mentioned by one of the presenters, of coming up with a reasonable way to estimate a positive mutagenicity, frequency from an in vivo TGR, and turn that into an acceptable intake level. I think that's a very you know. Great goal to work towards. But you know, there's still a lot of effort involved in that as well. But again, I would point that as a better direction for resources.
Moderator: Well, thank you. And then this is a general question for the panel. I see we're coming up to the end of our we're actually well over. But we're coming up towards the end of our panel discussion. And so you can have a little bit of lunch before the next session. So we heard about data being generated by FDA by industry. And you know, one of the challenges we have in the generic drug side is, you know, minimizing redundancy in data. And so I wanted to get this panel's thoughts on, regardless of whether it's CMC. Information or BE information or safety information. What are some opportunities for leveraging this collective knowledge so that we can focus our research needs. And also, you know, how can that information be used to make the progress. Some of the what you've heard today from our speakers.

Robert Dorsam: Hi, Bob Dorsam. So I think that there's a really big opportunity when there's data produced to publish so that it can be leveraged. So I would encourage anyone who's doing research in this area to please publish because that helps advance the field for the work that's being done by our FDA scientists. Certainly. They're publishing as well we're in this together to get you know what are safe levels for these nitrosamines also understanding how to mitigate their effects or their formation but publishing is key. Now, the second part is to then integrate that into a model which we see in in QSAR, for example.
So that then there's this understanding of how individual data impacts potency. And then we can use that for informing potency for a compound. The likes which we don't know. Yeah, the one that just comes in front of us right now. So I think we need to publish and then use that to expand. QSAR. And I, I do. You know, I respect and take the points of our colleagues here on the panel, that we really can't do a lot of carcinogenicity studies. No, that is true.
I think that we can choose some defined big ticket studies. Perhaps if they were to serve as anchors and then be used to expand QSAR. Perhaps that would allow. You know, more less conservative limits. And then, perhaps reduce the number of studies that would need to be done by. You know various individual manufacturers for their individual applications. So I think it's really publishing and then working it into our models. That would then help us predict potency for compounds that haven't been tested.
Moderator: Thank you. We have a question from our audience.
Audience Member: Yeah. First, I want to thank, all presenters and panelists and public speaker for the nice presentations and the wonderful discussion about the nitrosamine problem I want to move gears a little bit toward this endogenous nitrosamines. So the levels of endogenous, nitrosamines maybe like 20 thirtyfold, if not even more than the extrinsic one that's formed from drug precursors. So do you think we can consider the human factor as well as the food effect and mitigation, the risk of our nitrosamines in in our research.

Robert Dorsam: I take your point. That's a very valid point. That's you know. Perhaps the baseline of exposure, you know, from endogenous and exogenous that. The question being, What do the drugs addition to that overall nitrosamine exposure? What does it lend? You know. Is it substantive or not? And we don't really have that firm understanding of what is the basal exposure? So I think that that has been highlighted as an important research area.
I believe in the HESI roadmap as well as in past research. I think in the 2023. We talked about the importance of that as well. I think it remains an area where we need to learn more, because if we're going to, then say we can accept more nitrosamines in drugs, it would only be because of the relative risk is negligible. And we're not there at that point yet. So it does warrant further research.
Moderator: Thank you. I see we have another question.
Audience Member: Hi, yes, so my question is, the nitrosamine formation appears to be caused by many, many factors. So I'm curious from the public perspective, public panelists perspective. What is the actual predictable impact on reformulation of some of these drug products. How would reformulation actually significantly reduce the probability of nitrosamine formation? Especially when we've seen, you know, maybe environmental causes. Environment can cause nitrosamine formulate formation and also, practically speaking, you know, just for Metformin, for instance, you have a whole new class of oral antidiabetic medications, you know. Metformin still has a valuable role. But what's to say, you know. Requiring reformulation might promote kind of thinking from the industry perspective that it's not worth investing in that area so kind of like drug shortage perspective. The reformulation of these products to reduce nitrosamine formation to level that we still don't have a clear understanding of what the link to causing cancer is just that perspective. What's the overall thought that reformulation can reduce significantly. Nitrosamine formation.

Martin Ehlert: Okay, I can make some comment on that. Thanks for that question. You're touching on two things is our reformulation efficacious. And what market impact do they have? So to answer the first part, I would say that generally, yes, reformulations are efficacious. I certainly know examples where one can drop the level of nitrosamines formed during shelf life over a product by two to three orders of magnitude, putting it well within currently prescribed safe limits. So it does work.
The second part of the question, though, is unfortunately a bit messier. I can imagine older affordable drugs that have a relatively small subset population of patients and for which there are relatively few marketing authorization holders in the US market companies may decide it's not worth reformulating. That's part of the theme. Why, we're touching on ways to simplify post approval, change process for nitrosamine formulation. And I'm certain that there are companies making decisions to discontinue products that are otherwise beneficial for a certain set of patients. So that is an important consideration is that going to happen on large scale products with many patients. No, probably not. In that case they will be reformulated. They'll be available in the market. My concern is for more of these niche products. I'll leave that there if anyone want to add more.
Robert Dorsam: So largely. I agree right. The primary goal of much of this exercise is to make sure that that patients have medicines that they desperately need. You know, metformin, although it's an old drug. My wife's a physician, I feel confident if you told her she couldn't have metformin for our patients anymore. It would be, you know, not acceptable to her.
And so, just because drugs are old, don't make them unimportant. Part of the entire process that we, we think have to think through here is like any new drug, even right. What are the benefits and what are the risks? You try to optimize the formulation, to minimize the risk in every way possible. And make sure that the drug gets the full benefit. And so it's that balance that I think regulators and industry and doctors in the entire community are going to have to work through. So it's kind of a process, which is why we've been talking about it and working on it for several years. Now.

Dr. Matt Vera: I I would just add that as as the scientific understanding of this whole problem matures, and it has a lot in in the last few years. You know better risk understanding will hopefully lead to problems that to products that never need to be reformulated. Right? So there's, you know, the the reformulation issue is for a certain segment of sometimes very important drugs that that have a particular problem. But as our understanding grows you know, hopefully, we can sort of skirt the need in the future for reformulation. And and it's really important, I think. To understand how quickly the science of this is changing. And I think Professor Munson and and I think Naiffer's slides kind of showed that timeline that in in 2018, you know, we were talking about NDMA in the sartans right? And then quickly, we found NDMA in ranitidine, and we found NDMA in certain Metformin formulations, and even though they all generated NDMA, they did it in distinctly different ways, distinct from each other and distinct from the NDSRI scenario that we're talking about now so so as as that science matures. And and you've heard some of that from from Professor Munson. With this crystal defect issue from from Dr. Ashworth, and from from Dr. Ehlert, with with NOx and and ways to model and kinetics. I mean, we're getting better at this. And and I think, as as the the scientific landscape improves we have a lot of opportunities to completely avoid the need for reformulation or or shortages caused by this. You know, with the the present cohort of drugs that are sort of moving through the development pipeline.

Thank you. And then one last question from our audience members, yeah, this is regarding the reformulation products, where we have a well established, an animal to human PK correlation. In that case. As Dr. Tausif is rightly stating, with the multimedia equivalence has been established. We can also, can we look into a small group studies of dog or monkey kind of a

thing where we can show the equivalency? I mean, it's it's a bridging study. Kind of thing. Would that approach helps instead of a full fledged? BE studies

Bhagwant Rege: As we mentioned the risk, for, BE, it depends on the properties of the compound and the properties of the formulation. So where you have BCS class one and 3 products. We can certainly look at some alternative approaches, but direct, just submitting animal studies as evidence of BE. Perhaps in my mind, is not acceptable. You may have to incorporate it. Some sort of modeling platform to, and then further demonstrate using virtual BE, perhaps to demonstrate BE. But again, I'll let Fang elaborate.

Fang Wu: Yeah, in terms of modeling. Actually, I call back one's point actually, animal, we, we can use animal as a kind of a starting and the investigation. But final call would be on BE human BE. Or if you have the in vitro testing results, even using the IDES system and go to and have some permeability testing results and dissolution using bio relevant media. Those data can be incorporated into PBPK and use virtual subjects to do the virtual BE simulation that could give you some risk assessment on whether this change could cause failing of BE yeah, that would be my perspective, my.

Tausif Ahmed: I just want to add few comments this is the Tausif. So I think. I kind of echo what Bhagwant and Fang, you have said, see, rather than going for animal studies, which will again add one variability one approach could be rather than doing, a full fledged pivotal study. If we can do a study in small number of healthy subjects, a small subset, and then, do a virtual BE, or and link it with your in vitro and PBPK model. That would be more a prudent approach rather than bringing the variability of a animal. PK, which, again, is is questionable. So that's my perspective. Thank you.

Moderator: Thank you very much. That concludes our panel discussion. And thank you so much for the wonderful discussion on the nitrosamine impurities, and we really appreciate your knowledge sharing on the research and results and findings and insight on the research priorities.
And so with this, we're going to conclude this session. And I also have a message for the next session. So all the faculty and the public commenter for the next session. Please meet at the podium 10 min before the start of the next session. That means you need to be here at 12:35, and for the other audience. And we're going to resume at 12:45. Sorry about late.