



SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

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FDA, College Park, Maryland • Live Webcast

Real Time Captioning

Schedule

- 9:00 am-9:40 am Health Effects of PHOs and Trans Fatty Acids: Data from Clinical Trials - Martijn Katan, VU University, Amsterdam, The Netherlands
- 9:40 am-10:20 am Epidemiological Studies on Health Effects of PHO: Strengths and Limitations of the Available Human Data – Ingeborg Brouwer, VU University Amsterdam, The Netherlands
- 10:20 am-10:45 am Break
- 10:45 am-11:25 am Mode of Action and Dose-Response Evaluation of the Effect of Partially Hydrogenated Oils on LDL-Cholesterol-Michael Dourson, TERA, Cincinnati, OH
- 11:25 am-12:05 pm Dose-Response Assessment Approaches to the Analysis of Non-cancer Health Effects: Current Practices, Advice from the National Academies, and 2014 WHO/IPCS Guidance – Weihsueh Chiu, EPA ORD, Washington, DC
- 12:10 pm-1:00 pm Moderated Roundtable Discussion – Norbert Kaminski, moderator
Comments: DeAnn Liska, Biofortis Clinical Research, Addison, IL
All speakers

8:15 am-8:25 am FDA Welcome and Overview-Dennis Keefe, FDA, CFSAN, College Park, MD

If I can ask people to take your seats, I'd like to get started on time. Good morning to you well. My name is Dennis Keefe. I'm the director of the FDA's office of food additive safety. It's my pleasure to welcome you to our first series on colloquia on emerging toxicological sciences challenges in food and ingredients safety. The U.S. FDA is partnering with the Society of Toxicology this year to provide a series of four half day training sessions that focus on high-quality, cutting-edge, future oriented toxicological science. These sessions are intended to provide a forum through which FDA can engage any experts in toxicology from around the world. These colloquia are open to the public and offer the public opportunity to get the same information FDA is hearing. I want to be clear these colloquia are not intended to offer public forum to discuss or make recommendations to the agency on regulatory issues. We're here to talk about the science.

The first of these colloquia, the one you have joined us for today, focuses on complexities in evaluating human clinical and observational data for ingredient safety assessment and with the case study being partially hydrogenated oils. It will feature scientific experts from Michigan State, the University of Arkansas Children's Nutrition Center, the Free University of Amsterdam, the U.S. EPA and TERA, Toxicology Excellence for Risk Assessment which specializes in chemical risk assessments. The next three colloquia are being scheduled over the next seven months and will focus on topological studies versus computational modeling. Special topics and topological in points and risk assessment and risk management.

Before we get going, I want to make a few administrative announcements. There are restrooms upstairs that you passed on your way in that are available. During this session there will be cards made available to ask questions for those in the room. If you would write your questions down and submit them for the panel discussion. For those online I understand there is an opportunity for you to submit your questions electronically.

Keep in mind we have a very full calendar scheduled this morning and not all questions, there may not be time to address all the questions.

It is my pleasure to introduce Dr. Norbert Kaminski who is the president and professor of pharmacology and toxicology at Michigan State. He joined appointments in the College of human medicine and veterinary medicine. He will be our moderator today, so please join me in welcoming Dr. Kaminski.

[Applause]

8:25 am-8:30 am Welcome from SOT–Norbert Kaminski, SOT President, Michigan State University, East Lansing, MI

Thank you very much, Dennis, and on behalf of the Society of Toxicology I'd like to welcome everybody to the first as Dennis said four colloquia that will be cosponsored by FDA as well as the Society of Toxicology. For those of you who may not know, the genesis of this colloquium initiated with ideas about this kind of a series that came from Dr. Suzanne Fitzpatrick and Alan Rudman. I think it is a tremendous idea in terms of having a series of four of these colloquia that is really going to focus on the science. It is an opportunity not only for continuing education but also to allow the members of the Society of Toxicology and others who may be interested in these various subjects who may not necessarily follow the science to get an opportunity to hear some of the experts in the world talk on issues that are at the emerging forefront especially in terms of the food area. We are really excited about this colloquium series.

The other thing I need to do this is a slide of the organizing committee which really did a tremendous job. I want to take a few minutes to acknowledge them. James who chairs the organizing committee. Martin who will moderate the majority of this session. Brian Delaney, June from Kellogg's. Ronald, Kristi and Catherine. They have all done a tremendous job organizing this series very quickly. We just decided to do this probably late August and wanted to get this started quickly and within several months hold together a fantastic program for this first session.

With that, let me introduce our first speaker as well as moderator for the presentations. Martin received his PhD in physiology and biochemistry from Reading University. Martin and I first met around 1985 as I was finishing up my doctoral degree at North Carolina State. Martin had just been recruited by Ernie

Hobson to work in his lab as a postdoctoral fellow. After a few years at North Carolina State, Martin went on to the -- Institute ultimately taking a faculty position at the University of Arkansas where he is risen to the rank of Professor of professor of pharmacology and toxicology. He has also served as associate director for basic sciences for the Children's Hospital Research Institute and more recently associate director of basic sciences for the nutritional center they have there. Currently, Martin's primary appointment is in pediatrics and division of developmental nutrition. Martin serves on a lot of different committees. We served on a study section together and he also serves on a lot of editorial boards. Without taking up time from his presentation I'm going to turn the microphone over to Martin.

[Applause]

**8:30 am-9:00 am Setting the Case Study Framework -An Introduction to PHOs--
Martin Ronis, University of Arkansas Children's Nutrition
Center, Little Rock, AR**

The committee has charged me with kicking off this morning by setting the case study framework. Since most people who are in toxicology and regulatory science -- a lot of nutritionists are not familiar with toxicology and regulatory science. We are kind of in between. We have a lot of diversity in the audience. What I thought we would do is start off fairly basic and then get complicated. Most people are not lifted biochemists. Where are the slides? There we go.

I have no conflicts of interest. [Laughter] I don't work on partially hydrogenated oils, but I do work on fats and toxicological issues related to consumption of different classes of lipids. I actually do know something about the topic. We are going to start off by talking about basic lipid chemistry. When we start talking about the structure of fats and oils, we are really talking about triglycerides and triglycerides consists of a molecule of glycerol to which are attached three fatty acid moieties. Those fatty acid moieties can be quite diverse. They can consist of saturated fatty acids, mono unsaturated fatty acids or polyunsaturated fatty acids in which there are many double bonds and these can be conjugated or unconjugated. The problem with fatty acids by chemistry is that the nomenclature is confused and many different people use many different nomenclatures. It is very easy to get misled as to what we are actually talking about. There are essentially two ways of looking at a fatty acid. One is this -- designation which starts at the first carbon and the last carbon is -- and sometimes known as [Indiscernible]. There is a second nomenclature which is the numeric nomenclature and that starts at this carbon. This is carbon one. This is nine toward the end of the chain.

Physical properties of fats. The melting point, whether a fat is solid or liquid at room temperature is determined by the carbon chain length, saturation and unsaturated bond geometry.

In terms of the endogenous pathways in animals, animals convert carbohydrates to fatty acids and they do so through the generation of glycolysis and Krebs cycle and then a series of steps resulting in the formation of 16:0 which is palmitate. This undergoes elongation to form a stearate in which an additional two carbons are added. This is then desaturated to become oleate and the double bond is at position nine. These fatty acids are synthesized by animals plus a variety of dietary fatty acids which include a variety of polyunsaturated fatty acids which are essential fatty acids are converted into triglycerides. Because this is a primary biosynthetic pathway, animal fats including lard, beef tallow and dairy products are rich in 16:0 and 18:0 and generally solid at room temperature.

They are not the only source of fats and oils that are predominantly saturated. There are vegetable fats that are also predominantly saturated and these are predominantly coconut oil and palm oil. Coconut oil consists of completely saturated fatty acids but these are medium chain with the carbon link between 18 and 14. Palm oil is rich in palmitate 16.0 just like animal fats. The vegetable oils consist primarily of mixtures of mono unsaturated in polyunsaturated fatty acids so all of oil is mainly 18.1 with soy oil mainly 18.2. Canola oil is a mixture and corn, sunflower and safflower oil arming the 18th two. Fish oils are highly polyunsaturated and they are -- fatty acids. They start from three carbons to the end of the fatty acid chain.

Animals don't have the necessary enzyme machinery to make polyunsaturated fatty acids from oleate. Therefore linolenic acids are essential fatty acids and they are required in the diet albeit at low levels. These are the basis of biosynthetic pathways that lead to the formation of -- [Indiscernible]. This area of lipid biochemistry is becoming very complex but also very exciting. There are many novel bioactive lipids that are still being discovered that are part of this pathway.

What is a partially hydrogenated oil and what it is about? This is a process discovered sometime in the early 1900s. If you take a source of polyunsaturated fatty acids such as soy oil and global hydrogen through it in the presence of heating that this will undergo hydrogenation and ultimately form stearate so the double bonds would be lost. The addition of catalysts to catalyst to this process has resulted in the ability to be able to modulate how many of these transformations occurs in to what degree you get the formation of saturated fatty acids from unsaturated fatty acids in oils and therefore to modulate the physical properties of the final product which is a partially hydrogenated oil or PHO. You can see on the conversion there is a kink in the chain and that kink occurs at the double bond in oleate because in oleate the double bond is in the cis position. In the cis position the two hydrogens are on the same side of the carbon chain. You can also have the two hydrogens on opposite sides of the carbon trading and this is a trans double bond. One of the things that happened as a byproduct of the hydrogenation of polyunsaturated fats is in addition to forming Overlake acid you also form a lady acid -- elaidic acid. They also contain trans fats.

Why are they used and why is this process so popular in the food industry? One of the things about trans fats is the trans fat reduces the kink kink in the fatty acid and changes the physical property of the product. Products that contain partially hydrogenated oils is the happened into need the melting point. It results in partially hydrogenated oil products which are immediately spreadable such as margarine. There is an increased shelf life due to greater stability than polyunsaturated fats in the face of oxidation or popularly known as rancidity. They also have superior baking products and a pleasant feel to the mouth. Products that contain PHOs melt in the mouth with no waxy aftertaste. In 2006, 50% of oil was hydrogenated.

What are the trans fat isomers in PHOs? The trans double bonds are either a carbon 9 or a carbon 7. The major one is elaidic acid and large range isomers. There are a smaller number of 18-point to and five isomers at 9, 12 or 15. 15. In terms of partially manufactured hydrogenated oils, -- is up to about 45%.

How does that translate to fatty acid composition includes? If we look at soybean oil and partially hydrogenated oils, one can see the composition goes down dramatically. There is an -- up to about 24% and a smaller degree of 18.2. In baked goods this translates to about 27%. Margarine about 17% in shortening about 15% of the total fat.

What about consumption of partially hydrogenated trans fats? This is a moving target. We may hear something later about whether or not these values are still relatively accurate. It was suggested 5-10 grams per day resulted in trans fats from the United States. In North America, levels from 3-10 grams per day. Levels somewhat higher in places like Iceland and comparable in places like Australia and lower in countries in the Mediterranean where they primarily use olive oil as a lipid source. In Asian countries, it was really low.

The toxicological assessment of trans fats in the diet is complicated by the fact there are natural ruminant trans fatty acids. These are produced from Linoleic acid and linolenic acid. -- linoleic like -- linolenic like [Indiscernible]. There are also conjugated acids that are made called CLA's represent a small proportion of the trans fat produced by ruminants. The total ruminant trans fatty acids are about 5% of dairy product backs, and about 4% of beef that. The average -- ended with constitutes about 4-mix per gram of dairy products.

Small amounts of these conjugated linoleic acid -- one of these bonds is trans and the others are conjugated. CLA is sold as a dietary supplement.

From the perspective of toxicology and risk assessment of these components of the diet, the potential toxic effect we're going to spend most of today talking about is cardiovascular toxicity. One of the things that seemed should be fairly uniform about consumption of trans fats is there is an increased ratio of low density lipoprotein to high density lipoprotein. There also changes in lipoprotein particle size. Increased plasma triglycerides and overall dysregulation of -- that part of this involves -- and involves changes in the packaging of lipoprotein although the molecular mechanism remains somewhat obscure. There is also evidence of increased inflammation and increased cellular adhesion after trans fat consumption. There are a variety of more isolated reports that trans fats may increase of domicile of the city, insulin resistance, etc.

What are the challenges of the topological assessment of partially hydrogenated oil? These are the questions I posed to the remaining panelists in today's discussion and hopefully we'll get some answers as we go through the next series of talks. In terms of epidemiological observation studies of diet affects, the issue is complexity of diet composition. What do we measure, when do we measure and how do we measure? How good or bad are food frequency questionnaires in determining what we are actually consuming? Is measurement of chemical composition of foods and fluids a better measure and if so when do you do it? Can you extrapolate by chemical indicators to cardiovascular events and what are potential confounders? There other dietary components that may also play into these endpoints. What are the effects of weight and body composition and age and sex? How do we do with healthy worker affects in epidemiological studies? What about socio-economical status, smoking and drinking? How do we do with independent risk factors which apparently have nothing to do with trans fats and what is the accuracy of intake data?

When we look at challenges to interpreting clinical trials on the effects of dietary components, we have to ask how many meet the gold standard of placebo-controlled double-blind studies? What are the statistical limitations? Most of these are short duration studies with surrogate endpoints. With macronutrients such as trans fat which constitutes a significant portion of the macronutrient intake, what is the appropriate controlled diet? Do we use saturated fats or polyunsaturated fats? Do we use carbohydrates? Then there is the dietary control itself. Did subjects eat what they were supposed to during the study? Is there enough data to determine dose response, slope and shape? Is there a threshold for health effects?

We all know there is a push into in the toxicology and the regulatory community to include mechanistic studies in risk assessment. This becomes a real problem in the study of food ingredients. When we look at animal models to study food ingredients and cell culture models, there are obvious advantages just like there are for environmental or industrial chemicals. These assays and experiments are relatively cheap to do and easy to generate mechanistic data but can they be extrapolated to people? This is a really important question particularly when it comes to dietary components. There are enormous species difference in lipid metabolism and the development of diabetes. Do mechanisms identified in both models apply to humans eating diverse food sources and sites? Is the reductionist approach possible in relation to toxicological assessment of food ingredients when dealing with a complex mixture in which perhaps there are other biologically active ingredients that either work on the same pathways in a positive or negative fashion.

Then there is the issue of manufactured trans fats and ruminant trans fats. It has been suggested their help beneficial effects associated with ruminant trans fats. Improved body composition and cancer prevention. Are there differences in biological properties and molecular mechanisms underlying effects of trans fats in PHOs or ruminant trans fats? Are the reported differences which are primarily in animal models simply related to dose?

The rest of the morning is going to be spent by the experts in this field trying to answer those questions. We're going to have four talks followed by a moderated panel discussion. The talks deal with the data from clinical trials, the strengths and limitations of epidemiological studies, a dose response assessment approach in response to noncancer health effects which is a generalized introduction to the final top which is mode of action and dose response evaluation of the effect of partially hydrogenated oils on LDL/HDL. You we are lucky we have some of the world's experts in this area in the room to address these areas.

I would now call upon our first speaker to come up and talk about the health effects of PHOs and trans fats acids. This is Dr. Martijn Katan and got his PhD in 1977 and spent his career working on cardiovascular effects of cholesterol and trans fats. Is currently a professor at the Free University of Amsterdam and the world authority in this area and has been responsible for a lot of changes in the regular station of consumption of trans fats in many European countries including the large removal of industrial trans fats in countries like Denmark. He also has other claims to fame that make him somewhat notorious. A recent study of his found that if you don't filter coffee and you boil it they to increase your cholesterol burden. This is bad news for all of you out there who use French presses. Apparently you have to filter your coffee to get rid of the cholesterol -based ingredients. It is my great pleasure to welcome Martijn Katan to the stage to fill us in about all the studies over the last 20 years.

[Applause]

9:00 AM–9:40 AM Health Effects of PHOs and Trans Fatty Acids: Data from Clinical Trials - Martijn Katan, VU University Amsterdam, The Netherlands

Thank you Dr. Ronis for those kind words.

Are you a walker they asked me and I said yes.

I appreciate the opportunity to be here not just because I have great respect for CFSAN and for the FDA for what they are doing but also because this is a fascinating meeting where the toxicologists and the nutritionists are going to clash on how to determine whether something is healthy. I think very good things can come out of that clash. And there will be opportunities to talk about that. I am very happy to be here.

I'm going to talk about the data from clinical trials. This is my Facebook address in case someone has questions that won't come up in discussion.

My competing interests is we got some money and the special fats for our first studies 25 years ago from you will ever and materials from a company called lipid nutrition. I have no speaking fees, consultancies, equity, patents or expert testimony.

Here is the outline of what I'm going to talk about. I have three general background agenda points. That is the lightbulb proteins, LDL/HDL and their properties. A little bit of the background and history on trans fatty acids and how we get to where we are. The trials which I'm going to speak, how are these done? Then I will come to what I call the meat of my talk, the trials that have been done on trans fatty acids and their effect on lightbulb proteins and then I will give you my conclusions.

First LDL/HDL and heart disease. You have heard about the bad cholesterol, the LDL, and as usual we have more of the bad than the good. 50-80% of the cholesterol in our blood is LDL. Then there is the good cholesterol, HDL. HDL immobilizes cholesterol from cells and 20-40% in our blood is HDL.

Evidence that HDL protects against coronary heart disease. CHD. The epidemiological evidence is very strong. Anything that affects your HDL will affect your heart disease risk. Anything that can be wrong with the person given a lower HDL and a higher risk. They can be smokers, overweight, they can have a lack of exercise. It will lower HDL and increase the risk of heart disease. The genetic experiments of nature are much less definitive. The effect of mutations which cause your LDL to be low or high are inconsistent. Some seem to affect risk and some don't. It's not really very clear. The randomized control trials of drugs that raise HDL have also been inconsistent. The early trials seem to show -- and finally the mechanistic studies. These are very consistent. You can show that HDL immobilizes the affects to the cells -- from the body and hopefully from the arteries and back to the liver and out.

How useful is that type of mechanistic evidence? No one said that better than Walter Willett from Harvard. He wrote in his book, our understanding of biologic mechanisms remains too incomplete to predict confidently the ultimate consequences of eating a particular food or nutrient. I think that is the general experience in many fields in nutrition that the human body as a whole is just too complicated to confidently understand what will happen if we put foods at the top.

What does that imply for HDL and coronary heart disease? I think there is no justification for prescribing HDL raising drugs. I don't know what the FDA says about it, but I trust they will say something similar. There are much better ways to reduce your heart disease risk. On the other hand, there is a lot of circumstantial evidence that lowering HDL is not a good idea so caution requires we avoid lifestyles that lower HDL. There is no controversy about avoiding obesity, smoking and lack of exercise. There is an increasing consensus that we should maybe hold the carbohydrates a little bit and high trans diets is what were going to talk about here.

LDL, that is a lot simpler. The evidence raising LDL causes coronary heart disease is very solid and expensive. LDL predicts coronary heart disease risk. Over the full range of LDL levels, for instance studies in China 30 years ago, the researchers compared the LDL in the rural Chinese which was really low with other people in China and they saw the lineal -- you also find it in the country for if there is a high LDL and very consistent in all parts of society. With LDL the genetic evidence is also very consistent. You have probably heard about the menial -- there is many mutations in LDL which change LDL concentration. There is a fascinating mutation in the black community, about 2% had a mutation which lowers LDL and they have a much lower risk of heart disease.

We know drugs of various classes will lower heart disease risk if they lower LDL. Surgery on the small bowel which will divert cholesterol from your body and lower your LDL also lowers the risk is no longer a realistic mode of treatment but it seems it was the mode of treatment before statins and it did work. Diets that lower LDL also lower coronary heart disease risk.

If you replace saturated fat which raises LDL cholesterol, if you replace it by omega-3 fatty acids like soybean, corn or sunflower oil you will see -- in randomized controlled trials. Here are the data. By and large they lower coronary heart disease risk to the extent you would expect. They lower LDL by 15-20% - except for this one this was an outlier. If you included the overall risk becomes a little doubtful. This is the diet heart study done in the 60s.

What happens in this study in Australia? The subjects are people who survived a heart attack were advised to eat margarines rich in Omega six polyunsaturated acids. In those days, -- these people were steered toward consumption to large amounts of trans fatty acids. Let me explain the apparent outcome. This is direct proof that trans fatty acids cause heart disease, this was a very poor study. They didn't know what they were feeding them. It was a bad study. The office realized they never published it in a peer-reviewed study and only recently did this get dug up and published by other people. If you combine this with the data on saturated fat and Omega six PUFA the evidence is strong you can leave out the Australian study and then you see replacing -- will indeed reduce your coronary heart disease risk.

For trans fatty acids, why do I talk about trans fatty acids and not about partially hydrogenated oils which is the real topic of this meeting? I avoid partially hydrogenated oil because hydrogenated oils can contain any amount of trans fatty acids from 0-60%. If you fully hydrogenated oil you get the only fat guaranteed free from trans fatty acids because you saturate all of the double bonds. Hydrogenated oil is a very imprecise term and that is why I use the term trans fatty acids or if I'm in a hurry I will also say trans fat which is also a misnomer.

Here is a catalog of trans fatty acids. This is the most common one, elaidic acid and others similar to it which is found in partially hydrogenated vegetable oils and here is the trans double bond. Here is backs Senate acid which is typical of milk fat and other ruminants. Is also found in industrial trans fats and the double bond here at the 11 position so related to -- they did the definitive study -- vaccenic acid has the same effect on LDL and HDL and this study will soon be published hopefully. If you want the data in a hurry just drop me an e-mail. Then there is CLA conjugated linoleic acids. [Indiscernible]. This has a cis double bond in here the 11 trans double bond.

These are trans fatty acids made from omega-3 fatty acids. This is what happens when you overheat soybean oil. Even when you hydrogenated fish oil which is not done much anymore which was the

staple of the fact industry 100 years ago you get a cis bond here and a transponder year. All these fatty acids all do the same thing. They raise LDL and lower HDL. How does this work? Very differently.

What do we know from tryouts on this affect? The first trial -- didn't believe in HDL and LDL so he measured total cholesterol and saw the effect of trans fatty acids was intermediate between that of the [Indiscernible]. The cis fatty acid and butter with saturated fatty acids and also an elevation of triglycerides. A very influential study was also done in the United States. The United States is usually first in everything.

This was done at Procter & Gamble which at the time was the major producer of March rate in this country. They fed us subject first high on the cis fatty acid and split up the subjects of continued of the cis and 13 were switched [Indiscernible]. No effect whatsoever of trans fats on the blood lipids in that study. That was an influential study.

In 1985, the FDA report on trans fatty acids stated there is no clear evidence of adverse effects and that was based largely on extensive toxicological testing on laboratory animals and human studies in which the Procter & Gamble study was predominant. This was the basis for U.S. policy for quite a while saying there is nothing wrong with trans fats. These animal models fail to detect the adverse effects of trans fat as they failed to -- on lipoprotein's including the coffee factor which you don't have to worry about.

That set the stage for the study that appeared in the New England Journal in 1990 where we stated the effect of trans fatty acids on the serum lipoprotein profile is at least as unfavorable as that of cholesterol raising saturated fatty acids because they not only raise LDL cholesterol -- raised a bit of a stir.

Here is the effect of cis trans fatty acids in that study. The [Audio Cutting In/Out]. Here are the trans fats and the saturated fats. The trans fats raises LDL although in our study just a tad less than the saturated fats. In the effect on HDL you see a clear lower rate on the trans fatty acid. This is the type of evidence in which we rely largely for the effects of trans fatty acids on health so it's about time to tell you how these studies are done. How are these randomized trials done?

The first issue we have to deal with is a macronutrient is not like a pill that you can add on what top of people eat. If you advance subjects will gain weight which will also -- the total effect of a fact is the sum of what you add plus what you remove so the fact can take its place. How are we going to convert these effects into a single number for the effect of trans fatty acids? It sounds difficult but actually it's not. Am I allowed to ask a question to the audience? Anybody here from the West? Yes. You would say going to College Park -- it increased my altitude by 80 feet which in the Netherlands is called a mountain. [Audio Cutting In/Out]. In the case of altitude is just sea level. College Park is 69 feet above sea level and Amsterdam airport is 10 feet below sea level. We can say let's define altitudes in terms of sea level so we get a single number and we don't have to fight over it. You can also define it in terms of feet over the Empire State building or the bottom of the ocean, it has no effect on the difference. If you say Denver is 5000 feet over sea level, it may be 4000 feet over the top of the Empire State building. The difference will stay the same.

Altitudes are expressed relative to sea level. Lipoprotein levels are expressed relative to cis oleic acids. You can easily calculate -- we know the relative effects of all these fatty acids and carbohydrates. [Indiscernible]. I have taken saturated fat from butter. If you replace that by monounsaturated you get lowering. If you replace it by trans you get increase. I'm showing you this or you can decide what your favorite standard is. If your favorite is oleic, the effect comes a lot larger. This is how we express that.

How do we get these numbers? You put a group of people on a trans fat diet. You put a group of people on the trans fat diet because people may differ somewhat in their response to the diet all of the difference tends to be small. We get large differences we need to the study. If you retest the same person in the second study you still get large differences but they are all over the place. The correlation between the effect of diet in one study to another is relatively small because there is random error. Cholesterol goes up and down. That's why you want to have a large group of people to study to average out random error an average out remaining responsiveness. This is not a good trial because you measure before and after and all kinds of things can change besides the diet when you put people in a laboratory situation. They have to get up earlier in the morning. They realize they should exercise more. Something might be changing with the weather. This type of study we do not accept for our reviews. What we want is a concurrent control group and we measure the change of level -- in the treatment group. As we already have the blood we also measure fatty acids and no how were subjects have complied with the diet we wanted to beat. We get estimated precisely. We also have a period on the control diet where we stabilize people. We have the Delta here in the Delta here and we subtract that and that is the number for trans. Many of these studies are not done in the parallel way that the crossover design. This was our latest study where we measured people after three weeks on oleic acid then we switched them to CLA then we switched into industrial trans fat. Then we have a bunch of people in a different sequence. We randomize any secular effect of the weather or the latest fashion in diet or what has been -- all that averages out and all you have left is the effect of the trans fats.

For little bit of educational entertainment because we have been added at it for quite a while already and you have been up early. Here are the subjects in one of our tryouts. This was like 25 years ago.

They come into the laboratory at noon to get a hot meal. The card shows how much food they need because people need different amounts of food. Some people eat twice the amount that other people eat because they are larger or more active so that is wait out on the scales. That is why we express foods in percentage of calories. We express it relative to total energy so you get a much better correlation.

Here are the three diets in our trans fat study. This is the cis diet. This is one days worth of food. Here is the trans fat and the saturated day. As you see they are similar and there are small differences. We have more cheese to get the saturated fatty acids -- and stuff like that to make sure we really got [Audio Cutting In/Out]. We also allow them these roles and the composition of the roll world is identical to the composition of the total diet. What you have got here is the same as what you have got here. At least from a new trend point of view. If you're talking about attractiveness or convenience, it's different, but the question was you had to keep your weight so if you feel hungry, eat this stuff and you can stay on the same weight. We collect duplicates of all these diets. This is six days worth of food. They get different foods every day and we collect that in buckets. Then we grind it up. Do you notice growing fat has nothing to do with what you eat? This is exactly the same food as we serve them here but you're not going to get fat on this because it's not attractive or convenient or tasty so the science of obesity is a different feel than the science of nutrition. Anyway, we grind it up and analyze it chemically. We give some of the two rabbits to see what they do. They react totally different. Because we have the fatty acids we know exactly what went in. We know whether they complied and we measure the lipoprotein and we finally get to the nitty-gritty enter the result of tryouts on trans fatty acids.

We did a systematic review on that a couple of years ago and there've been other reviews and angle board is -- Ingeborg is working on the study. [Indiscernible]. A proper control treatment and proper design and proper control of food intake. They had to last two weeks or longer because we live by that time blood protein levels are more or less stable. No weight loss or gain because -- conclusions. You

have to exclude poor studies because they are biased and not neutral. Bias toward zero outcome. This is something very useful if your product is being studied. You can contract out the research and say I want you to study this but I'm going to give you very little money and time and give me a design in somebody will come up with the design. Then the outcome is zero because it's easy to get a zero outcome, just do a bad study.

To remind you what Dr. Ronis said. We have two types of trans fats from industrial hydrogenated oils and from ruminants. Here is the outcome of these various studies and this is a graph with three types of fatty acids. The industrial partially hydrogenated oils, the room in the trans and the other Roman it trans fatty acids and the conjugated manually acid. Focus on the open circles. As you see there is a spread. Most many of them find a significant effect on LDL cholesterol. There is a spread and very good reasons for this. If the study is done within 10 miles of the -- you will get a very large effect for reasons which have not been explained. There are of course other reasons. If you draw a straight line through these you see it goes up with the dosage and the dosage of percentage of calories from trans fatty acids and you see the same thing for the HDL which goes down also with the dosage with the trans fatty acids. Would like to express such data as a ratio of total cholesterol or total cholesterol related to HDL because that is the predictor of heart disease. Then you get these open figures similar to the LDL data and the ratio goes up as the dosage of trans fatty acids goes up and zero points all over the place. This one [Indiscernible]. You can express qualitatively as an effect -- 1 calorie percent on lipoprotein levels in 24 trials. LDL goes up, HDL goes up and the LDL/HDL ratio goes up and the confidence interval is fairly narrow. They are not going to change this picture because after the first 20 studies, very little is going to change. These straight lines all run through zero and this is something that has been hotly debated because we forced them to go through zero because we say the net effect of nothing is nothing. If you give people nothing you are going to have no effect. Can zero intake of trans fat raise LDL? If you have a before and after study, yes, you put people on trans fats and do -- you take the difference between this diet and this diet and eliminate that, how could you still didn't get an effect -- the only way to explain that would be that the researchers add something to the trans fat diet, for instance two fried eggs with bacon. If you do that with every diet you're going to end up at zero trans -- so you will have intercept in this curve. The lines should run through here or something and that would be the effect of the fried eggs and bacon. This is not responsible research. You have two types of fat, partially hydrogenated fat and the saturated fat and you balance the fatty acids so the only difference is in the trans or only at or whatever your comparator is. That is why we feel justified in saying the eight -- nothing must be nothing.

Other blood factors affected by trans fatty acids, do trans fatty acids have other adverse effects other than LDL and HDL? Yes they do. This is what we call the play atrophic -- speefifteen effect. The effect on endothelial function is a little bit inconsistent but they seem to reduce endothelial function. The effects on systemic inflammation and diabetes risk, etc., they are far far that is consistent on the effect on lipoproteins so I don't want to spend too much time on this. The rising triglycerides and lipoprotein adds to the risk of cardiovascular disease.

Conclusions. The first three conclusions are relevant for the topic at this meeting that I've taken the liberty of adding somewhere. About PHOs, trans fatty acids increase -- to change in fatty acids raises CHD risk. Affect is dose-dependent. We can never exclude that the line Rickles a bit but there's no indication for that. There are a number of conclusions I'd like to snuggle in because I think the relevant. Animal and industrial trans fatty acids have the same effect. Our conclusion is CLA supplements are unhealthy and that was shared by the food standards authority of Australia and New Zealand which looked into this very carefully and decided they didn't want these supplements on their continent. Even more general observation, a history of safe use does not guarantee safety. This stuff had been used

since the very early 20th century and nobody noticed it did something. Something that increases a very common disease after many years [Audio cutting in/out]. I had the same experience with the coffee factor. They never noticed anything until they started to do epidemiological research and trials then it turned out there was something in this coffee that raised cholesterol and caused a large number of heart attacks. The validity of talks a logical testing needs scrutiny.

That is my contribution to this meeting. Let's scrutinize. V-neck I'm grateful to my students and colleagues all over the world who made this possible because I only had a small part of it. I am grateful for your attention and you are not allowed questions. [Laughter]. You have to write them or you can always send me an e-mail. Thank you very much.

[No Audio]

9:40 AM–10:20 AM Epidemiological Studies on Health Effects of PHOs: Strengths and Limitations of the Available Human Data - Ingeborg Brouwer, VU University Amsterdam, The Netherlands

-- I consider observational studies of epidemiology and they sometimes say what Professor Katan was just presenting is intervention epidemiology. I'm talking about observational studies right now. I talked about the observational effects of partially hydrogenated oils. My competing interests, you deliver donated the margarine stop for our CLA study and lipid nutrition is still the same CLA they donated for that one study. I also do not accept speaking fees. I looked up the word equity and I don't think I have that. Consultancy, I did some for the European commission but no commercial consultancy and I advised the WHO committee that I do not receive any remuneration for that. I outline my presentation so I will explain about nutrition science and nutrition epidemiology. They will go into the results of the association of trans fat and death and the association of trans fat and coronary heart disease. Then I would say something briefly about partially hydrogenated oils and replacement of these oils by of the facts on the coronary heart disease risk. The possible association of trans fat with other diseases and what can be done to change the intake of trans fats? And then I'll end with some conclusions.

Professor Katan mentioned it is a challenge and it is unique. Dietary variables are very complex. They are of continuous nature. We all eat fat. We may eat different amounts of fat but we all leave that so we are all exposed. It is not like a drug or a toxin. They we have the problem of intercourse they should because we do not eat nutrients, we eat foods. Many nutrients are in the same kinds of foods so you get intra- correlation and intra- correlation between foods and also other kinds of contacting or intra- correlation is they have a habit of combining certain foods. You probably know the expression a fish has to swim which means a lot of people if they eat fish and drink a glass of wine with that and it's very difficult to distinguish between the difference of the effect of the glass of wine in the effect of the fish.

Another point is gradual changes over time. Eating better has evolved over the years. Often we just have one measurement of the diet. In this case it is also the content of the foods has changed over the years and that of course also affects the endpoints. These things you have to keep in mind when we continue talking about this. Another point is and that is of the crucial limitation of research is individuals have limited awareness of what they are eating. We are dependent on the memory or people who follow other people the whole day to see what they are eating but that also usually changes the habits of the person who is followed. There are different ways you can study the diet that if you do not provided and you would have to rely on what people say they are eating and it's not so easy to remember it and to write it down the whole day, you tend to either change your diet because if you have to write down

your complete diet for five days, by the fifth day, -- that makes it much easier to write it down but that is not what we want. It is extremely complicated to do research.

What is the difference between the observational and the intervention research on macronutrients? First of all, the observational. The advantage is real life, natural environment. People can do what they do. We can look at long-term differences. We can study large study groups much larger than when we try to control the diet and we can look at heart disease and points. You can actually see if people get ill or not. What is the downside? It is extremely complex and we have no control so there is a lot of confounding. People not only do other things in the diet but also people might take the stairs and other people might take the elevator and if that determines your health risk, the diet can be the same and we cannot pick it up -- the stairs or the elevator. The intervention, we can look at the actual effect of the treatment versus a placebo which is complicated in the case of diet, and we have more control because we can tell the people to come to our department and have the food and the more you control the better you know what goes in. The downside is they are usually shorter term and no real life and we are dependent upon compliance. Usually you have smaller study groups. We can also have smaller study groups because we have more control. Most of the studies, at least in the field of trans fat our own the intermediate endpoints. There are only very few studies in nutrition that could be done with actual disease endpoint and you would have to do a strong intervention long-term. The few studies that have been done are really good of course because they give you important information.

We come to the fact again were talking about macronutrients and this is something I would like to stress again which I already pointed out but I think it's very important people realize we're talking about macronutrients which is different than a normal toxicology or drug approach because macronutrients need energy. You can only say something about an effect of a macronutrient if you say what it is replaced by. If we look at fatty acids in diet and we change the fat in the diet been there are a few -- you can replace it by carbohydrates or proteins. You can replace it by other fatty acids. You can not replace it and then you get weight loss. I think that would be a great solution for many people. It's also great for your cholesterol levels. Usually we have no control over this and it's a combination of the above options if we look at real life in a laboratory situation. We can choose what to replace it by injured can recalculated, etc. This is important to keep in mind. What do we want to know? What does the intake of trans fat does on either death or cardiovascular disease?

The situation is as follows. You may recognize the picture. These are potato eaters. The nice thing about these potato eaters is there was no trans fat. If you compare it with the lower picture, you see people who get french fries. Let's assume they use the wrong fat to make the french fries, the difference between these groups of people is the trans fat. The one on the top, they were eating the boiled potatoes and the people on the bottom were eating fries with trans fat. We look at the people who eat the boiled potatoes and see how much disease they get and the people who eat the trans fat and we see how much they die. You have probably noticed more differences between the picture and the people eating the french fries. That is why it is so complicated to study this because it's not just a matter of looking at trans fat yes or no. If we look at trans fat yes or no, the crucial elements are we have to have a good estimation of intake. We also need to have a large enough intake range because if everybody is eating the same in the time of van Gogh when they were all eating the boiled potatoes, it is very difficult -- the intake range is too small to see any difference between this people who eat the potatoes and don't because they are all eating it. You need enough intake range to say something about the actual effect. The inter-correlation again, it is in the food. It changes over time. Very important for the trans fat because the Transpac amount includes have changed a lot over times so if you look now at studies which collect the data and by the end of let's say 2006 or seven, it can be a completely different

picture, a much smaller range of intake than if you look at studies that have been done in the 90s for example. Confounding is important in this because we do not know what other things these people are doing and we try to control this as much as possible but there will always be residual confounding. And another point that has nothing to do with the intake, but you have to have a good measurement of the outcome. The indication that it's usually quite straightforward and most times these figures are quite precise because it can be determined if somebody is still alive or dead and we have quite good registrations nowadays. With different kinds of disease it is sometimes more complicated and if you look at the causes of that it might be more complicated but it is important to have a good registration of that and I think for most of the studies I am referring to now that is not a major problem.

First of all, the association between trans fatty acid intake and death. There is one study that looked into that and that was the Regards study that had more than 18,000 participants. Their dietary intake data was from January 2003-October 2007. Keep in mind the timeframe because the intake will be much lower than it was in the 90s for example. Seven years of follow-up and in that follow-up time, 1572 participants died. As an intake measure they used the so-called block 98 food frequency questionnaire. It has been validated against the 24 hour recall and they had a correlation of over 53 which is not too bad. There only 98 items in the questionnaire that you may have missed in determining the intake. What you see in the figure is the quintiles of intake and the fifth quintile has the highest intake of trans fats per quintile and the trans fat intake in energy percentage is given here and so you see the difference between the fifth quintile and the first quintile.

This is the association with mortality. What you see is the quintiles of intake and you see the people with the highest quintile of intake more often die. The mortality rate per thousand in the fifth quintile was higher and there was [Indiscernible] over the quintiles.

Here I have shown the same but in a table. You see the mortality, 12.8 per thousand died -- the way we express this is what you see here in the first model. The relative risk or risk ratio for the people in the fifth quintile was 1.83 which means they had 83% more chance of dying than the people in the first quintile which has a relative risk of one.

The top model is the role model and what they did didn't other models was try to control for as many things as possible. The third model is the one which has taken most of the control and what you see here is the risk ratio has gone down. Apparently the people who have the high intake of trans fat also do a lot of other things wrong. The risk ratio is still higher in the fourth and fifth quintile than in the first quintile but it's not as striking anymore as it was when we looked at the role figures. That is to give you an idea about what confounding can do and how we control for these sorts of things.

That was looking at intake of trans fat and dying from the intake of trans fat. If we look at the Association of trans fat and coronary heart disease then we have more data than just on dying. That is the next thing we will do.

There are roughly two ways to look at observational data if we look at intake of trans fat and coronary heart disease. The first is the prospective cohort data. You have people and you measure the intake of trans fat and then you look at how often they get the disease.

Another way to look at it is the so-called retrospective case-control data. You have people, and some people have the disease and other people don't have the disease in the UC with the intake of trans fat is. There is a downside on this second part. Maybe the people have changed their habits when they got the

disease. You never know what came first and that makes it a bit more complicated and that makes it we have more confidence in prospective cohort data than retrospective case-control data. What you see is the publication that tried to summarize several studies that looked at intake of trans fats in the Association with coronary heart disease risk. He looked at an intake of what happens if you replace -- with carbohydrates with trans fats. He did that in two ways with perspective call for data and with retrospective case-control data. These have large confidence intervals so we are less sure about what is disease events -- for the publication because it is the same publications. You they use a slightly different way of calculating but they come up with roughly the same figure.

Another way of looking at it is you do not look at an average of energy percentage, but you can also look at the intake. See take the most extremes of the populations and the effect of TFA intake. What you can see here is there a different amounts but differences between the first and last quintile in this example was 4.3 grams per day. In the -- study it was 4 grams per day [Indiscernible]. What you will see again is you will see a similar picture and a similar figure. 1.22 is the risk the people in the highest group compared to the lowest group. That is for total CHD.

They had more data on fatal CHD. You see again a very similar figure comes out so it's 1.24 with a 95% confidence level.

Benson was the only one who try to look at Pacific the industrial trans fatty acids because all this does is -- on the other studies -- they all took [Unclear/Accent] it is very difficult because there is not much data out in the data that is out I have shown here in DC is very limited but again it is very much in the same direction as what I've shown before because I am convinced there is not really a difference.

Then we come to the risk of coronary heart disease. What I try to say -- what -- did is say you haven't oil with for example 20% trans fatty acids and in oil with 45% trans fatty acids and you replace it by other oils, does that help? If you look at their calculations it is worthwhile because this is based on the observational studies and then you see if you take out the fats with the high amount of trans fats than the risk goes down quite considerably. They did that for the observational studies. They also did that for the clinical trials and the affect on the life of proteins and -- and CRP into account. Then you also see it is worthwhile to replace these trans fatty acids. What you may also see is you take those pictures together that this is observational data and this is the trials on the lipoprotein that is not exactly similar. Of course there are some uncertainties in it and it seems if the affect or the Association you get out of the observational trials is larger than what you get out of the trials on lipoproteins. There might be more going on but it might also have to do with more uncertainty about these figures, at least I am convinced there is more uncertainty about these figures that these figures. The trials on lipoprotein have been so well-controlled and we have a number of very well designed and well-controlled trials which give a very convincing answer on the fact that trans fatty acids influenced LDL in the way we do not appreciate.

What about trans fat intake and other diseases? There is not that much out. There is some random studies -- the other thing is there is a little bit more on and maybe needs more attention if you decide trans fats -- that is the Type 2 diabetes. The health study has looked at that. Again you see the quintiles of intake of trans fat and you see the relative risk for diabetes risk. Although it is a bit higher in this group than in this group, the difference does not seem so striking but that is also again because the difference in intake is not that large. You do not expect very extreme differences that the P for trend is significant. They also recalculated what would happen if you replace the trans by PUFA or by carbs because PUFA versus carbon is going up -- if you replace the trans by PUFA then you get a lower risk.

You think we really have to be concerned about the intake of trans and diabetes but there is also other data out. For example, the -- cohort -- you see nothing. The verdict is not out on this yet in my opinion. For the coronary heart disease it is quite clear.

[Captioners transitioning]

There is a higher risk with those -- with those with higher increase. Then question is what can be done to change the intake of the Transpac or the [Indiscernible]

You can see that different countries and cities and parts of countries etc. have different ways of dealing with trans-fats and some new regulations and other different things -- This little country, that is where I come from work you may notice that it doesn't have any of these symbols. I think it is nice to explain that in my country there was no regulation or anything going on. What happened was we had what we called the polar model which means that you put people in one room and you let them talk for so long so that they agree on something. That is what happens. The scientists and people from mainly the margarine companies that there is something wrong with transfected so we have to do something about that. That is what happened because if you look at this picture then you see the composition of margarine in the Netherlands -- in 1980 nobody knew anything. In 1989, still unaware of anything going on. Then the studies came out and in 1995 there was already less trans fats in the margarine but it was still a challenge for the industry because it is still not easy to take it out. You bake your cookies with trans fat and then you suddenly take it out your cookies are totally different. It looks like something went wrong in the oven.

It is technologically quite a challenge to take it out of the margarines. But in 1996 they managed to do that and since then the trans fat in margarines is extremely low.

Other countries -- here for example do see fingers -- figures for 2005 and 2009. This is an example of a few products that [Technical difficulties] has tested a large serving of nuggets and french fries and biscuits and microwave popcorn. [Indiscernible - low volume] but that is my opinion. [Laughter

The microwave popcorn was always high in trans fat. In 2009 you see in these countries it was already -- it had already come down. For example in France, Germany and the UK in 2006 -- 2005 it was still quite high and then in 2009 it was almost gone. So it is possible.

In Denmark I think they were the first to really take action, at least the government took action and put a ban on trans fat. That was quite effective because if you look at the figure of the same three products, again, it is down here in Denmark in 2001 there was still a lot of trans fat and in 2005 already it was completely banished.

It is possible. Does it help to lower the trans fat intake and food? Yes it does because if you look at ritual into -- habitual intake is to be sent by the Dutch population in 1988 it had already come down quite a bit but what you see now, these are the latest figures and the P50 which is a photo trans fat and the percentage is only point .6% and it is not coming from partially hydrogenated oil but it is coming from [Indiscernible] it can go down considerably in a whole population.

This is a figure that is not specific for the Netherlands. You see this all over the world got you see the same thing happening. --, You see the same thing happening. My conclusion is that the higher intake of trans fat is associated with fatal and nonfatal cardiac heart disease and content of the of -- trans fats in

foods can be replaced by other fatty acids although it is a challenge for industry. This will lead to a lower intake of trans fat by the population. I think the industry has already done the challenge and knows how to get rid of trans fats. It is just a matter of if they are really willing to do it any further. Thank you.

[Applause]

And of course I would also like to thank all of the people that did research on this subject to made it possible for me to show all the figures. Thank you.

Dr. Ronis

So we are a little ahead of schedule but because we also simultaneously are testing on the web, we would like to keep to the schedule. At this point we are scheduled to have a break until 1045 -- 10:45 and so that is what we will go ahead and do. In the meantime if there is anybody in the audience who has questions for the panelist for the panel discussion or if there is anybody who is watching on the web has questions, please write them down and give them to the staff here in the hall or email them and we will take those into account during the panel discussion at the end of the discussion. Thank you so much.

10:20 AM–10:45 AM Break

Dr. Ronis

I guess we are going to get started again so if everyone could get settled down -- a reminder if questions come to you during the course of the next couple of talks, please provide them on a piece of paper to the staff. We will try and get to them during the moderator discussion at the end of this. For the second half of this colloquium we are going to -- the shift. With talked about the epidemiological and clinical data out there. Now we are going to look at the perspective of those response and risk assessment as it relates to dietary components.

To set the stage for that is our next speaker [Indiscernible] who is a physicist. -- Dr. Weihsueh Chiu who was a physicist. Dr. Chiu got his bachelor's from Harvard and his PhD in physics [Technical Difficulties] and then moved into the area of public policy in dose response. His interest is in mathematical modeling and for disconnects -- for apology -- it'd work the accounting office at conducting [Indiscernible] such as Agent Orange before joining EPA where he is currently the chief of toxicology pathways branch of the iris division at the National Center for Environmental Assessment at the EPA here in Washington DC.

He is going to give us a general introduction to does response assessment [Indiscernible] to the analysis of noncancer health effects. Advice from the National Academy and the 2014 WHO IPCS guidance. Dr. Chiu.

[Applause]

10:45 AM–11:25 AM Dose-Response Assessment Approaches to the Analysis of Noncancer Health Effects: Current Practices, Advice from the National Academies, and 2014 WHO/IPCS Guidance - Weihsueh Chiu, EPA ORD, Washington, DC

Thank you very much. Can everyone hear me all right? Okay. I am really pleased and honored to be here to talk at this colloquium. And I found the morning session really fascinating in terms of the richness of

the data and the history of how this was -- how the health effects of partially hydrogenated oils and trans fats came to be and what the current state of science is.

My job is how to translate this into a dose response analysis that could be used for some sort of a risk assessment context. I will provide some general principles as well as describing some ideas as to how they might be -- different approaches might be applied in the case of partially hydrogenated oils and trans fat. I don't have -- working for the US government I don't have a government statement but I do have a disclosure statement and that is that these views represent mine and they may not represent the official policies of [Indiscernible]

First double give a couple of examples of current approaches were noncancer [Technical Difficulties] deriving reference values such as a reference dose, as well as examples for residual risk or economic and if it's analyses. Then I will talk briefly about some recent and also perhaps not so recent advice from a national academies on how to conduct those assessments and then I will close with the just published in September, guidance document on the dose response assessment from the WHO/IPCS guidance. I will make some references to partially hydrogenated oils although I must be going that I am not an expert at any of those compounds.

Dose response assessment really needs to be placed in a larger context. This morning we heard about a lot of the research in terms of -- in terms of epidemiologic information but ultimately the point of risk assessment is to translate that information to something that could be used to make risk management decisions. My job is to focus on -- since I think we have a good idea in terms of the hazards -- that was really made clear this morning. To talk about those response assessment [Technical Difficulties] science and risk management how that part of the risk assessment can be done. What is the most important question? What is the risk assessment context for doing any risk assessment? Those? So many potential risk management context might be trying to establish an exposure level that is likely to be without appreciable -- appreciable risk. Another context might be given the current exposure route -- levels got what is the residual risk hold is the burden of disease -- was a cost -- we can do a cost benefit analysis as to how the different regulatory operations by change exposure and how that might change the incidence and effects in what benefits would result from that waiting that against the cost of the intervention.

First we're going to talk about the first one that is establishing a safe dose concept. Here I will talk about be IRI as Grimmett EPA -- IRIS at EPA where we talk about the [Indiscernible] and direct toxicity values looking at the available studies, -- deriving at [Indiscernible] [Technical Difficulties] the methodology is focused on deriving a level that is likely to be without appreciable risk -- a level that you can walk away and not worry about.

Just briefly for those who are unfamiliar with these approaches what do we mean by points of departure, symmetry adjustments and uncertainty factors? Point of departure -- at the lower end of the response relationship from which you start to apply extrapolations. We often cannot measure or do clinical trials or experiment studies at environment to levels that would be amicable to the EPA context or maybe food attitudes, the low levels that you can't necessarily measure in a study. We need to start to demarcate where the data stop and where we are starting to do extrapolation [Indiscernible - Intermittent Audio] starting with either a low -- LOAL or NOAEL or be MDL or be NCL -- BMDL or BMCL -- [Technical Difficulties] those is for basal metabolism differences across species. And then trying to use so-called uncertainty factor sort safety factors to try to derive a level that is of little concern. These include human variability and multi-human extrapolation factors which can be replaced with data

derived or chemical specific factors if you have a data on a particular compound and point and in a number of factors that more reflect the limitations in a particular data set -- if you're concerned about disclosure those exposure but you only have [Technical Difficulties] [Indiscernible - Intermittent Audio] what are if we were to apply this to the partially hydrogenated oils or trans fats? Here is a basic outline of our conceptual model where we have partially hydrogenated oils in food. We have ingestion of trans fatty acids. You have these changes in lipids. And then you have a change in the risk of cardiac events.

Some of the key questions are what is the endpoint that we are going to derive a point of departure for. What is the key event any mode of action context in conducting those response assessments? Meaning that this is a necessary precursor [Technical Difficulties] [Indiscernible - Intermittent Audio] toxicity, HDL, LDL, is there a ratio? Or is there some other precursor along this area that we should be looking at?

Second, are we sure that these lipids explain all of the toxicity? We heard this morning about other potential mechanisms and biomarkers for which there seems to be some strong evidence that there are mechanisms outside of just HDL and LDL that increase risk of cardiac toxicity -- code vascular disease. -- Cardiovascular disease.

And then you would take this data and you would derive a dose response relationship. And what level of the fact would we consider without appreciable risk? Obviously a 10% change in cholesterol is probably pretty bad. 5%, 1%, 0.1%? Is that negligible? There is untouchable -- judgment that needs to be made to apply the concept -- RfD concept to these continuous results.

This isn't the only possible response to dosage response is currently practiced. For things like residual risk and economic benefits analysis there is different approaches that maybe is epitomized by how EPA regulate air pollution. There we do sort of a dose response and economic benefits assessment together and we identify what studies are suitable for deriving a concentrating response versus mortality -- ozone versus asthma attacks or hospital visit. We derived health impact function saying that -- what change in the incidence of effects are we going to have if we change the regulation on air quality?

And for many of these endpoints that we can assign some economic value to whether it is viable and statistical life work cause of illness -- cost of illness such as how much is a cost to go to the hospital emergency room, we can monetize a change of incidents and derive economic benefits from reducing the level of exposure and then compare that with what are the costs of debt reduction.

This is really focused on comparing amongst different options as the impact of changing exposure at the population level -- how is this done? You take data on air pollution and exposure and health effects. You derive a concentration response relationship. Your this is the residual risks, where the zero point is fixed. It is relative to this point here. As you go up in ozone levels, you have more risk. And then these uncertainty bounds given that they have [Technical Difficulties]

The current ozone standard here -- is shown here. This is where most of the current expulsions are, where these black tick marks are. The policy context is then got maybe we want to revise the standard into this range here. We want to go down from 75 to 60 perhaps. Then we wouldn't have to consider it how much of it in terms of risk reduction we are going to get right reversing those reducing to different standards.

Because this is in the range of observation we can essentially interpolate this acknowledging that there are certain uncertainties in this confidence. This is essentially an interpellation exercise. It might be that

there are similarities with the case of PHO's. You have data like this in terms of change in LDL to HDL ratio as a percent of [Indiscernible] effect. Current exposures of perhaps several years ago are in this range. You are in the range of the data.

Than if we were try to make some policy change to reduce exposures from here to here or to shift this whole distribution of exposures to the left what would be the health impact of overall in the population for making such a change?

Basically how would various risk management interventions change exposure and then how would that change the overall health operation? This has been used. I am not an expert in this area so I just picked some things out of [Indiscernible] you can use this meta-analysis of clinical trials derived from -- the dose relent those response relationship in this same weight that those responses -- dose response relationships with ozone. Then you can develop policy relationships. In this particular paper, if they reduced trans fats by .5% or 1% this is a fewer number of cardiovascular desks -- deaths that they would anticipate based on this modeling approach.

Essentially there is no real need for extrapolation if we are in the range of the observed data. We are interpolating. We are not extrapolating. This is a similar diagram as before. Some of the issues that are what are the different [Indiscernible] actions including no further action, how do they affect the different trans fatty acids? Are they resulting those are the resulting exposures within the range of the observation of the data so that we can of confidence in our estimates of the impact. Again if we're going to use a particular biomarker doesn't biomarker us -- expert in all of the effects and also are there other health effects that would be included to do a mobile cost-benefit type of analysis. We might want to go beyond just carded -- cardiovascular effects. Just to compare [Indiscernible] they are for different purposes -- looking at exposure level at which affects might likely occur. In ecology it is based on toxicology data -- uncertainty factors are used to extrapolate across. What will the effect be if you had this exposure? In this is the walk away level. [Indiscernible] the purpose of the approach is to estimate -- be incremental change as you make different policy options.

There is actually those what I am going to talk about next is the way -- more recent advice and approaches that can actually unify this so that we can maybe have a single framework approach that can address both types of policy options. One of the issues that has been raised with reference values is that the uncertainty factors are not just uncertainty. They are a mixture of various adjustment factors and they are accounting for human a very -- variability and also because you are multiplying them together if each of these is conservative you may have compounding conservatism issues as you add more factors.

Many decision contexts like residual risk or economic benefit benefit from having more than just the conservative bound on the dose. Just by way of example if you had confidence found in the essential is meant depending on how you would [Indiscernible] different orders as to how you would prioritize different situations

[Captioners transitioning]

This brings us back to the national--response back in 2009 recommending incorporating background [Indiscernible] as well as uncertainty and a recent report [Indiscernible - audio cutting in and out] Bayesian methods depending on a focus on the decisions because that has a lot of topics that were discussed. Basically, beyond [Indiscernible - audio cutting in and out] looking at the mode of action and

mechanisms and and [Indiscernible] all of these together said and to bridge the gap and he walk away and safety approaches so for the [Indiscernible - audio cutting in and out] thinking about the vascular risk and some [Indiscernible - audio cutting in and out] in terms of the visceral level and mode of action of how this leads to that and then also in the context of the boundary in the study and the effects of [Indiscernible] and how it impacts the overall population. So these are modifiable factors you cannot change what you can't see [Indiscernible - audio cutting in and out] the lower LDL and LDL and the response relationships [Indiscernible - audio cutting in and out] toxicity including a lot of what [Indiscernible] has organized in focusing on content and points of days like--and points. There is a prominent--four [Indiscernible - audio cutting in and out] cancer and some things I feel have overshadowed a lot of these cool [Indiscernible - audio cutting in and out] in general. And uncertainty as this tran1*should think about [Indiscernible - audio cutting in and out] and you go from having a single approach to the able to characterize a single framework in response mission should. [Indiscernible - audio cutting in and out] so it replaces with a--does we do not select this--and also looking at uncertainty and that is certain fraction of the population of those in effect [Indiscernible - audio cutting in and out] the target of the dose response assessment--having that effect [Indiscernible - audio cutting in and out] interest species characterizing the working group as well as the magnitude of the effect--variation of [Indiscernible] specifying what population you are protecting. Is this a 95% of? [Indiscernible - audio cutting in and out] whole interest species--variability and that the variability [Indiscernible - audio cutting in and out] so really moving from [Indiscernible] providing quantitative through probabilistic test. Here's the exposure subgroups deleterious effect and these things can coexist& They [Indiscernible - audio cutting in and out] what you mean by deleterious success? And [Indiscernible - audio cutting in and out] moving to the DMI, the specific coverage the effect of a specific magnitude, and also account for a distribution so [Indiscernible - audio cutting in and out] [Indiscernible - audio cutting in and out] pick different levels of--not only needing to be characterized but also in terms of the magnitude and the incidents affect an array of decision [Indiscernible - audio cutting in and out] analysis. And [Indiscernible] be able to characterize that because ultimately these levels is difficult to verify it is .1% or zero cell-cell who focus on toxicology, [Indiscernible - audio cutting in and out] the same as using human data. The Mexico taking this into the fatty acids, to characterize individual does spots relationship for individuals--versus these cholesterol levels.

We are made to worry below this level so some of these--defined to uncertainty in this relationship this human variability derived in [Indiscernible - audio cutting in and out] strategic reference dust to the appropriate affect [Indiscernible - audio cutting in and out] current practices are not [Indiscernible - audio cutting in and out] kind of a safe level and supporting benefits risks to characterize certain variability in the fact there is a baseline variability in exposure across innovation and encouraging us to move across a dose response approach but more broadly [Indiscernible] this unified approach that can be used in a decision context some of the issues of fine reference dose methodology and what is the key and what level is considered to be--risk for benefits possible options and still within the data when we do the analysis and incorporate [Indiscernible - audio cutting in and out] other factors and how we can address the response assessment and what uncertainty do we have in the shape of the individual's response and how can we quantify this variability and not just sort of [Indiscernible - audio cutting in and out] what levels of magnitude and incidents of effect are appropriate for the findings of such a level. [Indiscernible - audio cutting in and out] as well as the other colleagues to work with on these issues raised thank you very much.

[Applause]

Dr. Ronis

Thank you for setting the [Indiscernible] in terms of the regulatory aspects. And in a more specific fashion we're going to have Michael Dourson come to us to talk about the mass dose evaluation on the effect of PHOs on the LDL cholesterol levels. Mike is the director of TERA a risk assessment non-for profit associated with SOT and he has worked very closely with big address with this specific topic.

[Applause]

11:25 AM–12:05 PM Mode of Action and Dose-Response Evaluation of the Effect of Partially Hydrogenated Oils on LDL-Cholesterol - Michael Dourson, TERA, Cincinnati, OH

Good morning to you all. [Indiscernible - audio cutting in and out] and by TERA and I think SOT is going to travel here, which is wonderful. I don't know of any [Indiscernible] but I do have a bias. I have a toxicologist and when he first got this assignment, we looked at the data and [Indiscernible - audio cutting in and out] a famous toxicologist from the FDA said it is easy if you could learn into steps. In 10 years. [Laughter]. Mike Folger has won the Lehman award the test called to report and he said a risk assessment is not a science and you can imagine I was not in adherence there is a logic problem or you fold in different sciences sorting doesn't epidemiology [Indiscernible] toxicologist, people that are chemists, it is small but we have it working with a larger team and I will share the slide at the end and of course that larger team fits within a larger group. So it really is not only us but [Indiscernible]. So you know the background the FDA published this thing [Indiscernible - audio cutting in and out] I'm not going to belabor this point. Nice work [Indiscernible - audio cutting in and out] and you see different things with this graph. Trans fatty acids and a huge intake and--to change the LDL. And one of the things that we recognize going in, there is an exceeding complex [Indiscernible - audio cutting in and out] you have seen this earlier. You have done some stuff with glaciers and environmental contaminants. This is more complex. The thing you can do here and I just picked this one up off of the [Indiscernible] software. Here is this regression here. Oh yes. Oh. The mouse? [Laughter]. I am sorry. I use a Macintosh. We don't use extra things. SOT of it is a common argument. This particular aggression has no biological basis and I think the point with these regressions that appear to have biological basis, is difficult to know if they're accurate or correct within a mode of action so the outline of our top that will be mode about should the evaluation [Indiscernible - audio cutting in and out] and laying all this out, the EPA guidelines folks are not unfamiliar with the mode of action. They're going to focus on LDL change and we're going to try to do dose response analysis. Of course some questions, and we're not talking about [Indiscernible]. Maybe we are. What is that pesky shape of that low-dose region. Does it matter? That is the typical bias that bring a risk assessment perspective to a nutrition question is not unique. I participated before in the University of [Indiscernible - audio cutting in and out] in 1999 on a contrition and toxicology risk assessment, those that name of the conference in which read to get the group together and it has not been done often in Evanoff has been done since we could do more of this. The other challenge, what is the TFA replacing? We have some talks this morning, especially professor Katan with the careful work that he knew exactly what was being replaced. Not all TFA is our equivalent. This is something we learned that you nutritionists have known for a long time. Mode of action evaluation. The good news is you don't have to know all of this this would be a mechanism of toxicity. It would be great to have this and for some chemicals we have a. Nitrate-actually know that in detail. Probably some other aspects of nitrate toxicity we don't understand that you don't have to know all of this that you should vote us on key events so that is what risk assessment people do now. We do multidiscipline teams and we focus on key events. Obligatory steps necessary for the end result in this case LDL change of coronary heart disease. So it is a critical aspect? What are the events by which they race LDL? What is

the evaluation of causality and modify the Hill criteria? Associations are nice, hypothesis generating, but they are not causal. The population of--is down on and the population of Stewart's. Good Association. Violation of human development. We have folders and and all experimental data to the mode of action but each time we did that, Lou ask ourselves, how is this relevant? Shape of the dose response [Indiscernible] another critical aspect. CLEC which urges the national Institute in safety environment environmental protection agency. I can say this as if it is English and acronyms. That is scary. But the point is this framework that this international body has worked on. You identify key events to the critical endpoint you look for experimental--not only in dose response but temporal. Those are the key ones and they're not the only ones but they are key obviously if you get a change in LDL and you take TFA that is not a temporal relationship. That is an obvious problem with the hypothesis that the cost changes but biological plausibility and other modes of action are important. We need to go through this framework. Submit here is a nice publication from 2009. Allen is a famous toxicologist from the United Kingdom. See the events are laid out. We don't have to go through these that we have a key event in their sequentially related and finally a toxicological event of concern. We focus on changing LDL that we talked about Prof. Brouwer it is a coronary heart disease that is equally valid. You can do both. Here is an example this comes right out of the US EPA they studied one of the key inventions, the formation of metabolites another key event was cell damage in real duration. They were able to demonstrate a threshold. I am not sure of this example but this particular key events. He has a threshold to one of the three events if you have a key event of Lee will have a threshold of some key event. Period

I am sorry my cell phone is going off to tell me time and I have to use my cell phone for time. Anyway this is the LC framework. And laying out the [Indiscernible] they see in a table in that particular way to see how they related to one another. Another thing that says it fits but until you lay this out in a framework and demonstrated fits, that is racetrack to get scientific credibility and risk assessors not the least of which other nutrition and health know the data perhaps better than risk assessors. Selling these out and framework is really important. So let's get the MLA for mode of action. [Indiscernible] is on the phones of by get in the hard questions I will go to John. And this mode of action can work if there is an increased LDL production by the liver. Liver releases a low LDL or a decreased clearance so after they are released a sequence of what else's forms and you get more or less a clearance by these other end points. And so these proposed key events are contributing things some of those you know quite well you can increase the LDL's increase liquidation of [Indiscernible] agency of the things here. You can get a decreased receptor activity so others may pick up from the blood and by doing that. Of it. That is one way to get rid of it but something in the diet like a decreasing receptor activity there are some thoughts behind they can get an increase that way. Here is a nice picture you have probably seen something similar. You have a liver here that is why to produce the LDL's. So you have a pool of these and you release some of the lipids from these tissues and you get the IDL and LDL and these get taken out by the liver and excreted or they can change the process with the HCL so looking at these boxes. Increased production a decrease excretion. Okay. So we look at the mode of action through the framework and focus on Bradford Hill and it is about the space in the project he hypothesis and you tested. And you do sequence of observable effects. Cause-and-effect relationship focusing on the key event and there is quantitative and qualitative aspects of this concordance. And we found that TFA has data consistent with the hypothesis that the mode of action for increase in LDL some of CRM, the data is consistent with that hypothesis is the increase of secretion rates of [Indiscernible - audio cutting in and out]. Activity of this enzyme is significantly higher in the subjects of this trans fatty acids. LDL is significantly low levels devoid of the specific activity. So we focus on some of the experimental animal work that is not directly relevant but from a mode of action understanding that it is and the TFA has increased this low propane--protein and some *in vitro* studies& increased production we talked about data consistent with decreased receptor activity through the receptor takes the LDL out of circulation. And you get reduced

expression of receptor. It get a released activity due to altered membrane would be. He did a TFA in that particular membrane it is not conformant to, it may not work as well and reduce receptor bindings of the IDL and LDL again, taking them out of the CRM and putting them into--like any good hypothesis you have to look at all data they are consistent with this hypothesis. For instance, the high TFA did not always raise plasma and--this particular enzyme activity is not higher science high in trans fats. This again is a decreased production. We have the other key event evidence inconsistent with this key event. Production contributes more to increase LDL levels meant to LDL clearance and you can see some specific things. Regarding a reduced membrane liturgy, there is no conflicting evidence that we found that the supporting evidence is *in vitro* that has limitations that we are showing you the data that our team covered that we think it supports or it does not. The TFA exposure does not always decrease of this binding. That is an important piece of data as well. It is difficult to establish so there are few relevant dose response data available for TFS. We did not find consistencies but you like to find the smoking gun. Many of the *in vivo* studies did not investigate the dose response to see these studies and will give you service the survey data in a minute. A lot of studies are just [Indiscernible]. There is a large variability and we will show you that. For or all of the who do not have a lot of data lots of times single endpoints investigated that we did not see violations. We did not see some where we have to change LDL's and afterwards a [Indiscernible] diet. Experimental design and indeed it would be a violation. That's good. So now we're going to talk about dose response shapes and I think-let me see where I am on my time. Well I think I am doing okay. If I am not, just yell at me. So a shape of the dose response curve does little you talked about any mathematical person they will say all models are wrong. Some are useful but they're all wrong. Threshold reverse affect. The safe exposure level. Risk for effect increases with a single molecule. Then people say there is a thing called virtual safe just the FDA client and it helps us step out of that it is presumed by work chemicals that interact with DNA values this assumption quite a bit actually. The idea of a threshold number of molecules must equal or exceed a threshold where you get the response. The point at which something starts. As it is pursuant to have applied for chemicals that interact with cellular processes where the organ or tissue [Indiscernible - audio cutting in and out]. Evidence supported for the threshold, evidence presented is LDL levels in clinical studies can be fit by linear regression. That is circular if you do not test all formulations. The individual thresholds individual variability means there is no population threshold that is evidence presented and lattice logically incorrect. It could be exposed over the threshold. That is her. Things like physiological limits. You have to consider both of those. If the dose response continues between the youth and states and individuals in the range of normal. We found that in the data. The events that we studied for mode of action are highly regulated. Plasma LDL levels described by non-limit-nonlinear pharmacokinetics. Every aspect with transport utilization appears to be subject to regulation and homeostasis. So the adverse effects would occur or perhaps occur Wicomico stasis is perturbed, one a threshold is reached or exceeded. That is our conclusion to mode of action and understanding and risk assessment is iterative and feedback is always welcome in these things. A goal of regression expanding the database used for the regression that has been published already. Improve the accuracy of the investigation playing greater--insurgencies listen, they value it the shape of the dose response curve if you can then use this data to inform our understanding of human variability. Models suggest shape of dose response curve but we these mode of action to draw conclusions so when we show you what we're going to do it a few minutes today to understand mode of action because all models are wrong and the model survey is to explain the biology city to have a better understanding of biology. The models were used our continuous difficulty--there are models that try to estimate thresholds. We did not use them. Theoretically the change resulted in order zero increase but we are assuming others are held constant and you know this is very difficult to do in this data set. And even if you do it doesn't suggest a threshold that doesn't exist, make sure you an example. We looked at lots of data. I am going to show you these settings all had one dietary group that had zero TFA. If you are a toxicologist, that warms the cockles of your heart. You can

see you can do this is a dose response and you could see it hasn't changed but you could do the amount of TFA. And when you plot these data you see it looks like they're going up. So what we notice is there are four data points on 07 looked at the data and it turns out in these studies there are 20 doses so there are two controls. That is how I think of a control. But there are two of them and we picked one. We could have picked the other but look at the variability in response to the change in LDL and there is no TFA between groups. So, how do you interpret this? Is this background eerie ability? People are different on the days. What is this variability? If it is somehow reflecting a background, what does this say about this change in this variability or this change that is outside this variability but not here.

This is sort of interesting--probably made public and somehow will be made available. And we really thank our colleagues for that. The criteria here, I'm going to move faster. Because I think I am running out of time. Including criteria a little bit different than--then it is not that there is this wrong and ours is correct that is not it at all. We're trying to do as much data as they can and this inclusion criteria this is a off comparison. They are more limited. Ours is more expensive. That counts as an advantage and a disadvantage read we have a broad inclusion criteria and makes it hard to do consistent comparisons. Now that I regression is different than regression because we will combine studies between exposure and response that we will try to address the effects of random error and small sample size. So studies with small sample size don't get weighted as much. And this is the health of our epidemiologist and modeler. So combine studies waiting for power elite is several analysis that were John adjusting things such as biological effects and the minute analysis we cannot address the dose response questions and what does begins to occur because for a couple of reasons, the models used to not have a threshold built into it and multi variant models, we are able to dig deeper surgeon [Indiscernible] make more sense. We had 44 data points and 22 publications. Each data point reflects the difference between the lowest TFAs group and a test group. There is a mathematical reason we did this because we have to get a variance on the radar change because if you don't have a variance on the response, the radar change, -the break of change, you cannot compare one study to the other. We took one study and Bruce Allen could do a better job of explaining this potent the lowest group as a comparator group that we tested each other group again set. We did linear and nonlinear. Power models. These are just generic models and these data may not reflect the data on the underlined basis and you are not limited to these models. You can use other models although I would not recommend the PowerPoint thing. Future evaluations. There are other ways you can look at this data. Results. Finally we get the results. Unfortunately we have limited high-quality low-dose data. Large input data particularly low TFAs and -take let us know this is not the primary determinant of the change in the LDL's. Back to the original graph where there is a lot of variability of zero. The model explains 70% of the variability when you put a parameter and that accounted for variability at the zero dose. If you took that her imager away, then he will not only explained 40% of the variability. That is not all that good. While statistical difference between the coefficients of one wishes a model that gives us a Steve dose curve which is related to pharmacokinetics and 3.6 which is a slope consistent with a threshold. You cannot distinguish those two. Modeling cannot inform an understandable shape in the low-dose range and if you do a threshold model, how do we get there. We do know how to do that and I said these other things so the threshold is not different statistically. To make here is the low-dose data. This is the hill criteria with a value of one. That is one parameter. There is these--here's a criteria user consistent with data. If I show you a figure with everything. These are consistent with the data. This is a regression. With all of these data and this might be similar to others and again, what we have the x-axis is a change so there is no zero TFAs no other Grapher we had a true zero. This is not a true zero. This is the change versus the change in the LDL which is the parameter after.

Here's all the data. Again, we have Excel spreadsheets to show the data and comments from our colleagues at some point it is a release to the appropriate processes but this is this week that we came up with this data. The size of the circle means is a better study so this has better control, the smaller the point. Less certainty. We have healthy populations. Open circles on healthy populations, in the dark circles we force this regression to go through zero. There is an argument about this. We forced it to go through zero. If what we did was include a parameter that looked at the variability of zero and that parameter allowed us to go one standard deviation away from this particular [Indiscernible] 30. And when you use this parameter, you get 70% of the variation. And of course using the hill criteria the parameter eight was one and if we use the hill criteria parameter 3.6 we can distinguish of this curve will look different. It might be a little bit more this way but the point is, there's a couple of different ways you can model it. We cannot distinguish the models so depend on motive action to help inform our extrapolation.

Summary. Data insufficient really applied modified bacteria for evaluating a mode of action. But it nonlinear dose response is expected. Why? Because of some of the background information on motive action that we talked about before. There is nonlinear kinetics. There is high regulation of some of the events. And it looks like it appears in a homeostatic range. There is large variability. The regression ways the studies by degree of answers take and it improves our understanding of a dose response. This is good. Other people should look at this data and do regressions. Of the risk assessment, techniques can aid in understanding offer new perspectives and hopefully that is what we have done with you she you paying attention and we welcome your questions. Thank you.

[Applause]

12:10 pm-1:00 pm Moderated Roundtable Discussion – Norbert Kaminski, moderator

Okay. Thank you for that interesting and provocative presentation. I am sure there will be a lot of discussion about it. So [Indiscernible - audio cutting in and out] moderated and we will kick off with a bit of a summary SOT of okay.

So you'll start off with a few introductory remarks from DeAnn Liska [Indiscernible - audio cutting in and out] she is working at Washington University for many years and a representative from the food industry and was a director at the Kellogg Company so she is going to put the mornings remarks and to a [Indiscernible - audio cutting in and out] perspective and take a few minutes to do that and then the floor will be open to return questions from the audience and from the web audience and I will be passing that on and we will take it from there and we will see how long this will go on.

So if I could get all of the panelists back to the front.

Comments: DeAnn Liska, Biofortis Clinical Research, Addison, IL

Thank you to the speakers for a great series of presentations that will set us up for a good discussion on the initial question. So we were asked initially, to look at the evidence and the area then takes that correspond to current intakes. One of the questions that we heard today was something about current intakes for the intakes of TFAs. In terms of that, on the evidence that we were looking for evidence in the 0 to 3% intake range because the FDA has published evidence that current intakes are more like .5% at the highest intakes and the current intake of the TFAs currently. That is one of the aspects who are looking for that, we looked across that arrangement and the discussion today for the clinical data

initially and some of the epidemiological data and one of the questions is, when you look at the data, how does that reflect current intakes and when we look at the quintiles, most of the current intakes are in quintile one and two. So hopefully in the discussion we will talk more as we move forward how to take the evidence that we currently have into perspective with current intakes and in our second series of discussions, we were talking about dose response and how to look at the effect of what is going on now and worry may want to go in the future and we brought of the current exposure as [Indiscernible - audio cutting in and out] a much of the evidence as you saw in part two is usually looking at a percent changes that we had some discussion about clinical trials away to perform clinical trials and there has been some discussion about what that comparator should be and it is a changeover the comparator so early on we had a discussion by Dr. it and about the comparator being the [Indiscernible - audio cutting in and out] I'm interesting in this understanding of the comparator because most of the evidence we sign compared to-then you seek the effect that we are seeing at the high levels but one of the questions I had during this presentation has been, this is because the system is decreasing because the transit is increasing, what are we looking at in this effect and it is an assist ratio or about the [Indiscernible - audio cutting in and out] or the assist that is a good question about the comparator. And that comes back to trying to relate this evidence to policy or making a change. What are we asking people to do? Are we asking them to increase certain types of oils or are we asking them to decrease [Indiscernible] across-the-board and I think as we look at the evidence, we need to understand those aspects we start thinking about how to apply that to the population. In terms of our evidence that we looked at, we found that there was limited evidence the low level intakes excepted primarily control groups and I think about the right control group when you're looking at low levels in intakes of trans fats and how to do those studies in the future is very important. I am leaving this with a couple of questions but I am primarily interested in the application of this evidence of the higher intake levels to the lower intake levels because that is what we have been looking at overall and I want to bring it back to that as well and the key comparator that was brought up in the first discussion that we had about what that comparator should be if we are asked a question if you go from current intake levels and you decrease those levels, what should take its place in what to do we think that change should be in terms of the CHD risk and that is ultimately the key question and we saw different approaches to that today but I don't want to take a lot of panel time to bring it back to how we assess the current evidence and especially if it relates for the intake were we are currently standing. So that would be my major question.

[Captioners transitioning]

Dr. Kaminski

I think you can join us up here on the panel. I think it is been a really good morning. We have had very excellent presentations, starting out with Martin's presentation on really setting the stage for the rest of the morning, talking about the framework and give a yes an introduction to PHOs. And Dr. Katan talked about the health effects of PHOs and trans fats based on clinical trial studies that he performed. And actually, Dr. Katan when I saw the slides of your milkshakes that you are creating with your food, I think it led me to an interesting postulate and that is maybe that the secret to weight control is eating food that tastes bad. [Laughter]

We then had a presentation by Dr. Brouwer telling us little bit about epidemiology and the health effects of PHOs. And we had several talks focus now on dose response assessments by Dr. Chiu and finally a presentation on linking the mode of action as well as dose response to the evaluation of PHOs. What we are going to do now is I have a stack of questions. I hope I can read them as some of the handwriting, I have to admit, is going to be a little bit challenging. Some of these questions are directed to the panel and some are directed to specific members of the panel.

What would be helpful as even if the questions are directed to a specific individual if other panelists want to follow up with a comment, I think that would be appropriate. I am going to start with the first set of questions here which are for Professor Brouwer . The first question is has the rate of CHD gone down in the countries that have reduced trans fat?

And then has overall fat intake also decreased? In the follow-up I guess to that also, it is all part of that first question, are there any data or data yet available or incidence of CHD or mortality from Denmark since their ban?

Dr. Brouwer

Yes, there are figures from Denmark that shows the coronary heart disease in the country has gone down over the years. I think you should always interpret this data very cautiously. Because of course a change in TFA is not the only thing that has changed in such a country. There are indications that incidents of coronary heart disease has gone down for example in Denmark, but I think that over the years so many things change that it is very hard to pinpoint that and say it is because of the TFA I know that that seems [Indiscernible-heavy accent] for example does that. Or maybe smoking went down as well, I don't know. I don't know all the other factors, I only know that it went down.

Dr. Kaminski

Dr. Katan did you want to follow up?

Dr. Katan

Yes, [Indiscernible-heavy accent] I think studied this in depth in the Netherlands. They noticed an unexplained fall in CHD in the years after trans-fat intake suddenly lowered by the big companies taking it out of food but of course limitations, which Dr. Brouwer indicates, always applies to this kind of thing. It is compatible but it is not hard proof.

Dr. Chiu

I think also, I remember reading a couple of abstracts where people try to use the data to predict how much would have been expected in terms of reduced cardiovascular disease. I think there was one in Ireland or Northern Ireland, I cannot remember, but there was a model that seemed to predict and it seemed to validate that the numbers were consistent for being enough of a predictor of the model.

Dr. Kaminski

Okay. The next question is also for Dr. Brouwer and one thing we need to keep in mind is these questions did come in throughout the entire session. Actually after I asked the questions it might be something that Dr. Dourson might want to jump in on as well. Do the relative risks for combined cohorts for CHD consider mode of action of trans fats to guide regression?

Dr. Brouwer

Can you repeat the question? It's a bit complicated.

Dr. Kaminski

Do the relative risks for combined cohorts for CHD consider mode of action of trans fats to guide regression?

Dr. Brouwer

So do you mean predicts the change in LDL for example, that would also predict the change in risk and coronary heart disease?

Dr. Kaminski

I think the question was getting to his mode of action considered in your analysis, I think if I understand the question correctly.

Dr. Brouwer

Yeah, well, that's very difficult to say. Just through doing models for certain things, for example, LDL concentrations are in your line of evidence so you think that the trends are causing a raise in LDL and that raises causing coronary heart disease, you do not adjust to the doctor of course because otherwise you raise out the effect of the trans fat. The models take into account different fatty acids in the diets and differences in the diet. Taking into account body mass index and age, those things that you're expected to find in the line, you do not adjust for. And in that sense you take into account that you think that the effect might be via LDL and of course you don't take into account things you do not know.

Dr. Kaminski

Dr. Dourson and Dr. Katan?

Dr. Dourson

Well I think the question might be getting to the point where in your work, if the regressions are based on linear regressions, was the mode of action thought about and you picked something other than a linear regression. I guess I think the question is getting at this idea of circular argument, if you use always linear regression with epidemiology data, then one conclusion is that the response is linearly associated with those. But that's of course with a linear regression. I think that might be it.

Dr. Brouwer

I did not just show linear, I showed the difference between the highest quintile and the lowest quintile. It is not just a matter of linear regression, you show what the risk is in the highest quintile versus the lowest quintile.

Dr. Dourson

The other quintiles would be with the threshold, is that what you are suggesting?

Dr. Brouwer

I don't know that, I just have the data but there is not much data in the lower range which is absolutely true. You can think of a lot of things, but if it is not there, it is important to realize that. It is also important to realize that might be a weakness. If it is not there is also not possible to fill up that gap. For example in the data that you showed in the lower doses are not very useful for showing effective lower doses. You should accept the dose data isn't available.

Dr. Kaminski

Okay, Dr. Katan

Dr. Katan

No, you go first.

Dr. Liska

I had a question about the linear regression --

[Indiscernible-muffled speaker]

Dr. Kaminski

Dr. Katan, why don't you go first?

Dr. Katan

Yes I worked on mode of action of dietary fatty acids on lipoproteins for about 20 years, and other people before me worked on a 40 years and other than that there's people before them, but I think here that we are talking about things in a different ballpark. I have a clock, and can take it apart and can take all the parts out and look at them and put them back together again and understand how everything works, and if I have an iPhone and I want to understand how it directs me with Google maps to the airport, I can take it apart with a screwdriver and look at it with a magnifying glass and I will not get anywhere. It is a totally different ballpark and the same thing goes for the comparison between say the effects of certain chemicals on DNA mutations and then what we are talking about here with diet and lipoproteins. So many brilliant people have tried this and have gotten nowhere and we have so many explanations and they all seem valid and they don't allow us to understand this. Until we can take a human being apart into its constituent macromolecules and look at them and put them back together again into a working state we should be modest about mode of action.

Dr. Liska

I just wanted to ask about the regression if it took into account the change in the other fats. For instance, if there was a decrease in [Indiscernible] and there was a decreasing LDL and if there was an increase in that alone in terms of comparative diets, how would we do that so that we know what we're looking -- what we're looking at is trans and not a result of changing other fats in the diet?

Dr. Brouwer

That is the point, this is not a drug, it is nutrition. It is impossible to say anything about macronutrients as I explained, unless you say what it is replaced by or if it is not replaced, you get loss of weight because of energy. You have to say what it is replaced by and there is no real placebo. You can only say okay if we compare trans fat with [Indiscernible-heavy accent] and if we compare trans fat with whatever, it's this. I think the not nice thing we trans fat is it is that compare to anything so whatever you replace it by, the effects on cholesterol metabolism is worse if you take trans than anything else. And that makes it in that sense easier than some of the other factors in the food.

Dr. Kaminski

I would like to follow up a little bit on this mode of action question because I think some of the data presented in the first two presentations were very convincing at least at higher doses with increased trans-fat, there certainly seems to be an increase in LDL. I know that Dr. Dourson provided some hypothetical modes of action that could explain that, for example, a decrease in uptake or an increase in LDL production. I am not an expert in this area and I am wondering if the folks on the panel here to give us a little more insight as to what the current thinking is for that increase based on some of these scenarios. Do we have any insights as to which one of those, or is that several of these that are involved?

Dr. Katan

My impression is that people have given up on this. The Nobel prize winners for the LDL receptor looked into this for a while and I have discussed it extensively with them and what I remember is that they said stay out of it. So Bruce Spiegelman, he looked at the effects on gene expression and he found very possible effects in one gene in another gene and it can explain what happens to the LDL receptor. This is the one part that we really understand thanks to Baron Goldstein. But saying that something affects the production of LDL really means very little because there could be 99 different sequences and they are all interacting. This is the way that we looked at it in the 1970s, production in metabolism, but it has not helped.

Dr. Dourson

I will add a little bit because I think what may be that you're getting to Dr. Kaminski is this idea of high-dose effect, low-dose what is going on? We have looked at the data and I do not think there's any question from a risk perspective that we have a high dose of fats so the question is what is the low dose response? And I think the answer is what is our understanding of the mode of action? You can regress response in many different ways and then the mathematical models will give you different answers and we cannot distinguish them.

Dr. Brouwer

Can I make an analogy that is maybe familiar to you or toxicology people? I have done quite a bit of work on fish, and if you look at epidemiology for example, we see that those people who live fish live longer and are healthier and have less heart disease. The toxicology comes up and you say yes there is metal mercury in the fish. And I say yes, apparently if you look at the whole fish, the fish is still doing better and it is better to eat fish, even if the metal mercury is in them.

But that does not mean that you should allow the metal mercury to be in it. If there were less metal mercury in it it would be even better because we all know metal mercury isn't good for you. And I think that is sort of a similar situation with the TFA. If you take low doses of metal mercury, you cannot show anything. You cannot show that it has negative effects. That's the problem with TFA. And that is the problem with this as well, it is difficult to show but it is not a reason to say that we accept it is in. In my opinion, I'm not into politics or anything so I don't have to set thresholds or whatever, I just said let's make sure that we get this as low as possible.

Dr. Liska

I think this is a very pertinent question, what is the fate of the low dose of the trans fat? We say that it is energy production so it means it is destroyed at some level, is it being incorporated into the membranes and when? That is something that you might think happens only a higher intake level over a longer timeframe so how do we understand that and how much the body can handle? I know that in other situations of nutrients at low levels of intakes the body has mechanisms for managing those but if you go up higher you do see an impact of other pathways. So I think that gets to mode of action and also gets to what happen at low dose levels in terms of the fate of the TFA because we call it a nutrient because it can generate calories. That we say there is no positive role for it so is it truly a nutrient or is it truly a negative entirely like mercury and totally toxic? I don't know if we could agree upon all of that.

Dr. Ronis

It certainly isn't an essential nutrient and we do know that much. Trans fats are not essential fatty acids but they are natural components of the diet. What we are parsing here is a philosophical difference in terms of what we do with industrially produced trans fat. We will never be able to get rid of trans fats and will never be able to ban trans fats from the diet because they are a natural component of the diet. It does come down to dose response at the end of the day. I think the evidence we've seen today has been fairly clear that in high doses there are effects on lipoproteins and there are effects on cardiovascular mortality. The question becomes is there a dose in terms of added diet that that affect would become negligible from a public health perspective relative to the amount of natural trans fats in the diet? Or do we take the philosophical approach of do no harm which is rather than adding to the natural component of trans fats, which we presume is also bad, but not at a level that is high enough to produce toxicity, do we want to add to that burden? It comes down to a philosophical question and rather than dancing on the head of the pin the bottom end and in asking questions about whether or not at low doses there is significant effect or not a significant effect. We have to look at this from the bigger picture perspective in terms of public policy and look at it from a philosophical context of do we need these things? And if so what is the justification for putting them in the diet if there are alternatives?

Dr. Kaminski

Dr. Katan?

Dr. Katan

Can I come back to my neighbors question about incorporation into membranes because there we know quite a bit, fatty acids that you get incorporated into body fat in its various membranes of your body. So this happens with at least the fish oil, omega-3 fatty acids, it happens with the first gram that you take. If you give someone 1 g, it will show up in the red blood cells. For the trans fatty acids there seems to be again, more or less a linear relationship, and you could bring the same objections to those regressions. Whether the incorporation and membranes matters at all, we do not have a clue.

Dr. Ronis

I would like to echo what Dr. Katan just said, we are what we eat. Whatever we do in terms of changing diet composition is reflected in the changes of body composition and that is certainly true for dietary fats. Trans fats are treated much the same way as other fats that are incorporated into fat metabolites, not just triglycerides and other fat metabolites incorporated into the body. When the level of intake it is, whether natural or through industrial chemicals, it will be incorporated in it will produce and affect. The only question is at what level does that affect become adverse?

Dr. Chiu

I've had questions trying not to get to how many angels are on the head of the pin. Just help to put this in context, from a mechanistic point of view of trans fats more potent at increasing bad cholesterol relative to other fats? Is there a quantitative potency difference or is it a truly qualitative difference and that there is a completely different mechanism by which the change of LDL? If it is just quantitative it is like the relative potency of your different fats. First of all you would want to have the fats with lowest potency or bad effects. It is really the whole mix of fats that are producing the bad effect. It is more of a relative potency question rather than a threshold type of thing.

Dr. Katan

I can answer that with an anecdote in 1988, [Indiscernible-heavy accent] rushed into my office and said look what we found was trans fatty acids and he showed it to me and they raised LDL in the lowered HDL and I said to have you mixed up the data somewhere. Fatty acids don't do this. He never mixes up anything. But yes, trans fatty acids are unique there's no other fatty acids that does that and no other nutrient. Carbohydrates lower HDL but they also lower LDL. Proteins will lower LDL little bit and raise HDL little bit, alcohol raises HDL but not much effect on LDL and fatty acids none of them does this. It is a weird effect.

Dr. Chiu

Is it a single mechanism for both affects or could it be they are like alcohol or one is compound on the HDL and they are like another compound on the LDL? Or maybe we don't know.

Dr. Katan

Is Google maps a single effect compared with talking to my wife on what you call it? On Skype? Is it the same mechanism or is it a different mechanism? I do not think that you can answer these questions in these terms. There could be 2000 odd mechanisms interlocking

Dr. Chiu

My point is, is this a cumulative risk issue? Or is their benefits related to well assume that your percent fat intake and total percent energy from fat is the same, we are talking about what is accumulative impact of that mixture. If we changed the composition of that mixture incrementally we have an incremental change in the channel to affect?

Dr. Katan

Do you mean additive or interactive? We can explain a lot of effects of diet on health by adding up the separate effects. They could be second-order interactions but they do not seem to be major.

Dr. Liska

And discussion and the literature about sad that in trans fat things somewhere on LDL but not similar on HDL, there has been other discussion of literature that the effect of trans fat on HDL is not shown except at higher intake levels, like 5 or 6% energy levels. So I just wondered this trans fat, when we're looking at it in 3%, is it really similar to saturated fat in that level? Are there other fats within that level of 3% or below that may be similar or do we really know this trans fat, well, I'm still not sure I understand is trans fat is negative or no. In what we're seeing as the cis-fat effect from the data that I've seen. I don't know if we really addressed that, is it negative trans fat, is positive cis-fat, it a ratio, what we understand about that whole picture?

Dr. Katan

Let me take the last point first, because we have addressed it but just maybe not clearly. Trans fat will increase your LDL to HDL ratio no matter how you put it into the diet. Whichever caloric source you take out will always increase your total HDL cholesterol ratio if you put in trans fat. Does that answer your question? It does not have to be replacement of cis, it doesn't have to be your replacement of polys, if you replace saturates, if you replace carbohydrates, if you replace proteins, if you replace alcohol, you always get the worst total HDL cholesterol ratio in the trans fat.

The other point was about effects at low intakes on HDL. We cannot be sure until we do experiments on zillions of people at very low intakes. But the general picture is yes, that also happens all the way down to zero but of course the effect is a lot smaller and nobody can prove the shape of the curve with total certainty. You can always use a computer program and it will throw some other curves. So who can say? The simplest interpretation is yes it works proportional to dose.

Dr. Dourson

I do want to pick up on Dr. Chiu's point about timing. When our team looked at the data, we were doing 44 different studies, different dietary groups. We took the dietary group with the lowest TFA and use that as a reference and the reason we did that was to get variability in the y-axis so we could do a meta-regression. We took that and did a comparison to other dose groups in the study and as I understand it they were equally fat. Now there were different amounts of different kinds of fat but we took all the studies together. The variability that you see in the data reflect human variability of course, and variability in the underlying composition of fat that was switched out. Probably other things that Dr. Hayward would understand better than I do. So to your point Dr. Chiu we have a comparison of different dietary groups in different TFAs and it was switched out and everything was equal fat, however we do not have a lot of difference in time. The investigators are doing great work that we do not have a lot of time sequence. So we are not sure about that part of your question. And I apologize that this was not clear.

Dr. Brouwer:

Maybe the time question, more time can take because this was the issue...

Dr. Katan

I do not get it. What is the time question? Whether raising your LDL will change her intake of trans fatty acids.

Dr. Dourson

No. pardon me, the time question was to Dr. Chiu's point, if you've done a study for 30 days or 50 days, but what if you carried on for 30 years? How do you make that judgment? And of course we do not have those kind of data.

Dr. Katan

We do not have studies on trans fatty acids going beyond I guess a few months or something.

Dr. Brouwer

Maybe eight weeks.

Dr. Katan

What we see with trans fatty acids is the same time course as with all the other fatty acids and we do have very long-term exposures. For saturates and polyunsaturates we have seven year exposures in the Veterans Administration trial. In three or four years exposure in the Finnish Mental Hospital trial, and we ourselves have done studies ranging from weeks to months and to half a year. The effect is largest in the first week, and it was kind of equilibrate after two weeks and then there's a little bit left after that and then at three weeks it is all done. Here we can use the mechanics and it is a very good agreement with turnover time with LDL because it has a lifetime of a few days. That would fit. And we do not see the effect going down again after many years, as long as the diet is there the LDL level is there.

Dr. Dourson

And we do have the chronic epidemiology studies that Dr. Brouwer referred to, but that does not measure LDL change. So Dr. Chiu that is a good question, I do not have the data to do long-term LDL change other than studying other parameters perhaps.

Dr. Brouwer

But we know that if you keep on the same diet, you have a stable cholesterol level for a long time and you can that will determine quite a few of the risks for coronary heart disease. I think the way that the data has been presented for the lower values by Dr. Dourson, I think that is not a proper way of using data in the lower range. These studies have been carefully set up. I think that you must explain the design and what is crucial and the design is that you take your comparison against your control so that you know what you're exchanging. You're exchanging the trans fat for the monounsaturated fat or whatever is in the control and you can recalculate that, for example, monounsaturated fat. So just by using the data of the control group, that is not a proper way of looking at the values. Because then you're more or less showing a study on what is the variability in cholesterol and time. And that is not the issue that is at stake here. Because here you would like to know what do small doses of trans fatty acids do? I think that might be an important question, because as I already explained, if you do not have the data you cannot show it. So it is not possible to use that data for that purpose. I think it is a pity because we simply do not have it, we can say something about higher doses we can extrapolate to the higher doses, and we can show that it seems to be dose response related, the more that you eat the worse the effect becomes, but this is my opinion, it is not the correct way to go.

Dr. Dourson

I apologize for not making it more clear but I think that is exactly what we did. We showed differences in TFA between diets and how it affected change in LDL. Again we have more extensive information in the Excel spreadsheets and at some point that will be made available and we do invite people's thoughts on it.

Dr. Brouwer

The point is you have the data on what is in the diet but there is no control in that period. There is no control, the design of these studies are very carefully done in the sense that you control the diet, for example for three weeks on monounsaturated fatty acids, but the rest of the fatty acids and carbohydrates stable and then compare it with higher trans intake and the rest of the diet also is stable. That is a fair comparison to make. If you use the data differently [Indiscernible-low volume].

Dr. Liska

Having been the one that got the original evidence map I just want to address that because I'm not sure you did understand because some of the studies did not have controls they had comparators and they did not define a group as a control. So that was where you would look at it for instance there was one study that we use a lot where she compared everything to butter and if you say what was the control? There was one group that was industrial oil that had a low level of TFA against some others that were clearly margarine. And then there was PHOs. So what will you call the control when she had done the study and reported it so we did not use the term control, we used the word comparator. [Indiscernible-low volume]

Dr. Ronis

This is the essential problem with trying to take these principles to diet, there is no such thing as a true control. When you change one component of the diet you change another component of the diet in the same way. It makes these kinds of comparisons extremely difficult to do. I have a question for the two nutritionists on the panel, just to set this in more context perhaps, there is a good deal on the studies have shown where there is a high-level trans fats there is a risk of cardiovascular disease but in the context of changing other components of the diet which are changed or are definitely much more variable between populations and between individuals in terms of what diet they choose to eat, what is the relative importance of this change relative to cardiovascular risk compared to some of the changes in the diet? Such as for example changing the amount of saturated fat versus the amount of processed carbohydrates you eat? Or changing the fat source that you use for frying from polyunsaturated fats to olive oil? Or the effects of fish oil supplementation which is now becoming very prevalent in the population? What are the relative changes in the risk of cardiovascular disease in HDL and LDL, in relationship to those, very common manipulations of the diets, relative to the effects that we are talking about today?

Dr. Katan

Well in part, that is a value judgment which I leave with great confidence to the FDA. The comment I want to make is that it is a lot easier to replace partially hydrogenated vegetable oil in the populations diet with unhydrogenated oils than to make these other changes like reducing salt, reducing saturates, these are tough things to do while the trans as you see can disappear from one year to the other. It is just a matter of industry saying okay, we're going to put in something else and say look it is done. The effect on coronary heart disease rates, there are uncertainties in that because the effect based just on LDL or HDL is clearly smaller than the effect on seen in the associations in the epidemiological studies. My guess and it is just a guess, the truth is somewhere in between. We know there is more than LDL and HDL, maybe triglycerides or other things, but these are not negligible effects. The question of course is how low should you go? That is very much a value judgment.

Dr. Kaminski

One more comment and that I have to get back to some of the other questions.

Dr. Chiu

I comment is related to applying toxicological principles to nutrition and I think one of the key differences is the concept of homeostasis. What brought this to mind is that comment that you are what you eat. If you have a stable diet you have a stable LDL and HDL ratio and if you change the diet all these things change. So essentially, homeostasis is not that each person has some set point of LDL, it is just a product of what they consume and their other habits. So what we're talking about here is homeostasis is not a control mechanism, it is more of a reflection of the total nutrition.

If you change that, you change the person's homeostasis, they are still homeostatic, but the parameters of the homeostasis have shifted. So that is very different than traditional toxicology were we say the liver is in the normal stage in all the hepatocytes are functioning, but you've killed cells in your altering homeostasis, but is a completely different concept than here eared here even the baseline homeostasis carries with it long-term cardiovascular disease and again a low baseline homeostasis you have a lower risk and if you have high you have a higher baseline risk. For toxicity there is no threshold for homeostasis here. Every state of homeostasis carries some risk. It is just a matter of where do you want to be in that parameter range?

Dr. Katan

This is an interesting point. There is no homeostasis in the classical sense of the concentration of plasma cholesterol. There's homeostasis of sodium and potassium and lots of things, but there is no homeostasis of LDL cholesterol just that there is no homeostasis of the amount of plastic in the ocean. Cells regulate their internal cholesterol very carefully and they kick anything they don't want out into the fluids. And that is why LDL is not uniquely but remarkably sensitive to environmental effects, you cannot change your sodium concentration in the blood by eating more or less salt.

Dr. Kaminski

That was a very good exchange. But I'm going to go back to a few of these questions before time runs out. The next question is for Dr. Katan, are any of the CHD affects of trans fatty acids identified and well conducted animal studies?

Dr. Katan

Some people think they can do that. The pioneer of the adverse effects of trans fatty acids is [Indiscernible-heavy accent] had various animal models, some people think enhancers you can reproduce some of that but I'm quite doubtful. I'm quite doubtful about animal models of this whole field. Because they have often been discordant with the human data and also because it is very easy to construct an animal model. If you say I want an animal model where I get this and this effect of this and this stimulus, sure, we can do it. In my mind as an outsider that is one of the essential parts of toxicology, we see that this and this causes cancer in humans and could you construct an animal model so we can fight it? But that is the risk of animal models. If you want to see something, sure, create it in a mouse. I do not think that we have models that re-create this reliably in animals.

Dr. Ronis

Martijn, do we have any evidence to suggest that primate models might be good models for this – relative to rodent models. I've seen one primate model out there, do think it is any good?

Dr. Katan

I think it's no good, and there's something weird. Do you know that primates are not sensitive to statins? If we had not tried the original statins in dogs we might never have had it, it is amazing.

Dr. Dourson

I just want to add a little to the commentary here. The animal model, and what little I have studied of that is appropriate to say, it doesn't replicate humans for LDL but you can use animal models to study underlying modes of action. And even using animals that don't use the same mode of action, you can glean information on what might be relevant. Animal models have their place, like all models they are wrong, some of them are useful. Again, we get the report out on the mode of action we have introduced some animal will models to help us explain the mode of action. I would encourage you folks to read it and give your thoughts on that.

Dr. Kaminski

The next question is for Dr. Brouwer again. Can you elaborate on what you thinks the relative CHD risk is for individuals between the first and third quintiles in the cohort studies that she presented?

Dr. Brouwer

The relative difference in the relative risk? In most studies, they are fairly consistent in that most studies show at approximately 22% 23% higher risk for the highest intake group, adjusted for other factors, compared to the lowest.

[Indiscernible-multiple speakers]

[Indiscernible-heavy accent] there is a difference in intake between the first and the third quintile is often only small. The distribution is not normally [Indiscernible-heavy accent] which makes it easier to pick something up in the fifth quintile than any other quintiles. I especially show a problem that [Indiscernible-heavy accent] and the people who are in the fifth quintile are not just eating more trans but there also do a lot of other things differently and in this case, wrong.

Dr. Liska

I have a follow-up, maybe it is a question, we saw something about modeling the risk in the change of TFA. If we are consuming something in the diet that is quartile two and we're trying to shift by decrease to quartile one, how do you use that? How would you use that in modeling? Right now we've looked at changes in modeling or changes in CHD risk based on quartile five to quartile one but we are not consuming at quartile five. Maybe that is not even relative [Indiscernible-low volume] and I think that is where the question comes, but is more quartile three to quartile one, but if the range we would be looking at today. [Indiscernible-low volume]

Dr. Katan

We do not have the data for trends in CHD directly, but we do have a huge amount of data on LDL and CHD. And if you take data from say 1 million people or so, you see a perfect straight-line for risk against incidents from very low to very high LDL levels. [Indiscernible-heavy accent] if you have enough data points if you have the hazard ratio against LDL concentration, and it is a perfect straight-line from top to bottom.

Dr. Kaminski

The next question is the linear progression in the final presentation indicates an increase in LDL concentration of approximately .08 mmol per liter or perhaps three or four milligrams per deciliter. Is there an increase in CHD risk for rise in LDL concentration of this magnitude? I think that is to you Mike.

Dr. Dourson

I'm sorry, was that to me?

Dr. Kaminski

It said the final presentation, so I think it was you. I will read it again.

Dr. Dourson

That's okay, I think I've got it. Again, I don't think anyone has a question with a high-dose effects might be for TFAs as they will increase LDLs and there's some associations with LDLs that might be very good with coronary heart disease. The question is what is a low-dose extrapolation or interpolation. I would encourage our colleagues interested in that to study the mode of action of the various modes of action for this in order to get a grasp on that. Because plotting [Indiscernible-low volume] or the LDL data we have done, it is helpful, but the mathematical model cannot answer this question.

We have already demonstrated that we could have a model with a hill parameter of 3.6 which looks very much like a threshold and one that has a parameter of 1.0 that does not, and we cannot distinguish between these two choices and two of many choices perhaps. So understanding mode of action is very important. As far as that specific question I would have to defer to my medical colleagues if there was a slight rise and that would cause the medical concern. That's important but that's outside the realm of the toxicologist. If others want to answer or help answer that I would be happy to defer.

Dr. Kaminski

Dr. Katan I think you wanted to comment.

Dr. Katan

In drug trials of cholesterol lowering, you see that lowering of LDL by 1% reduces risk by I think 2%, Ingeborg? It's about that. What you see in the epidemiology is 1% difference, 3% difference in risk. So that is easily explained by longer exposure. Exposure in trials is on average two years. Halfway in a four year trial. With epidemiology it's often decades. 1% change in LDL will change an individual's risk by small amount but on a population level this would be a lot of heart attacks. The same is with blood pressure, one or 2 mm will cause a small change for a person but a large change for filling up hospitals.

Dr. Brouwer

It's always a matter of risks of course.

Dr. Dourson

As a risk person I need to push back a wee bit. I think, and perhaps I am wrong, that you're making these projections of a small increase of risk with a small increase of LDL on the basis of the linear regression from effects that you see at high-dose. Again I do not think there's any question about the effects of high-dose but if you presume a linear regression you assume linear regression, then indeed you will get effects at any dose. So in order to do that I would encourage you to understand mode of action, and you know it better than I do in this regard, to help you understand what the appropriate regression is. If it is linear, then so be it. Our study of information led us to say that we cannot make that determination and that it was more of a mode of action understanding. In our mode of action understanding led us to believe that homeostasis and other regulated parameters suggests that the concept of the threshold for adverse effect. But again I am willing to talk to folks about this.

Dr. Brouwer

I think this is purely based on what we know about LDL. A raise in LDL or lowering in LDL, that is independent of what is causing the raise in LDL or decrease in it.

Dr. Dourson

So just a rejoinder. We have information on LDL slight raises in low areas with cardiovascular disease. Or is it association that goes from high-dose to low?

[Indiscernible-low volume]

Dr. Dourson

I'm sorry?

Dr. Katan

This is a graph showing the relation between cholesterol and heart disease.

Dr. Dourson

Okay. Could you explain this to us please?

Dr. Katan

Maybe we should do this over lunch.

Dr. Chiu

Direct the point is we are in a range of observation, from people with very low LDL to people very high LDL and there is a linear relationship with cardiovascular risk and most people are in the middle somewhere. So if you shift the whole population, then the people with very low data slightly bigger than very low and the people that are high get a slightly bigger than very high. What I am hearing is that you can measure the difference from 1% shift in LDL to a shift in the overall incidence of heart attacks.

Dr. Dourson

And these are statistically significant differences in the low area?

Dr. Katan

They probably have P values in the range of 00000001 because the numbers are so large and the P values don't mean all that much. This is a straight line, as straight as it gets here I will show it to you over lunch. It goes from the bottom to the top of the LDL range worldwide.

Dr. Dourson

Thank you.

Dr. Kaminski

Let's move to the next question, this one is for Dr. Katan. It says here that TFA increased CHD risks. You also should data to indicate that TFA raises LDL levels. Can you explain further on what you think the true CHD risk is, particularly at 2 to 4% of energy level or range assessed in the US?

Dr. Katan

Yes, that is a difficult question because if you calculate risk, purely from LDL is much lower than say 22% increase that is seen in the epidemiological studies. If you add in the HDL and the lipoprotein A and triglycerides, you get just over half of that so you get to say a 10% increase in risk for 2% energy change trans fatty acids. I think that is fairly plausible. I would say that there is 22% mixture that other factors. It is reliably higher than just the LDL effect.

Dr. Kaminski

I have been saving this question towards the end. And I think that everyone can weigh in on this one. After the fact, toxicology and epidemiology studies show increased risk for cardiovascular disease from trans-fat intake. My question is, is there a take-home message that can guide future safety assessments of new food components that can be used to more effectively minimize risk prior to human exposure?

[Laughter]

Dr. Katan

I assume new food components refers to say additives or stuff like that, right? The question really is please explain in four minutes how we should do toxicology. I cannot improve on what is being done. I just want to say that the certainty that we can derive from human experiments is much higher than the certainty that you get from giving something to rats for three months and then opening them up and looking at their entrails and dividing something by 100. There is a bit of the ritual here. People are worried and we want to show that we take it seriously so we do these studies we put it in the computer we come up with a number and we control that number and we check on the food so that people are less worried. In the back of our minds we think what we keep levels very low. It is probably not a big thing. That is my take on toxicology [laughter] but Dutchmen are notorious for being blunt so if you want to kick me go ahead.

[Laughter]

Dr. Dourson

Not at all. Thank you. That is a great question, the way I would approach it as a toxicologist is I would say what kind of study could we do that is short-term that focuses on a likely mode of action for whatever you're going to introduce into the diet? What might it do? You could start with studies on similar compounds, there's whole bunches of people who have done that kind of work. But eventually you get into maybe an *in vivo* and lots of *in vitro* first, but in *in vivo* system where you can look at mode of action and once you understand better about mode of action then take the step of getting the animal model that is reasonable and stuff it into *in vivo* whole animal. I would start with the whole idea now of mode of action, push that idea of mode of action first. What can we glean from the studies on that? And maybe that would help you with introducing this, if that was the question.

Dr. Kaminski

One last question I'm going to ask because it is one that also interests me, this is to Dr. Brouwer, what are the age-related effects of trans fats? Do we know anything about that?

[Indiscernible-low volume]

Age-related, yes. Do we see the differences in how people respond at different ages to trans fats?

Dr. Brouwer

I think that is very difficult to answer because the studies have been done on adults. There is hardly any studies in children that have been done, there are a few I know. The problem is those are not of the best quality so it is difficult to say if there is a specific action in children, and for adults with studies have been in the age range of somewhere between 18 and very old. Maybe some people have looked at specific age effects, but I am not aware of those.

Dr. Chiu

Is there anything that we can glean from other fats in terms of different effects at different ages or from other dietary components?

Dr. Katan

Yes. Age as such has no effect on the responsiveness of people to saturates and polyunsaturates and dietary cholesterol. But initial cholesterol level does so people with a high initial level tend to respond

more than people with lower initial level. Very curiously people who are overweight or obese respond less than people who are lean. This is something that we observed in the early 80s and it is been confirmed at various places and we have models of course and mechanisms, but we don't really understand it. But by and large, that is about it. And the difference is that people often see responsiveness between different groups or trials are largely random noise and not reproducible.

Dr. Kaminski

Thank you very much. I would like to begin by thanking all of the speakers. I think you just did an outstanding job, I think the presentations were great.

[Applause]

The other thing that I wanted to do was also thank the FDA for hosting this. It was really a terrific venue. We appreciate all the people that came in person and also all the people that participated. I also wanted to thank the SOT for helping to organize this as well as providing the link for the website. And I believe that we will have this also recorded and on our website for those who would like to listen to it again or direct one of your colleagues to listen to it as well. So again, thank you very much for your attention, and again to all the speakers and everyone involved in putting this on. Thank you very much.

[Applause]

[Event concluded]