Nitrosamines as Impurities in Drugs; Health Risk Assessment and Mitigation Workshop

Moderated by Anne Painter

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Virtual Public Workshop

Food & Drug Administration (FDA)

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Reported by: Irene Gray

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A P P E A R A N C E S

List of Attendees:

Anne Painter, Host

Dr. Aisar Atrakchi, Panelist

Dr. Gerhard Eisenbrand, Panelist

Dr. Soterios Kyrtopoulos Ph.D, Panelist

Dr. Joseph Guttenplan, Panelist

Dr. Mark Cronin, Panelist

Dr. Errol Zeiger, Panelist

Dr. John R. Bucher, Panelist

Dr. Jerry M. Rice, Panelist

Dr. Stephen S. Hecht, Panelist

Dr. Richard H. Adamson, Panelist

Dr. Michael DiNovi, Panelist

Dr. Sruthi King, Panelist

Dr. David Keire, Panelist

Dr. Deborah Johnson, Panelist

Dr. Timothy McGovern, Panelist

Dr. Robert T. Dorsam, Panelist

C O N T E N T S

P R O C E E D I N G S

MS. PAINTER: Hi, everybody. Thank you so much for joining day two of our workshop on nitrosamines as impurities in drugs. Before we get started, we just wanted to do another reminder of the house rules for today's workshop.

As a reminder, everybody who is not speaking, please keep your phone on mute. And all attendees will be muted, and only panelists will have the ability to unmute themselves.

If you are using the video feature, only the panelists that will be engaging in today's discussions will have their video feature turned on. If you are not speaking, please have your video feature turned off.

Regarding any questions and discussion, please submit any questions that you would like to have answered by using the Q and A feature to the bottom center of your screen in Zoom. Any questions that you do submit, we do have a team of moderators who are monitoring the Q and A chat box. If you see that your question has been dismissed, that means it

has been received by the team and sent to the moderators for review.

If you are a panelist who will be speaking, please utilize your chat feature, as the host will prompt you when it is time for you to present. When using the Q and A chat box, the moderators and the workshop host will work together to monitor the questions, and they will make sure that they are addressed as time allows during the workshop.

And additionally, today's meeting presentation and recording will be made available on the webpage later this week.

Thank you.

DR. ATRAKCHI: Good morning, good afternoon, and good evening, and welcome back to the second and last day of the workshop on nitrosamines as impurities in drugs. We had a productive and informative day yesterday and look forward to the discussions today. We continue with the remaining questions, starting with question five, the last one under Exposure and Risk Assessment.

Question five: Should the regulatory

limits for nitrosamines listed for food and water, or amount formed endogenously be considered in determining the acceptable intake of nitrosamines in drugs?

Considering the abundance of nitrosamines, in and around us, should these amounts be taken into consideration to determine an acceptable intake in medicines. Keep in mind, one takes a drug as a patient to gain benefit to treat a disease or illness. It is not a choice that one makes. And the American public expects their medicines to be safe.

However, as a personal choice, we eat how much we eat, and what we eat and drink -- is our own choice. Some authors -- and also, from yesterday's discussion -- stated that endogenous production of nitrosamines and their precursors are a more important source of exposure than exogenous intake.

It is also important to keep in mind that in order to estimate the dietary intake, it is not only of nitrosamines, but in addition we need to estimate nitrates and nitrides, and not focus only on

diets that are rich in these compounds -- i.e., not a biased assessment.

So, with that, I would like to start with Dr. DiNovi.

DR. DINOVI: Yeah. Good morning. And thank you. So, this question has bothered me -- I guess is the right thing to say -- since we first started discussing it, because it is just -- there are lots of things, here, that are -- it is not obvious on the surface.

As an exposure assessor modeler -- purely as a numerical person, I would say the answer is, no. Because the regulatory limits do not actually impact the inputs into my exposure assessment models.

But clearly, a bigger answer has to be, yes. And as we discussed extensively yesterday, as more and more information becomes available about the endogenously formed nitrosamines, that would certainly impact how you choose to pick an acceptable intake, which is, of course, a risk management mitigation metric.

The limits themselves -- it sounds

contradictory but setting a limit does not impact exposure very much. Unless you are in a position where many -- and I am thinking in terms of food, which is my world. Unless you are in a position where many of the products would become unacceptable at a level that you have chosen for safety -- so then, you are brining in all sorts of economic impacts -- which I do not think we are discussing, here -- the limits are typically set in a way that balances the ability to measure the numbers -- for example, at -- although, I do not think that is particularly a problem, here -- with the cost, both in time and effort. We discussed this briefly yesterday, on getting here.

So that is why you can hear in my voice there is a hesitancy to just say -- you know, yes or no -- which is my normal choice for yes/no questions. But you clearly are going to have to take especially the endogenous formation into account when you get to the end, if the endogenous level is as we have heard three orders magnitude higher than the drugs, there would be no impact in picking an acceptable level from a numerical risk assessment point of view.

As you said yesterday, Dr. Atrakchi, you do not want them in there at all. So, the CDER's goal is to get them out. And that is a separate question from my world modeling the risk. And I will be interested in hearing what my colleagues have to say. So, I will pass it along, now. Thank you.

DR. ATRAKCHI: Thank you, Dr. DiNovi.

Dr. Kyrtopoulos? You are on mute. We could move to Dr. Eisenbrand and go back to Dr. Kyrtopoulos in a moment.

DR. EISENBRAND: Yeah. Thank you, Dr. Atrakchi. This is Gerhard Eisenbrand speaking.

Well, I have made this -- I have commented on this point yesterday, already. And again, I would like to repeat that in my opinion, it would be a pragmatic way of using the dietary exposure, for instance, as a point of reference for the potential to validate or evaluate the potential risk coming from drug connected exposure.

And while I, personally, would prefer to use, for the time being, the dietary exposure, is that it seems to me that although the data are, sort

of, 10 years old or some about that -- the database about the dietary exposure -- it still is valid, in a sense. And of course, one can confirm this -- I think, within a very reasonable timeframe, to be sure that the date had not moved very much, if at all. I would personally guess that they may, today, be even somewhat lower than before -- than 10 or 15 or 20 years before.

But this is a relatively good database. We have a lot of data there. It is data dense, and it allows a -- what I would call a commonsense approach, saying -- well, we have this -- in my opinion, really more or less unavoidable exposure. I mean, you have, perhaps, a certain liberty -- degree of liberty to modify it by your personal preferences. But still -- I mean, taking a reasonable medium amount of -- which we know is really below point five micrograms a day -- it is around point two, depending on the country where it has been measured. So that is a database that is quite solid and could be used. I think the intravenous risk of formation is much more -- in my opinion, much more difficult to quantitate in terms

of -- you have a lot of, perhaps -- ability by the health status and nutritional status, and so on. So, my plea would be to not disregard this nutritional exposure as a potential point of reference to -- you know, evaluate the risk from -- in a -- one -- it is a common sense approach, in my opinion.

So, I think, that is what I want to say, here, to this point, again. Thank you.

DR. ATRAKCHI: Thank you, Dr. Eisenbrand.

Dr. Kyrtopoulos?

DR. KYRTOPOULOS: Can you hear me, now?

DR. ATRAKCHI: Yes. We can hear you.

DR. KYRTOPOULOS: Ah. Okay. I am please.

So, I have started saying, before, that I agreed with what Dr. DiNovi had said, to a large extent, in that we have this exposure from the diet. Possibly, we have a much larger exposure endogenously. We do have certain reservations, as we discussed yesterday. But it does seem that there is significant endogenous exposure. So -- and there are two ways in

which one can see this background -- let's call it -- exposure in relation to the question we are discussing.

On one hand, one can say that there is this very large amount of exposure that is coming from sources other than the one we are interested in. And these sources -- the drug contaminants, are adding just a little bit to what is already a large number. So, there significance falls, in weight.

On the other hand, given that these background exposures -- especially the endogenous exposure -- if it worth -- for whatever value it may have, larger or smaller -- should be seen as contributing to the background incidents of cancer in the general population. I think that this was already stated, yesterday, by one of the panelists.

For example, taking the numbers that we are currently discussing -- assuming, for instance, that the mean -- average mean endogenous exposure holds true to the number of 144 micrograms per day that I mentioned yesterday -- that would seem, based on the Peto bioassay, to be -- to correspond to

something like a one percent lifetime exposure to that -- would be equivalent -- give rise to about one percent liver tumors. So that would be the contribution of dimethylnitrosamine to the background incidents of cancer. Which, overall, in humans, is, of course, much larger. It is in the order -- 30 to 40 percent.

On the other hand, traditional risk assessment considers this acceptable exposures. Those exposures, which are predicted to result in an incremental lifetime risk of one in a 100,000. So, from that point of view -- it starts from the general incidents and counts what gives rise to an incremental risk. In other words, ignoring the -- this background incidents and the associated exposures.

I think that in principal, that would be the correct way to go about it. And this becomes even more valid if we assume that maybe we are over estimating endogenous exposure. If real endogenous exposure is not in the thousands of micrograms that we are currently suspecting, but it is lower, then it becomes -- the weight of the incremental exposure --

it becomes more significant, conceptually.

So, I think, that in principal, we should not be taking into account this background exposure, whether it is coming from endogenous sources, or from food, or whatever else.

On the other hand, having said that, I do recognize the need for an expediency for a practical approach, given everything that was said before -- that people have to take med -- these medicines. They have no choice. And it is a question of balancing risk and benefits.

So, I -- my overall final, kind of, position, would be that we should aim at estimating what is acceptable based on risks calculated without taking into account the background exposure. However, we need to be flexible in view of the particular expediencies which exist. That is it for me.

DR. ATRAKCHI: Thank you, very much.

Dr. Rice?

DR. RICE: Good morning. In listening to the views of the previous speakers, I have thought carefully about my own position on this question,

which some might consider simplistic. But I keep returning to the fact that the physiologic effect of environmental exposures to a given agent is a cumulative result of all systemic exposures to a agent that one may experience. And I think, in consequence, that one really must consider the amounts in food and water in the context of what one may decide is the acceptable intake of nitrosamines in drugs.

The balance of evidence seems to be that the amount consumed by drugs -- consumed in drugs is miniscule -- or, at least, very much smaller than what one experiences from intake in water -- and especially, in foods. And I think it would send a confusing message to consumers -- citizens in general -- to try to tell them that the body somehow knows whether a given molecule -- any given nitrosamine comes from a drug taken by necessity or in a food taken voluntarily. Once it enters in the body it is all the same irrespective of origin. And therefore -- very strongly believe that you must look at the totality of environmental exposures in determining acceptable limits.

The amounts formed endogenously are, to a certain extent, a different issue. Ideally, you should consider that, also. But we have -- as Dr. Krytopoulos has just outlined, that seems to be a moving target. And while it might be helpful to make an effort to consider these -- again, to provide perspective in determining how much concern they give to small impurity in a drug preparation.

The net result is that if we start giving much different limits for what is consumed in food versus what is in drugs, it would seem -- I think, to most people, to call the entire risk evaluation process into some question. So, I say we should not prove it. Thank you.

DR. ATRAKCHI: Thank you, Dr. Rice. I just would like to make a -- maybe, one or two comments on that.

In foods, in general, we are not necessarily ingesting NDMA, NDEA, and other of the more potent nitrosamines. We are mostly taking the nitrates and nitrites of the food. It is when the food is cooked, that is when the nitrosamines are

present.

In recent dietary assessments -- in recent study in 2019 and 2020, the amount of -- the mean NDMA in foods was present at point one parts per billion. And the total nitrosamines -- there were about eight of them -- was present at point two three parts per billion. Very low amounts in foods. And this is all the result -- I would imagine -- from the progressive and advancement in food preparation, and so on, over the last 20 years.

So, the amounts from foods, and the natural amounts in vegetables, spinach, and others, they are very low. And in terms of calling the -- what we have in drugs -- as very, very miniscule amounts relative to what we are ingesting, again, I would like to emphasize that drugs -- we take drugs in the anticipation that we are taking the active pharmaceutical ingredient that is responsible for treating the disease or the illness. We do not expect the drug product -- or the drug substance to contain impurities that have no benefit to the -- or contribution to any benefit to the patient, and into

that tablet or pill that we are taking. So, it is a very different assessment.

And, also, we have -- as regulators, we have GMP quality to follow for what is in a drug. It is expected. We all expect the drugs to be clean, pure, as much as possible. So, it is a little bit different type of assessment.

Although, I do agree that -- we all, I think, agree that we are exposed -- around us, there is a lot of nitrosamines present. It is all relative. And I think you have -- you are saying the same thing. Again, I am not talking about the endogenous. The endogenous has so many variables. Whether it is the bacterial colonization, the nitrosation in the gastric of patients, different than those are who are healthy. That is not what we are talking about -- what I am talking about now. It is more of the exogenous exposure. So, just something to think about. And then, we can discuss this a little bit more, later on.

Dr. Guttenplan, please?

DR. GUTTENPLAN: Yeah. I am more of a laboratory researcher, than involved in regulation. I

do not disagree with anything that has been said so far. I just have one other view point.

And that is, suppose one did not have any exposure, either from exogenous agents, or endogenous formation. How would one regulate the nitrosamines that are present in drugs? Would that be considered a risk, or not?

And so, I think that is another way of approaching the regulation. Suppose we just disregarded the probably higher amounts that would be present in the normal dietary intake, and normal environment of the general population.

And that is the extent of my comments on this.

DR. ATRAKCHI: And we do -- I mean, to answer your question, if we are not going to take into consideration anything else outside the medicine, then this will be controlled under what ICH M7 -- and we have other -- that is for mutagenic impurities. And we also have other guidance's that are for regulatory -- for regular impurities that are not mutagenic. So -- and the complication, here, with

nitrosamines, as we all know, is because there are -- we are surrounded, almost, by them, in food and in the environment. So that is why this question is, should we take this into consideration. Otherwise, we can just go and follow some of those guidance's that we have, and we can calculate the risk/benefit in that sense.

But that is a very interesting and good point.

Dr. Bucher?

DR. BUCHER: Thank you. I think I mentioned this in my response to question three, yesterday. And I agree. And I think Dr. Kyrtopoulos laid this out very nicely.

In my view, we should not consider either the endogenous levels generation, or the amounts in food and water, with respect to determining the risks of the incremental increase in risks into the nitrosamines in drugs.

And I based this upon an argument that Dr. Kyrtopoulos also mentioned. There is no reason to believe that somehow the nitrosamines that are being

generated endogenously are not carcinogenic. I think there is a good case that could be made that a considerable amount of the, quote, unquote, "spontaneous tumor load," in the population -- which Dr. Kyrtopoulos correctly indicated runs between 30 and 40 percent -- could be due to contaminating nitrosamines in our own bodies, generated internally.

And if this is the case, then we really do not have a baseline on which to make a judgment about how much additional cancer risk could be posed by even the existing levels of nitrosamine contaminations in drugs. But we do have ways of estimating the risks associated with this, using the TD 50 calculations in the carcinogenic potency database, that I mentioned yesterday.

And I think it is appropriate to go ahead and use the one in 100,000 risk level as an acceptable increase risk due to contaminants in drugs. So, I would be in favor of ignoring, and not taking into consideration, the background endogenous formation, or the contributions of exogenous exposures through food and water, when calculating the risks of

nitrosamines in drugs. Thank you.

DR. ATRAKCHI: Thank you.

Dr. Adamson?

DR. ADAMSON: Well, I am with -- I would be in favor of saying you have to consider the amounts that are formed endogenously, and the amounts in food and water. And I want to bring up a point.

If you go to a delicatessen, or buy already cooked ham, or various other cooked things, those are all are already cooked foods, and probably have -- despite the fact they probably have an anti-oxidant in the preparation -- they probably have a small amount. So, you can buy foods, I think, that have small amounts of nitrosamines in them, in a store, in a delicatessen, and other places.

So, I think, you also need better measurements, as has been brought up numerous times, of the endogenous amounts. But we should also remember there are other endogenous carcinogens that contribute to the background. We make a lot of formaldehyde in our body. We make a lot of ethylene oxide. We have isoprene. We have a number of other

things. They all contribute to the background cancer.

And the amount that is present in drugs that we take, of nitrosamines, is miniscule, compared to the amount -- and probably adds -- you probably could not quantify the additional -- if you saying that 40/50 percent of the population -- let's say 40 percent comes down with some type of cancer, you probably could not quantify the additional amount. It is only a number because of risk assessment -- the addition of five or 10 cases per 100,000.

However, we should also remember that most people, when they get older -- my age, or starting in the sixth decade -- they are poly-pharmacy. They do not take just one pill. They do not something just for blood pressure. They take something for anti-acid. They take something for gout. They take something for numerous things. So, the drugs do give you a benefit.

But the amount of nitrosamines, in my view, should be whatever is feasible, with regards to also determining the cost of the drugs. So, those are the three points that I would like to make. We are

poly-pharmacy in the drugs. That the endogenous amounts do -- and food, do overwhelm the amount that we take as drugs. But we ought to regulate to the point where it is feasible concerning the cost and the benefit. Thank you.

DR. ATRAKCHI: Thank you, Dr. Adamson.

Dr. Zeiger?

DR. ZEIGER: Thank you. Hold on a second. Unfortunately, there are very good, logical positions -- logical arguments on both sides of this issue. And what I keep coming back to is that are our endogenous exposure -- that any health effects from nitrosamines would be based on the sum total of our exposure to nitrosamines, which includes exogenous and endogenous, as well as what is in the drugs.

But, the -- and we know the endogenous exposure is highly variable -- is affected by diet, by the genetics, by lifestyle, and many things. But everything contributes to the additivity of the response. And so, to some extent, this implies a need for more concurrent accurate -- more current, accurate human data on endogenous formation.

But even if the incremental increase is miniscule, I think a good argument can be made for considering the endogenous and exogenous -- other exogenous exposures to it.

You know, that said -- you know, it may not make a difference. You know, the added exposure from the drugs may not affect the overall risk assessment for that particular nitrosamine, or for the nitrosamines in summary. And I think maybe that would be a way to address it. What is the potential risk -- tumors per 100,000 -- cancers per 100,000 based on the exogenous plus the added drug. You know, showing there may be no difference, or there may be a difference. We do not know. But we just need more data on the endogenous levels.

But we cannot just say that -- you know, saying just looking at the -- what is in the drug, we cannot just say that, that is our only risk factor. Somehow, we have to include any communications on risk -- any calculations on risk, based on the total exposure. Whether that total exposure is just one percent more than the endogenous,

or 10 percent more than the endogenous.

But as I said in the beginning, one can make a good case for either approach, looking only the drug levels, or looking at the total exposure levels. Thank you.

DR. ATRAKCHI: Thank you.

Dr. Hecht?

DR. HECHT: Right. Well, we have very good data on nitrosamines in food and the exogenous exposure, particularly for the more common nitrosamines, like dimethyl and dimethylnitrosamines. So, we can really use that data in part to address this question of additional risk, as all the other speakers have mentioned. And chances are that the levels that you get of dimethyl and dimethylnitrosamine -- where we have a lot of good data -- from pharmaceuticals are probably quite low compared to the diet. But -- you know, the calculation has to be made.

But we do not have good data, in my opinion, on endogenous formation. It may very well be that the endogenous levels that are formed, are really

far greater than the amounts that you are exposed to through drugs -- as are also -- already been suggested. But I really do not think we have very good data on that.

And the other thing we have to consider are the structures of the nitrosamines. Most of what we have been talking about -- and a lot of the data in food is based on dimethyl and dimethylnitrosamine. But many of the structures of the nitrosamines that are found in drugs are quite different from that. So, I am not sure that we can actually make a blanket risk assessment by considering all nitrosamines at the same level of risk, because they have different biological activities.

In essence, I think that in some cases the nitrosamine exposure in drugs can be avoided by careful monitoring of the manufacturing process. And I do not think we should get into the situation where we are saying, oh, well, it does not matter because -- you know, you get so much exposure from food, that -- you know, so what if we generate some nitrosamines in the drug manufacturing process. No.

This is not correct. In most cases, the generation of nitrosamines during the manufacturing of the active pharmaceutical ingredient can be avoided by using correct chemistry.

That is all I have to say.

DR. ATRAKCHI: Thank you, very much, Dr. Hecht.

I would like to see if anybody else on the panel, who would like to add to this question?

DR. EISENBRAND: I would like to add a little bit more to that. If I am allowed.

DR. ATRAKCHI: Absolutely.

DR. EISENBRAND: I cannot agree more than with what Steve Hecht said. I think it is really important to recognize that we have a relatively solid database, in terms of exogenous exposure for food. And that is why I think it is a pragmatic process to take this as a point of reference. Mainly, because we do not really know how high or low the risk of the endogenous exposure side is. But this, of course, is a matter of future research.

But, Dr. Atrakchi, you brought a very

interesting point into the discuss. And that is the good manufacturing practice. And if we -- you know, leave a little bit at the moment, the discussion about the potential risk aside, and just concentrate, as -- by -- also said, just now -- on the good manufacturing practice, then we can say, well, according to good manufacturing practice, we can produce drugs without -- or with an extremely low miniscule contamination by n-nitroso compounds. And that, actually, would be, in my opinion, a good way out. Just concentrate on this. We did this in Europe before, for the cosmetics. Where it was realized that the exposure after mitigation measures had been taken, really approached miniscule amounts. So, we could concentrate on the good manufacturing principals, and say, well, according to good manufacturing principals, you can avoid nitrosamines conditions in the environment or in the fabrication environment, and so on, and can come down to limits that should not be exceeded. And that, I think, is a good way out, if we take this as a measure to follow.

But I think one point that should not

be forgotten, is that we also have a potential risk of endogenous formation of nitrosamines compounds from drugs. So, if you have, for instance -- the drug is really clean, but that has a structure that cannot be nitrosated endogenously, then we need to avoid that risk. And that is why it is now almost 40 years ago that WHO introduced this nitrosation as a procedure, just to get a rough measure about the nitrosatability of drugs. And this, I think, should not be forgotten -- that we also have to take this into consideration, in terms of regulation, as well.

Thank you, very much.

DR. ATRAKCHI: Thank you.

Dr. Keire?

DR. KEIRE: Yeah. I guess, I -- you know, when this nitrosamine -- when the nitrosamine started showing up in drugs, a lot of us at the FDA -- you know, were thinking along the lines of, well, okay -- you know, what is the level of exposure from drugs relative to diet, and -- you know, we got in contact with our colleagues at CFSAN, we dug into the literature, and a couple things strike me from that

whole process.

One is -- and I think it has, kind of, been referred to already -- that there is quite a wide range of values for the exposure from diet to nitrosamines. I mean, and -- you know, you might expect some level of variability because of the diet you might eat in South Korea, versus -- you know, a western diet, versus -- I do not know -- a lot of sausage in Denmark -- whatever it is. You know that there is a range of what you might consume in a given country.

And then, also, there is this aspect of the different measurement. And I think that there is a complexity to making measurements where you are -- you have to make sure that you are not adding nitrosamine in the process of making the measurements, right -- either from the preparation of the sample, or the measurement technique itself.

And so, I guess, I think I want to propose that I think there needs to be better data, right -- and newer data. And maybe with a standardized processes across many diets, right? The

drugs go in -- are distributed globally in lots of different populations with many different diets. And so, we really want to try to regulate -- you know, like, exposure to nitrosamines, you really have to think of it globally, across all those diets. And then, I guess, trying to come up with values, based on different measurements techniques, with -- you know, unknown suitability for the particular measurement.

You know, all these questions came up in that process. And so, I think it is a really difficult landscape to make a judgment about -- you know, what is the real level of exposure. And I think it does need a new focus.

As well as -- you know, the endogenous measurements -- you know, I think there needs to be better -- new and focused measurements that are doing this in the careful way. Again -- you know, down at this parts per billion level -- you know, trace measurements are -- can be challenging to do correctly -- you know, because of contamination issues, or other issues. So, I think there really is a need for good data with -- or new data with

standardized methods across many diets, to get a real baseline value. Thank you.

DR. ATRAKCHI: Thank you. And this is assuming that -- I mean, we did get a little bit different opinions -- or approaches, in the sense that, yes, we do need to take into consideration the diet and the exogenous exposure. But certainly, we also heard that, maybe, that should not be considered if we are simply, based on -- you know, we need the quality drugs, and when possible, these nitrosamines should not be present in drugs.

So, it is all relative. And it is all important things to take into consideration.

I do like to -- there was as discussion from yesterday that -- about this endogenous exposure in the past, versus the current situation. And I believe Dr. Eisenbrand, and Dr. Kyrtopoulos mentioned that there were good data -- very good -- there are good data that -- evaluation -- to update the evaluation of exposure to endogenous levels of nitrosamines. Can they -- can we use -- assuming the endogenous exposure is primarily driven by diet, is it

reasonable to use the current dietary intake levels in comparison to the past assessments to make a similar adjustments from those past measurements, until we get more of this new data that we can collect in the future? Can we extrapolate from those to the current status?

Anyone -- would like to comment?

DR. HECHT: Yeah. I --

DR. DINOVI: Dr. Atrakchi, it is Mike -- oops. Sorry.

DR. HECHT: Okay. Go on.

DR. DINOVI: Do you mean levels of nitrate and nitride in food now, versus the past?

DR. ATRAKCHI: Well, yeah. I mean, there was a discussion yesterday, that where there are a lot of -- there are good data from a while ago, where endogenous levels have been measured and determined, but they are old. And we need -- based on -- things have changed. The dietary -- how we eat, and what we eat, also, slightly have changed. So can we use the previous -- and everybody is telling us that we need more current information -- current

measurements of the endogenous levels compared to the past. But this will take some time. Can we use the old data and extrapolate from that for the current time, to see where we are in those amounts and measurements? Nitrates are -- obviously, we are -- it is mostly nitrates and nitrites that we are -- that are present in the diets. And the nitrosamines are present, but at very, very low levels in the fresh fruits and the fresh vegetables.

Did I --

DR. DINOVI: Yeah. Nitrate exposure is a thousand times higher than nitrosamine exposure. That is for sure.

DR. HECHT: Sure. I have some data on that.

DR. ATRAKCHI: Go ahead, Dr. Hecht.

DR. HECHT: Yeah. There was quite a bit of work some years ago on endogenous formation of nitrosoproline. It has been discussed yesterday. Of course, nitrosoproline is excreted unchanged, and it is not metabolized at all. So, the -- it is a good monitor of endogenous formation. And Mirvish, and

colleagues carried out a series of studies where they measured the amount of nitrosoproline in the urine of people who had ingested -- as an example, 400 milligrams of nitrate and 500 milligrams of proline. And this yielded about nanomoles of nitrosoproline in urine. That is a yield of point six times 10 to the minus third percent. So, that -- and I think that data is reliable. There is a lot of other data on nitrosoproline from 20 years ago, or so, that, I think, are quite reliable.

So, that gives you an idea of the extent of endogenous formation in one relatively favorable circumstance.

DR. DINOVI: So that is a very low conversion percentage, right?

DR. HECHT: Correct.

DR. DINOVI: Yeah. That is interesting. Thank you.

DR. ATRAKCHI: Thank you. Anyone else to comment on this?

DR. ZEIGER: Yes. You know, we have known for many years that diet -- you know, has a big

effect on cancer incidents. So, if we are going to do any studies on endogenous formation, maybe, they should be done -- not just take a few hundred people and measure it, but set it up, almost, as an epidemiology study. We are looking at effects of diets and different dietary regimes on nitrosamine formation. You know, vegetarian versus meat eater -- people high levels of fish -- people eating high levels of fruits and vegetables. You know, because this will all of -- this -- all of this will effect whatever endogenous levels we have. So, to just measure random -- people at random, I think will not give a fairly good range -- or fairly accurate range.

DR. EISENBRAND: Yeah. That has been -- actually, that has been carried out quite a bit -- this epidemically logical research by European and -- groups and also by groups in the United States. And -- you know, the main -- one of the main questions was -- that you have the highest levels of nitrate, you have in vegetables -- certain vegetables are really high, because they just accumulate nitrate.

And so, the highest intake of nitrate comes definitely from consumption of vegetables. However, fresh green vegetables have a protective associated -- at least, the consumption with a protective -- for instance, what mainly has been in focus in these investigations on colon cancer incidents. So, colon cancer incidents is -- there is a low incidence associated with enhanced consumption of green vegetables.

If you would think that nitrate in this case plays a role, then one has, really, to reconsider -- and has been reconsidered that. Because a possible explanation is that many anti-oxidative and inhibiting agents -- inhibiting to the n-nitrosation reaction, are also co-consumed in green vegetables, like phenolics and many other vitamins -- vitamin C, vitamin D, and so on -- and B.

So, that is the reason. And that is why I think the whole subject of endogenous formation is really very complex, and really, very complicated. It is not a simple way to really come to a point where you can say reliable, this endogenous formation is x times higher than what you get from exogenous

exposure. The suspicion is quite realistic that it is that way. But it is so many complex inference parameters going on, including the physiological state of the people -- the health state of the people -- inflammatory situation -- infections, and all these things that drive the formation -- the endogenous formation of nitrate.

And that is the main reason why I think that we have the best and most reliable data density with the analytics of the exogenous formation -- the exogenous occurrence of nitrosamine components in food. And I would, of course -- as I said yesterday, I think it is a good idea to re-check this -- to re-visit these levels. My suspicion is that they are even lower today. But it should be -- of course, it needs to be measured again -- the actual data as they are -- yeah.

But, still, I think the argument -- the most -- main argument is that we have a much bigger data density -- of good data, also -- despite of the -- all the analytical problems that have been mentioned, already by Dr. Keire.

But there are good data, because many of the more recent surveys have been carried out with -- especially, proof against artificial formation of nitrosamine components during analysis.

DR. ATRAKCHI: Thank you. Dr. DiNovi, would you like to comment on this -- in particular, what Dr. Zeiger was saying about conducting epidemiological studies on the -- what the diets and what people eat, and so on, and more controlled than just simply taking random. My understanding with CFSAN, you have fairly interesting software that where you can choose and determine what -- down to what a person -- how much a person can eat -- let's say a specific type of cheese and determine the content of whatever it is we are looking for -- whether it is nitrosamine, or something else -- fairly interesting models that CFSAN uses.

DR. DINOVI: Yeah. In fact, the numbers that you quoted earlier -- when you were talking about the exposure, came from work that one of my colleagues did -- Dr. Lee -- he is listed in the -- his papers are listed in the references. So, people

can take a look at those.

And the other thing that -- I mean, we do not do epidemiology type studies, at all, in my office -- and somewhere in the Center for Food Safety there are epidemiologist. But it is not us. The one thing that we do, do, that is related to what we are talking here, is we can look at time course of intake -- our food consumption databases go back, at least, to the late 90's. So, you can look at 20 years of data, and look at the trends and see if it is going up and down. So that is a question we can answer fairly easily -- whether or not nitrate -- and I am going back to nitrate and nitride level, which are -- you know, easier to get. That, we can take a look at.

But I do want to comment on one of the other questions -- and it is about the confounding things when you look at in vivo stuff. For example -- and I am just taking a look at some of Dr. Lee's data, here -- the single highest food sources for pre-formed nitrosamines are smoked meats, smoked fish, pureed and grilled meats. And the problem there is, all those, also, are

significant -- take that word with a grain of salt, but there are all good sources of polyaromatic hydrocarbons, and other carcinogens. You know, we, in the food world, know that grilling food makes it taste great, but it is also much more, air quote, toxicologically dangerous than boiling, steaming, or microwaving your food, right? So those things would have to be taken into account if you want to take a look at any of those things and see if you can tease out the nitrosamine contribution to whatever carcinogen -- carcinogenic endpoint you were looking at. But certainly, we would be more than happy to contribute to looking at these time courses of these things.

From a purely numeric point of view, thought, it is probably -- no -- I am going to go beyond probably -- it is certainly less than an order of magnitude change, if it is changed at all.

Thank you.

DR. ATRAKCHI: Thank you.

Dr. Kyrtopoulos?

DR. KYRTOPOULOS: Yes. Thank you. We

certainly have very good data on nitrosoproline excretion. I think these are the data we were mentioning in your introduction earlier, that -- we have reliable ES measurements and lots of studies.

What the nitrosoproline studies really told us, referred to -- concerned the conditions which influence endogenous nitrosation. They told us what the hosts capacity -- of different hosts, depending on the shelf conditions, and so on -- how they influence endogenous nitrosation. They told us about what intakes influence nitrosation, and so on. However, in the context that we are discussing now, I think that what -- which has to do with the role of endogenous exposure and how it should be assessed in the context of overall risk assessment for a particular chemical -- let's say NDMA -- here, we need studies which will somehow -- I do not exactly how that would be achieved -- give us reliable data on the endogenous formation of the particular compounds.

So, I can certainly see the weakness of what we have now, in terms of the analytical reliability of the data -- whether there may be

concentrations of NDMA in urine and blood, which are very old measurements -- or DNA adducts, whose shortcomings I mentioned yesterday. And I know that -- I believe that today we have -- we do have the technical capability to acquire much more reliable numbers. So, I think this is what we need, now, in order to be able to answer the questions that we have been asking. How serious is the endogenous burden of a particular nitrosamine towards the overall cancer risk. And, I think, for this, we need proper measurements. And I agree that they have to be done once we have set up the systems and we have found the proper design of the studies. I think we need, kind of, epidemiological studies, because it is very clear that we understand very little -- the determinants of endogenous nitrosation. We do not really have time to discuss this in detail, now. But I mean, there are papers in the literature where people try to give -- for example, anti-oxidants, inhibitors of nitrosamines -- ascorbic acid, or other chemicals -- and they saw results, which were not always what they had anticipated. They deviated from what one would

have predicted in one way or another.

So, I think the system -- this system of in vivo nitrosation is affected by a lot of factors, which we do not understand. So, until we get to the point where we can understand them, what -- if I had the technical capacity -- in other words, the right instrumentation to measure reliably what I want to measure, I would just collect a -- you know, a population of people, and measure what -- estimate -- carry out my estimation of endogenous nitrosation. And then, try to make sense of what the determinants may be, and what we -- the range of endogenous formation, and so on.

Thank you.

DR. ATRAKCHI: Thank you, very much. Any other comments from the panelists?

DR. GUTTENPLAN: -- yeah.

DR. ATRAKCHI: Yes?

DR. GUTTENPLAN: In a way, maybe, repetitive, but I think from what we talked about yesterday, we are below the threshold for non-linearity with exogenous and endogenous

nitrosation. So -- and the result -- the carcinogenicity and genotoxicity of nitrosamines is additive under these conditions. One should be able to calculate, then, an incremental affect of the nitrosamines and the drugs, irrespective of the endogenous and exogenous exposure, resulting in nitrosamines.

It is probably not measurable because it is such a small difference compared to what is there. But it should still be calculable.

So that is repetitive. But that is one way of looking at it.

DR. ATRAKCHI: Thank you. Anyone else?

DR. EISENBRAND: Just to state that I am totally in line with what Dr. Kyrtopoulos said. I think the -- going into the research to really address and quantify, wherever possible, the endogenous nitrosation potential, is very, very important for the future. But it is, in my opinion, quite, a big task. It is not really trivial. But we have -- today, we have the records and the means to approach that -- and I think it is very important to get quantitative

figures. But we do not have them, now, at the moment. At least not reliable ones. We have indication that there is, perhaps, substantially vivo formation, which may be exceeding the ex vivo exposure quite a bit -- the exogenous exposure quite dramatically, perhaps. But we need to confirm that. We need to really check it, re-check it, have the right methods to measure it. So, this will be an undertaking that will take time.

And for the time being, as I said already -- and I do not want to repeat me, again -- I think a good way of putting the endogenous potential of formulation of nitrosamine components in perspective to the exogenous exposure, or the diet.

And the other point I would like to make again is that, if we have good manufacturing practice rules set -- technically, to produce drugs without -- or with very, very low nitrosamine contamination, we should do that irrespective of a risk consideration, in my opinion. That, as I said, we did that for the cosmetics in Europe.

DR. ATRAKCHI: Thank you, very much, Dr. Eisenbrand. I think you -- I was about to more of

less summarize what seems to be the answer -- or comments on this question. But you just did it very nicely.

Mainly, overall, I believe is that it is very important to determine accurate the endogenous formation of nitrosamines. But this is require -- will require time and fairly sensitive and highly sophisticated analytical methods. And that should eb done -- need to be done. But it is in the future.

In the meantime, consideration for exogenous exposure to nitrates, nitrites, and other sources, as well as nitrosamines, that -- we have data from the past, and we could more or less reliably use some of this information, as well as, not terribly old data. However, more going back to the GMP, it comes down to the chemistries, and to the manufacturing. So, we do need, with respect to pharmaceuticals, and medicines, and drugs, to start with -- it is really the chemistry and the controls of the manufacturing that needs to be considered, until all these data are collected. Then, we can compare what is the levels that we ingest on a daily basis from the environment

around us, versus what is present in medicines.

If anybody disagrees with this quick summary, please go ahead and -- I know there were different opinions of how to do this, or -- but I think the overall -- there is pros and cons to both. Whether it is the exogenous, the endogenous exposure, as well as what is in the drugs, ultimately it is the drugs need to be under control for GMP for any types of impurities. And in this case, particularly for nitrosamines.

If no other comments, I would like to go to question six. Okay.

So, now we move to the chemistries of nitrosamines. Question six is one of two chemistry questions. In the absence of data and based on identified differences in nitrosamine chemistries and reactivities, can read-across for structural similarly to related compounds be used for nitrosamines? What are the key parameters to consider when conducting QSAR assessment for nitrosamines?

There has been a tremendous advancement of in silico modeling and software engineering to

predict many aspects of biology and chemistries, including chemical structural similarity. With a diversity of nitrosamine behaviors, both in vitro and in vivo, what are the pros and cons, and what is the extent of reliability for using QSAR to predict nitrosamine mutagenicity and/or their reactivity?

I would like to start with Dr. Cronin.

DR. CRONIN: Thank you. And there has already been a lot of discussion of chemistry and structural activity right from our first lecture yesterday, from Professor Eisenbrand. And let's just think for a moment why we want to do that.

And the advantages of going to structural activity, read-across QSAR, that in theory, at least, it is quick. It should be cheaper than doing, certainly, the longer-term animal tests. And it may not require animals.

You know, disadvantages; these are predictions, these are inferences, so we do have less certainty. And especially when we come to use read-across, we need some expertise. And already listening to the discussion in the last day or so, I

really appreciate the expertise already in this area.

My background is in, in silico toxicology. And I have taken the opportunity in the last couple of weeks to get a feel -- or refresh my opinions and understanding of this particular area, with regard to nitrosamines. There is a lot that has been going on. Particularly, very recently. And I am going to try to capture some of that, and, also bring together some of the information that we have already had.

Now, the question asks about read-across and QSAR, and I am going to deal with those separately. And I am going to start with read-across. And for those of you not familiar with the term, very briefly, read-across -- we are trying to infer an activity -- here, it might be the toxicity -- and that could be ultimately the carcinogenicity, or even a potency. We are trying to infer that for a molecule we may not know very much about -- or very little about -- from a similar molecule that is data rich -- or indeed a group of molecules that are data rich. And we have got a lot

of expertise in this, going back -- well, at least, 30 years -- and particularly with regard to regulatory use of read-across, and more specifically, probably in the last decade, how we can accept this, and the uncertainties associated with that. And I am thinking particularly of what has happened in Europe with REACH. But there is lots of other examples in the U.S. and Canada, and elsewhere in the world.

And read-across, itself, covers a number of techniques, which particularly in the last decade have been developed quite considerably, starting with the simple use of analogs -- and I will discuss that in a moment -- through to what we mean by chemical similarly.

So, I think there are three issues we need to think about with read-across, that are going to be immediately important for you for nitrosamines. And firstly is, what do we mean by data rich molecules? And, here, we have this term where we want to infer from data rich molecules, and actually -- as we have heard -- we are probably quite lucky for the nitrosamines, compared to many other chemical classes,

as we have many carcinogenic data supported by mutagenetic data publicly available, and available within corporate and regulatory arenas.

There are some really nice reviews in this area. There is a very good review, recently, in 2020, from Andrew Thresher, from Lhasa Limited, who takes you through the carcinogenic and mutagenic data, and as well, I am aware of the ad hoc working group led by LeadScope and Lhasa in this area.

In doing some background, I had looked at the publicly available resources. We have already heard reference to Lhasa Limited CP DB website. I just put a nitrosamine structure in there, and I got 140 structures out associated with carcinogenic data, to give you some idea of the data resources.

I also searched nitrosamines in the OECD QSAR tool box. And there are several hundred publicly available mutagenic data. And you will get these from -- or those kind of numbers from other resources. For instance, the US EPA chemistry dashboards, maybe ChEMBL and PubChem, as well.

And I also realize there is probably a

lot of data that have been made available -- or are available and could supplement the publicly available data from corporate backgrounds. I know the -- or I suspect the Lhasa LeadScope initiative has been able to tap in those.

So, with regard to data, therefore, we have many potential so-called data rich source molecules that we can read-across from. That gives us a strength -- or a position of strength.

The thing I would say, where are the data? Are they in a format that you can use easily for read-across? Are they in a correct informatics type format. The tool box -- the OECD QSAR tool box is one way of doing that. But may not be ideal for all sorts of purposes. So, FDA may need to think about how they are going to use those particular data resources.

Second issue with read-across is the concept of similarity. And similarity really is a very, very subjective opinion about whether two molecules are similar or not. And I know we have measures of similarity. But at some point, you are

going to have to make a decision that something is suitably similar, that you can have confidence in reading-across your toxicity.

We have got loads of guidance. There is lots of guidance -- for instance, from OECD, EFSA, ECHA, US EPA, and various industry bodies. And they give us several ways of defining how we can identify similar molecules.

For nitrosamines, the obvious way of doing this is saying it has the n-nitrosamine structure in it. But in practice, that would be too crude a measure to do read-across from. So, we need to undergo a process of sub-categorization. And that allows us to be quite discriminatory -- or it gives us the opportunity to be more discriminatory, at least. And to do that, we probably want to pull in our knowledge of reactivity, our knowledge of chemistry, and go to the data and get this information structural activity from the data.

So, again, I really appreciate some of the work that I have seen very recently from the ad hoc group. It was presented by LeadScope at SOT, a

couple of weeks ago -- to identify many of these types of structural activity relationships and put them into terms of structural alerts. And particularly, potency alerts can be based around chain lengths, substitution on the alpha and beta carbons, electron withdrawing groups, and so forth.

So, now, we can go from just a nitrosamine structure, through to much more subtleties about structural activity. And that really links in structural knowledge, to reactivity, to our understanding of mechanisms of action.

Final aspect of read-across, which has probably caused the most problems in Europe, in terms of REACH, is the justification of read-across. So, in other words, we build a category, we have identified analogs, we do the read-across, but then, we have to justify why that is acceptable.

We are back around into this -- the previous argument about structural activity, is, of course, a fantastic basis to start from. And we are also doing things like supplementing our arguments with new approach methodology and NAM type data. It

would be in vitro data, further in silico data, and chemico data. So therefore, maybe, if required possible to supplement these read-across arguments with a relatively small number of quite cheap, rapid tests to help us understand similarity, help us justify a hypothesis.

The reality I am learning is, it is -- we have greater certainty -- or we have less problems with certainty if we are reading across a positive, a strong carcinogen in this case. You are going to need more evidence, in reality, to be able to read-across a negative, or low toxicity compound. And we heard the argument yesterday at the end of the session about what happens if you have Ames negative compounds.

And the benefits of read-across, I think it gives you an ability to test hypotheses. And we heard again -- going back to the -- if we have a negative Ames test, we can then test hypotheses -- well, what do we know around that area of chemistry? What would we expect? Does it all fit together? And it gives us a rational way of organizing data. So, we are not just dealing with molecules, necessarily, in

isolation.

Moving onto QSAR, and more formally that is quantitative structure-activity relationships. And those -- there is a continuum from where read-across finishes and QSAR starts, it must be said. But they are more commonly thought of quantitative predictions on the basis of physical chemical properties structural descriptors. And they can be very, very varied. Clearly, we do not have time to go into the full gambit, here. But they can go from a very small number of molecules -- basements and some regression analysis -- which is where we started in the area -- right the way up to a very large number of molecules -- big data types of molecules where in we have to use -- and go up to machine learning -- artificial intelligence type methods.

For nitrosamines, I think we want to focus in on the smaller and more subtle types of models. I cannot see -- we do have large global QSARS -- as I call them -- coping with -- or including many types of molecules and many types of affects. But I think they will struggle with nitrosamines for

the subtleties we know that are in the chemistry and the activity cliffs.

So, my recommendation for QSARs -- if we are going to build them around -- for instance TD50 values, which would be very complex -- or mutagenic data -- would be to just look at nitrosamines as a group -- as the largest group -- and maybe even in -- to focus in on some of those structural activity relationships, and build quantitative models, there.

With regard to the kinds of descriptors that we are going to use -- well, I am a biologist, and I believe in mechanisms, and I believe our models should be relatable back to mechanisms of action, and also understandable. So, the question to ask ourselves is -- well, what is controlling the activity that we are trying to model?

Clearly, I have seen some relationships and QSARs attempted for -- to simply trying to correlate TD50 to log P. And without any surprise whatsoever, those relationships do not exist. So, it is more than our classic log P parameters. And of course, it must related in some ways to reactivity.

So, we need to think how we can parameterize reactivity. And I have not seen any good published QSARs in this area that they -- there may be some available. But surely, we can do quantum chemical level calculations to understand and model those particular steps that we know are controlling potency.

So, the last thing I would like to say about QSAR is, in terms of formulating the model itself -- and QSAR works best when you have a large variation of activity, going over several orders of magnitude. And ideally, that we have got a spread of data across that, so we can form a strong model. What I fear may be the case with QSARs for this -- for nitrosamines is, we have heard, repeatedly, we have got a lot of high potency nitrosamines. So, you may get a situation where you have a large group of data -- which are high potency, and then, relatively fewer data, which are lower potency. And this gives us some problems in modeling. There are ways around it. But just be very cautious with that.

So, to summarize, given the model and

given the problem of nitrosamines, given the current data resources and mechanistic knowledge, I think you can do read-across, you can do QSAR. My preference would be structural activity based rational read-across supported where possible by the data we have. And that is my comment for the moment. Thank you.

DR. ATRAKCHI: Thank you, very much.

Dr. DiNovi?

DR. DINOVI: So, I have to admit this is not my field, so I really -- I cannot say too much, here. But I certainly would not disagree with anything that I just heard. So, thank you.

DR. ATRAKCHI: Thanks.

Dr. Bucher?

DR. BUCHER: So -- well, I want to compliment Dr. Cronin. He has really done a terrific job at capturing the complexities and I must say, the -- his predictions about the issues related to read-across and QSAR models with respect to nitrosamines mimic, I think, our thinking, and what we have been able to do just in looking at this problem

over the last couple of weeks. Recognizing that people have been working in this area for 40 years, now, and trying to come up with good models to -- for classifying and predicting potency for various nitrosamines.

We have been looking at this only for a couple of weeks. So, I challenged our chemo informaticist, here, in the program, Dr. Kamel Mansouri, just to take a quick look at this, applying some of the new tools with machine learning, and something called the OPERA database, as a database of predicted physical chemical properties, and cross-referencing that to the carcinogenic potency database, and the loss of data what was just discussed. And it is very preliminary efforts. He was able to generate some predicted TD50s -- or potency estimates that did look reasonable, applying these machine learning and multiple regression models to the QSAR data and physical chemical properties. And as I said, incorporating the carcinogenic potency database numbers. He did this looking at the TD50's for over 700 substances in the database that have QSAR

ready data -- if you will -- and rodent TD50 data. And he also did this in models that were restricted only to the -- about 120 nitrosamine -- the rodent data TD50s, as well. And what he found is exactly what Dr. Cronin predicted.

The results of the five-fold cross validation studies that he did were somewhat promising, but the models needed additional work to better discriminate the very potent carcinogens -- the very potent nitrosamines, from ones that would be considered weak or negative. Additional information from the mutagenicity studies -- or QSAR and physical chemical properties for non-carcinogens -- which are not heavily represented in the carcinogenic potency database -- could improve these models. And of course, one problem with this is that it will not be addressed by additional work -- is the variability and the imprecision and the extremely -- in the experimentally derived TD50s have already been discussed. And this, of course, stems from the wide variety of study design and performance aspects of the rodent cancer studies.

So, the bottom line is, I think the answer is, yes, it is possible to get a general prediction of carcinogenic potency. How useful these numbers might be, remains to be determined. And just as Dr. Cronin predicted, the vast majority of the TD50s of the nitrosamines in the database, are, sort of, overwhelming the information from the less potent and the very, very potent carcinogens. So, we really need, unfortunately, to have a better spread of higher TD50 -- or lower potency carcinogens to look at to correctly model these chemicals. We do not have that in this case. And that is something that we are going to have to -- need to try to do some additional work, I think, to try to compensate for that aspect of the database.

So, that would be my comment.

DR. ATRAKCHI: Thank you.

Dr. Eisenbrand? Dr. Eisenbrand, I think you are muted.

DR. EISENBRAND: Sorry about that. I was quite impressed with the contributions until now. And I was very interested in. I may throw in some

further aspects that may be considered.

As far as I understand, this read-across validation exercise is going to find out ways to get reliable information about potency, including the extremes -- the very highly potent, and the non-potent -- or even, not ineffective. And that would be a little bit -- my point I would like to make, what about using our present day information we have about the non-carcinogenic nitroso compounds? And then, ask mechanistically minded, what makes them non-potent -- what makes them ineffective? And one can easily see that there is, of course, several layers of answers to that. Or maybe -- potency or the interaction with the nucleotides in the DNA. But there is, of course, a lot of metabolic action going on. And there we have activating, and disactivating metabolism. And I think mechanistically, it would be very important to input this information in.

For instance, we have -- of course, a very trivial molecular modifications -- the alpha C branching and the tert-butyl group. If that is attached, then the compound is not active, at all,

because there is no -- if there is alpha C hydroxylation on the other side, which may be a methyl group, then there is a split off, and the tert-butyl compound is not an affective -- agent. So, that is very easy, actually.

But what about, for instance isometrically substituted nitroso compounds. We had a series of investigations, for instance for n-methyl nitroso pyridines being either positioned -- substituted in two, three, and four positions. So, there is all three isomers have been subbed. And if I were to ask the specialists which one they would predict to be carcinogenic, I would be interested to hear. But -- so, just to make it short, it is just a two compound that is the two isomer. And the full nitro is a group attached to the two position of the pyridine that is carcinogenic and mutagenic, where as the three and the four are not. And they are -- the answer is, again, there is an equilibrium between activating and disactivating metabolism.

And this not only true for this -- for these compounds, but also -- for instance, the example

I showed yesterday, which was cimetidine, which was mutagenic in the hamsters. But turned out to be non-carcinogenic in vivo in the rat. Again, because of disactivating metabolism.

So, I personally think it would be a good idea to use all the negative examples -- the examples of negativity we have and study why they are negative. And of course, as I said, they are trivial answers, but there are also answers that need to be really investigated in detail. So, I think that would be a enrichment of the whole procedure. That is for the preformed nitroso compounds.

My second suggestion would actually be, we need some system of -- some predictive system to get grips on the nitrosatablity. How easy is a given compound -- maybe, of a new structure -- how easy is it nitrosatable? And I mentioned, yesterday, in my presentation, there is a richness in literature on that. Especially, Richard Loeppky has done a lot of work showing that there are specific structures that are very, very easily nitrosated -- tertiary amines -- and I showed yesterday, also, the example of

motapizone and metamizole. The one easily splitting of -- very easily splitting of dimethyl nitrosamine. The other one forming in nitroso compound that is not carcinogenic.

So, I think this second point to study the nitrosatablity, I think is important. It has been realized in the early times already. And that is why I mentioned the NIP test of the WHO, stemming from the early 80's -- which is a rugged test. But it very simply and chemically shows an information -- gives an information about the nitrosatablity. I think that is something that pharma will need. A simple test -- if there is not a in silico prediction systems that are reliable enough, which I do not see at the moment -- but there might be. And then, to use something like that.

Thank you.

DR. ATRAKCHI: Thank you, Dr. Eisenbrand. Very good points.

Dr. Hecht?

DR. HECHT: Yes. So, there are certain aspects of the structure and activity relationships of

nitroso compounds that we understand quite well, that have already been mentioned, just by Professor Eisenbrand, including alpha substitution. So, if you substitute the alpha positions, then you are going to inhibit the alpha hydroxylation metabolism. And probably decrease the carcinogenicity.

The other aspect is the polarity of the compound. So nitrosoproline is a great example. It is not metabolized. It is not carcinogenic. It is excreted unchanged. But there are other examples where if you substitute with a carboxy group, there is still activity maintained.

I am not optimistic that read-across and structure activity relationships are going to lead to predictability of nitrosamine carcinogenesis. Just look at the review by Kreuzman and Stewart, published in 1984. Look at the table in the back of over 200 nitrosamines and their target tissues and activities and tell me whether predications can come up with these answers. I really doubt it. I am not optimistic, at all.

That is all I have to say.

DR. ATRAKCHI: Thank you, Dr. Hecht.

Dr. Kyrtopoulos?

DR. KYRTOPOULOS: I am afraid this is not really my field. And clearly, we are surrounded by great expert. So, I thank you, very much. I will pass on -- thank you.

DR. ATRAKCHI: Very good.

Dr. Zeiger?

DR. ZEIGER: Yes. Thank you. First of all, I appreciate -- definitely appreciated Dr. Cronin's introductory comments on this. It laid out the strengths and some of the weaknesses of the problems.

I look at this. There are a number of aspects to it. I remember Willie Lijinsky's early work in carcinogenicity, showing that relatively minor structural changes could lead to quite large difference and carcinogenic potency, and also, organ specificity. This is something that somehow would have to be worked into any QSAR or read-across system that we adopt.

Secondly, it has already been mentioned

by a number of people -- the high proportion -- the low proportion of negatives -- you know, in the database -- of clean true negatives in the database, makes it a problem. Otherwise, you are just stuck with -- without those negatives, you are just stuck with a possibility of a -- as was mentioned, a nitroso structure automatically gives you a conclusion of mutagenicity and carcinogenicity, which may not be all true.

And I am concerned -- you know, when I deal with QSAR systems -- of the quality of the literature that is used to train and develop those systems. And I have had the advantage of being able to work in the past with fairly pure -- you know, very pure nitrosamines synthesized by Lijinsky. But I have noticed that in the literature, there are -- outside of that, there are many of -- many potential problems.

One thing that just came to me when I was preparing for this symposium -- this talk, was that looking at -- for example nitrosodiethanolamine, which supposedly is a non-mutagenic nitrosamine. We tested it and published the data back a number of

years ago, that it was weakly positive in the Ames test. But then, looking further, I saw that the analyzed purity of our sample was only 78 percent. Would not take much of a potent nitrosamine, within that 22 percent impurity, to give us a positive result.

So, I think whatever data are used for any read-across, or QSAR, really has to be addressed -- the people who are using it have to address the quality of the studies -- or the purity of the nitrosamines. Because a small amount -- a small impurity of a potent nitrosamine will change all the in vitro reactivities of -- in vitro responses of a supposedly non-potent nitrosamine.

All that said, I think -- unless we want to do carcinogenicities -- you know, analyses -- carcinogenicity studies on all potential nitrosamines, we have to develop our read-across and QSAR systems for this. Thank you.

DR. ATRAKCHI: Thank you, Dr. Zeiger.

Dr. Rice?

DR. RICE: Having never worked in this

area or attempted what appear to be very formidable challenges in developing the assessment prediction of activity for nitrosamines, and using structural similarities, I do not think I can add anymore to what has previously been said. But I would second Dr. Hecht's caution about the complexity of the available data for nitrosamines, and hope that future improvements in this direction will shine more light in what remains to be a challenging area for prediction.

I have nothing more to say. Thank you.

DR. ATRAKCHI: Thank you. I have some questions to bring up. But I would like to know if anyone else from the panelists would like to comment on this question?

Okay. So, Dr. Cronin, one of the questions presented is, read-across analysis can be very subjective. What methods can be used to more objectively identify the most relevant analogs for risk assessment of a data poor in nitrosamines?

DR. CRONIN: Well, that is the killer question for read-across. Thank you for whoever asked

that one. And there is no simple way of asking -- answering that.

Just to point out, if you are unfamiliar with it, there is the so call read -- European chemical agencies read-across assessment framework sets out a number of criteria in which to evaluate your read-across. And also, there is quite a -- well, several papers on understanding uncertainties in terms of read-across. We have co-authored something recently, and there is more historic work, for instance, from Proctor and Gamble and various other authors, as well.

I think what we need to think is, we are going to start with the chemical structure. So, we need to understand, firstly, what the difference is between the chemical structure in our target molecule, where we have few data, or no data, an a-source molecule. If you understand the differences in chemical structure, the question you are actually asking, therefore, is -- are the differences in chemical structure significant enough that you cannot perform read-across? So, are the differences in

chemical structure going to change the toxicological profile, both in terms of toxicodynamic -- which is what we have been talking about mainly. But also, toxicokinetic, which is, sort of, the lost child of read-across in many ways. And we have not done a very good job, up until now, doing that.

So, we certainly have been looking at different approaches to doing that. One can do that in terms of just understanding the chemical structures, themselves. Do they have the same -- most fundamentally, the same functional groups. Is the arrangement around, the n-nitrosamine -- which we have heard is so important -- is that the same. If it is not, is that going to affect reactivity. And gaining that knowledge.

And then, also with any changes -- for instance with chain lengths -- if we have a basic analog that may be varying just in terms of a chain length, how will they affect bioavailability and toxicokinetic? So -- and we can also add into the mix, things like chemical similarity and so forth.

So, at some point, you need to make a

decision. At some point, you need to be able to say that the differences are not sufficiently significant to be able to not to read-across. And you may also need to go and take some measurements. Now, these could be in vitro measurements, they may be further calculations -- but to support your arguments to perform that.

And one thing I would just like to put in your mind -- and I do not -- it may come up later, is the process of problem formulation. We have been thinking about just jumping in with QSAR and read-across. And we have not really been thinking about, well, how are we going to use that. And what I would recommend is that, if the agencies are going to use read-across, then they stand back and think, okay, what is the problem we are trying to solve here with read-across. So, it could be, very rapid screening and prioritization. It could be a much more detailed risk assessment. And there are different criteria for acceptance of those points. And your levels of uncertainty will be -- acceptable uncertainty would be different. So, put that in the back of your mind and

have some pre-defined criteria. And at some point, you have got to assess the situation you are with read-across and your analog and say, does I meet -- do we have sufficient confidence to be able to do this. If not, why don't we, and how would we get more confidence.

So, there is no easy answer. It is a process of intelligently using read-across in many ways. Thank you.

DR. ATRAKCHI: Thank you. The other questions is, is there enough data on higher molecular weight nitrosamines -- for example, more than 350 deltons -- to assess the mutagenicity, carcinogenicity or un-tested nitrosamines in this size range?

DR. CRONIN: Well, I will start off by answering that very simple, that I do not know. But we have access to the data. And that would be quite a straight forward question to answer. But I cannot answer it definitively, yes, or no. You would have to go through the data resources.

And, again, as I said, with the problem formulation -- before you ask -- before you go and

interrogate the data, what do you mean by sufficient data? I mean, that is quite an interesting and leading statement to start off with. So, have some ideas about what your expectations would be, and what the answers would be that you would want to answer them.

Thank you.

DR. ATRAKCHI: Very good. Both positive and negative examples of mutagens, carcinogens are needed in order to develop a QSAR model. Are the available negative data sufficiently robust for this purpose? That is, were the examples tested at high enough concentrations?

DR. CRONIN: I am going to answer the first part, in terms of QSAR methodology, and then, defer the second part, in terms of experimental design, maybe, to the experimental toxicologist.

So, one thing I meant to say, and I forgot to say, in my description of QSAR is, we can have two types of QSARs. One is a categoric approach, where we might just predict activity. So, whether a substance is mutagenic, or not. And this is really

what we are referring to, here.

The second kind of QSAR is more what I was eluding to in my initial comments, which was that we were -- we are modeling at potency. Which we can do, as well, in theory, with mutagenicity. And there are some QSAR for mutagenic potency. At which time, we would not include the negative data.

So, I would just put those points in your mind. But certainly, we do need high quality negative data that we are certain of to develop models.

And I would -- I will pass it over to the experimental toxicologist to answer. Thank you.

DR. ATRAKCHI: Thank you. Anyone would like to take that part of the question?

DR. BUCHER: So, this is John Bucher. I -- you know, it has been stated earlier that greater than 90 percent of the studied nitrosamines are positive for carcinogenicity. And this is a -- this represents a huge problem with respect to the balance of positive and negative data that you need to create decent models.

So -- and the other problem, of course, is that the simple nitroso entity, in and of itself, derives so much of the bioactivity, that you really need to have strong confidence and extensive data to be able to have the R groups on that nitroso be determinative, and override this --

DR. ATRAKCHI: Dr. Bucher, we lost your audio. Dr. Bucher? Maybe, check your mute button, again. Okay. Maybe, we will get back to Dr. Bucher when he finds out -- hopefully, resolves the issue.

Next question is, are there areas of nitrosamine chemical space for which we have too little data to make a QSAR predications?

DR. CRONIN: That is a really good question. And how to define nitrosamine chemical space is the first question in this. And I guess, what we -- if this was a more general question in more general areas of chemistry, I would say, okay, tell me all the structures that you have which contain a nitrosamine, or the particular class of nitrosamine, or what you are interested in, and think about your domain. Which structures are in that domain. And

then, I would map on the data to that.

So, I would admit -- and I have just been simply looking at substances for which there are data. And what you would need to do to answer that question is go and find -- or consider all structures that would contain a nitrosamine group.

I mean, one way you can start doing that is just -- as I said, I went to OECD QSAR tool box, which is one tool of many. It is really downloadable. You could draw out all of the nitroso compounds in that. You could do the same in the US EPA dashboard or go to ChEMBL or PubChem. And do a search for nitrosamine compounds. And then, just map on the data for that, and see where you have good coverage, and where you are lacking coverage.

And you need to think what your criteria are for coverage. Do you just mean in terms of the structure that is the basis for reactivity? Or are you looking at all kinds of physical chemical properties.

So, I do not know the answer to that question. I suspect there will be areas where we are

quite data rich and got quite a lot of data -- probably do not need to do much more testing -- and areas where there will be distinct lacks of data.

Thank you.

DR. ATRAKCHI: Thank you. Another question is, it has been mentioned that the stability of the reactive intermediates -- or intermediate should be considered when predicting nitrosamine potency. Such calculations can be computationally intensive for complex molecules. How can this information be practically incorporated into a model for mainstream use?

DR. CRONIN: Again, really good question. And the -- what is great about that question is, it is implying the mechanistic interpretability of it. And there is no easy answer to that. If you got complex molecules and it is going to take a couple of weeks to do a calculation -- potentially, it could do -- then, that is the reality.

The problem, of course, is, if you have a relatively large data set -- even 10 or 20

compounds -- then you could be in -- it could take quite a long time to create a model. The only way around that is to try and use parameters that may be less computationally expensive. I mean, I refer to quantum chemical parameters as you go down the areas in -- back into some empirical calculations -- you may be able to get results that are as good, or even use very trivial 2-D structure based type approaches. But, again, they just do not capture the subtleties of the activities cliffs or at all give out specific reaction mechanisms.

There is no easy answer to that. If you get a high quality model that is based around a metabolic route -- or a reactivity route, and you got a good basis for that in calculation -- going down the levels of calculation does not -- it will make it cheaper and quicker, but it does not make it better or as good. Thank you.

DR. ATRAKCHI: And we will take one more question. The -- in reference to Dr. Cronin's comment about challenges with justifying read-across assessments, any thoughts on what could be cheap and

rapid data to supplement and support read-across? Anything other than Ames test? Something that could provide quantitative data that could be correlated to LD50 values in an absolute or relative sense, versus a compound with a well-established TD50?

DR. CRONIN: I am a bit caught out by the LD50 comment. And I am probably taking that out of context. We have done a little bit of work with cytotoxic data, and there may be some benefit in that.

As I said, in read-across, we are building up knowledge and using NAM data. And in other areas of read-across, we have certainly been pulling in data from ToxCast and the high content data, metabolism data, and also, in silico predictions.

But what -- I think that is a great question, and I will pass it over to the experimental toxicologist and see if they can answer it -- so, in terms of are there rapid quick win types of in vitro or other assays that can support our read-across argument.

DR. ATRAKCHI: Thank you. Anyone from

the panelist would like to take this?

DR. BUCHER: This is John Bucher. And I must apologize. My computer -- I dropped the signal last time, right after I stopped talking. So, if you asked me a question, I apologize for not answering.

With respect to a follow up, there, I -- the only thing I can think of is, there are a number of in silico models for metabolism and for -- you know, rapidity of excretion, and things of that nature, that might be useful to incorporate as second level steps, I guess.

DR. ATRAKCHI: Thank you, Dr. Bucher. Any other comments?

Okay. It is 11:02. We will take a 10 minute break, and we will be returning 11:10, or 11:12. Thank you.

(off the record)

DR. ATRAKCHI: I think we should go back and -- before we begin with question seven, there are a couple more questions on question six.

The first question is, there is a difference between QSAR and SAR, but they are being

used somewhat interchangeable. SAR is an approach that has been shown to be health protective when suitable analogs are identified. If nitrosamines with existing data can be mapped to major groups than a nitrosamine with limited data could be mapped to the appropriate classification based on structure. The scientific community would greatly benefit if a group of experts could help map the structural space of nitrosamines and associated supportable limit. Can this be done?

DR. CRONIN: I will start on that. I agree with the statement -- I am reading it, now. I certainly agree with that. I think the term, SAR, in the question, is -- to what I was talk about with read-across. And this is actually quite similar to -- I spoke a little bit about -- there is an ad hoc group being led by Lhasa Limited and LeadScope, and that has a number of industry and academic members. I am not part of that, I should add at this point. I have just been reviewing their work. So, that is part, at least, of what they have been trying to do -- is identify these groups in terms of structural

alerts that are associated with reactivity that we can understand the reactivity. And also, seeing within those groups, there seems to be some variations of potency, which are unsoundable and predictable. And that is quite all right, because what you would be saying is for a new molecule, you would be putting it into that structural group, and then, seeing where it lies in read-across terms. That might just be called a trend analysis, or even a small QSAR.

But I agree with the sentiment. And yes, I believe as long as we have got rational ways of grouping molecules, that is achievable.

DR. ATRAKCHI: Thank you. The next question is, along with the QSAR assessment, can we consider metabolism assessment of complex nitrosamine impurities, which would account to the bioactivation of nitrosamine into reactive diazonium ion that result in carcinogenicity? For example, if a compound gets converted into diazonium ion easily, it would be more potent?

DR. CRONIN: There is a couple of different aspects to this. Again, I am reading the

question. There is a couple of different aspects that would -- I would highlight.

If we are dealing with complex nitrosamine impurities, I am assuming -- I am not sure what that means, off the top of my head. So, I will give two opinions. It could be a complex structure -- a large structure with much complexity in it. Or, it could be a number of different impurities in that way.

So, yes. It is possible to trade models for bioactivation. Some of the discussion before the break was around that. And indeed, they might be quite some hefty models, if they are going to be used computationally. But we should be able to calculate conversion into the reactive diazonium ion. What, of course, we need, there, is some kind of -- is some benchmark against -- so we need some data. We cannot, necessarily, just create a model de novo, without data to base the model on and understand the model.

So, my question -- if you were to come to me with that question, I would say, well, do we have data on which to base the model in the first

place. And I do not know the answer to that, off the top of my head. So, in other words, for nitrosamine -- I am sorry -- diazonium ion conversion. I suspect there probably are some kinds of data that we can use.

The second part to the question, if compound get easily converted, would it be more potent? I assume so. But I am not a particular expert in that area, and I would pass that over to other toxicologists to answer.

DR. ATRAKCHI: Thank you. Anyone would like to answer the -- that part of the question?

Okay. The other question is, should SAR/read-across focus on carcinogenicity endpoint, or consider separately the mutagenicity metabolic activation calculation by activated nitrosamine, or repair of alkylated DNA?

DR. CRONIN: That is a really great question, and I like that question a lot. It shows a lot of insight into what we are trying to do.

The history of read-across -- or probably the last decade of history of the

read-across, particularly in Europe has been to cope -- or to provide information for REACH endpoints, most specifically. So, in terms of the REACH legislation -- and again, I am just saying these comments from memory -- what we are trying to do -- or what we are assuming is that our read-across would be -- or have the same level of scientific competence that the animal test on which it is replacing would have.

The other implication, in terms of using read-across and in silico methods for REACH type endpoints is, that they should be relevant -- or they should be replicating the actually regulatory end point. So, that is why there is read-across attempted for carcinogenicity. And to me, these very complex toxicological affects are the most challenging to try to attempt read-across.

You go back to your same arguments that we had before about what level -- for a complex endpoint, what level of reliability and confidence can you have on that, on the basis of your understanding of the similarity and dissimilarity between two or

more compounds. And, it may be that we can never get there definitively for carcinogenicity, and we do need to add in other test data, as we were discussing before.

However, the -- when we -- and I hate to use the word simplify -- but, when we are looking at slightly less complex endpoints, such as mutagenicity metabolic activation and alkylation, and so forth, then we are cutting down the possibilities for differences between molecules -- because clearly, here, if we are talking, certainly, about in vitro data, then we are eliminating a lot of the variability, in terms of toxicokinetic, and those issues -- and focusing in particularly on -- in API terminology, the molecular initiating event, and whether or read-across argument holds up in terms of the molecular initiating event.

So, yes. We can apply SAR and read-across to all of those. I would say we can apply it to all of the endpoints and effects that are listed by the questioner. And it may be, then, you need to think, well, how are you going to use that

information.

So, if you get a good read-across, you can do a good read-across. For instance, for alkylation -- well, how does that relate to your ultimate safety assessment and risk assessment? So, that becomes a different question.

So, to answer the question, yes. We can do read-across. But I would also caution how are you going to use that information.

Thank you.

DR. ATRAKCHI: Thank you, very much.

Anymore comments on this question from anyone?

Okay. We can move to question seven, which is the last question. And it is focused on pharmaceutical manufacturing.

Question seven; Nitrosamines can be formed during manufacturing of the Active Pharmaceutical Ingredient, and/or the drug product. What are possible approaches to consider in order to reduce nitrosamine formation during manufacturing? Can nitrosamines be eliminated completed from the API

and/or the drug product?

We heard from this -- yesterday, from Dr. King, on the evolution of the contamination incidents with -- of medicines with nitrosamines. Is it possible to eliminate nitrosamines from drugs, and particularly, in the final drug product, which is the entity will be taken by the patient? We know nitrosamines could form during various stages of drug manufacturing process. They could form during storage, during packaging. What are the parameters, or steps that could be followed to either prevent their formation, or reduce their amount to minimum, and maintain safe medicines?

I will be asking my colleagues from the FDA to present on this -- or to answer this question -- to present on the challenges that we have with regard to manufacturing and the elimination or the minimization of nitrosamines.

Dr. Keire?

DR. KEIRE: Thank you, Aisar.

So -- I mean, in the simplest terms -- you know, any step in the manufacturing process of a

drug substance -- or drug product that leads to the conjunction -- the brining together of a reactive amine -- a secondary or tertiary amine and a nitride -- or nitrous acid, can lead to nitrisation chemistry, and the formation of nitrosamines. So, that is the simple part, right?

And then, if you think about the range of different drug products and the complex chemistries that go into making them -- you know, then suddenly -- you know, that gets a little more complicated. But I would say, that -- you know, it is not a nullable. All these difference processes can be examined with this nitrosamine lens, right? You know, looking for back -- what -- you know, steps that bring these factors together. And you know, one of the control aspects would be to -- you know, change that process, so that you are not bringing those two components together, if it is possible.

You know, another approach is to think about that process where -- you know, maybe there is a step -- an unavoidable step in the synthesis of a particular active pharmaceutical ingredient, that --

you have that step early in the process, and then, you provide subsequent purging capacity with other steps in the process before you get to the purified API, that remove nitrosamines. And of course, that has to be established -- you know, either through measurements or other ways of knowing that you are -- you know, reducing or eliminating the nitrosamines from the process.

So -- but I guess I do want to make a point -- is that -- you know, in some of the initial findings in drugs of nitrosamines -- you know, they actually had nothing to do with the drug itself, right? That -- you know, in one particular example, DMF -- dimethylformamide, is a solvent, in which the reaction was carried out in. And that was heated for a long period of time, near the boiling point of that solvent. And, of course, it is known that when you do that you get the formation of dimethylamine from DMF. And then, in subsequent steps in the process, there was a quenching reaction, which involved nitride.

And so, what you had is a side reaction of a side reaction leaving to the -- leading to the

formation of NDMA. So -- you know, that is, kind of, a -- the example of one part of the complexity, right?

And then, the other part is that we have been surprised with -- is supply chain, right? There were certain manufacturers that were using recovered solvents, and they were -- the solvents were recovered in a way that led to their contamination by nitrosamines. So that when they were used in the synthetic process, where there was no risk of nitrosamines, they were present because they were in the recovered solvent used.

And then, the third, kind of, major consideration I the stability of the drug as formulated. So, there have been a couple of examples where the amount of nitrosamine increased over time, ranitidine is an example of that. And that is a degradant of the API.

So -- you know, all these things have to be considered in the manufacturer and the formulation, and the testing, right? You have to -- you know, if there is risk, you have to test and you have to see if your process has purge capability, and

you have to know your supply chain is clean. But you know, if you have all those things going, it is entirely possible that you could eliminate nitrosamine contamination from the drug.

I mean, there may be cases where you may not be able to completely eliminate it, but you can certainly assume over the shelf life of the drug -- you know, in real world condition, that the drug is stable, so that the amount of nitrosamines does not go above an acceptable amount. And in that case, it would be safe for patient use.

So, I will stop there.

DR. ATRAKCHI: Thank you.

Dr. Johnson?

DR. JOHNSON: So, hi. Yeah. This is actually a really good question, and it is one that I think regulatory agencies and manufacturers have been struggling with for the last two years. I have been involved with the agency on the nitrosamine task force for about two years.

Some of the things that, I guess, we have learned since the very beginning, when we first

saw these, it was -- you know, in the sartans, and as Dr. Keire had mentioned, they were really, kind of, surprises. They were side reactions. And as we have gone on over the last two years, we have seen them pop up in a lot of different places, and our understanding of, kind of, how this happening, I guess, has expanded.

I do not think we still truly have a -- you know, a complete understanding of all the possible ways. But from just the perspective of what I have seen as a chemistry assessor and being on this task force -- some of the things that I have noticed that -- directly related to the question, now -- what can a company do to help -- you know, minimize the formation of these -- or could they be eliminated completely.

So, some of what I have learned over the last two years is that in order to get a nitrosamine, you have got to have precursors. So, you need a secondary -- or tertiary mean, and you have got to have a nitrous aiding agent. And I think the one we have most commonly seen is nitrous acid. But there

are some others that could potentially be there. And the tricky part we found is -- as Dr. Keire alluded to -- a lot of times this is happening completely unrelated to the chemistry that is involved in actually making the API molecule. These are side reactions that are happening, kind of, unnoticed. And so that makes -- you know, a control strategy -- or understanding it difficult sometimes.

So, I guess, some of the main places -- and just, kind of, reiterate what Dr. Keire said -- some of the main places we have seen where nitrosamine formation is occurring and things that you can do are, one, when you have got a synthetic scheme, where you have got both the nitrous aiding agent, and an anime -- so that could be amines there that are -- aren't as -- anime bases -- tertiary anime bases -- they could be secondary amines -- they could be a tertiary or secondary anime functionality on a starting material, or an intermediate. So, they are in the same synthetic process. So, the worst case scenario is when they happen to be in the same step.

So -- I mean, the easiest way to

prevent this would just be to eliminate one of those. So, if you eliminate one of the agents -- one of the precursors, then, theoretically your risk of nitrosamines should -- you know, go away. But that is not always possible. It is not always possible just to eliminate these. There is some chemistry that just -- you know, requires a nitrous aiding agent, or requires anime bases. And sometimes it is just unavoidable.

So, if you have a case where they have to be used together, and you cannot -- you know eliminate one of them, it would be great if you could potentially not use them in the same step. If that is not possible, then -- you know, if the anime functionality is all on a starting material, or intermediate, you might consider -- you know, a protecting group on there. But, being aware that even if you have small amount of an unprotected anime, it is going to still react. So, you still -- if the risk is there, you have to think about what is going to form. And then, you have to have a strategy of determining whether or not it did not. If it did, to

what extent.

If you cold -- if the risk is that there is a nitrous aiding agent in one step, and the anime in several steps later, one thing that we found was industry was being surprised quite frequently they were getting nitrosamines, but if you looked in the synthetic scheme, they were not actually in the same step. They were actually separated by multiple steps. And there were actually -- you know, several workups in between. And what we discovered was that things travel through your process a lot farther than you think.

And so, what I tell industry when I am talking to them, is you have to think wholistically. You have got to look at the whole process. You have to think about what is in there, and you have got to understand how it is traveling through your process. So, even if they are not in the same step, they could potentially be traveling through and interacting more than you think.

So, the other thing that we were surprised on in this -- discussed in the sartans case,

the -- there was nitrous acid that was deliberated added -- it was actually nothing to do with the chemistry. It was actually a cleanup step, because they -- there had been a reagent that was using that was needed to be destroyed. Nitrous acid was just there to basically destroy that reagent. Unfortunately, they had also used some tertiary anime bases.

And so, a lot of companies -- you know, they were able to use that outside the process, because it was just a waste management step. And so that was simple. And we all thought, well, that is great. They have removed one of the precursors. Things should be great. And yet, they were still seeing some nitrosamine formation. Obviously, much less. But it was still there. And after further investigation, it turns out that one of the reagents, sodium azide, has a considerable amount of sodium nitride in it. So, while they thought their process was nitrate free, it actually was brining in quite a bit.

And so, that is another tricky part --

is you got to understand where these nitrous aiding agents -- or amines might be getting into your process as impurities. So, again, a wholistic thinking. What exactly is coming into your process? What is in it?

In the case where these are coming in as impurities, a lot of times people were able just to put -- you know, a limit on that reagent for that particular impurity, and they were able to show that when controlled at these levels, nitrosamines were not really a big issue.

Another one that we have seen a couple of times -- and again, nitrous acid is the -- probably the most common nitrous aiding agent we have seen. We have also seen some nitrosamines forming because some APIs had some residual amines in them, and then, they were put through a fluid bed drier. And this is a known issue with the malting process. You know, if there is nitrous oxides in the air in the malting process, they know they can get nitrosamines. Well, the same things with a fluid bed drier. On a couple of occasions, we have seen -- where there were some residual amines in the API, and then, what was put

through the fluid bed drying process, they actually were picking up nitrosamines.

So, again, it is a -- kind of, an exercise in thinking wholistically about the whole process. Not single steps, not just single reagents, but thinking about how the whole thing comes together, and how it all interacts.

Another place we picked up nitrosamines -- and sometimes they are actually intermediates -- or they are actually -- you know, on the starting shelf. So sometimes they are part of the process. And in that case, it is a little easier to control, because you know it is there. And then, it is just a matter of making sure that you have the control strategy that -- you know, controls those at an acceptable level.

And, as to the question, can we just eliminate them entirely, that is our hope. For the last two years, that is what we have been hoping -- is can we just eliminate them entirely. And in some cases, yes. They have been, by eliminating the precursors. Sometimes people have added just extra

purification steps, and they have been able to get those levels down to where they are either undetectable, or below a level that we are concerned.

But these are good questions. They are ones we are still dealing with. They are ones we are still learning about. And the longer we go, the more we learn.

But, anyway, that is what I have to share. So, thank you.

DR. ATRAKCHI: Thank you, very much, Dr. Johnson.

Dr. Dorsam?

DR. DORSAM: Well, good morning, and thank you. So, this is a fantastic question. And the first part of the question regarding what are the possible approaches to consider in order to reduce nitrosamine formation, I do have to say that I look to Dr. Keire and Dr. Johnson for answers to that question frequently. And they have outlined many of the different routes of formation, as well as mitigating strategies in this response, but also in many other forums.

Coming at this from a pharm/tox perspective, I would say that the two real key approaches -- I have two things that come to mind. Number one is awareness. And what Dr. Johnson said is that there is a need for a wholistic approach to one's drug product. That is a key element, because it is not one particular root cause. There are several potential contributors. And so, I think awareness that there are many potential places where nitrosamines might be forming in a product specific manner. That awareness is key to then, mitigate. And mitigate would then go towards -- you know, where is the root cause, and modifying the factors. So, I think, full awareness is a critical first piece. And then, mitigation, thereafter.

And I focus a little bit more on the second part of the question. Can nitrosamines be eliminated completed from the API or drug product? And I think that in some cases the answer is, yes. Nitrosamines can be eliminated. Although, we have -- eliminated is really not detected according to an assay that is appropriately sensitive. So, qualifying

it that way, we have seen that it can be eliminated from some drug products and APIs. I think it is accomplished through identifying the risk, understanding the chemistry, and then, making adjustments to reduce the risk of formation. And it is has been a credit to good science and good manufacturing -- good drug manufacturing, that we have been able to see nitrosamines can be eliminated in some cases.

I focus a little bit more on some of those other cases that we are still working with right now. There are some drug products where mitigation presents more challenges. And in that case, then we are looking more heavily at that risk/benefit scenario for a particular drug and a particular population.

In those cases where that mitigation is more complex, we are working in pharm/tox very closely with our chemistry colleagues, with our clinical colleagues, drug shortage staff, as well as compliance colleagues. In order to really understand the feasibility of reducing nitrosamines in these complex cases -- to understand the medical necessity of a drug

for a particular population. And then, not only the medical necessity, but what is the appropriate balance of risk from the impurity, and the benefit of that drug. So, this is something that is multi-disciplinary in nature. It is complex. It is very drug specific. And we engage in that sort of discussion on a case by case basis.

I think it remains to be seen whether some products can fully eliminate nitrosamines. But we are certainly going to continue on working in a multi-disciplinary fashion, and with international regulatory bodies, to really ensure that we are maintaining that right balance of safe drugs. Again, really trying to inform the risk, because I am coming at it more from a pharm/tox perspective.

So, whether they can be completely eliminated or not -- our focus is to inform the risk. And I think that we are looking to identify what is a one in one hundred thousand cancer risk for these drugs. We have heard about this over the last two days. We have posed some questions to the panel that we are grappling with on a frequent basis. We have

plenty of data to support that these are impurities that are potent carcinogens across many species. And we will continue to use all available data to identify what is the risk of a particular compound.

In cases where there is carcinogenicity information, we will use that data to inform what is a safe level for a drug. We will use data -- you know, in a surrogate approach to identify where there are imperfect carcinogenicity data. We are continually focused on how to appropriately characterize the risk and potency of compounds, and both for cases where there are plenty of data, as well as where there is none. So, we are going to do the best with all available information for those cases where nitrosamines cannot be eliminated.

So, we are focused on risk characterization. We will maintain a one a 100,000 cancer risk threshold for these impurities in drug products. We will certainly take the advice of the panel into consideration, interact with our national regulators in order to continually move through how to grapple with these nitrosamines as a broad issue,

which impacts a bunch of the -- many different drugs. Because, ultimately, we are going to keep our focus on acceptable risk, in light of all the available information.

And so, with that, I think I have finished my comments, for now. And I will pass it back to Dr. Atrakchi.

DR. ATRAKCHI: Thank you, Dr. Dorsam.

Dr. McGovern?

DR. MCGOVERN: Yes. Thank you. And good morning. I think the previous responding -- you know, really covered the issues well. You know, and again, coming from a safety perspective on this issue, and -- you know, and similar to Dr. Dorsam, really more focusing on the latter question of -- you know, can nitrosamines be eliminated. And I think as we have heard -- you know, with sufficient time and effort, yeah. Probably in most cases nitrosamines can be eliminated. And some of those cases that effort is -- or the required effort is going to be less than other cases, you are also dealing with issues such as -- you know, as we have talked about contaminations

in solvents, what water sources you are using -- you know, possible presence in excipients can be a complicating factor. And sometimes -- you know, it is unavoidable in order to be able to synthesis your API. And sometimes, as Dr. Johnson had mentioned, some changes that are made to try to avoid nitrosamine formation, may have unintended consequences that -- you know, lead to issues.

And then, of course, as analytical techniques improve -- you know, where you once thought you did not have any nitrosamines present, and the lower you look, the more likely you may be to find them at -- you know, very low levels. So, the question can really be -- you know, do they need to be completely eliminated in all cases in order to maintain patient safety? So that overall risk/benefit concept that Dr. Dorsam was -- you know, speaking to.

And at a certain point, the effort to eliminate nitrosamines may exceed the corresponding impact on patient safety. Given the multiple way in which people may be exposed to nitrosamines -- you know, we have talked about over the last day and a

half, or so -- you know, such as food, water, polypharmacy being another one -- it seems reasonable that drug sponsors implement appropriate quality be design -- or GMP approaches in order to -- you know, try to avoid the presence of -- or formation of nitrosamine impurities -- you know, as best they can -- and with due diligence, in terms of the supply chain, as well.

However -- you know, as we have seen, these impurities may be difficult to control, or predict, even, in certain circumstances. So -- you know, we need to balance the efforts required to control impurities versus the overall impact they would have on patient safety, so that we are not -- you know, over expending resources unnecessarily on this particular issue, when they could be better used elsewhere, in terms of -- you know, the public health issues involved with drug development.

As Dr. Dorsam mentioned -- you know, we have identified acceptable intake levels for various impurities, to date. More will likely be generated in

the future. And we have done a lot of work in this area in conjunction with regulatory partners around the world. In general, if a sponsor has demonstrated a reasonable effort to avoid or control these impurities, and the impurities are controlled within the AI limits as indicated in -- you know, our guidance and other regulatory guidance documents -- that level of control -- you know, certainly seems to be reasonable.

We have had -- you know, discussion over the last day and a half -- also taking into consideration -- you know, TD50 approaches, versus benchmark dosing. We can consider to take a look at that, and consider -- you know, those differences, and what impact they may have on -- in terms of acceptable levels. As well as -- you know, assessment in conjunction with -- you know, dietary intake levels, and endogenous production. And certainly, we have talked about -- you know, what data gaps may be present in those cases.

So, I think -- but you know, certainly is a start, those -- if you know, impurities are

controlled within those AI limits, that seems to be -- certainly, be a relatively safe level of control to being with. But certainly -- you know, if steps can be taken to completely avoid the presence of the nitrosamines, I think that is better for everybody. But as we all know, that is not always possible.

So, thanks.

DR. ATRAKCHI: Thank you, Dr. McGovern.

Dr. Hecht?

DR. HECHT: Yes. I think the key factor that was mentioned earlier is awareness. And if we have awareness of the possibility of nitrosamine formation, I think in most cases it can be mitigated. I think the sartan example is a good one -- where there was not sufficient awareness that when using sodium nitride to destroy the excess azid that was used to make the triazole in the -- that is in the drug, that -- you know, nobody thought about the fact that nitride can lead to nitrosamines by reacting with a small amount of impurity in the solvent. So, I think, now, we have awareness of this possibility, which should lead, presumable, to much less of a

problem.

I suppose there is some cases where, perhaps, the formation of nitrosamines cannot be avoided, due to the particular procedures that are used in the drug manufacturing process. In that case, might have to take other approaches, such as improved purification methods. But we have the tools to detect nitrosamine impurities. And very well, that whole -- and presumably, at this point, we should have the awareness to avoid their formation.

Thank you.

DR. ATRAKCHI: Thank you, very much.

Dr. Adamson?

DR. ADAMSON: Yes. Thank you. I would agree with the previous commentors. And I would say that it basically involves good manufacturing processes and practices. And good manufacturing practices include quality management, sanitation and hygiene, building and facilities, the equipment that is being used, the raw materials, the personnel -- and that includes awareness -- the validation and the qualification of the people that are manufacturing.

And it has been mentioned by Dr. Dorsam and echoed by Dr. Hecht, but complete awareness. So, look at the chemical reaction that is taking place. Look at the method of synthesis. Look at the reagents. Look at the solvents. Is there nitride in the solvents? If you are using water, look at the nitride level. And as been mentioned, avoid solvent dimethylformamide, which -- if possible. Certainly, avoid dimethylamine, if possible. And you can -- necessary, if you are recovering the solvents for efficiency, effective recovered solvents. And I cannot emphasize enough, check the contaminated equipment. That needs to be check, is all.

And then, also, avoid processes as has been stated, involving secondary and tertiary means, particular with nitride and other acid conditions. And then, also, check all batches -- and add a medium batches, if possible. And check the status of the -- not only the API, but of the finished product, and check it under adverse conditions, such as excess of heat and moisture. And I think if you do this, you will have checked most of the boxes that are necessary

in order to avoid nitrosamine formation.

Can it be eliminated completely from API and the drug product? That certainly is the goal, but I am not sure in some cases if it is necessary. And then, as previously said, you need to look at the amount, and -- that is present, and you need to look at the risk/benefit of the drug that is being used.

So, those are the comments that I would say.

DR. ATRAKCHI: Thank you, Dr. Adamson.

Any other comments from anyone else on the panel?

DR. EISENBRAND: Yeah. This is -- Eisenbrand speaking. I totally agree with what has been said. And I also agree that I think the driving principal behind mitigation is awareness. And that is what my colleague, Dr. Hecht, has already lined out.

It is extremely important to be aware of all possibilities. One example that has been mentioned by Dr. Johnson was that in a drying process, the air that is being used is not purified, but it is just environmental air containing nitrogen oxides.

And then, we get in a problem you may not have been aware of before.

But there are also some very straight forward things. For instance, the drug diclofenac has normally been formulated as a sodium, or a potassium salt. But in some instances, I do not exactly know for what reasons, it has been used and marketed as a dimethyl or a dialkylamine salt. So, that needs to be checked whether this is really necessary, or it is just probably a me too approach.

So, there are several of these things that can be used. But there are other totally unexpected sources of potential contamination. And one maybe also the use of nitrocellulose in packaging or printing, because it has been found out earlier, already, that nitrocellulose is a very strong nitrosating agent. We came across that because it was found in Europe that -- in cosmetics, the nail lacquers are very often formulated with nitrocellulose. And of course, they contain nitrosamines if they are secondary amines. Yeah.

So, there are expected and unexpected

things. And it is awareness of costs that is driving this mitigation. But one has to be open, also, to potentially unexpected things to scrutinize and mitigate nitroso compound formation during manufacture up to formulation and packaging.

Thank you. That is my --

DR. ATRAKCHI: Thank you. Any other comments?

Okay. There are some questions from the attendees. One of them is, as part of the nitrosamine risk assessment exercise, pharma companies are reaching out to the manufacturer of excipients asking for presence of potential nitrosamines in their excipients. Are these -- are there available data on levels of nitrosamines in excipients? Surely GMP process for APIs should guarantee absence of potential formation of nitrosamines, but the same should be applied to excipients that are much higher in quantity in the drug product. I am interested in the opinion of the experts on this.

Who would like to start?

DR. KEIRE: I guess, I can comment a

little bit. I think that -- you know, the main concern with the excipients is actually the presence of nitride. I do not think we have had any reports of excipients containing nitrosamines. Although, again, if the excipient -- particular excipient contains a secondary amine, and also nitride is present, then it is a possibility.

But the main concern I think that we have had, is that there is nitride present in these excipients -- or other amines that could be oxidized by some means to nitride. And then, that could lead to some chemistry with the API when it is formulated.

I do not know if Deborah, or others want to add to that.

DR. EISENBRAND: Could I ask a question? Is the excipient -- is that filling material? Or what is it? I mean, is it mainly starch, or is it inorganic material? Could you just explain a bit? I mean --

DR. KEIRE: Yeah. I mean -- yeah. There are many, many different excipients. You know, cellulose binders, magnesium stearate, coatings -- you

know, that are -- but for the most part they are not amines -- they do not contain amine. So, they -- you know, as you said, starch, cellulose based binders, that --

DR. EISENBRAND: Oh. Okay.

DR. KEIRE: -- those are the major components. But others should feel free to weigh in as well.

DR. EISENBRAND: I ask this because, we came across years ago that some -- certain inorganic materials -- for instance like bentonites or -- contain nitrogen oxides. So, they appear to be -- as we found at that time, as a source for artifactual nitrosamine formation. They can nitrosate amines. And maybe, they can also nitrosate drugs. So, I think it is important to know that is not the nitrosamine content, per se, that may be relevant. But it is the nitrosating possibility -- those things.

DR. KEIRE: Yeah. And I think that has been our -- you know, our major concern -- focus -- you know, question that we have been trying to ask.

You know, I guess I should say that

there is still a lot unknown, here, right? You know, we are really -- you know, working with the manufacturers and they are doing root cause analyses, we are trying to do -- you know, get better understanding about what is present in these excipients. And you know, in terms of these, kind of, other process related impurities in the excipients themselves, and how that could lead to chemistry in the solid state -- in the tablet -- you know, where -- or in the process of formulation.

So, it is a -- there is a -- still a lot of unknown in this area. We are still learning.

DR. ATRAKCHI: Thank you. The next question is, if nitrosamines are eliminated mostly from the API and/or the drug product, will there be any way to determine whether nitrosamines will form as the drug product sits on a shelf or in the person's body?

DR. KEIRE: Well, I guess I can speak to the first part of that. I mean, the -- you know, the drugs are put on stability testing, right? So, in that, there are, kind of, standard conditions for

that -- you know, 40 degree C, 75 percent humidity, is an example of an accelerated stability condition. And that -- or they are -- you know, put in -- they are just stored at room temperature, at the temperature that they are going to be -- you know, distributed in, in some supply chain. And for the period of time, over the shelf life of the drug, and -- you know, they would be tested periodically, if there was a risk for nitrosamine formation. And so, with that data -- you know, the agency could say, yeah, this is a -- we -- you know, we would approve this drug for this period of time -- you know, to be on the market, right? They would have an expiration date on it -- you know, established with those data.

DR. ATRAKCHI: Thank you. Any other comments?

DR. JOHNSON: Yeah. This is Deborah. Yeah.

And so, there is another thing -- you know, it -- when something goes on stability -- you know, your analytical methods are good for what you are looking for. So, some of the problem, these are

forming in drug products. And you know, if people are not looking for them, and they are really low in level, and they miss them.

But another place you might pick this up is during product development, where you were doing your drug excipient compatibility studies. Especially with -- you know, excipients that are known to have nitrite contaminants. That is another place that you could be looking to make sure that -- you know, you are not picking up nitrosamine formation, there. And you may have to explore several vendors, because -- you know, not every vendor is equal, they may not have the same process in making the excipient. So, that is just another place that you could be looking in -- again, in a proactive manner -- a wholistic manner, to -- you know, you are, kind of, building the quality into the product. Is -- you know, up front, is there a potential for this API -- especially if it has got a secondary or tertiary amine functionality, or you have residual.

You know, a lot of times some of these amines carry over because -- you know, they were used

in the process. If you got residual amines in the process -- you know, are there some excipients that are known to have these nitrite -- you know, contaminants -- you know, do some work up front to ensure that you are -- you know, kind of, picking things up early.

DR. ATRAKCHI: Thank you. Any other comment on this question?

Okay. The next question is, is it possible that nitrosamine impurities in drug products will keep on increasing during the shelf life of the drug product? What if some impurity is below the limit of detection, or tolerable levels, at the time of packaging, and it increased above the tolerable levels after six or 12 months, when it is reached to the consumer? How can you assure stability after a drug is in the marketplace?

DR. KEIRE: Yeah. I think I, kind of, answered this question earlier. I mean, the -- you know, the -- we have to be given data that says that the drug is sufficiently stable, such that -- you know, it -- talking about nitrosamines -- that, that

nitrosamine limits succeed in specified limit over the shelf life of the drug. There has to be data that would show that before the drug was allowed on the market. So --

DR. ATRAKCHI: Okay.

DR. KEIRE: Oh. And I guess -- sorry. The other point there is detectible. You know, it would not be detectible at the initial state, but then it would be detectible later, right? And that goes -- that speaks to the point of appropriate analytical methods to detect the presence, or the absence of something.

So -- and that is a -- you know, we -- you know, the FDA and also, actually, international regulatory partners have, kind of, established limits in guidance documents that say what they would call appropriate, right? I mean, one of those is, kind of, a 30 part per billion bar that has been set. I mean, of course, that might be difficult with a drug that is -- analytically, that might be difficult with a drug that has a very high dose. But for most drugs, that is going to be quite a load limit. And so that

is the approach that is being taken for -- you know, defining appropriately -- appropriate detection limits to say something is absent.

DR. ADAMSON: Yeah. I think the manufacturers might also want to consider the possibility of adding an anti-oxidant to the drug finished product.

DR. ATRAKCHI: Thank you.

Next question is, can stoichiometric ratios between amine or nitride levels be used as a rational to exclude a nitrosamine formation in drug product, considering that sometimes amines and nitrides may be concurrently present in negligible amounts in associated to unfavorable conditions for nitrosamine formation?

DR. KEIRE: Well, yeah. That is a bit of a complicated question. I think it depends on the -- yeah. It depends very much on circumstance. You know, I -- you know, you do need certain ratios to get some chemistry to go, right? So, you know, it -- certain amounts of nitride and amine may be necessary to get significant reactions under -- you know, a

particular condition. So, it is a little bit difficult to answer that question, because of the -- we need -- kind of, need a specific case to talk about, there.

DR. EISENBRAND: Could I comment, just for a second, on this?

I think it is worthwhile for the manufacturers if they are facing such a question of stability -- the one thing, I think, is very clear, if it is only very small, tiny traces of nitrosatable compounds, then, I think it is obvious that there is no risk. But one should also consider under -- you know, if make a false condition storage testing -- storage stability testing, one should think of having such a condition where you have environmental and NOx concentrations. Because, we have found out -- and many others, later, also -- that there is an interaction between solid material that contains nitrosatable and NOx, of course. And you can get them -- the question of stability, then, needs to be widened by this consideration. That it may be that the drug is sitting in a car -- or sitting somewhere

else -- or you have it in a bar, where it is smoking, and this sort of thing. And then, you have the exposure to NOx. And maybe, you can solve the whole thing by using the right packaging, or course. But one should look at this.

DR. KEIRE: Yeah. I mean, I think there is a big focus on -- you know, real world conditions, right? You know, the hot mailbox, the glovebox in the car -- you know, the human bathroom. You know, I mean, if you think about those things -- you know, what does it experience in the truck in the middle of summer in the southern U.S., right? You know, so -- you know, before it gets to the shelf.

So, you know, I mean, you cannot completely model all those conditions. But you know, you try to get appropriate data that can be predictive of stability under a variety of conditions. And you know -- and those are -- you know, have been, kind of, internationally harmonized in ICH documents, about -- you know, which conditions are standard conditions that can be used to establish stability.

DR. ATRAKCHI: The next question is, is

there any information regarding azocolorants and/or colorants with amine groups? Also, colorants normally have a non-negligible quantity of unspecified impurities.

DR. KEIRE: Yeah. I mean -- you know, I -- if the colorant contains -- you know, diethylamino group, or a secondary amine that might be reactive -- you know, that -- you need to look at it through that awareness lens, that we have been talking about, as a potential source of nitrosamine.

I mean, generally, the colorants are there in very small amounts. But you know, again, it is a case by case, kind of awareness question. And an understanding of what is going into the drug. But --

DR. ATRAKCHI: Thank you. Is good risk assessment enough? When should testing for nitrosamines be triggered?

DR. JOHNSON: So, I will give a crack at that. So, if you look at the FDA nitrosamine guidance, it has several steps. And the first one is to do the risk assessment. And it clearly says that if -- when you do the risk assessment, if you do

detect a risk, then you should do confirmatory testing. So, that is confirmatory testing.

So, if you do the confirmatory testing, and you use appropriate sensitive methods, with appropriate limits of quantitation in detection, and you do not detect these nitrosamines, then, you have confirmed that, yes, it was a risk that -- you know, your process has mitigated it, and -- you know, the product does not appear to be at risk for nitrosamines.

But if you do the confirmatory testing, and you are actually detecting these above the limit of quantitation, then, clearly, your process is creating them. And then, you have to move into the realm of the control strategy. And you have many control strategies open to you. And one of those might be -- you know, testing -- you know, putting a limit and a test forward in a starting material or intermediate -- something upstream. Or, you always have the -- you know, the option of testing the API.

So, there is some different ideas of testing. There is the confirmatory testing that has

to be done when you have identified a risk in your risk assessment, that does not necessarily -- you know, result in -- you know, normal -- you know, routine testing in the API.

DR. EISENBRAND: Dr. Atrakchi, may I have one further addition -- information to the former question about the azo dyes?

DR. ATRAKCHI: Yes, please

DR. EISENBRAND: Yeah. The Scientific Committee on Consumer Safety of the European Commission in 2012, I think it was, issued two opinions about nitrosamines in cosmetics. And the first one dealt with azo dye constituents of cosmetics, and -- the risks -- so this is publicly available information to that. Thanks.

DR. ATRAKCHI: Thank you. Considering the surprise, in quotes, nature of materials carrying through more steps than we through, or from coming in with other materials, do we think we need to start testing API routinely?

DR. JOHNSON: I will answer that one, if I can.

So, our goal with the nitrosamine guidance was never intended to tell people they have to test every single batch of API all of the time. You know, that is, kind of, the quality by testing. And I think we are encouraging people to have quality by design -- to build quality into the process.

And so, I think what we have learned throughout this process is, there was just some assumptions that materials were being removed from a step during the workout. That -- you know, nitrites in some of these that they were water soluble -- they were being removed. And I think what we learned was they are not being removed as thoroughly as one thought.

So, again, being wholistic -- realizing that potentially there are these materials -- you know, precursors in the process, then if you understand how they are being purged, then -- you know, final API testing is not necessarily -- you know, you do not have to do that. You might just have a in process control to make sure your nitrite level is below a certain level, or an amine level is below a

level. You might know that -- you know, there is potential for a particular nitrosamine forming in an intermediate step. And again, that could be controlled upstream, using an option three approach -- you know, as an API -- or as an IPC, or an intermediate specification.

So, there was never any -- you know, expectation that people would have to test everything. What we just learned was that things in the process were carrying through more steps than people thought, and that you cannot assume. I guess, that was the bottom line that we learned. Just do not assume. You have to understand the process and the purging capability completely, and then, move from there.

DR. ATRAKCHI: Thank you. Does every nitrosamine demand equal levels of removal or avoidance?

DR. KEIRE: Well, I think I will hand that to my pharm tox colleagues to -- I think -- you know, they have established limits that, kind of, point to the -- I mean -- you know, NDMA is at 96 nanograms as the AI limit, versus NDEA being at 26.5.

So, there is distinction between those two. And, as well as others. So -- but, I will leave that to my pharm toxicologist to weigh in.

DR. MCGOVERN: This is Tim. I can jump in. I -- currently, the identified limits we have in place range from 26 to about 96 nanograms per day. So -- you know, given the uncertainty in all these calculations, you can argue there is really there is not a lot of difference in that at those levels.

So -- you know, there is some differentiation in the approach taken. Of course, those are not every nitrosamine. Those are NDMA, and NDEA are probably the two with the most robust databases. There may be some others out there, as well. Certainly, if we are dealing with a nitrosamine that has yet to be identified, where we identify a significantly higher acceptable intake. Then, yeah, you could -- you know, obviously, you would treat those somewhat differently than you would with the current ones we are dealing with.

DR. DORSAM: That is right. I emphasize that. To say that we have tried to be

mindful about each chemical -- each compound as it comes in, to identify what is the risk associated with that compound. And so, I think, if -- you know, not all nitrosamines are created equal, and there are -- you know, some that are more potent, some that are less potent.

We do know that there is need for care with this class of compounds. And so, we will use whatever data are available to assess the risk for a particular compound, realizing that the risk for this compound may be different than NDEA, or NDMA. You know, we are trying to go mindfully compound specific for each of the products that we are encountering a nitrosamine issue. And I think that we will continue to do that.

DR. MCGOVERN: And as Dr. Dorsam mentioned earlier, you would also -- can take into consideration -- you know, what the drug product is, what the proved indication is for, and potential impact on drug shortages -- you know, especially for severe disease indications.

DR. ATRAKCHI: Thank you. I do not see

any more questions. Does anybody else notice other questions that maybe we can answer at this time?

DR. ZEIGER: This is Errol Zeiger. I have one quick question -- or comment. And it is based on my lack of knowledge about manufacturing logistics. But it can always -- one possible take away from all these discussions is that every manufacturer really needs to have an analytical laboratory to support them. And are there good -- are there analytical methods that they would have to obtain -- have to use, because -- you know, at the levels of nitrosamines that are being measured, one cannot use just any analytical method, but essentially what FDA would consider the state of the art effort. So, is this going to be a requirement -- you know, for manufacturers?

DR. KEIRE: Right. I mean, I think they have to have the technology, and the appropriate analytical tools to make the measurement -- you know, that is fit for this purpose, right? And which is down in the part per billion -- you know, part through million range. So, this is trace analysis that does

require instrumentation.

And you know, we -- you know, the FDA has provided, I think, to date, publicly posted on the website 11 different methods. And certainly, the European OMCL have also provided methods -- made them publicly available on really a wide variety of platforms. I mean, the -- you know, many of the FDA methods -- you know, we, kind of, transitioned over time form using GCMS approaches to LCMS -- high resolution LCMS approaches, because we had that equipment available, number one, and it was best and most sensitive that we had.

And I think, every manufacturer -- you know, has the access to these kinds of labs, or they have contact research organization where they have testing done. And that is the expectation. You know, the first thing you need to do is to have this analytical target for what you need to measure. You know, pharm tox provides us with that underpinning of -- you know, you need to control this particular impurity at this level. You know, whatever it is -- 96 nanograms. And then -- you know, with that

information, the analytical chemist is developing methods that are appropriate for that.

But -- so, I think there is actually a pretty wide range of technology that is available. I do not think that this a real limiter for most pharmaceutical manufacturing firms. So, the technology is there. The methods are available -- publicly available. So -- you know, and that is -- you know, one of the things -- the reason we do that is to, kind of, speed this process. We -- you know, if there is a question if there is a risk, we want them to make measurements and give us the data so that we can be assured that their product is of appropriate quality.

And -- oh. And I guess, one other thing. I want to -- I guess, often times there are too much focus on the ATI, and the synthesis. I think -- you know, we do want to emphasize that the drug product -- you know, and the formulation of that drug product really needs to be considered, as well, because we have cases where the API has no nitrosamines, but then, when it is made into a

formulated product, then nitrosamines appear. So, this is not only on the API manufacturer side. It is also in the drug product manufacturing side.

I will stop there. Thank you.

DR. ATRAKCHI: Thank you, Dr. Keire. One more question. Is -- can the panelists comment on the formation of API related nitroso impurities? What factors contribute to these impurity's formation? Is this a common occurrence across all products? And this is a -- the nitroso group is attached to the API, itself.

DR. KEIRE: Right. You know, I think if you look at the structures of --

DR. EISENBRAND: Ranitidine.

DR. KEIRE: -- many -- yeah. Ranitidine. Yeah. If you look at the structures of many of the drug substances, they have secondary amines that have the potential to react and for nitroso drug substance related impurities. So, this is also a consideration.

And the same question needs to be asked about the drug substance -- the molecule itself,

whether it can -- has the potential to form a nitroso compound.

DR. ATRAKCHI: Would you say this is a common occurrence among -- the last part of the question -- is this a common occurrence across all products?

DR. JOHNSON: So, if I could make a comment. When you are looking at the potential of the actual API forming nitrosamine, I mean, you can really look at the structure. If you have an API molecule that has a secondary amine -- I mean, you have to consider that a nitroso -- you know, API -- you know, degradant might form, because you have got a secondary amine. Especially, if you got one where the alkyl groups are tied back, and the amines is -- you know, really, really out there.

The -- you know, if it has got a tertiary anime -- well, ranitidine had a tertiary anime in it. I would think just from an organic perspective that a secondary amine functionality is probably at more risk than a tertiary amine. But, if the molecules got those function -- those amine

functional groups on it, then you have to consider this, because some of the excipients may contain nitrous aiding agents. Or -- you know, if you are doing fluid bed drawing in the API or in the drug product, again -- you know, if there is NOx available, it is possible.

So, you can literally look at the API molecule and do a real quick risk assessment just based on the actual structure.

DR. ATRAKCHI: Thank you. So, I think to summarize the -- really, this question, it is awareness, and it is wholistic approach. To me, it is all about chemistry. And we have the state of the art tools in the 21st century, and very sophisticated techniques that could eliminate -- or, at least, extremely minimize the levels of nitrosamines in pharmaceuticals. And certainly, the evidence for the sophisticated techniques, at least in the late 90's and early 2000's, is the food manufacturing and processing. It has -- it is a success story that really led to extremely lowering the levels of nitrosamines in foods. And I believe this can be

achieved with drugs, as well.

We are close to 12:00 -- I am sorry. Go ahead, Dr. Keire. Please.

DR. KEIRE: Well, I guess I just wanted to add to that. I think that is a really pertinent comment. I mean -- so, we are not the only industry -- you know, the pharmaceutical industry -- that has had to manage this -- you know, not having nitrosamines present in the product. And I think -- you know, that Dr. Eisenbrand has already -- you know, presented on the malt kilning step in beer. And you know, I was actually reading about this. And it used to be that -- you know, there was microgram per liter of nitrosamine -- NDMA present in beer because of this particular step. But then, when there was knowledge gained about this step and this process, then they ultimately eliminated that. And you know, more recent surveys of beer show almost no nitrosamines.

And so, I think -- you know, it just is -- you know, the food and beverage industry has gone through this transition the pharmaceutical industry is now facing this same task. But it is not

unachievable. Other have -- you know, succeeded in eliminating or minimizing the presence of these things. So, I remain hopeful that the pharmaceutical industry can be the same.

DR. ATRAKCHI: Fully agree. Thank you, very much.

It is almost 12:30. We will take the lunch break from 12:30 -- or from now, until one o'clock. And we will resume -- we have some other questions -- just follow up questions from yesterday, as well as today, that we can provide to the panelists to answer. And then, we can wrap up.

Thank you, very much. Enjoy your lunch.

(off the record)

MS. PAINTER: Good afternoon, good evening. We are back. It is 1:01, here on the East coast.

DR. ATRAKCHI: Welcome back for the last part of the second day and final day of this workshop. What I would like to do now, is we have some of the questions from the attendees, that

includes both from yesterday's discussion, as well as today. I would like to go over those. And that way we have a full discussion of everything that has been -- that is interesting and important to everybody.

The first question is, can you see a correlation between nitrosamines and radiation safety? Would the linear no threshold model for radiation safety be applicable for nitrosamines moving forward? Therefore, for drug manufacturer, we essentially have to eliminate the potentials for nitrosamines formation for a drug to be considered safe. Or, essentially, also, adopt the as low as reasonably applicable principal.

Who would like to start this discussion? Anyone?

DR. EISENBRAND: Maybe, I start?

DR. ATRAKCHI: Yes.

DR. EISENBRAND: I think there is a difference between radiation safety and the dose response towards nitrosamines. I would say for pharmacologic reasons, there should be somewhere in

the dose response curve of nitrosamines, a threshold where the dose is so low that no measurable -- let's say, DNA damage is to be seen. And if we agree that DNA damage is a very important point used as a biomarker for the potency as one may say -- yeah -- reflecting to biological activity on the target DNA, then we -- I think, now adays, will have the means to find out such a dose level, where DNA damage is negligible -- in a sense that it is just diving in the background DNA damage we have anyway. And this is a possibility that definitely deserves research and investigation. And I think with today's instrumental analysis means, we can do that. We have done this, for instance, for acrylamide, where one easily can follow down the dose response down to a level where the ancillarisation, in that case dives into the background of -- into the biological background, more or less. So that is a difference to -- you know, radiation, in that sense.

DR. ATRAKCHI: Thank you, Dr. Eisenbrand.

Anyone else would like to comment on

this?

DR. EISENBRAND: Just perhaps, to add on it, that is why I think a no risk level would be the -- as low as reasonable achievable would be the way to go in this question.

DR. HECHT: I basically agree with this. But I also recall the conclusion of the -- of bio assay of dimethylnitrosamine and diethylnitrosamine by Peto, and Grasso, and others, in which they concluded there was no evidence of a threshold. So, I do not know that we have the data to really establish a no risk level for compounds like dimethyl and diethylnitrosamine, as an example.

DR. EISENBRAND: Mm-hmm. Yeah. I totally agree, Steve. I think, this would, perhaps, cover dose ranges that are below the manifestation of tumors. So, in other words, one could, perhaps, follow down by studying DNA alkylation to further down dose levels, until one reaches, perhaps, somewhere a level where it really is insignificant from the physiological background of DNA alkylation. That is what I mean, actually.

DR. HECHT: Right. Yeah. I agree.

DR. EISENBRAND: By the way, Dr. Atrakchi, when I -- there was also a question concerning the acceptable intake estimates -- the default acceptable intake estimate -- estimated some type of -- this alluded to the big difference between nitrosodimethylamine and nitrosodiethlyamine. And actually, I think this shows the difficulty of relying on the TD50 when determining such estimates, because it would mean -- it would reflect a more than -- about a three times higher potency of diethylnitrosamine, as compared to dimethylnitrosamine, if you look into the pository of 96 nanogram per day for dimethylnitrosamine, versus 26.5 for methyltyrosine.

And that is why I favor, really, the BMDL 10 values, because if you look into the BMDL 10 values, they reflect much better. The vicinity, so to speak, of the biological effects of dimethyl and diethylnitrosamine, of course, still showing that diethylnitrosamine is the more potent with BMDL value of 18 microgram per kilogram, versus the BMDL 10 of n-nitrosodimethylamine of 27. But it is a much

smaller difference as the one from the default acceptable intake estimates, based on the TD 50 -- just as a general note of caution from my side.

DR. MCGOVERN: Dr. Eisenbrand, this is Tim McGovern. Just a follow up question regarding benchmark dosing approaching.

I think a good argument is made for using the entirety of the dose response curve as the benchmark dose uses. We get into some subjectivity and variability as -- you know, as you use that data, then, to support calculation of a PDE -- permissible daily exposure level. And the application of uncertainty factors -- and depending on what level of uncertainty factors you use across the various factors that are applied, you can end up in a wide range of permissible daily exposure levels.

And you know, often times -- you know, especially with nitrosamines, they can be -- even in a fairly conservative approach, you can up in an PDE that is -- you know, significantly -- you know, in least in order of magnitude greater than the AI calculation. Do you have any comments, or any of the

other panelists, in terms of -- you know, using that approach and the application of uncertainty factors, and differentiation or the difference in the PDE versus AI levels, that are the outcome of those calculations?

DR. EISENBRAND: Yeah. As I mentioned already, I think that unsafety or safety factors are default factors. Like, the AI values. And maybe the BMDL values are -- you know, considering the much more important portion of the dose response curve, as far, of course, as they are available. That is just the case for very few of the nitrosamines, like ethyl -- diethyl and dimethylnitrosamine. And, you know, that is why I think these BMDL 10 values reflect better the potency differences, as the AI estimates derive to.

DR. ZEIGER: This is Errol Zeiger. I might say, just as an aside, the genetic toxicology community has been moving more and more towards the use of BMDL for in vivo comparisons. There are some recent publications by Ilsey -- I do not remember who the first authors were -- that show that the BMDL was much more stable and useful than LOEL, or other

measurements. And the group at Health Canada, led by Paul White, in Ottawa, has been looking at BMDLs as a very effective for comparing related chemicals, such as polycyclic aromatics in vivo. But also comparing responses in different tissues -- you know, from the same animal. So, hopefully, in the future, more and more gene tox data will be coming out as -- with BMDL calculations, instead of just LOEL, or just -- you know, maximum response calculations.

DR. EISENBRAND: No. I agree. EFSA -- it -- the same, actually -- favoring the BMDL process.

DR. ATRAKCHI: So, in general, how would the results compare, that are available for the same compounds -- how do they compare between the BMDL and the TD 50? How far are they off? Or are they comparable?

DR. EISENBRAND: No. They -- the AI estimate reflect the TD 50 values in a way. You know, they are derived by exultating to one in a hundred thousand risk. And there is a big difference in the AI values between dimethyl and diethylnitrosamine, not reflecting the true biological difference and potency.

They are much closer. Of course, n-nitrosodiethylamine is more potent. But not by a factor of three.

DR. ATRAKCHI: But I also think the values -- the limits will go much higher for the -- for -- maybe not -- I mean, maybe not all nitrosamines, but the NDMA and NDEA, if you use BMDL. They are probably going to be even not in the cohort of concern if you use the BMDL. And that is a fairly big jump from what is indicated in M7.

DR. EISENBRAND: No. The way out -- and so for instance sabers, is that they say we do not take any acceptable intakes for these compounds -- for the cohort of concern compounds. We just calculate -- or -- yeah -- derive the distance between the 10 percent, or you may take a five percent BMDL, where you -- and the consumers exposure. And in a general -- a very general way, they say if the distance is more than 10,000, then there is no concern. Very -- as a -- over the sum --

Here, we are not totally at the 10,000. We are between -- I would say, 6,000 and 10,000. But

there is a big difference, still, between.

DR. ATRAKCHI: Right. There was a question earlier this morning along these same lines for NDMA and NDEA. The question is, can one of you address why NDMA is considered one of the most concerning nitrosamine impurities based on discussion during the day, day one, when it currently has an AI of 96 nanogram per day, versus NDEA and others that have a lower AI, at 26.5? If NDMA is of a greater concern, why does not -- why it does not have a lower AI?

DR. EISENBRAND: Yeah. That is what I have just addressed with my remark to the AI estimates and the BMDL 10 models that actually are, in that case, more realistic.

DR. ATRAKCHI: Right. I just thought I should read the question from the attendees. Thank you.

The next --

DR. MCGOVERN: And this is Tim. I think it --

DR. ATRAKCHI: Yes?

DR. MCGOVERN: -- might be safe to say that they are both highly potent. And you know, with these types of calculations, I would suggest there is not a big difference between 26 and 96 nanogram per day, in terms of potency, because there is a lot of uncertainty around those values to begin with.

And -- you know, I guess, in terms of endogenous levels, maybe more of the focus is on NDMA just because that has been the more commonly observed of the nitrosamines.

DR. EISENBRAND: Yeah. That is really fair. I think -- I agree, totally. But you know, this raises such questions. Let's see -- these estimates. I agree with you, that, that is not a big difference, actually.

DR. ATRAKCHI: Certainly, considering the dosimetry and the tumors that they both induce, fully agree that these numbers are not very meaningful between the two. They both are very potent mutagens and carcinogens.

The next question is, can panelists comment on the use of incorporating normal variation

in DNA repair gene expression in humans into risk assessment models? Anyone would like to comment?

DR. KYRTOPOULOS: Can I respond? This is Kyrtopoulos. Can you hear --

DR. ATRAKCHI: Yes, please.

DR. KYRTOPOULOS: Yeah.

DR. ATRAKCHI: Yes. We can hear you. Thank you.

DR. KYRTOPOULOS: Yeah. Okay. Well, first of all, first question is, which DNA repair genes we are speaking about. Among the nitrosamines, which are found in drugs. MGMT is one that we discuss a lot. And it is relevant to carcinogenesis by the methylating or, maybe, the ethylating agents, also. But we do not know it is all in connection with carcinogenesis by other longer chain nitrosamines, which are found as impurities in drugs.

It is quite probable that their pre-carcinogenic DNA damage is -- may well be repaired by other mechanism -- like, the excision repair mechanisms.

So, in contrast to MGMT, which is just

a single gene, excision repair involves multiple steps, multiple genes, and there, one has to start asking which genes to use are markers of repair efficiency.

The other, and probably more important issue in my -- problem, in my opinion, is that whereas in simple animal models we can see, clearly, the affects of DNA repair variation. Animals overexpressing MGMT, for instance, are resistant to NDMA carcinogenesis, and conversely MGMT knock outs are much more susceptible -- the -- in the case of carcinogenesis in man, in human populations, it is much more difficult to come up with relationships between the moderate -- I mean, the normal variation in DNA repair and a carcinogenic susceptibility. There are rare cases where there are, again -- there is genetic lack of some genes, which result in serious cancer susceptibility. But those are fairly extreme cases. Especially for -- I mean, in the case of smoking related cancers, there is some knowledge about the variation excision repair and its impact on susceptibility. But, if we speak about nitrosamines,

we cannot really point to a specific cancer which we believe is caused by nitrosamines, so as to be able to investigate in a quantitative way, the relationship between the variation in DNA repair and risk, so as being incorporated in risk assessment.

So, I am afraid this is just not possible. Thank you.

DR. ATRAKCHI: Thank you. No comment. I will go to the next question.

My understanding is that the use of linear extrapolation from TD 50 to one in 100,000 risk assumes that there is no impact of DNA repair on the dose response curve. If that is the case, why would the setting of less than lifetime limits be based on an understanding when repair is exceeded?

DR. BUCHER: Oh. If I -- maybe, I can take a stab at this one. I think those two are actually unrelated. I think that the issue of less than lifetime involving some concepts of where repair processes might be exceeded were in those situations where there was quite a large potential dose of nitrosamine given for a very short amount of time.

And in our -- in my discussions of this, and in our -- what I think we were talking about, was given situations where nitrosamines might be present in drugs at the acceptable intake level, or not much higher for short periods of time -- that these kinds of concepts would not necessarily apply where DNA repair processes would be exceeded.

So that was the way I took that question.

DR. KYRTOPOULOS: Yes. May I follow up on that?

DR. ATRAKCHI: Yes, please.

DR. KYRTOPOULOS: Yes. In fact, I think I pointed out rather the opposite. That we are in the dose -- in the linear dose area of the dose response when we are speaking about exposure levels, which include exposure coming from the environment and endogenous sources, even if we are over estimating that kind of exposure. Plus, the exposure that would come from the drug if we had imposed the standard risk assessment level -- 10 to the minus five -- that was well within the linear area -- really, a part of the

dose response curve -- so that even if were to exceed that within certain limits, obviously -- but even if you were to exceed that, we would still be -- by applying the LTL approach, we would still be in the dose response -- in the linear part. And that is why I had said that from the point of your DNA damage, LTL -- application of the LTL approach would be permissible. The reservation I expressed was that we do not how the other factors which affect cancer risk -- other biological processes, like -- sorry -- cell replication, DNA adduct formation -- how they respond to increase doses. But from the point of view of DNA adduct formation, we have -- suddenly, we are not suggesting by applying LTL we would be risking running into conditions of exhaustion of repair. No.

DR. ATRAKCHI: But the conclusion from what you said, Dr. Kyrtopoulos, is that with all these reservations and concerns you have, the conclusion is that it is not a good idea, because of these other unknowns that involved. It is not a matter of the exceeding the DNA adduct formation, but it these others --

DR. KYRTOPOULOS: Yeah.

DR. ATRAKCHI: -- factors that we do not know about. Therefore, the less than lifetime approach is not a good approach to use for nitrosamine. Is that correct?

DR. KYRTOPOULOS: Yes. Yes. That is right. I -- if for reasons of expedience one has to use it, it should be used with great cautions, because we do not know how these other biological factors, which are -- make important contributions to the overall cancer risk. Very -- and let's remember -- I mean, we saw some data, yesterday, focusing on the liver, coming from the rat studies. And the liver is the main target for -- of -- let's say dimethylnitrosamine in the rat, and also for -- both in terms of DNA adducts. And -- but also, in terms of carcinogenesis. But that need not be the -- so, in all species. In fact, for example, in the Pattes monkeys, which we have looked at, we see extensive DNA damage, extensive accumulation of O6 methylguanine in many of the tissues. So, if we extrapolate our discussions -- or, rather open up the framework of our

discussions to other tissues, we can see that we are introducing a lot of additional unknowns. How various biological processes in operate in different tissues.

So, we have too many unknowns. And this is why I said that one should take the decision to apply the LTL approach hesitantly. And if they -- we do, it should be with great caution.

DR. ATRAKCHI: Thank you. That is much clearer, at least in my mind. But in that case -- so the O6 adduct formation as biomarkers, may not be -- well, by itself -- on its own, is not appropriate. We have to incorporate many other parameters with a DNA adduct, in order to make it make sense. Does that --

DR. KYRTOPOULOS: Yes. Ideally, this is exactly as you describe it. In practice, of course, we often have to use -- we can only use a limited number of tools, which have certain -- satisfy certain characteristics. And we do tend to use DNA adducts -- especially, if there are others which are involved in -- on the biological pathway, like O6 methylguanine does -- is mutagenic. So, it is thought that it is actually directly contributing to the

generation of the mutation in oncogenes, or whatever else, which eventually drive the carcinogenesis.

So, if we have the opportunity to use it -- and this single biomarker, yes, we should use it. But we should keep in mind the limitations in its use. Yeah.

DR. ATRAKCHI: Very good. Thank you. Anyone else would like to comment on this?

Okay. Next question is, so far it seems that we do not have assuring data for less than lifetime to be safe with higher nitrosamine levels, even with short duration exposure. Do we know thresholds for adduct formation -- or of DNA repair?

I think we probably answered that question.

DR. KYRTOPOULOS: Yes. I think so. And, in fact, I pointed out that we have no evidence as far as O6 methylguanine is concerned of any -- a threshold. If you -- I went into the literature today, actually, because I had seen this question. And I looked up some papers which claim that they see non-linearity in the dose response. It turns out that

every time you see this claim, it is because they used high doses, which were generating so much O6 methylguanine that they were depleting the repair enzyme.

So, if we are speaking about exposures at the levels that we are speaking about -- these low levels, there is no good -- no evidence of a threshold for adduct accumulation.

DR. ZEIGER: Well, actually, there has been some shown of -- a group at University of Swansea led by George Johnson, looking at low levels of alkylating agent, was an inducible DNA repair enzyme. I do not remember which particular one, because I do not have the paper handy. But they did clearly see a threshold --

DR. KYRTOPOULOS: What -- yeah. I remember --

DR. ZEIRGER: -- of a mutation, and of an adduct formation -- or adduct persistence.

DR. KYRTOPOULOS: Was that an O6 methylguanine adduct?

DR. ZIEGER: I think it may have been.

But I -- the paper came out a couple of years ago, and I just do not remember the details.

DR. KYRTOPOULOS: Yeah. Yeah. I do not remember the details. But anyway, other papers which I tried to look up today, it was clear that they were using doses which, in my opinion, were very likely to have been impacting on the methyltransferase levels.

DR. ZEIGER: Oh. Agreed. One of the major problems we face in the field is a lot of the experiments are done at the high dose -- in the high dose, subtoxic range, whereas we are trying to extrapolate -- or draw conclusions from low dose administration, as we would see with nitrosamines.

DR. KYRTOPOULOS: Yeah.

DR. ZEIGER: And we know that once we get to the high dose, we are often not in a linear relationship. But we have to somehow draw a straight line down to zero to do the risk assessment for low doses.

DR. ATRAKCHI: Very good. Thank you. Does the less than lifetime considerations which are

based on extrapolations of wrath data, take into account what Dr. Zeiger said, concerning the inducible enzymes? MGMT is known to be inducible in rats with increasing dose, whereas it is not in humans.

DR. KYRTOPOULOS: I have to come in here. May I? I am sorry. I do not want to monopolize.

DR. ATRAKCHI: No. Please, go ahead.

DR. KYRTOPOULOS: -- yes. Again, this is a question that drove me to look at the literature today. It is true that in the early days, there was a lot of work done to look for evidence of inducibility of MGMT in experimental animals. And this came because -- you know, the first methyltransferase that was discovered to -- which was repairing all this methylguanine was found in E. coli. It was called the ADA gene. And that was a highly inducible gene.

So, people started to look for equivalents of the ADA gene in animals. And there were papers where people administered diethylnitrosamine, for instance, to rats, and they did find an increase in liver MGMT. But do you know

the doses they used? One paper used five milligrams per kilo, and another 100 milligrams per kilo.

With dimethylnitrosamine, the one that I have found from the old papers, which have found some induction, the lowest concentration -- dose that had been used was one milligram per kilo of dimethylnitrosamine for four weeks.

When we did our study, where we exposed rats through the drinking water to doses of dimethylnitrosamine at the levels in the range which have been used by the -- in the Peto bioassay, we -- excuse me -- and we had exposed for up to 180 days, we found a small increase in liver MGMT, between 15 and 30 percent after 120 days of exposure to, at least, 220 micrograms per day. For a human, that would correspond to 15 milligrams per day for a 70 kilo human.

So below those doses, we have found no evidence of a change in MGMT. So, I take these claims of inducibility of MGMT with a big pinch of salt.

DR. ATRAKCHI: Very well.

When exposure is considered, the low

dose range for nitrosamines? It is a big question. What is a low dose range for nitrosamines, I suppose to induce cancer?

DR. KYRTOPOULOS: Well, I would say -- comparable to those likely to be experienced by humans? Having in mind that a lot of the experimental studies use higher doses, which may distort the picture. For me, that is the primary criteria. And coupled to that, I would say, following on from previous discussion, non-MGMT depleting.

DR. EISENBRAND: I agree totally with Dr. Kyrtopoulos, because we discussed this before, and it was always in relation to human exposure, more or less. That is low dose.

DR. ATRAKCHI: So, I mean, is there a number? What is a low dose?

DR. KYRTOPOULOS: Sure.

DR. ATRAKCHI: A range of low dose?

DR. KYRTOPOULOS: Yeah. If you -- if you look into, as we discussed yesterday -- when you look into human exposure, you end up with at max one microgram per person per day. That is a low exposure.

And that is what we discussed yesterday. And that was meant, actually. At this level, you will not find any induction, or any -- be it repair, or be it a metabolic enzyme. I do not think so.

DR. EISENBRAND: Yeah. I would even go a bit higher. I mean, even within the limits of the possible endogenous exposure that we, kind of, estimate with the tools that we have, which goes up to, maybe -- even a milligram total -- in total. That is a few micrograms per kilo, I guess -- tens of micrograms per kilo. If you compare this with the experimental studies which are being carried out, it is very low. Okay.

So, I would -- personally, I would call even those exposures low in a practical sense. But, I mean, this -- we should not attach to the use of the word, low, any significance in terms of risk associated with it. It is more low in terms of the mechanistic considerations that go -- that we go into, in order to assess the risk.

DR. EISENBRAND: I agree.

DR. ATRAKCHI: Is there any possibility

that activation occurs by enzymes, other than P450 and in organs other than the liver?

DR. KYRTOPOULOS: When we gave MDMA to Pettes monkeys, we saw large levels of methylation in the liver -- the stomach had even higher levels than the liver -- bladder, esophagus, pancreas, uterus, all had high levels of methylation -- and every other tissues we looked at measurable levels of methylation, in contrast to the rat, in which we see methylation primarily in the liver, and much less in the lung, the esophagus and the bladder. I leave -- blood is always methylated. But let's leave that out.

Now, why is it -- where is -- why is there this big difference? Is it that there are other enzymes in the tissues of the monkey? It is a possibility. My own working hypothesis is that, first -- liver, first pass clearance in the monkey may be a little bit less efficient than it is in the rat. So, a bit more dimethylnitrosamine escapes, and has the opportunity to reach post hepatic tissues.

When we gave dimethylnitrosamine together with -- sorry -- with ethanol, which is an

inhibitor of P450 2E1, which is the main enzyme which metabolism dimethylnitrosamine -- we found a small decrease in the methylation in the liver, but an enormous increase in post hepatic tissues. In the esophagus the methylation went up by 20 times.

Again, I do not know whether that means that metabolism in these post hepatic tissues is being carried out by other enzymes. I suppose the ethanol dose that we gave did not kill 2E1, it just suppressed it sufficiently to allow MDMA to through.

But, yeah. I do not know. I believe that in the rat esophagus, an enzyme which metabolizes dimethylnitrosamine have been discovered by Peter Swann some years ago. But -- I mean, there are other people much more expert on metabolism than myself, here. So, maybe they want to comment.

DR. HECHT: There is plenty of data from organ culture studies that extra hepatic tissues can metabolize nitrosamines.

DR. KYRTOPOULOS: But this is why a 2E1 see -- or by a different mechanism?

DR. HECHT: I am not sure whether -- I

do not think in most cases we know what enzymes are involved. There are -- P450 is no doubt. But you know, there is plenty of data from organ culture studies with a variety of nitrosamines, that demonstrate ability to metabolically activate.

But for example, in the esophagus -- the rat esophagus, which a common tissue affected by nitrosamines -- methylbenzyl nitrosamine is a good example. I mean, we know that the rat esophagus can metabolize methylbenzyl nitrosamine to get an O6 methylguanine and other DNA adducts. But we do not really know the enzyme involved.

DR. KYRTOPOULOS: I think, Paul Kleihus [ph] has shown that it is mediated -- and he has also said that is made inside of P450, if I remember that correctly.

DR. HECHT: Probably a P450. Yeah. I do not think we know which one, though.

DR. ATRAKCHI: Are there certain genetic factors that are known to create higher levels of endogenous nitrosamines?

DR. KYRTOPOULOS: I do not think we

know anything about the factors which affect -- or almost anything. We know a little bit. But we really do not understand the determinates of endogenous nitrosation, whether they are environmental or genetic. At least, I do not know any such factors.

DR. ATRAKCHI: Yeah.

DR. HECHT: I think we know a little about diet, and --

DR. ATRAKCHI: Yes.

DR. HECHT: -- formation. I think there is a data on nitrosoproline inhibition by ascorbic acid, for example. I am pretty sure that data exists. In terms of the genetics, I do not think so.

DR. ATRAKCHI: Right. Would it be prudent to determine endogenous levels of nitrosamines in placebo groups in clinical trials for comparison with drug effect?

DR. KEIRE: So, yeah.

DR. ZEIGER: So -- quickly, I would just say, yes, because you are not measuring the drug affect against zero background. You are measuring

against the endogenous background.

DR. EISENBRAND: Yeah. I --

DR. KEIRE: And you should do --

DR. EISENBRAND: Sorry.

DR. KEIRE: Sorry. Yeah. You should do it -- you should do a crossover study, actually, on the same people, right? You know, so control as much as the biology as you can in the measurement.

DR. EISENBRAND: Yeah.

DR. KEIRE: Absolutely.

DR. EISENBRAND: That is a good idea, actually.

DR. KEIRE: Yeah.

DR. ATRAKCHI: Yeah. I think it is a must. The question is, probably that the number of molecules defines the of reactive diazonium or carbonium ions form. Does this -- doesn't this define the potency?

DR. HECHT: Yes. It should. And, I think, again, if you go back to the Peto study, you see -- a linear dose response, I think, down to very low doses, which is consistent with that.

DR. CRONIN: Yeah. I think this question may relate to -- there was some discussion at the end of yesterday's session, on whether we should be using weight -- or molar -- so nanograms, or micrograms, or molar values. So, yes. I would agree. It does. And it does bring into mind the use of the correct units for setting limits.

But regulatory units tend to be by weight, rather than by molar units. But for QSAR modeling, for instance, and for comparing between molecules, we would prefer to use molar units.

DR. KEIRE: Yeah. I mean, I guess, the comment I want to make is that, as a chemist, I am very comfortable with either of those, right? And molar units is just fine. But, I guess, in terms of public communication, people do not understand, sometimes -- you know, those terms. Or they do not think that way. And so -- but they do understand a weight. I do not even know if they even understand what a nanogram means, in terms of weight.

You know, but it is -- you know, in terms of communication, weight is easier to understand

by a bigger group of people.

DR. ATRAKCHI: TD50 and the CPDB does consider timetable data, whereas this is normally not done for the BMDL. How does this affect comparability?

DR. BUCHER: So, I looked at this question, and I -- it puzzles me a little bit, because I am not sure what timetable really referred to in either the enervation of the TD50 methodology, or in BMDL.

The Peto's group in developing the TD50s came up with a lot of corrections for comparing studies that had short period of dosing and various periods of observations. And maybe took into -- difference in, whether tumors were considered incidental, and not necessarily fatal -- or considered fatal. Things that we have also used in NTP and earlier days.

I think, though, that most of the studies, now -- certainly, the one that we have done at NTP, have shifted over to doing -- calculating an individual survival based risk for each animal,

depending upon when it died in during the course of the study, whether it was during the study, or at the end of the study. And these risks that would then be taken into consideration in determining a survival adjusted incidence factor -- which is then the appropriate metric used in BMDLs.

I do not know that this is in any way shape or form possible to retrofit on the TD50 calculations. But I have doubts -- serious doubts. But that is the only way I could understand that, that question might be interpreted.

DR. ATRAKCHI: The panel suggests that we should control nitrosamines in pharmaceuticals to zero exposure. However, proving zero is impossible given nitrosating agents -- nitrites are in water, excipients, so forth. Also, to show the absence of nitrosamines require an analytical limit. Shouldn't we be focused on the determining safe level of exposure, versus zero?

I think we have discussed this. But anyone would like to comment on this question?

DR. KEIRE: I guess I would say that

zero is preferred. When we cannot get that, we will take it below the acceptable intake.

DR. ATRAKCHI: Yes. I think that is the whole point -- is they should not be there. And I do not know if zero is a very absolute number. But --

DR. MCGOVERN: I guess, the main issue is -- it is difficult to prove zero.

DR. ATRAKCHI: Right.

DR. MCGOVERN: It is all based on your analytical --

DR. KEIRE: They are not --

DR. ATRAKCHI: It is all just the analytic method.

DR. KEIRE: They are not detectible by the appropriate analytical method.

DR. ATRAKCHI: Exactly. Right. Good.

The use of -- sorry -- if an API could potentially form a nitrosamine, wouldn't it be observed in toxicology studies, because endogenous formation is more probable than exogenous conditions? That is the question.

Any of the toxicologists or biologists

can answer this?

DR. EISENBRAND: I think that is -- if I may -- that is very difficult. We normally do the tox test in rats, or in animals, at least. Or -- and they are, of course, quite big differences between the rats and humans, in terms of nitrate, nitride, and nitrogen oxide metabolism, so to speak. So, rats, for instance, do not circulate in saliva nitrate as far as we know. And there are other differences.

So, I would not take this form an animal experiment as it guaranteed a nitrosation does not happen in humans. And that, of course, depends also from the nutritional status. You have a totally -- normally, a very different nutritional status between a rat experiment -- or rat populations and humans. Especially, with a -- of nitrate from vegetables. But.

DR. KEIRE: Yeah. So, I recall a comment that I heard that humans are a poor model for rat physiology. So -- you know, this is always going to be a gap that is present. You know, and the practical matter is sometimes you cannot test -- you

cannot do toxicology tests in humans. So --

DR. ATRAKCHI: The next question is the use or surrogate compounds has been discussed in relation to API related nitroso impurities, which could potentially form, in terms of setting limits and evaluating their carcinogenicity affect. When it comes to determination and control of such API related nitroso impurities, is the use of surrogate compound in method development, validation, and quantification, recommended? If yes, what aspects would you recommend that should be taken into account regarding this election of the appropriate surrogate, in terms of uncertainty factors, or setting method parameters, and etcetera?

I guess, this is specifically in relation to an API related nitroso. What surrogates, I guess, would be appropriate in setting the limits.

DR. CRONIN: I am not sure -- quite sure who -- or the surrogate means a read-across analog, which takes us back to the discussions we had earlier. In which case, it is one way we can demonstrate formalities of mechanism action and

structural similarity. Or whether there is some thoughts of the surrogate may mean it has the same drug action -- similar API. In which case, is a very different scenario.

That is how I start to think about answering this question.

DR. ATRAKCHI: Thank you. I think the next one -- I will read it. This is -- it is a follow up from yesterday's discussion about whether acceptable intakes for more complex higher molecular weight in nitrosamines should be addressed differently than low molecular weight nitrosamines, such as MDMA and MDEA. Specifically, in the case where a novel higher molecular weight nitrosamine is encountered in a drug product for which the carcinogenic potency is unknown, should the default AI -- 18 nanogram per day for EMA, or 26.5 nanogram per day from FDA -- be applied on a molar basis, rather than a mass basis -- parenthesis, 259 picomoles equals to 26.5 nanogram of NDEA -- to account for active site of NMO group, rather than the molecule's mass? AI based nitrosamines can have two to five times molecular

weight than the simple nitrosamines.

DR. HECTH: Sure. On a molar basis. It makes sense. It is the nitroso group, and the carbons that are alpha to it, that are critical in determining whether there is going to be activity or not. And the rest of the molecule, will -- sure, will play a role in the activity. But I think, at a first approximation, you would want to go on the molar basis, not just by molecular weight.

DR. ATRAKCHI: Any other thoughts? Thank you, Dr. Hecht.

DR. CRONIN: Yeah. I -- again, we are back to considering the whole question of the limits, and the unit in the limit. And really, to answer this question, you would have to say, is the -- my understanding of the question -- I apologize if I am misinterpreting -- is the limit, in itself, protective, and is that the most conservative limit we want, regardless of whether it is calculated in nanograms or molar value.

So, that is how I would think about it, just in terms of being precautionary.

DR. ATRAKCHI: Right. The next question is, it appears from the discussion that read-across, QSAR, and Ames data will not be sufficient to classify novel nitrosamines as low or no carcinogenic potential. What studies would the panelists recommend to definitely classify a novel nitrosamine as low or no carcinogenic potential?

DR. CRONIN: Again, I hope I was not that pessimistic about how we can use QSAR and read-across. I think there are possibilities for classifying a compound using good read-across, supported by good QSAR modeling, which could be modeling of the metabolic step, for instance, and Ames test data.

I think the caution I had was that we required more evidence -- more certainty to able to classify something as no or low toxicity, regardless of any point. So, I think there are possibilities to do it. I think we can be guided by the structural activity, here, as well as other information. Otherwise, you will be in the situation where everything you want to classify as no, or low toxicity

will have to be tested. I think we can move away from that paradigm.

DR. BUCHER: So, Dr. Cronin also mentioned the fact that problem formulation is very important in that kind of situation. And I would agree that -- you know, you have to, sort of, play out the whole possibilities associated with a potential contaminant that you are -- that is not recognized yet as to whether it is a carcinogen or not. Look at the potential concentrations of the material, and then, make some expert judgments based on -- you know, what is known about the chemistry of the material.

But I -- you know, as an organization that does two year bioassays, faced with the potential problems of many, many nitrosamines coming down the pipe, it is just not an attractive solution.

DR. ATRAKCHI: Any other questions that I may have missed, or any that came from the attendees -- my colleagues who are following on those? Or any other questions from the FDA to the panelists?

I think, if none, it is a -- we are a little bit early -- earlier than with the time allowed

for this workshop. But I think to make things -- there is one question.

If a novel nitrosamine tested negative in the Ames test, what modification to standard protocols would you recommend to make sure it is an Ames negative?

We have discussed changing the S9 from the rat to mouse or hamster. Any other recommendations?

DR. ZEIGER: So -- yeah. This question has come up before. Right. And if I saw a nitrosamine function on the chemical, I would do a quick test using just TA1535, TA100, and rat liver S9. That seems to be the most useful combination for detecting nitrosamines.

The rat is less sensitive -- rat S9 is less sensitive than mouse S9. And I think, also a bit less sensitive than hamster S9. And all the nitrosamines that I am familiar with are positive in the TA1535, TA100 series of strains.

So, that would be a quick and easy test. You know, S9, two strains. If it is negative,

there, I would go to the wider test battery. But if it is negative there, I would not expect it to be positive in the wide test battery, unless there was some other functional unit on the -- some other mutagenic unit further down the molecule that is not associated with the nitrosamine function.

DR. ATRAKCHI: Thank you, Dr. Zeiger.

DR. GUTTENPLAN: I have a comment.

DR. ATRAKCHI: Yes, please.

DR. GUTTENPLAN: So, probably 30 years ago we reported that the potency of nitrosamines in the Ames test can be greatly increased by dropping the PH. It turns out that the intermediate -- the hydroxy -- alpha hydroxy nitrosamine is much more stable at acidic pHs, and therefore, you get much more permeating into the bacteria.

So, if you want to start playing with conditions of the assay, dropping the pH to six, or six point five, for a nitrosamine, should greatly increase the sensitivity. And --

DR. ZEIGER: Dropping the PH to six would kill a lot of the salmonella on the plate.

DR. GUTTENPLAN: It works. We did it.

DR. ZEIGER: Okay. Because --

DR. GUTTENPLAN: It is published.

DR. ZEIGER: Okay.

DR. GUTTENPLAN: They were only exposed during the pre-incubation. And then, of course, they get -- it gets diluted into the top auger.

DR. ZEIGER: Okay. We have experiences where once you go down to six or below, you are getting toxicity from the pH affect, whether it be a pre-incubation, or a plate test.

DR. GUTTENPLAN: Yeah. I have to go back and look at the publication. Maybe, we went to six point five, rather than six.

But anyhow, it is out there. And it was confirmed by a group in Japan. And -- so it is just if you want to play with conditions, you can probably make the Ames test more sensitive. And as Dr. Zeiger mentioned, hamster S9, is certainly better than rat S9, and probably better than mouse S9.

DR. ZEIGER: Well, in my experience, the mouse was better -- equivalent or better than

hamster. It is -- but this is primarily with a series of cyclic nitrosamines. I have not compared them to any extent with the aliphatic.

DR. GUTTENPLAN: Yeah. I think there is literature out there on that.

DR. ZEIGER: There probably is. Yeah.

DR. ATRAKCHI: Very good. Thank you, very much for a very comprehensive and valuable information that you all have provided us to consider, and to think about.

I think, to summarize, mainly, is that in terms of nitrosamine classification, the parameters we suggested -- or proposed -- or put on the table, they all are important, whether it is the potency -- we should take that into consideration -- the chemical structure, as well as reactivity. These are all parameters that are important for us in order to make a classification of nitrosamines.

But, also, what is considered -- whether a classes specific limit -- whether it is the 18 nanogram per day, suggested by EMA, or any other number -- that could be used, as well. But it needs

to have some basis why if one number fits all.

We also discussed the issues of risk assessment of multiple nitrosamines in one that will be present -- or found in the drug substance or drug product, and we understood that the -- they need to be treated, basically, whether it is one, or 10, or five. They need to follow whatever the conservative approach as one potent nitrosamine -- whether it is the NDMA or the NDEA, because the affect is additive.

We also discussed the less than lifetime approach, and there is a great deal of information there to consider for the less than lifetime consideration with nitrosamines.

And the QSAR -- the QSAR is really the -- probably the future. But there are many gaps that needs to be addressed for the QSAR of nitrosamines, in particular. Things to consider is reactivity -- is the behavior of the diverse nature of the chemistries of nitrosamines in vitro and in vivo, and the slight modification in structure that could lead to significant different in biology of that particular nitrosamines.

But it is a tool that needs to take a lot of things into consideration, and a lot of the data that is available, out there. But I believe there is more to do with the QSAR and the read-across.

The topic of endogenous, whether it should be considered -- the exogenous as well as -- and/or endogenous levels of nitrosamines when we assess the risk assessment for the nitrosamines as impurities in drugs. The endogenous formation is -- seems to be, based on the discussions of the last two days -- is very important parameter that needs to be addressed, and it is lacking in good and accurate information.

But that is something for the future, that should be -- that studies should be designed and should be considered and should be conducted. It is not something we can do immediately and use the information from that.

In terms of the other exogenous -- the dietary -- that is another one that depending on the diets, different parts of the world, different parts of the same country -- it is also a difficult -- but

it more manageable than, perhaps, data from the past, as well as data that can be collected and some -- a lot of publications do exist -- could be considered.

But ultimately, the way that I think it ended -- the discussion on this -- is that we do need to pay attention. If we ignore both of those big contributors to the nitrosamines, then it is the quality of the drug -- of the medicines that are taken by patients that needs to be considered, in terms of limiting the amounts of nitrosamines in the pharmaceuticals -- in the medicine.

And again, the last two questions were on chemistries and manufacturing. And it became evident that nitrosamines -- it is all about chemistry. It is all about -- from the beginning, the awareness and the wholistic approach to understanding what is the synthetic pathway -- what is the particular molecular that one want to synthesize and manufacture. There is a lot of -- it is not only it. But it is everything around it -- all the excipients, all the temperatures, all the pH, and whatever the intermediates -- everything needs to be considered

along the path of formation of that API, and ultimately, the drug product, as well as beyond that. It is the post marketing, the packaging -- how is it -- where is it going -- in what conditions it is going.

So, I think, in my mind, there is a great deal that can be done and controlled right from the beginning on the chemistry and in manufacturing, before we get to the problems of -- we found nitrosamines in drugs and how do we do risk assessment. It need -- all of this needs to be addressed before we try to mitigate and see, well, what can we do, can we go to one in hundred thousand, can we go less than that, because this drug is essential, it needs to be on the market, and needs -- we cannot take it off.

In my mind, there are a lot of tools. There is a lot of things that can be done to prevent and minimize the formation of nitrosamines in drugs before we get to all the way and trying to see -- and try to really grapple on the issues of what can we do, it has to be there, it is going to be there, what can

we do, and what is the risk for the patients.

With that, I think, we really appreciate your contribution and your participation in this workshop. It has been valuable for the agency, and for the attendees to listed to your expert scientific opinions on the topic.

Thank you, very much. And this workshop, as we indicated in the beginning, it is recorded, and it will be available by the end of this week, as well as the two presentations, and Doctor -- both, Dr. King, as well as Dr. Eisenbrand, and Kyrtopoulos slides will be available at the end of the day today.

Thank you, so much, for participating.

DR. KEIRE: Thank you, Dr. Atrakchi, for your -- great moderation.

DR. ATRAKCHI: Thank you.

DR. KEIRE: Yeah. And the panelists, you guys are all great. I appreciate you brining all this knowledge together. It is very helpful. Appreciate it.

DR. ATRAKCHI: Thank you.

DR. KYRTOPOULOS: thank you.

DR. ATRAKCHI: Thank you, all. Bye-bye. Thank you.

(Whereupon, the meeting concluded at 2:12 p.m.)

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