formation of this amide bond.

Well, if you look at the amino acids in the food system, there are already a couple that have amide bonds present in the side chain. One of these is asparagine and the other is glutamine. Now, amino acids during typical cooking conditions undergo a process of decarboxylation followed by deamination. If you look at this asparagine side chain, it looks very similar to acrylamide. What is known as the structured aldehyde of the amino acids would form this aldehyde here, and there is the possibility that there is a side reaction going on where we can form acrylamide from asparagine. You will see, as I show further on, that this is a small part of the reaction. Glutamine here just has an extra methylene group but there is the possibility that under typical cooking of food where you get decarboxylation followed by deamination it might form some type of rearrangement of products which could lead to a conceivable small portion of acrylamide formation.

[Slide]

To address this we developed a model system. In this case we tried to make it like a potato chip. We took potato starch and water. We were able to heat this kind of like a dough sheet, and to this system we could add a variety of amino acids, reducing sugars and a variety of other ingredients which could include inhibitors. After this we could take it to a frying process and then measure acrylamide in the finished product. This could also be baked and we showed in our studies that baking can result in the formation of acrylamide.

So, the elegance of this model system--we have to make sure that our system is inert itself. Here we looked at a potato starch system and we went through the frying process and got less than 50 ppb acrylamide. To our potato starch we started adding reducing sugars such as dextrose. We take our potato starch, we add in asparagine alone and we start to detect acrylamide formation. However, the combination of dextrose and asparagine gives us significant amounts of acrylamide formation here.

We also looked at other amino acids, such a alanine, aspartic acid, lysine, threonine and glutamine. We can actually detect a small level in glutamine, 156 ppb versus asparagine with 9000. So, about one percent the level is formed in glutamine compared to asparagine. However, arginine, cysteine, all these other amino acids do not form detectable levels of acrylamide. You can see from this that we kind of felt that asparagine is really the source of acrylamide.

[Slide]

How does this relate to food systems? Well, what about amino acid composition of potatoes? We looked at that and approximately 50 percent of the amino acids in potatoes are in the free state, which means it is not incorporated into the protein. Out of that, asparagine is roughly half of the free amino acid content. So, it is conceivable that potatoes have such a high level of free asparagine that that could be the source of acrylamide formation in potato products.

[Slide]

Other reactions were carried out with using free amino acids such as asparagine. We wanted to find out whether protein-bound asparagine could also participate in the formation of acrylamide. So, as an analog, you can purchase N-acetyl asparagine where the alpha-amine group is tied up to this bond, here, mimicking a protein analog. We reacted this with dextrose to see if it formed acrylamide. The results were that no acrylamide formation was observed. So, from our understanding, we felt that all we need to be concerned with is free asparagine because that is what is the precursor to acrylamide formation during heat in the food system.

[Slide]

We know that asparagine is required for homolysis and dextrose is also required. So, we looked at a dose-response curve from dextrose. If you look at a potato, to get a rough estimate, asparagine is actually 1.25 percent in a potato. The dextrose or reducing sugars is about 0.5 percent in a fresh product when harvested, but as

product is harvested, usually in the late summer early fall, it may go into storage and be stored for quite a period of time and the level of reducing sugars will actually increase in potatoes. So, you can see that as you increase your level of free reducing sugars you actually increase your level of acrylamide. So, we know that you need not only asparagine but a level of reducing sugars in potatoes.

[Slide]

Are there other carbonyl sources that can form acrylamide? Some recent work speculated that the formation of acrylamide from asparagine, the structured degradation reaction--structured degradation reaction is implicitly explained, actually a di-carbonyl such as, in this case, glyoxal reacting with the amino acid causes the reaction to proceed. We also showed that glyceraldehyde, 2-deoxyglucose and ribose are also efficient at forming acrylamide in food systems.

People who are familiar with the Maillard reaction understand that the typical browning

reaction involves first a reaction of a carbonyl amino acid. If you use a molecule such as 2-deoxyglucose where it is C2 here, you do not have a hydroxyl group. This prevents the molecule from undergoing the rearrangement. So, this actually lets us know that all we need is to Schiff base the formation for the formation of acrylamide. This is also verified by reactions we did, lipid aldehyde such as decanel, and Dr. Adam Bakowsky at Health Canada also published about octynal, another lipid aldehyde that can react with asparagine to form acrylamide.

However, people may ask is lipid oxidation contributing to acrylamide formation? I would think not because if you look in the food system, typically the reducing sugars are probably on the order of about one to two magnitudes higher than the lipid aldehydes. So, I think what we need to be concerned with is level of reducing sugars.

[Slide]

To prove that, initially we are thinking that the asparagine actually going into acrylamide

is the side chain here. Just to make sure we can prove that this is going on, you can purchase isotopes which can be incorporated and enriched in either ^{15}nitrogen or ^{13}carbon. So, we carried out experiments just to confirm that this is where the source of carbon nitrogen is coming from in asparagine.

[Slide]

For the initial experiment we used amide-labeled asparagine so it is ^{15}N here. Reacting with dextrose, we should form acrylamide where we have a ^{15}nitrogen at the amide bond. Acrylamide has a molecular weight of 71. We are going to be monitoring and any unlabeled acrylamide would show a mass at 72. However, since we are incorporating ^{15}N here, we think we should be detecting this at a mass of 73. And, this is what we see. We do not detect any unlabeled acrylamide. Really 97 percent of the total acrylamide response is at the monolabel, suggesting that this amide nitrogen is being incorporated into the acrylamide.

[Slide]

We did further studies where we labeled the alpha-amine nitrogen. Again, this is the source of the carbon nitrogen so when we add dextrose to form acrylamide we should get a mass at 72 here so any detectable levels of acrylamide were the unlabeled acrylamide, again suggesting that this nitrogen is not being incorporated into the acrylamide formation.

[Slide]

Next was to verify where the carbons were coming from. So, we purchased a uniformly labeled asparagine where all the nitrogen and carbons are labeled. In this case we got this nitrogen label and these three carbons and we should see an increase of four mass units and we should be detecting acrylamide at a mass of 76. Indeed, from our analysis all we could detect was the acrylamide at 76. You see in this chromatogram that we also monitored at 75, 74, 73 and 72 for any other molecules. So, from these experiments we concluded that this side chain of asparagine is what is being incorporated into the acrylamide.

[Slide]

From these studies we were able to form this following mechanism of acrylamide formation. The alpha amine group of asparagine here is a mucophilic attack on the carbonyl source, forming glycocyamine. As you are driving away the water, you get the formation of Schiff base. So, actually this process is favored under reduction of water in your cooking system. After this, as heat is applied we get decarboxylation that forms as intermediate which rapidly degrades to form acrylamide, os hydrolyzed to form beta alanine amide, and beta alanine amide itself can undergo elimination of acrylamide. We showed that we can heat this under typical frying conditions and it will be able to decompose even in the absence of sugars to form acrylamide.

So, this is kind of a proposed mechanism. How can we prove that? Well, utilizing LC/M mass we are able to do that. What I will show you on the next slide is where we are going to be monitoring our carbonyl source. In this case we

use dextrose so we can monitor that at a molecular weight of 180. We are going to be monitoring asparagine. And, as we heat a product out we are going to be looking at the formation of Schiff base, the beta alanine amide and the acrylamide to prove our mechanism.

[Slide]

Here are the first monitoring intermediates in acrylamide formation. In this case, we use just regular asparagine reacted with dextrose. You can see that at our initial time, zero seconds, we get a response for dextrose and a response for asparagine. At intermediate time, 180 seconds, we actually start to see our first intermediate Schiff base being formed. As we heat this on to 270 seconds we have actually depleted all our source of asparagine, dextrose. The Schiff base is gone. We get an extra intermediate beta alanine amide and also acrylamide. So, we can monitor these intermediates in this reaction system.

Just to confirm that they were there we

actually used isotope labeled ^{13}C and ^{15}N molecules and we can see the corresponding shift to mass units. In this case we are monitoring asparagine at 133, with these all being labeled, and incorporation of six mass unit difference. We monitor at 139 and we can see the increase so the acrylamide has gone from 72 to 76. The beta alanine amide has gone from 89 to 94. So, we have confirmed that these intermediates are actually formed during the reaction process.

[Slide]

Next is understanding acrylamide formation in food products. All these studies I have been showing you right now are a model system so we need to prove in a real food system that asparagine is the source of acrylamide. Questions arising--is asparagine the only precursor to acrylamide in heated foods? What about other potential sources of acrylamide, methionine, glutamine, cysteine or acrolein? These have been postulated by people to maybe provide a minor amount of acrylamide. In our model system, we think we have disproved this fact

and have shown that they are not sources.

But another way to do this, we decided to do selective removal of asparagine from a real food product with the enzyme asparaginase to address these questions because we felt like if we could have asparaginase in this real food system degrade all the asparagine and look at acrylamide formation we would show that that reduces acrylamide formation and asparagine is the source of acrylamide in a real food product.

[Slide]

Asparaginase, this enzyme, will hydrolyze the amide bond of asparagine utilizing water and will form aspartic acid. If you remember our initial model system that I showed you, we analyzed aspartic acid's ability to form acrylamide and it formed undetectable levels. So, we feel like if we do convert asparagine to aspartic acid it should result in reduction of acrylamide formation.

[Slide]

So, here is our real food system. We took washed, Russet bake potatoes purchased from the

local grocery store, boiled for one hour, and then we blended the flesh on a one to three ratio with distilled water. We have two plots here, one as a control and the other one we did with enzyme asparagine-treated, carried out for 45 minutes at room temperature. Then we microwaved this at two minute intervals for a total of ten minutes. This is a highly cooked product to maximize acrylamide formation. Both the control and asparaginase treated products were dry and brown after the step.

To make sure that the enzyme was working correctly we analyzed for change in asparagine and aspartic acid. This is our control sample and, as mentioned earlier, free asparagine is high in potatoes so you have a nice peak response here for asparagine and a smaller response for aspartic acid. In our asparagine-treated sample you can see where the asparagine has been depleted. We depleted about 88 percent here and the aspartic acid is subsequently increased. So, we know that in our system here the reaction was carrying out the way we expected.

Next was to monitor for acrylamide. We can see in our control product we have 20,000 ppb acrylamide. Asparagine is treated down to 164 so we actually got greater than 99 percent reduction in acrylamide by using asparaginase. So, we feel that this experiment is able to prove that asparagine is the mechanism for acrylamide formation in a real heated food system, in this case being the potato.

How does this relate to other foods? In our studies we looked at the yield of acrylamide from asparagine. We deduced that the yield was less than 0.5 percent. Dr. Adam Bakowsky at Health Canada also showed in his work that the yield was about 0.1 percent. So, we have taken these numbers and looked at a variety of food products. If you look at the amount of free asparagine in the starting food products and you correspond to the yield of about 0.1 to 0.5 percent, I think that will compensate for all the level of acrylamide that has been detected out there. So, we feel that asparagine is the source of acrylamide formation in

all food products.

[Slide]

Acrylamide precursors are ready to intervene. We know that asparagine is important for this formation and also reducing sugars. Typically in food systems reducing sugars are glucose and fructose. Some foods will contain sucrose and in the cooking process will undergo hydrolysis to form glucose and fructose.

How is this affected in potatoes? Well, the level of asparagine and reducing sugars actually varies by the source of potato. I think there are many people out there in potato processing areas who are monitoring asparagine in a variety of potatoes and also looking at reducing sugars, and we know that in storage conditions, as potatoes are stored for periods of time, the level of reducing sugars will increase. If you look at a product in the early fall, it will probably have a low level of acrylamide but as people start to use more potatoes that have been stored for a longer period of time the acrylamide will potentially

increase.

[Slide]

In conclusion, asparagine is the major source of acrylamide formation in foods. Carbonyl source typically in food systems is going to be reducing sugars as required in the reaction. Oil oxidation products and starch do not appear to be significant factors in acrylamide formation.

One thing I forgot to mention when I was showing you our model system where we took our potato starch and added amino acids and fried it, we also looked at fresh oil versus an oxidized, aged oil to see if acrylamide formation was affected and there was no difference. So, we were able to conclude that oil quality such as oxidized oil did not significantly affect the level of acrylamide formation.

So, that is the conclusion of my talk. Thank you.

Questions of Clarification

DR. MILLER: Thank you. Comments or questions?

DR. BUSTA: You were generating acrylamide in a microwave in a water system which wouldn't be over 100 C. Right?

DR. ZYZAK: Yes.

DR. BUSTA: I thought we required a higher temperature than that to generate acrylamide.

DR. ZYZAK: I mentioned that in the microwave system it was dry and brown, and there are results out there that you can form acrylamide during microwave conditions. What we found is a big factor in the level of acrylamide formation is the moisture of the product. So, if you do microwave something and you still have a higher moisture content, it is probably okay. It is when you get down to low moisture content that you drive that reaction. As I showed the mechanism, as we remove water from our Schiff base you get the decarboxylation step. So, I think finer moisture content is a critical factor in acrylamide formation in food products.

DR. BUSTA: Are you saying temperature is not?

DR. ZYZAK: Temperature is. I think it is a combination of both. You need to have a low moisture environment to get that Schiff base and you need to get heat involved to get the decarboxylation step going on. You do need both of those going on, but we do see that at 100 degree C you can form acrylamide.

DR. MILLER: Johanna?

DR. DWYER: I think I am right that ascorbic acid is a reducing sugar and is present in some foods.

DR. ZYZAK: Correct.

DR. DWYER: Is that a significant factor? For instance, my ancestors ate a lot of potatoes and I want to know if I have gene damage.

[Laughter]

DR. ZYZAK: I think you were talking about ascorbic acid and four carbon sugars can participate in this reaction, but I think if you look at the level of reducing sugars, such as the glucose and fructose, they are about half a percent and can range up to two percent in a potato. So,

that far outweighs the level of ascorbic acid in there that is enough to facilitate the reaction.

DR. MILLER: Yes?

DR. MEHENDALE: I was wondering if you have tried any carbonyl blocking mechanisms in your reactions.

DR. ZYZAK: Yes, the typical anti-browning reason are sulfites. All this would be simple if we could add sulfites to solve the problem but it didn't work and you can only add a pretty low percentage of sulfites in a food product, like if you buy dehydrated potato products I think it is less than a percent or something like that, the level of sulfite you can add in there. Since you already have a couple of percent of reducing sugars we didn't see any benefit to adding sulfites.

However, we also looked at another amino acid like lysine. We added lysine in there to block the carbonyl source, dextrose in this case, that was ineffective. However, if you add the amino acid cysteine, you can actually decrease the level of acrylamide formation. The question is, is

the cysteine reacting with the dextrose tied to the carbonyl, or is it reacting with the acrylamide once the acrylamide forms? It is actually a later part of the reaction so it is complexing with the acrylamide.

DR. MEHENDALE: I have a follow-up question. You know, in some old literature a gentleman by the name of Serami has done a lot of work on di-cosylated end products for aging.

DR. ZYZAK: Yes.

DR. MEHENDALE: It seems to be that it was carbonyl groups of dextrose that are involved.

DR. ZYZAK: Yes.

DR. MEHENDALE: So, it seems to me like there may be some potential for either blocking or reacting the carbonyl groups with other things.

DR. ZYZAK: Yes, you bring up Dr. Anthony Serami and I did my graduate studies with Dr. John Baines, who were kind of competing with each other so I am very familiar with his work. We also did studies by adding one protein and other things in our model reactions. Maybe we can add a protein

source that will either react or just tie up the reducing sugars. We weren't very successful at that.

We did try adding a protein source to our model system and didn't seem to have a significant increase. But, again, in that case we were using something off the shelf like a relatively inexpensive source. I mean, you can go out and buy some yeast products which may have a high content of glutathiamine. It is a very pricy product. So, if you want to try to reduce the level of acrylamides, you could probably incorporate a source of protein which may have a high concentration of thiol groups in there which are known to be very active with acrylamide once it is formed. People have done studies looking at amino acids. Amine groups will react with acrylamide but not very readily where there are high thiols.

DR. MEHENDALE: So, how long can we keep potatoes?

[Laughter]

DR. ZYZAK: I think many people in the

industry are also looking at that, you know, how is acrylamide going to be affected as later in the season we are using older potatoes? I think we are all looking at that now and I think many people are addressing that so it will come up in the future I believe.

DR. MILLER: It depends on how you cook the potato.

DR. ZYZAK: Sure, yes.

DR. MILLER: Any other comments? Yes?

DR. TORRES: Are there any other food systems where there would be a lot of free asparagine?

DR. ZYZAK: Yes, asparagus has a high level of free asparagine I believe. I believe at the subcommittee meeting Dr. Lauren Jackson showed some data that the level of free asparagine is high in almonds. I think it is high in legumes, beans, bean products. Asparagine actually is used by plants as a source of nitrogen storage system. So, most plants utilize asparagine to store the nitrogen for further use as energy or convert it

into protein. So, I think we are kind of stuck with this because, you know, that is the way plants are going to grow so they are going to use nitrogen as a fixation source until we can use some biotech and utilize some other source of nitrogen.

DR. RUSSELL: I was just wondering is there much of a difference between white potato and sweet potato in the acrylamide formed under similar conditions?

DR. ZYZAK: You know, I don't have that data. We haven't done that experiment but I think definitely there is activity in that. We, ourselves, know that the variety of potato will affect the level of acrylamide because different varieties of potatoes will have a varying factor of asparagine in them and reduced sugars so we are also looking at that.

DR. MILLER: It seems to me that most root vegetables cooked at high enough temperatures should be excellent sources of acrylamide.

DR. ZYZAK: Yes. We actually talked with kind of a potato professor in industry just to

understand more about how it is using asparagine so we can get potatoes with a lower source of asparagine. There doesn't seem to be a lot of information out there about that. You know, I was specifically told that roots are different from tubers so I don't know all the botanical aspects of that but it can be different whether it is a potato versus a carrot.

DR. MILLER: Well, if it stores asparagine as a nitrogen source then, depending on how it is cooked, it will have a high level of acrylamide.

DR. ZYZAK: Exactly, yes. I think any source of product out there that has free asparagine, if you cook it under conditions where you are going to drive off the moisture and heat it up, you are going to get acrylamide formation.

DR. MILLER: Certainly the big concern would be legumes as well.

DR. ZYZAK: Yes.

DR. BUSTA: Is this information readily available to anyone who wants it now?

DR. ZYZAK: Which information? What I

just presented? I think it is going to be up on the website so anybody can download it.

DR. BUSTA: How about before this?

DR. ZYZAK: Yes, actually the JIFSAN--you know, you have heard the struggle between people whether you are in academia and there is a need to publish--I think Procter & Gamble is a great company to work with. Actually, at the JIFSAN meeting back at the end of October I presented the mechanism and I told people we identified these intermediates and I informed people we used the enzyme to confirm that. At AIOC we showed mechanism formation, which was in late September. So, once we felt confident and we knew this was the mechanism we have been trying to be forthcoming to the industry and the academic people, releasing the information.

DR. MILLER: Terry, do you have a comment you want to make?

DR. TROXELL: Thank you. If you look at the spectrum of foods in which we find acrylamide, you are tracking foods that contain enough

asparagine and glucose to form acrylamide so we are talking about wheat products, corn products. So, it is not just tubers and so on.

DR. MILLER: No, no, that is the point I am trying to make.

DR. TROXELL: Exactly. Might I ask a question of the speaker?

DR. MILLER: Not a good idea! Johanna?

DR. DWYER: I was just wondering, I think I followed your chemistry but I wasn't sure about instant mashed potatoes. Would those be high because of the extrusion product?

DR. ZYZAK: You may have small levels.

DR. DWYER: I am talking about the instantizing process.

DR. ZYZAK: Yes. In industry I think most of the mashed potatoes you buy from the shelf actually have sulfites in there but there is still a level of acrylamide. We are even monitoring our flakes, our starting material, and during the flaking process of potatoes they go through cooking and they are mashed and they undergo a spray-drying

process and, yes, there is actually a small amount of acrylamide. I think it is probably around 100 ppb but you also have some precursors there too which are formed, such as the Schiff base. So, during the formation of these dried potato products you do have a small amount of acrylamide and probably some precursors.

DR. MILLER: Depends on how they are cooked again.

DR. ZYZAK: Yes.

DR. MILLER: Other questions or comments? Thank you.

DR. ZYZAK: Thank you.

DR. MILLER: Our next speaker is Dr. Robert Brown, substituting for Dr. Steve Saunders, from Frito Lay.

Reduction Strategies

DR. BROWN: I feel very fortunate to be here today. There have been some very good presentations this morning and it has been nice. I don't have a handout. We will have to print one off. Anyhow, I feel fortunate to have heard the

particulars this morning. There is some interesting science going on and it is amazing how far we have moved forward in a short period of time on this. FDA and other groups have really moved forward quickly.

[Slide]

I also feel very fortunate to be standing in here for Dr. Steve Saunders because Steve is not only my mentor and my colleague but he is also a good friend of mine. Unfortunately, Steve was not able to be here today due to unforeseen circumstances and I know that he wishes he could be here to be making this presentation today, and he wanted me to convey to all of you his sincerest regret for not being able to be here in person to make this presentation. I am a nutritionist and I was attending a nutrition meeting in town, and he asked me if I could step in and present this information for him. I am sure I can't really substitute for Steve but I am going to give it a shot. That said, I want to present the slides that Steve sent to me and the notes that he provided for

me.

[Slide]

That was an excellent presentation from Procter & Gamble today, very compelling information on the formation of the acrylamide passing through a Maillard reaction product and that is what we have here so I can just skip past this.

[Slide]

If we look at the first intermediate of the reaction between asparagine and glucose or reducing sugar, we see this intermediate. When the typical Maillard reaction product is formed that typically has an energy activation level of about 25050 kilo calories per mole. These products that are formed are the typical browning colors and the flavor compounds that are formed in the typical Maillard reaction product.

As David mentioned, there is a second pathway, a minor pathway proceeding through the Schiff base and going to decarboxylation and beta elimination and proceeding to acrylamide. We have done some work in our laboratory on a model system

similar to what P&G has done, and we have estimated that the energy of activation of acrylamide formation is on the order of 70 kilo calories per mole. So, you see, it takes more heat energy to form this compound and it is more of a minor pathway as compared to that going to the Maillard reaction products.

[Slide]

Clearly, our first insight then is that in a chemical pathway leading to acrylamide is a low yield pathway with a higher activation energy. This will be demonstrated in a couple of slides I have coming up to show a difference in concentration between reactants and the products in this reaction.

[Slide]

If we look at a summary of the data on acrylamide values in food, you are all very familiar with this data but I want to make a couple of points about the different concentrations of acrylamide in foods. First of all, if you look at the foods on this table you will see that there is

a wide variety of foods that contain acrylamide, and across these different foods there is a huge range of concentrations of acrylamide found in these food products.

Additionally, even across and within a category of food there is a very, very wide range of acrylamide formation. You will see in some foods that the range of the acrylamide can be as much as two orders of magnitude. So that is quite a bit.

The third thing is that undoubtedly we are going to uncover more food products that are going to contain acrylamide, and I think the data that was presented by Procter & Gamble makes it clear that we probably can find those foods quickly by determining the concentration of asparagine in those foods and looking at potentially the cooking process, and then looking at the level of reducing sugar in those foods.

[Slide]

This slide looks at those different foods that were on that list. We did a food consumption

survey and we looked at this information to look at all foods that contain acrylamide to get an idea of what the impact on the American diet would be. If we look at this list, this list shows that approximately 38 percent of total calories consumed in the American diet are foods that contain acrylamide. As you look down the list you see that, of course, many of the nutrients at about that same level also are coming from foods which contain acrylamide. The variation in micronutrients is dependent on the type of food, some of which is fortified. For instance, many of the bread products are fortified with iron, and such. So, that level of iron would be higher than you would expect as a percentage of the calories.

[Slide]

Clearly, this insight tells us that the acrylamide question really is affecting a large fraction of the American food supply and is something that we have to be concerned to understand.

[Slide]

When we began our thinking at Frito Lay about means to look at the issue of acrylamide formation and how one might begin the investigation of reduction of acrylamide in food we organized our thinking in these three areas: One could look at removing the reactants, either the glucose or the asparagine; one could look at disrupting the reaction to form acrylamide by a number of means; or one could look at removal of acrylamide once it is formed in food products.

Finally, at the end of the talk today I just want to talk a little bit about the significance of the study of exposure to acrylamide, which should be an interesting endeavor that we are looking forward to.

[Slide]

If we start with looking at ways to reduce the reactants, first of all, this data is coming out of Dr. Mottram's lab at the University of Reading in the U.K., and if we look at this, this was mentioned just a few minutes ago, the difference in acrylamide that is potentially found

in different potato products and, in this case, what we see is that if we look at the baking potatoes as compared to the King Edwards potatoes we will notice that in the raw state we find essentially no acrylamide. In both potatoes, when they are boiled, in other words the heat stays below 100 degrees C, we see no formation of acrylamide. But once the products are fried we see the formation of acrylamide. In this case we see a ten-fold difference in the formation of acrylamide between the typical baking potatoes and the King Edwards potatoes.

Clearly, what we would anticipate from the information we just saw from Procter & Gamble is that the level of reactants is probably different between these two, whether it be more reducing sugars in King Edward's potatoes, though those were not measured in this particular study, but that would be a good assumption that we might make.

DR. MILLER: Just a matter of clarifying something, which is the potato generally used to make chips?

DR. BROWN: There is a variety of potatoes used in all industries. Frito Lay uses a proprietary potato and I am not sure of the exact variety name of that one. We call it the Frito Lay variety and it is called a chipping potato, and they tend to be lower in reducing sugars but, as was mentioned, as potatoes are stored over time the levels of reducing sugars rise.

[Slide]

In this slide if we look at the asparagine in various crops, as was mentioned just a couple of minutes ago, in fact the level of asparagine does vary quite significantly across crops. As you look at this, there are many crops and food products that have quite a high level of asparagine. In this case, you see in some of the products that there is a huge range of asparagine level in potatoes, looking at 0.5 to 10 milligrams/gram. In other products such as asparagus there is a huge range in the level of asparagine. Then, if you look at wheat the range can be 100-fold difference between low level wheat and high level wheat. So,

what we have is a system that has a lot of complexities in it and you can't look at one solution that is going to work for everything.

Just to mention too, this table was compiled by the Food Research Institute and they have a list that goes quite a bit longer than this, but just to know that we have a very complex problem involving the entire food supply.

[Slide]

In our laboratory we did a model system and we looked at the substrate concentration and applied the reactants, both glucose and asparagine, and looked at the change in concentration with the formation of acrylamide. What we see here is a second order reaction where the maximum level of acrylamide is formed when both the substrates are in fairly equal concentration. Whenever one of the substrates is at a reduced concentration you see that there is quite a large reduction in the level of acrylamide formation. This is an interesting insight for us because at this point we were able to understand that you need to look at your

specific food product and make the determination which is the reactant that is highest in that food product and that is the one that is probably going to be the one reactant that you want to go after in that particular food product. The equation on the bottom of the slide describes the fit of the surface plot.

[Slide]

Then, the insight is that the reaction is a second order reaction and that the concentrations of the two reactants need to be in fairly equal concentrations to get maximum acrylamide formation. The reaction becomes very limiting for that reactant that is at lower concentration. So, in the case of the example that Procter & Gamble brought up when the reducing sugars are at much lower concentration than the asparagine in potatoes the reducing sugar is, in fact, the rate limiting step on the formation of acrylamide.

[Slide]

The next area that we want to look at is what is the possibility of disrupting the reaction

of acrylamide formation. If we go back to the data from the U.K. and we look at the bottom there and we look at what is the effect of cooking on acrylamide formation, if we look at the data on the bottom there, you look at frozen frying potatoes, French fries, and you look at a cooked product having approximately 3500 ppb acrylamide, then overcooking the product increases the level of acrylamide by four-fold. This demonstrates very clearly the time-temperature relationship to the formation of acrylamide and is something that gives us insight into some of the things that we may begin looking at to reduce the formation of acrylamide.

[Slide]

In our laboratory we wanted to validate our model system and this is similar to data that was done by Mottram et al., in their lab in Reading. In Reading, they showed that there was an inflection point of formation of acrylamide at 120 degrees C.

I would be interested in the data that was

presented on microwave cooking, looking at the moisture level of the products that were done in the microwave, what the real temperature was at the surface of the product in formation of acrylamide and looking at the browning reaction. If there was a browning reaction that was actually taking place in the microwave, I think that the surface temperature may have been higher than 100 degrees, but that may not be the case. I don't know if they had measurements of the surface temperature but that would be interesting to know.

But in our model system in the laboratory, if we look at this in the model system we have no formation of acrylamide at temperatures under 110 degrees C. The reaction really starts going as you go above 120 degrees C and then is exponential in the increase in rate of acrylamide formation. I think this data is important because, as Terry mentioned earlier today, the study to look at surface temperatures and being able to probe, temperature at the surface is going to be very critical. And, the information from Procter &

Gamble regarding moisture content--in most food systems, as you know, if the moisture content is high you cannot drive temperatures beyond 100 degrees C. As you drive off the moisture, that is when your surface temperatures can actually reach above 100 degrees C and that is where you will see the formation of acrylamide beginning.

[Slide]

We plotted a kinetic model of the formation of acrylamide over temperature in Kelvin here, and if you look at the formation of acrylamide in this kinetic plot what you see is that the inflection point appears to be around 120 degrees C. From that point the rate of acrylamide formation is very rapid. The rate on the bottom of the chart, on the right-hand side there, is approximately 175 degrees C and that is the typical baking temperature for most food products. As you can see by that temperature, at 175 degrees that is well above the temperature to drive the formation of acrylamide to a maximal rate.

[Slide]

The insight, therefore, is that acrylamide formation is extremely temperature dependent and occurs well below temperatures needed in typical baking or frying operations. It is probably not possible to cook products without any formation of acrylamide. The surface temperature studies that will be undertaken I think will be very interesting, looking at whether one could modify the final surface temperature of products by alternate cooking methods.

[Slide]

We have also investigated the pH dependency of acrylamide formation in foods and we found that at pH under five acrylamide formation is severely inhibited. Even at the pH of six there is some significant inhibition of acrylamide formation and as you get towards neutral pH you see that the acrylamide formation is maximized at a pH of around seven.

[Slide]

A very interesting idea in the whole disruption of acrylamide formation is can you come

up with an inhibitor of acrylamide formation similar to the case of vitamin C added to reaction to inhibit the formation of nitrosamines. It is a very exciting idea to think that we could come up with something like that, but in this case the whole Maillard reaction process is one of amino acids forming the color and flavor compounds that we expect in our cooked foods and in this case we are trying to inhibit a single amino acid reaction rather than the whole cascade of free amino acids that would react.

However, one other point on that is that the inhibitor would have to be food safe and it would have to be approved for use in foods. I think we are all hoping that a similar simple story can be developed, and we have seen published reports and we have heard from Procter & Gamble today about some of the potential ingredients that could be added to foods to inhibit this reaction. Rosemary flavonoids have been reported to inhibit the reaction of asparagine going to acrylamide but we haven't seen data on that.

There was, of course, the interesting JIFSAN conference that we just heard about, and the use of the amino acid cysteine to inhibit this reaction. It would be interesting to look at what levels of cysteine could inhibit the formation of acrylamide and how could it be added to foods. Obviously the wide number of foods we have would require different mechanisms to incorporate cysteine into a surface of a product that would try to inhibit this reaction because there would be no need to have the inhibitor throughout a product if, in fact, the formation of the acrylamide was occurring only on the surface of the product where the browning is taking place.

In our model system we have also studied other inhibitors that might be functional in this area, and we have looked at divalent and trivalent cations and found that they also inhibit the formation of acrylamide. However, it takes a large amount of the divalent or trivalent cation to have this inhibition come into place. It takes approximately one equivalent of cation per mole of

reactant to inhibit the acrylamide formation. We are not sure how these could be practically applied to foods. It may not be successful in all types of food because, again, of trying to get the cation in the area to inhibit the reaction.

[Slide]

If we turn to the kinetic model of Wedzicha and Mottram we have a pretty interesting array of areas that we might look at to try to develop a mechanism to inhibit the formation of acrylamide. As we see here, if glucose is in fact in a particular reaction system, the rate limiting step, there would be means to force the reaction of glucose, to deplete the glucose by other reactants that might react with glucose such as other free amino acids that would compete against asparagine for the formation of this reaction. Also, pH, temperature and time variables are other potential things that we might look at. If we can look in the cooking process at some way to control the final surface temperature of the product as another means of looking to inhibit this rate of formation.

As we learn more about these reactions and these kinetic constants out of the lab, we hope to learn much more where the best place to attack this issue will be.

[Slide]

Finally, I want to talk just a couple of minutes about our attempts to actually remove acrylamide after formation in food products. In this case we have tried a couple of things. The supercritical CO-2 is very effective in removing acrylamide. Of course, it removes everything and completely destroys the product.

[Laughter]

UV light is something that we had high hopes for to be an effective use, a fairly simple technique to cause the acrylamide to polymerize and effectively eliminate the hazard. In this case we take ground product. We expose it to all levels of light. I think we came up with this, remembering back when we used to make polyacrylamide gels and we thought this was something that, you know, was really going to work. We exposed to product to

wavelengths of light ranging from UV to red and essentially we have found no effect at any wavelength that we have tried to date. Potentially the level of acrylamide is too low in food products for this to be effective.

[Slide]

Finally, I am going to talk just a little bit about the toxicology of acrylamide and its presence in the food supply.

[Slide]

On this first slide is data that was also developed for us looking at the food consumption survey and mirroring that to the data on acrylamide content of foods consumed in the American diet. If we look at the total here and then if we look at the red line on the bottom, which is essentially drawn on the X axis, that red line on the X axis would be the standard risk assessment line that would be developed under standard techniques of developing risk assessment for average daily exposure. As you look at that line you can see that it is orders of magnitude, at least two orders

of magnitude lower than the exposure of acrylamide in the U.S. food supply. You can see that no one food is going to be the bulk of that. Assuming that we can eliminate the content of all potato products, fried potatoes, other kind of cooked potatoes, mashed potatoes and potato chips and we remove the acrylamide from all those products, we are still in the range of two orders of magnitude too high with the total acrylamide concentration in the diet using standard risk assessment techniques to form this line.

This is where I think the research on toxicology, looking at the low concentration versus high concentration p450, 2E1 metabolism of acrylamide is going to be very important to understand the difference in low and high concentration of acrylamide in the diet.

[Slide]

Steve gave me some food for thought here. Everybody is probably not that hungry right now but I didn't have any lunch so I still have some room for food. The whole idea of carcinogen in food is

not a new one to us, and is something we have dealt with before if we look at cooked meats and such. We also have the NAS report and the Ames/Gold as other references to talk about that.

Also, as we evaluate acrylamide we have to understand that humans have been cooking foods for millennia and we have been exposed to acrylamide for all those years. So, we need to understand what is going on and what is happening or not happening with acrylamide exposure low dose versus high dose. We need to understand the toxicology there because it is going to be very important to us.

As Terry said this morning, there are going to be no quick fixes of this issue because acrylamide is going to be so widespread in the entire food supply. We are going to have to look at different bullets. Instead of one magic bullet for all foods, there are going to have to be different bullets for different food products as we move forward to look for means to address this issue.

[Slide]

As we begin doing feasibility analyses of what kind of intervention we can have in the food supply and what we are going to do, we really need to understand the whole kinetics and the removal of substrates from food products. We need to understand each individual food, determining whether in this case the asparagine or the reducing sugar would be the rate limiting step for the formation of acrylamide.

Also, if we look at low temperature intervention what would be required in the development of new cooking techniques that could potentially reduce the level of acrylamide in foods. For some foods it will be impossible to develop these low temperature techniques. In other foods it may be possible to use two-step cooking where you heat at higher temperatures before moisture levels are driven down and then lower temperatures as the food product is drying out and temperatures on the surface can increase.

Again, there are no magic bullets here.

We have to look at individual potential solutions to each of the problems, and there is absolutely no precedent to the kind of ordered magnitude of intervention we have to have in the food supply from the processing to the cooking to the growing of foods in our food system. It is dependent on what we find in the toxicology and how far we have to drive the acrylamide level down.

[Slide]

Then some final thoughts here, the issue affects a large portion of the food supply. Lowering acrylamide clearly in one food is not going to do much to lower the overall level of acrylamide exposure in the population. We need to understand the toxicology here so that we know what our target is in this case.

Clearly, as was mentioned this morning, foods cooked at home and foods cooked at restaurants are going to be a significant source of acrylamide so foods that are processed by the manufacturer are taken into the home and then baked out, for instance as apple pie. Those foods are

going to have a large potential exposure to acrylamide so we need to look at methods that would also address those.

If we look at what does the future look like, given the magnitude of the change in the food supply that could be represented by this, we need to really understand the nature of the low dose hazard of acrylamide to humans, and we need to really look at the impact of any proposed interventions and the consequences, if there are any unintended consequences to the public health. In going forward, as we begin to study the toxicology of acrylamide, we need to be simultaneously looking for all the interventions that are possible to be driving the acrylamide level down so that we are working on both ends of the spectrum, to both lower acrylamide concentration in the food supply and to understand the real significance and the real health effects of acrylamide levels in the food supply and on human health. Thanks very much.

Questions of Clarification

DR. MILLER: Thank you. Comments or questions?

DR. DWYER: Thanks, Bob, for a very, very interesting talk. I just have a question that is sort of silly but I was wondering does free asparagine have a taste in food so if you took it out it would taste different?

DR. BROWN: I don't know. I am not a flavor chemist so I am really not too sure. Thanks very much.

DR. MILLER: Thank you. We are ready to take a break. We are a little early again. I will tell you what, why don't we go ahead with the exposure assessment, Dr. Robie? We will go ahead and have that and then we will take a break after she finishes.

Exposure Assessment

DR. ROBIE: I am going to be referring to the handout you have in your package. I am going to be presenting the exposure assessment for acrylamide for FDA as prepared by Dr. Michael DiNovi and myself.

[Slide]

I am going to start out the presentation by going through some history, then I am going to move right into our exposure estimates and the model that we used, the assumptions that we made and the future work on it, and then the results and the interpretation.

[Slide]

You have already seen this a bunch of times. Less than a year ago a group of Swedish scientists reported the occurrence of acrylamide in food, and in their report they included a preliminary exposure estimate. This was based on acrylamide data in about 100 food samples. They split that into eight food categories. They may some assumptions about the foods for which they didn't have acrylamide data, and they calculated a preliminary exposure estimate of about 0.7 mcg/kg body weight per day for a 60 kg individual.

[Slide]

Then, about two months later, two months after the Swedish scientists publicized their

findings, there was an FAO/WHO consultation held to discuss the issue of acrylamide exposure through the consumption of food. They also performed some exposure estimates. They used the same residue data as the Swedish scientists used in conjunction with some food consumption data from several national food consumption databases. They used a couple of different approaches to exposure estimates but the bottom line they reported was a range of about 0.3 to about 0.8 mcg/kg body weight per day, which is in agreement with the Swedish scientists.

[Slide]

That is all the history I am going to bore you with. Now I am going to go on to how we approached calculating the exposure to acrylamide. Really we approached it in the same way that we would approach the exposure to any additive or contaminant or naturally occurring substance in food, following this general equation.

Basically, we need information on the concentration of the substance that we are

interested in, in the food. Then, we need information on the food, and we need to know how frequently it is consumed, and we need to know how much is consumed and when it is consumed. We get the frequency and portion size from the food consumption databases and the concentration data for acrylamide we get from the laboratory. This expression, this multiplication takes place for every food, each individual food, the concentration of a food, times the intake of the food. This is summed over all of the foods that would contain the substance to get estimated daily intake of the substance for an individual. Then, this information is summed over individuals to get an EDI for the population. Again, this is the way we do it for every food additive, everything in food. So, there is nothing different there.

[Slide]

Food consumption surveys--I mentioned that we are going to be getting a lot of our data from them. We use three food consumption surveys. Let me explain them, the differences between them and

why we use three.

The first two that we use are CSFII surveys. We use a three-day consumption survey and a two-day, and each has about 20,000 participants. We just wanted to see the comparison between these two. This is going to help us evaluate the robustness of our model. Of course, in making this comparison we have to have knowledge as to how the number of days of the surveys is going to affect the outcome, the final answer that we are going to get. So, that is what I have in this last bullet. I was just going to leave this up here and then it was explained to me last week that this might not be intuitive so I am going to spend a couple of minutes explaining what this means.

The percent eaters, the percentage eaters value that we get from our food consumption survey is the percentage of eaters that report eating or consuming food on any one day of the survey. So, the longer the survey, the better chance we have to get higher percentages. We capture more of the eaters the longer the survey duration is.

The intake for the eaters is considered overestimated for a shorter survey because the intake for the eaters as reported from the survey is reported as an average over the days of the survey. So, the more days of the survey, the lower the intake for the eaters, the mean intake for the eaters.

[Slide]

We also use an MRCA 14-day survey. Again, we had the data; we wanted to make the comparison and test the robustness of our model. After saying what I just said about how the difference in the number of days is going to affect what we expect to see from the survey, I need to point out that this is a 14-day survey but it is 14 days of reported frequency. So, the percent eaters is going to be higher. The participants in the survey, every time that they consume the food, they report it as a consumption occasion. So, we are going to get percent eaters but they don't report the amount of food. The amount of food we get from the USDA Nationwide Food Consumption Survey so the frequency

data from MRCA is linked to this food consumption survey and this is a three-day survey.

So, I am going to talk about the differences between the CSFII surveys and the MRCA surveys because, again, we are going to be comparing them and we have to have knowledge as to what the differences are between them to make valid comparisons. There is a lot of difference in the food groupings between the two surveys. The CSFII food coding system allows us to be a lot more specific about the foods that we choose. The MRCA survey has big, broad categories. For example, for the CSFII survey we were able to separate crisp breads from crackers and other salty snacks, whereas for MRCA we weren't able to do that and they were all grouped together. When we get to the data and you see the tables you will see how the categories are changed slightly.

Another difference in the surveys is the time periods over which they were carried out. For the MRCA we are talking about early to mid-'80s, and the two-day CSFII survey was carried in the mid

to late '90s. Certainly, food consumption patterns and habits have changed over that time. Whether or not it is significant enough to affect the model is debatable but it is a difference that merits mention.

[Slide]

I am not going to spend a lot of time talking about this because our office is not actually doing this. This is work that is being performed by Dr. Clark Kerrington with the Office of Plants, Dairy Foods and Beverages Risk Assessment Division. He is looking at taking the two-day CSFII data, our most recent data, and expanding it to longer than two days. We really want to model product exposure and using a two-day, three-day or even a 14-day survey may not be that appropriate. I mean, it is data that we have so that is what we are using right now but Clark is going to be working on adjusting this two-day CSFII survey data and that is work that is in progress right now.

[Slide]

So, we are back to our exposure equation. This is basically the same equation that we had a few slides ago. The food frequency and portion size are combined here from the information that we get from the food consumption survey. Again, we multiply that by the concentration of the substance that is in each individual food. This is summed over food and summed over individuals. This is typically done at the mean so we arrive at a point estimate. The result we get is one point. This approach is useful for substances only in a few foods or when the EDI and the ADI to TDI in this case are very different from each other. In the Frito Lay presentation we saw that the ADI and TDI are not that different from each other so these two things don't really fit for acrylamide. Acrylamide is in a lot of foods so we decided to use probabilistic modeling.

So, instead of just using the means and getting point estimates we are able to use the entire distributions for the food consumption and also for concentration data. I am going to talk

about this in a lot more detail in the next few slides.

[Slide]

Probabilistic modeling is an iterative process and for each iteration, as I said, we use the whole food consumption distribution so each iteration is a random sampling of the food consumption distribution for an individual food. In a similar way, the computer will randomly sample the distribution for the acrylamide level that we have so we are not just going to be using an average acrylamide value that we have for each food. We are going to actually use distribution and then we are going to again apply the percentage of eaters to the result we get from multiplying the food consumption to the acrylamide level.

[Slide]

I mentioned random sampling. I am going to show an illustration of what that is. This is known as Monte Carlo sampling. To be strictly correct, Dr. DiNovi and I used a variation of Monte Carlo sampling called Latin hypercube sampling.

But for purposes of what we are talking about today, which is illustrating how the food consumption distribution is sampled, this sampling is certainly appropriate.

What we have is the cumulative probability distribution for a given food from zero to 100 percent. So, from zero to one the computer generates a random number for each iteration. It generates a random number from zero to one. So, in this case, this is where the computer is generating a random number for this iteration. This random number is used to sample the food consumption distribution. This is the generated value for food consumption that we got for this iteration. The acrylamide concentration distribution is sampled in a similar way. Again, those two numbers for each iteration are multiplied together to arrive at the acrylamide intake that we will get from consuming that much of this particular food.

[Slide]

For this expression I am going to be using AA for acrylamide throughout this presentation. I

believe this is probably the first time you have seen this. This is just the same exposure equation that we have seen, just reworked a little bit. The food amount, the information that we get from the food consumption surveys is here. We are going to view this as one iteration. This is what is happening for each iteration for each food. The acrylamide level that is randomly sampled from the acrylamide concentration distribution is put there. Then, these two things are multiplied together to give us the amount of acrylamide that this iteration or virtual consumer will be exposed to by eating this amount of the food containing this amount of acrylamide.

Now, I have been saying that we are applying the percent eaters to this, and the way that we do that is also in each iteration and either a zero or a one is multiplied by the amount of acrylamide that we have decided we get from eating that food in this expression, here. One is in proportion to the percent eaters. Let's say a certain food is reported as having 80 percent

eaters, for 80 out of 100 iterations this number is going to be one, and this food will be considered to be eaten by this iteration, this virtual consumer. The other 20 times this number will be zero and there will be no contribution to the total acrylamide for this iteration or this consumer from this particular food.

Again, this expression is for a food for an iteration. The values are summed over all the foods and then we have combined all of the iterations to arrive at distributions for acrylamide intake, which I am going to be showing later on in the presentation.

[Slide]

This is what we will refer to as the first page of the handout, virtual consumer number one. I just want to illustrate the point that we showed on the last slide a little bit better by actually showing you the output that we see on the computer screen when we run an iteration.

I just have the first seven rows reproduced up here. You have the whole table. The

food consumption of the eater is the result that we get from sampling the food consumption distribution for this iteration for each individual food and then the acrylamide concentration, again, is sampled from the acrylamide level distribution for this particular food. These are multiplied together and, again, they are multiplied by either zero or one depending on whether or not this food is considered to be eaten by this consumer for this iteration. So, we either have a zero here or we have a number for acrylamide contribution to this particular eater. These are all summed then to arrive at the value of 0.49 for this particular eater or this particular iteration.

We have provided you a couple of additional virtual consumers in your packet. I think there are three more virtual consumers just to give you an idea and make sure everybody understands the point of what we are doing here and the results that we are getting.

[Slide]

We did 25,000 iterations. I am going to

explain some of the assumptions that we made in the model. The first one is that there has been no accounting for correlations between food choices, either positive or negative. Examples of that could be if you look at virtual consumer number four, virtual consumer number four is shown as consuming both oven browned and restaurant French fries on the same day. All of this is entirely possible as an example of a negative correlation. A positive correlation could possibly be between peanut butter and bread or coffee and toast. These are things that we haven't included in the model. It is not that it can't be done; it can be done but historical knowledge has shown us that really it won't make that much difference and won't have that much of an effect on the bottom line result that we are going to get from running the model.

Another assumption that we made, or what we wanted to make you aware of at least, is that we have used the food consumption distributions only from zero to the 99th percentile. The 100th percentile we saw what we considered to be some

pretty irrationally high values. I know that is a strong word but 13 liters of coffee for one person in one day not only seems like a lot of coffee but it is also 11 liters higher than what is reported at the 99th percentile. So, we didn't want these numbers, which we considered irrationally high and possibly reporting errors or calculation errors of some kind, to interfere with our model. Another example of that is cookies, 620 grams of coolies per day was reported at the 100th percentile by the CSFII two-day survey and the recommended portion of cookies is 30 grams, and also at the 99th percentile this value of cookies was 130 grams. So, you know, it is a five times difference between the 99th and 100th percentile. So, we just went ahead and took off the 100th percentiles and used from zero to 99.

DR. BUSTA: Why did you use zero?

DR. ROBIE: It is possible that people consumed zero. I see what you are saying, to just cut off both extremes of the distribution. There is no zero percentile? Good point.

[Slide]

Some of the limitations that we see in the model include the surveys and the laboratory data which are two inputs for getting information from the surveys about the food consumption. We have already talked about these. We have talked about the duration of the surveys. Two or three days, even 14 days to model product intake is not an ideal situation. And, the food classifications, I have already talked about, especially for the MRCA. They have very broad groupings. It would be better if we could separate the groups as much as possible.

A primary limitation of the model is laboratory data. You have heard people talk about the laboratory data all day and I am going to do it again. Some of the samples that we have of food types are represented by fewer than five data points from our laboratory and we see that as a limitation. Most of these are the samples that are lower consumption and don't contain a lot of acrylamide. We have tried to focus on the ones

that we think are going to have a big impact on the total overall intake for the population.

Another model limitation that we see is variability in acrylamide levels in different foods. Again, you have already heard people talking about this all day today. What we do see is that as you go down this list we see greater variability. There is a significant amount of lot-to-lot variability, even more brand-to-brand variability. Different products can be included in the same food category. I don't want to pick on potato chips here but we have a lot of potato chip data so we have definitely seen this. We have seen differences in lot-to-lot, brand-to-brand and product-to-product. If you have a baked potato chip versus a fried potato chip, they both have acrylamide. The differences between the two are pretty drastic.

Then, there is always the problem with the foods prepared at home and, again, you have heard about this. We do have data on toast and we have data on oven browned French fries but these are

samples that were prepared in our laboratory following package instructions and I don't really think we have probably captured the variability that we are going to see in people making it at home to their color and taste preference.

[Slide]

Just one more slide and then we will go to data. We wanted to make you aware that we did apply some factors to some of the food types, typically the foods that are consumed as liquids. The analytical technique that we are using makes it a lot easier to test these as solids than as liquids as they are consumed but, of course, they are reported in food consumption surveys by the survey participants as liquids as they drink them or eat them.

So, for the last three on this list, instant coffee, dry soup and dry cocoa powder, the calculating effect was pretty straightforward in that we are taking the amount of the dry powder that we are going to then add a known amount of water to and dilute and consume the entire amount.

So, whatever is in the package, you are consuming it all.

For ground coffee it is not so straightforward because when you make coffee you don't eat the grounds. We know how much acrylamide is in ground coffee. What we need to know is how much acrylamide is in coffee as consumed so we can compare it with the food consumption data that we have. We use a value of 24 for this. This has been experimentally derived by Dr. Musser's lab. They measured the acrylamide in the ground coffee. They made coffee from it and then they measured the acrylamide in the coffee as consumed. They are still working on fine-tuning this number, if it is 25 or 23, but we are right there with the preliminary value that they have given us, I am sure.

[Slide]

As I said, we used three food consumption surveys and data from those and we did it for two populations. So, really you are going to have six slides for these. Instead of tabulating all 30

foods for all three surveys for both populations, I am going to go ahead and just show two slides just to show you all the foods that we are considering, and then we are going to go on to the survey-specific information. Actually, these are results from the CSFII two-day survey for the two years and older population. The data that you have here is the mean population for acrylamide intake for this food. This is restaurant fried French fries; this is oven browned French fries. These are sorted in order of their contribution for acrylamide, the contribution that they are going to have on the total acrylamide intake for the population. And, we have cumulative percentiles too.

So, restaurant fried French fries will contribute 15 percent acrylamide to the total population. Oven browned French fries will contribute another 13 percent. So, the cumulative percentile there is 27 percent, and so on down the slide to soft bread. We pick it back up again at corn snacks and then down to soup mix. Here are

the last, cocoa to breaded fish and then donuts to multi-drinks, with multi-drinks having the least contribution to the total population acrylamide intake of 0.37 mcg/kg body weight per day. I know that these look like they have no contribution. I decided to only go out to three decimal places on the slide but they do contribute, just not very much.

[Slide]

Now we are going to look at data specific to the populations in food surveys. Let me refer you to page six of the handout. The way that these are ordered is just from oldest data to most recent data. There is no other significance to the order that I will be showing you.

These are the results of the MRCA 14-day survey two years old and older population. I have up here the foods contributing five percent or more individually to the total acrylamide population of 0.48. These are eight foods. You have the rest in your handouts. For each food we have the percent eaters, the mean food consumption for the eaters,

the acrylamide concentration for that food. If we multiply the mean food consumption of the eaters by the mean acrylamide concentration for each food we get an eaters only acrylamide intake, which we then weight using the percent eaters to get a mean population acrylamide intake for that food. Then we sum all these to arrive at a value of 0.48 mcg/kg body weight per day as the mean acrylamide intake for the population using this survey data.

[Slide]

This is the same table for the CSFII three-day survey. Again, the foods that we have chosen to tabulate on the slide are the ones that contribute five percent or more individually to the total mean acrylamide intake of the population which, for this survey, is about 0.32. It is a little bit less than what we saw for the 14-day survey but, again, over 14 days we are going to have a lot greater percent eaters. That is why this value is lower here than for the MRCA 14-day survey.

The other thing of significance to look at

on this slide--actually, I was going to show you the percent eaters difference. Look at the percent eaters for potato chips here. It is 18 percent for this survey and it is 76 percent for MRCA 14-day survey. So, the difference in the percent eaters can be very significant for some foods.

The other thing to note on this slide as compared to the slide for the MRCA data is that these top foods that each contribute five percent or more are the same for both surveys. The order is changed a little bit but the top contributors are the same for both surveys.

[Slide]

Then I have the same slide, which is the next page in your handout, for the CSFII two-day survey. The bottom line here is 0.37 and we saw 0.32 for the three-day survey. It is a little bit higher, which is expected, but everything is pretty much in the same ball park. Again, the top eight foods are the same. The order may be a little bit different but the top contributors are the same no matter which survey you look at, which shows us

that our model is very robust.

Another thing I just want to point out is that all these values are very consistent with each other that we have gotten from these three surveys, and also they are consistent with previous exposure estimates which I talked about on the first couple of slides of this presentation.

[Slide]

We are going to go in the same order of surveys for two- to five-year old population. That is on the next page of the handout. The first things that are probably pretty obvious to you are that we no longer have eight foods; it is seven foods because the coffee dropped off. Two- to five-year olds aren't drinking a lot of coffee, which is good. Each of these foods, these tops seven foods, are again contributing five percent or more individually to the mean acrylamide intake for this population and it is the same seven foods as we saw on all the other slides, except for coffee.

Another thing that I am sure you have noticed is the fact that the mean acrylamide intake

for the population is about twice what it was for the two years old and older population. This is expected when we are talking about data on a kilogram body weight basis. Children tend to eat about half of what adults eat but they weigh about a fourth of what adults weigh. So, the factor of two is an expected result for what we see here.

[Slide]

Then, for the CSFII three-day survey data for the two- to five-year old population, again the coffee is gone and the top seven foods are the sane. The order is moved around a little bit. Again, the value that we have here is lower than we had for the 14-day. Again, we are capturing fewer eaters, and it is also about twice what we saw for the CSFII three-day survey for the two years old and older population.

[Slide]

One more table of data, the CSFII two-day survey, and we see the same trends as we saw for the two years old and older population. Again, this mean acrylamide intake for the population is a

little bit higher than we saw for the three-day survey but with a shorter survey that is expected. Again, the top seven foods are the same.

[Slide]

I have shown you tables of data, lots of tables of data. Now I am going to show you a lot of distributions to go along with those tables of data. On the data tables that you saw we showed you mean acrylamide concentrations and mean food consumption and a mean result but we really don't get out just a mean; we get distribution of acrylamide intakes. This is for the MRCA 14-day survey two years old and older population. Acrylamide intake is on the X axis in units of mcg/kg body weight per day. The mean, again, is about 4.8 mcg/kg body weight per day. This occurs about the 70, 75th percentile and that is the case for all of the survey results, all the distributions that I am going to show.

DR. MILLER: Just to clarify, we are talking here about population means; we are not talking about eaters?

DR. ROBIE: Right, we are talking about population means. I am sorry if I misspoke.

Another thing of note, the 99th percentile we have shown here is 0.91 mcg/kg body weight per day, just about twice the mean for the population, which is an expected result.

[Slide]

This is the same looking type distribution for the acrylamide intake from the CSFII three-day survey data for the two years old and older population.

[Slide]

For the CSFII two-day survey for the two years old and older population.

[Slide]

Then we will go on to the two- to five-year old population. One difference of note here is that we have expanded the scale to six. We cut the scale off at three for the two years old and older population. We have already discussed how we expect to see a doubling in the population mean intake when we are talking about two- to

five-year old population when comparing to a two years old and older population on a kilogram body weight basis.

[Slide]

Here is the acrylamide intake distribution for CSFII three-day survey two- to five-year old population.

[Slide]

And the two-day survey. So, we haven't seen anything unexpected. Again, all the surveys agree with each other and the previous exposure estimates, and that is all I am going to say about that.

[Slide]

The table that you have in your handout to go along with this slide I believe is on page five. What we have done here, just to round out the whole picture and put things in perspective, is to show you how much acrylamide an eater would get from consuming one recommended portion of any of these foods. We are applying the mean acrylamide concentration. We are multiplying these by

recommended portion sizes. These are not portion sizes that we got from any survey; these are portion sizes from 21 CFR 101. This is the labeling section of CFR, the food labeling section. These are the portion sizes that you are going to see on labels. These are recommended portion sizes.

What I have tabulated here are the top eight foods that you kept seeing consistently in the previous tabulated data. You have the full list in front of you, and these are also in alphabetical order.

I guess the thing I want to point out here first of all is that this is wrong in the copies of the slides. This is 3.2 and the actual number is 2.0. It is correct on page five of your handouts. Something else to note is that certain foods that we saw having large contributions appreciably contributing to the total acrylamide intake for the population, notably breakfast cereal and soft bread, there is really not a lot of acrylamide per portion for these foods. These are frequently

consumed foods and highly consumed foods.

[Slide]

We also ran some "what if" scenarios. Probabilistic modeling lends itself very well to carrying out these types of scenarios. What we are talking about here is looking at the effect of a chosen mitigation measure on the population acrylamide mean, final result. So, we have chosen some foods and food groups and set the acrylamide level of these foods and food groups to be zero and we ran the model. I am going to show you the results for several food groups.

It is important to note, however, that the foods are still included in the model. We are just assuming that the acrylamide can be removed from the foods. To remove the food from the model and consider it not being in the diet anymore we would have to consider what it would be replaced by and the implications behind that. So, that is not anything that we did here. We just set the levels to zero in the foods.

[Slide]

These are the results for several different food groups for the CSFII two-day survey two years old and older population. Remember, the population mean result for the acrylamide intake was 0.37. We have assumed zero concentration of acrylamide in French fries, both types of French fries, both the restaurant fried and oven browned French fries, and recalculated the mean to be 0.26 mcg/kg body weight per day.

We also ran it assuming that acrylamide would be removed from snack foods, and in snack foods we included potato chips, corn snacks, popcorn and pretzels. The mean was reduced to 0.31 mcg/kg body weight per day.

We did the same thing for breakfast cereal and coffee. The bottom line here, and Terry mentioned this in the morning, is that no one food is contributing the majority of the acrylamide to the total population of acrylamide intake that we are seeing in any of the surveys.

[Slide]

For our future work I have already

mentioned the modeling of longer-term food consumption that Clark Kerrington is working on for us to more accurately model chronic intake. We will continue to run "what if" scenarios based on the technological capabilities in industry. Also, we are going to analyze the sensitivity analysis of our model. Sensitivity analysis will allow us to determine the sensitive inputs for the model which, in turn, allows us to identify important uncertainties and that is going to help direct our future efforts in our exposure estimate.

[Slide]

In summary, we have seen that the mean population acrylamide intakes that we got from three survey are consistent with previous exposure estimates. The greatest contributors to the mean population acrylamide intake, the top eight or the top seven for the children, is the same for all surveys. We see that some of the foods that have lower acrylamide levels do contribute appreciably to the overall mean population but, again, this is because they are commonly consumed foods. On the

"what if" slide I just showed no one food or food group accounts for the majority of the mean population acrylamide intake.

Thank you very much for your attention.

Questions of Clarification

DR. MILLER: Thank you, Donna. Comments or questions? Yes?

DR. BUSTA: If you ran the "what if" scenario on the two- to five-year olds that had a mean of 1.0, knowing that the coffee wouldn't be there, would it be a similar kind of reduction or would it be more dramatic? These range somewhere between 35 percent reduction and less than that.

DR. ROBIE: Let me find the data.

DR. BUSTA: I can look for it. Down to 0.84, so go down from 1.0 to 0.84. You are talking about removing French fries?

DR. BUSTA: Breakfast cereal.

DR. ROBIE: Breakfast cereal.

DR. MILLER: Johanna?

DR. DWYER: Thanks for a very good presentation. I am sort of hung up on one thing,

and that is this business of using food consumption surveys of different lengths of time, two, three 14 days, and the issue of a person reporting they didn't eat it because they didn't eat it on that day when what you are really trying to model is chronic consumption. It could be that they do eat it but they don't eat it the day that you observe them or they report on.

It is my understanding that the group at Iowa State has been doing some statistics, as they always do, and have developed a method that may help to adjust distributions in that respect. Now, I understand that, particularly when you get up to the 90 percentiles, they can be up to the 95th percentiles. It is also my understanding that a group at the National Cancer Institute, Dr. Dowd and his colleagues, are working on something called propensity to consume that basically is just--I hope he is not here--a guess as to how often you consume something that you are telling the observer, and that has now been approved for use in NHANES. Could you tell me in your modeling if you

are taking that into account, adjusting in any way for the artifact because of those issues?

DR. ROBIE: The longer-term food consumption modeling that we are talking about that another scientist in our office is working on, I believe that is about the same as the IOC. I don't understand the intricacies and I didn't talk about it too much about it today, but this is something that has been done before and been published. I believe this is along the same lines as what you are talking about. This is just taking a little bit more time. We went ahead and did what we could with the two- and three-day surveys to see what results we could get. Of course, we are not stopping there. We are considering this preliminary. But I believe that what we are doing with these data here, these two-day data to expand over 365-day period is similar or the same as what you are talking about.

DR. DWYER: Well, if you are eventually going to be using the NHANES survey for looking at hemoglobins and all these other things and trying

to relate it to consumption it might be worth talking to the people at NCHS to find out if, on the instrument they have, they have 120 foods I think where they are asking for propensity to consume, if those eight foods that you care a lot about from the standpoint of acrylamide are included in that list. If they are not, perhaps it is worth talking to them and seeing if there is a way to get them.

DR. ROBIE: Thank you.

DR. MILLER: If you did the same analysis you did on the population of eaters only, would the top seven foods change any or would the distribution change any?

DR. ROBIE: Well, we can look at one of the tables because on your tables we do also have a column of eaters only. I am just going to put up one of the tables for anybody who doesn't have the handouts. If we were to sort on the eaters only column, certainly the foods that you are seeing right now are not going to be the same ones if you sort if by that column. We have looked at that.

The breakfast cereal is where it is because the percent eaters is high, especially for the 14-day surveys, 37 percent. So, I guess the answer to the question is yes, they would sort differently.

DR. MILLER: It just seemed to me that there were enough differences between the two, just quickly glancing at these curves, that you would get a different pattern of what the top seven or eight might be for people who are eaters and also, of course, the total intake is going to be considerably higher or at least would be significantly higher.

DR. ROBIE: For eaters only, yes, that is true.

DR. MILLER: It would be interesting to know how much greater the intake is for eaters only.

DR. ROBIE: Well, it is not really appropriate to add the eaters only values. If each of these categories was 100 percent eaters then that would be appropriate, or if we knew that they were the same eaters. That is why we have added

for the mean population.

DR. MILLER: Right, I understand. Anybody else?

DR. DWYER: I have one more.

DR. MILLER: Yes, please.

DR. DWYER: I guess the first thing I would ask if I were coming off the street is the Swedes came up with an estimate of about 0.7 and then FAO came out with an estimate of 0.3 to 0.8. You are coming out with estimates that are toward the lower end. Is this because you are from a fast food nation or is it because of some defect in the model, or is our model better and were those early estimates simply imprecisely high?

DR. ROBIE: Well, I'd like to think our model is better but I can't necessarily say. I can say for the Swedish data, again, this is a very limited data set, only 100 food samples for only eight food categories. They made a lot of assumptions and predictions for the foods that they didn't have data for, but they assumed that they had acrylamide, things like meats and vegetables

and fruits. They went ahead and assumed that they had the same average level of acrylamide as the food they did test. I believe that is why their value is so high.

As for the results from the FAO/WHO, it is hard to know reading the report. Again, they used the same residue data so we are talking about a limited data set to start with. It is not really clear from reading this report what they have done about the foods that haven't been tested at this point, if they also made assumptions or if they assumed those to be zero and ran the model. Again, there are several different food consumption databases so it is possible that the value is higher for Sweden and lower for another country and that is how they came up with this range. Their lowest number in the range is 0.3; our lowest number is about 0.3, and we go from about 0.3 to about 0.5.

DR. MILLER: Dr. Torres?

DR. TORRES: What is the body weight difference between the U.S. population and the

Swedish population? I imagine it must be significant.

DR. ROBIE: I can't say I know the answer to that question.

DR. MILLER: It really isn't that much different. Anybody else?

DR. TORRES: One last question, if you were to run the computer model a couple of times what would be your position about the value obtained? Is it going to be pretty reproducible? The question I want to ask you is how confident are you of the numbers that you are getting?

DR. ROBIE: We are very confident in the numbers. The number of iterations we have chosen is 25,000 and Dr. DiNovi and I ran it once just to see how long it took to converge. It was 5000 iterations and we have run it several times and get the same results.

DR. MILLER: We will take a 20-minute break and be back here at three o'clock.

[Brief recess]

DR. MILLER: Given the nature of the

product and how widely is used, why don't we see pizza on this list?

DR. ROBIE: Somebody else asked me the pizza question. We don't have laboratory data on pizza. We have some data on Boboli pizza crusts where they detected above the limit of detection. I can't remember the actual residue levels. Forgive me, I don't have the data right in front of me and there are quite a few data points. But I know that Steve Musser's laboratory has tested the Boboli pizza crust. I don't think we have tested any like take-out pizza from any pizza chain or anything like that.

DR. MILLER: Well, what kind of data did they get?

DR. ROBIE: For the Boboli pizza?

DR. MILLER: Yes.

DR. ROBIE: I think it was non-detect. It might have been detected but under the limit of quantitation for the method. We deemed that the values weren't high enough to really have enough data. We are talking about a Monte Carlo method so

we don't want to add something that has just a few levels that non-detects or are under the limit of quantitation.

DR. MILLER: But you have large numbers of eaters.

DR. ROBIE: Well, we would assume we have large number of eaters. I can't necessarily say that without looking at the survey data. You would assume I guess that there would be a large number of eaters but I don't want to say that unequivocally without looking at the data.

DR. MEHENDALE: To follow up, you know, crust is one aspect and also cheese and the crust, of course, is relatively low in moisture and so on.

DR. BUSTA: In one table it said 33, the crust. It is really low.

DR. MILLER: That is interesting. Given that it is basically a flat bread, you would expect to find a high concentration. Thank you.

DR. ROBIE: While I am up here, I just want to point out an error on the last two tables of the handout that you have. It says two plus

population and it should be two- to five-years population. That is one of the hazards of cutting and pasting. I apologize for that error.

DR. MILLER: Thank you for your patience. Dr. Tim Fennell, RTI International, will talk about adduct studies.

Adduct Studies

DR. FENNELL: Thank you very much. I am very pleased to be here. I would like to thank the organizers for inviting me, and I would like to thank you all for your time.

[Slide]

What I am going to talk about, what I was actually asked to talk about are adducts of acrylamide, and metabolism is one of those things that is inextricably thrown in there so I am going to talk about metabolism also.

I am going to review some of the general concepts of metabolism and pharmacokinetics, hemoglobin adducts and DNA adducts, and then get into a little bit about the history of metabolism of acrylamide, hemoglobin adducts and DNA adducts,

and some of the recent studies that we have been doing and where we are going.

[Slide]

I would like to acknowledge a number of my collaborators. I used to be at CIIT and while I was there I worked extensively with my wife, Dr. Susan Sumner. I would like to particularly call attention to a couple of people. One is Rodney Snyder who has moved to RTI with me, and Burham Ghanayem at NIEHS who has collaborated with me on Cyp 2E1 null mice. I would also like to call attention to the various sources of support I have had from CIIT and from the acrylamide industry, and in particular most recently SNF who is currently funding some of my studies.

[Slide]

Going back just to give you a little bit of history, I started work on acrylamide in 1989. I had never been to a meeting on acrylamide until last year.

[Laughter]

It just shows you how much interest has

been generated since April of last year. Since August of last year I have been to five specific meetings on acrylamide.

[Laughter]

That is what it is like to be in vogue.

Acrylamide is a reactive chemical. It undergoes Michael additions. As we have heard, it is very reactive with sulfinyl groups. It also reacts with amino groups. It is extremely reactive with proteins and reacts very slowly with DNA.

[Slide]

I am going to be talking about a number of different kinds of labeled acrylamide and I just wanted to go through and review for everybody what I am talking about. For unlabeled acrylamide, we usually use that for pharmacokinetic studies for measurement of chemicals. For chemical measurements with things like mass. spec. For radio-labeled acrylamide we use 2,3, ^{14}C acrylamide. The labels are in the vinyl carbons. Usually we have a small percentage labeled used for metabolism disposition, pharmacokinetics, adducts--you name

it, we can do it.

The limitation here is how hot you can make this, how high a degree of radioactivity. It has a tendency to polymerize. So, that is always one of the big concerns. You can't make it tremendously high.

What we have done a lot of studies with is uniformly labeled 1,2,3 ^{13}C, ^{3}C acrylamide which is essentially 100 percent labeled at each site. We use this for metabolism disposition studies and also for adducts. We developed at CIT a method for analyzing metabolites by taking this material, giving it to animals, collecting the urine and looking at metabolism in the urine using ^{13}C NMR spectroscopy and we can use that to find and characterize specific metabolites even if we don't know they are there until we go looking for them and we can measure them.

[Slide]

One of the other things we need to talk about when it comes to metabolism is glycidamide. This is the epoxide metabolite of acrylamide. It

is formed by oxidation. It is a reactive epoxide. It reacts with protein and it also reacts with DNA. This is the big concern from the standpoint of carcinogenesis. This reacts with DNA and can cause mutations.

[Slide]

So, when we are dealing with metabolism and pharmacokinetics, generally most chemicals undergo metabolism to things that are more water soluble and less toxic. While most of the metabolites are unreactive and heavily excreted, some are more reactive. The problem with reactive chemicals or metabolites is that they can react with micromolecules, with glutathione, and they can disrupt all kinds of cellular processes that can lead to toxicity or carcinogenicity.

When you have metabolism by more than one route, and we actually do have that with acrylamide, you may have one route that will give you the reactive metabolites and the other that will give you stable metabolites. Then, you can have those reactive metabolites undergo further

metabolism so that life gets complicated.

What we really want to know is the balance between the various metabolic processes, the relative rates, and these can be an important determinant in toxicity and they can differ between species and between high and low doses.

[Slide]

So, when it comes to risk assessment, we want to know about metabolism and adduct formation so we can understand relationships between exposure and internal dose. Here, internal dose is something that pharmacokineticists think of as area under the curve in blood. It is the amount integrated over time at a particular site.

For dose response--we have already heard about dose response and linearity. Do we have linearity in range of effects? Can we compare our internal dose measures with effects that are generated in bioassays? Do we have differences between species? Can we use measures of dose for reactive chemicals or metabolites to improve other studies such as epidemiology studies,