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FOOD ADVISORY COMMITTEE AND DIETARY SUPPLEMENTS SUBCOMMITTEE

FURAN MEETING

Tuesday, June 8, 2004

1:55 p.m.

Bethesda Marriott
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C O N T E N T S

P R O C E E D I N G S

Welcome and Introductions

DR. MILLER: I think I would like to get started to enable us to finish on time and give people a chance to make their planes, and so on.

First of all, let me welcome the new members of Food Advisory Committee meeting for this afternoon's session, which will deal with furans and the data necessary in order to estimate the risk of furans in food.

For the record, when I call your name, I going to introduce the new members of the committee. This meeting is being held in conjunction with the Contaminants and Natural Toxicants Subcommittee of the Food Advisory Committee, and there several members of that committee that will be sitting with us in our deliberations.

When I call your name, will you please just repeat your name and the institution with which you are associated.

First, Dr. Acholonu.

DR. ACHOLONU: My name is Alex Acholonu, Alcorn State University, Mississippi.

DR. MILLER: Dr. Aller.

DR. ALLER: Marion Aller with the Florida Department of Agriculture and Consumer Services.

DR. MILLER: Dr. Gray.

DR. GRAY: George Gray with the Harvard School of Public Health.

DR. MILLER: Dr. Lee.

DR. LEE: Ken Lee with Ohio State University.

DR. MILLER: Dr. Chin.

DR. CHIN: Henry Chin with the National Food Processors Association.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: I am Joan Chesney. I am Professor of Pediatrics and Infectious Diseases at the University of Tennessee and also the title you see on the roster at St. Jude. I am also here representing the FDA Pediatric Drug Subcommittee.

DR. MILLER: Thank you.

Since we have some new members, we are

required to repeat the discussion of conflict of interest for this particular issue on furans.

Linda Reed, who is Acting Executive Secretary of the Food Advisory Committee, will read them.

Conflict of Interest Statement

MS. REED: Good afternoon, everyone. As Chairman Miller indicated, I am Linda Reed, the Acting Executive Secretary of the Food Advisory Committee meeting. I would like to welcome everyone and particularly our member from CDER.

I need to read the conflict of interest statement into the record again.

The authority to grant permission to borrow Special Government Employees currently serving on an advisory committee in a sister center, in this case, the Center for Drug Evaluation and Research, is granted to the Associate Commissioner for External Relations, Mr. Peter Pitts.

Relying on that authority, Mr. Pitts has signed a memorandum granting permission for Dr. P.

Joan Chesney to serve as a temporary voting member for this portion of the meeting concerning furan on June 8, 2004. Dr. Chesney will represent, as she just indicated, the Pediatrics Advisory Subcommittee of the Anti-Infective Drugs Advisory Committee.

Because of the breadth of topics to be discussed at this meeting, all of the members and temporary voting member have been screened for any and all financial interests associated with regulated industry.

Based on this review, FDA has determined in accordance with 18 U.S.C. Section 208(b)(3) to grant general matters waivers to Dr. Marion Aller, Dr. Douglas Archer, Dr. Johanna Dwyer, Dr. George Gray, Dr. Norman Krinsky, Dr. Margaret McBride, Dr. Sanford Miller, Dr. Robert Russell, and Dr. Carolyn Waslien.

The granting of these waivers permits these individuals to participate fully in the matters before the committee. Copies of the waiver statements may be obtained by submitting a written

request to the agency's Freedom of Information Office, Room 12A-30 of the Parklawn Building.

In an effort to enhance consistency within FDA, the agency has recently adopted a policy whereby all public commenters will be asked to report any personal financial interests that could be affected by the committee's deliberations. A copy of the policy was provided to any individual who registered to make comments at this meeting. Additional copies of the policy may be obtained from the registration desk.

Similarly, we have asked all of our guest speakers to complete a financial interest and professional relationship certification for guests and guest speakers to identify any potential conflicts of interest.

Dr. Don Forsyth and Dr. Glenda Moser will be the guest speakers at this portion of the meeting. Both have indicated they have no financial interests in the food industry.

I would like to thank for your attention and I will turn the meeting back over to Dr.

Miller.

Thank you.

DR. MILLER: Thank you, Linda.

As a matter of procedure, each of the speakers have been assigned a time for their presentation, and in order for us to make certain we get through the presentations, and most important of all, the discussion, I intend to be as ruthless as I can in keeping the time.

We have several limitations on our time. For one thing, we have to be out of here by 6 o'clock at the very latest. Otherwise, as I indicated this morning, we may be involved in somebody else's wedding.

Also, there are some of you who have planes to catch, and in order for the committee to complete its business, which will be explained in just a moment, it is important that we stick to the time schedule.

The first presenter is Dr. Nega Beru of the FDA, who will provide the background and discuss the charge to the committee.

Opening Remarks

DR. BERU: Thank you, Dr. Miller, and good afternoon. My name is Nega Beru. I am the Director of the Division of Plant Product Safety in CFSAN's Office of Plant and Dairy Foods.

My purpose here today is to provide you with some of the background on furan in foods to set the stage for the scientific overviews that will follow immediately.

I will also lay out what input we are seeking from the committee.

The structure of furan is depicted on this slide. It is a 5-member O-ring with two double bonds. It goes by a number of names as shown on this slide also, has a molecular weight of 68, a melting point of -85.6 degrees Celsius, and a boiling point of 31 degrees Celsius.

This last property, it is fairly volatile, may be important with respect to how much furan consumers are exposed to in foods as consumed.

Furan is a colorless liquid that is used in some segments of the chemical manufacturing

industry. It is used, for example, as a solvent for resins and in the manufacture of lacquers.

It was the subject of a 2-year bioassay by the National Toxicology Program in 1993. As a result, it is listed in the Department of Health and Human Services report on carcinogens, because it was found to cause cancer in rodents in the NTP study.

Furan is formed in food during traditional heat processing techniques, such as cooking and canning. Its mechanisms of formation are beginning to be elucidated, and there appear to be a number of them.

Later in this session, Dr. Don Forsyth from Health Canada will present to you their studies on mechanisms of formation of furan in foods.

The discovery of furan in foods is not new. Furan has been reported in a small number of foods starting as early as the 1960s, although very little quantitative data exists in the literature.

Furan was found in coffee, canned meat,

baked bread, cooked chicken, sodium caseinate, hazel nuts, soy protein isolates, hydrolyzed soy proteins, rapeseed protein, fish protein concentrates, and caramel.

What is new here is that FDA has developed a quantitative method to measure low levels in food and has found that furan forms in a wider variety of foods than previously thought including in some baby foods.

In addition to FDA, Health Canada and NFPA, together with some of its members, are investigating furan levels in foods, and, in fact, FDA, Health Canada, and NFPA are also currently collaborating In a round robin evaluation of the method that was developed by FDA.

FDA's finding was made during investigations aimed at confirming a report in the scientific literature that furan forms when apple juice is irradiated. As part of that investigation, a number of non-irradiated, but processed foods were also evaluated using a semi-quantitative method.

In the exploratory survey we posted on the web on May 7, we used a more refined quantitative method. FDA initially concentrated on foods that appeared to have high levels during the initial screen using the semi-quantitative method. FDA also analyzed foods that didn't necessarily have high levels in the initial survey, but could potentially result in high exposures based on consumption data.

For each type of food, foods were obtained from two to three manufacturers, and, in addition, to get at the lot-to-lot variation, two lots were examined per food.

Foods that were tested include baby foods, such as apple juice, applesauce, sweet potatoes, carrots, and green beans, infant formulas, both liquid and powder, and adult foods, such baked beans, soups, chilis, spaghetti sauce, tuna, coffee, and chicken broth.

Over 160 samples were tested in the exploratory survey including replicas of the same brand or product, and the results ranged from

nondetectable to approximately 100 parts per billion furan.

Right after my presentation, Drs. Kim Morehouse and Jeremy Mihalov will present more detailed results of the survey, as well as the exposure assessment that was based on the results.

FDA made the data collected in this exploratory survey public on May 7 by posting them on the FDA's web site. At the same time, we posted on the web a detailed description of the method used to analyze the food samples, as well as a set of questions and answers on the issue of furan in foods.

FDA also issued two notices in the Federal Register on May 7. One was to announce a call for data on various aspects of furan in foods, which I will go into a bit later. The other was to announce this very meeting of the Food Advisory Committee and the Contaminants and Natural Toxicants Subcommittee.

When we announced the data to the public, we did so with a number of message points. Of

course, we said that finding furan in foods is a concern because based on studies in rodents, furan is a potential carcinogen in humans.

At the same time we made it clear that furan certainly did not appear suddenly in food, its occurrence in food has been reported before. What is new here is the discovery in a broader variety of foods than previously thought including some baby foods.

We also said that this discovery is not an immediate public health concern. This was based on our preliminary exposure assessment and a National Academy of Science's review of the toxicology of furan done for NASA, and this review is in your briefing books, which concluded that, one, the weight of the evidence suggests that furan is an indirect carcinogen, and, two, calculated and no observable adverse effect level of 80 mcg/kg body weight per day.

Nonetheless, we said that there are many questions that must be answered to improve the risk analysis. Thus, we said that we intend to conduct

an expanded survey including foods as eaten in order to determine exposure and risk to consumers more accurately.

We also said that we will look at what additional studies are needed to determine furan's potential risk to human health, as well as studies on mechanisms of formation and reduction methods if the risk assessment warrants such studies.

Finally, we said that we will seek input from our Food Advisory Committee and Contaminants and Natural Toxicants Subcommittee on what data are needed to fully assess the risk posed to consumers by furan in foods, hence, this meeting.

We intend to evaluate all the available data including input from this meeting, and develop an action plan to address the issue of furan in food. The action plan will certainly include an expanded survey of foods, but may also include mechanisms of formation/reduction in foods, as well as toxicity studies to address mechanism and dose response.

In the call for data we issued on May 7,

we asked for data in several areas. With respect to occurrence of furan in foods, we asked for data on the particular foods in which furan occurs and the levels in these foods, the formation and occurrence of furan in home-prepared foods as opposed to, say, manufactured foods, and on environmental sources of furan in which a typical consumer is likely to be exposed.

With respect to mechanisms of formation, we asked for data on possible mechanisms of formation, as I mentioned earlier, we wrote a letter here about studies that Health Canada conducted on mechanisms on formation.

We also asked for data on variables that enhance or mitigate furan formation in foods, on the stability or dissipation of furan in foods, and on the effect of post-production practices, such as consumer heating of canned foods on the furan levels in foods.

With respect to toxicology of furan, we requested data on mechanism of furan toxicity, mutagenicity, and carcinogenesis, on reproductive

and developmental toxicology, and on metabolism of furan in vivo including characterization of any reactive metabolites, and the role of such metabolites in producing furans adverse effects including carcinogenesis.

We also asked for data on the diversity of furan pharmacokinetics in humans or the alteration of furan metabolism as a result of dietary, medical, or environmental interactions, and data on whether sub-cytotoxic doses of furan produce any adverse effects, such as a change in enzyme activities or ATP levels.

Importantly, we asked for data on the effects of furan at doses lower than those used in the 1993 NTP study in order to accomplish the following objectives:

1. To establish a dose-response curve for the various toxicological endpoints.

2. To determine whether furan toxicity, including carcinogenesis, is a threshold dependent event.

3. To determine whether the carcinogenic

activity of furan is secondary to its hepatotoxic effects.

Last, FDA is also asking for data on the mutagenicity of furan in the TA100 strain in the Ames test, and the behavior of furan in other in vivo assays for mutagenicity or toxicity.

In the Federal Register notice call for data, we asked that data and comments be submitted to FDA by July 9, 2004. We also said that we would share with the committee and the subcommittee any data or comments we received by June 1.

To date, we have not received any data on any of the areas we specified in the Federal Register. We did, however, receive one comment. That comment was from Dr. James Coglin [ph], president of Coglin & Associates, a consulting firm on food, chemical, and environmental toxicology and safety.

The comment describes work done on various heat-induced heterocyclic compounds including furan as antioxidants and urged the committee and subcommittee to consider the beneficial health

protective effects of such compounds in evaluating the safety of furan in foods.

This brings me to the charge and the question we are posing to the committee. This, by the way, are found in Tab 2 of your briefing packages.

The Food Advisory Committee and Contaminants and Natural Toxicants Subcommittee are being asked to provide input on data that would be helpful for further evaluation of the potential risks posed by the presence of furan in foods.

Essentially, this is the question we are asking the committee. Taking into consideration the data needs already identified by FDA in the Federal Register notice requesting data on furan, and the presentations you are about to hear at this meeting, are there any additional data that are needed to fully assess the risk of furan in foods?

With that I will end my presentation. I trust this will provide an adequate background for the more detailed presentations that follow, and I thank you for your attention.

DR. MILLER: Are there any questions for clarification? Dr. Dwyer.

DR. DWYER: I wasn't clear from the data needs if you are also considering doing home-cooked foods, for example, if I made a sweet potato pie at home, are you planning on doing those, as well?

DR. BERU: I think in the long run, we want to do that, and perhaps even consider adding furan to the total diet study. Certainly, we have done some preliminary work on home cooking in terms of what dissipation of furan may take place during normal home preparation of meals of canned or jarred foods, and Dr. Morehouse will present some of those data later.

DR. MILLER: Dr. Callery.

DR. CALLERY: Are you planning to also do the Ames test on metabolites of furan, especially metabolites that may have some predicted toxicology?

DR. BERU: Well, at this point we are sort of in a data collection mode. We want to see what work has been done out there, and certainly we

intend to do what we can to fill the data gaps including those studies.

DR. MILLER: Thank you.

We next have three papers dealing with overview of furan in foods, the first presented by Dr. Kim Morehouse from FDA. Ten minutes.

Scientific Overview of Furan in Foods

Analytical Methods/Occurrence

DR. MOREHOUSE: Hello. My name is Kim Morehouse and I am a research chemist with the Office of Food Additive Safety, Division of Chemistry Research and Environmental Review. My collaborators on this project have been Ms. Patricia Nyman, Mr. Timothy McNeal, and Dr. Gracia Perfetti.

Today, I am going to present some data that we have obtained on furan in foods and sort of explain to you why we got into this in the first place, even a little bit more than what Dr. Beru has presented already.

As was noted earlier, during our investigation of the possible formation of furan by

ionizing radiation, we noted that heating the sample caused an increase in the amount of furan that was detected.

This increase was not due in an increase in the volatility of the furan, but rather was indeed due to generation of furan.

We also noted the presence of furan in pasteurized apple juice that we had purchased locally at a store, but that furan was not present in apple juice that we prepared fresh in our laboratory.

This led us to investigate the presence of furan in heat-processed foods, and we started looking at various foods. Originally, we were just looking at it from the standpoint of comparing radiation treatment to heat treatment of foods, so we were doing a very random sampling of products. Basically, I just went through the store, picked up samples off the shelf that were canned and pasteurized products, and this was a quick semi-quantitative determination. We weren't as determined that we had to have exact numbers, but

rather an order of magnitude because we were just trying to say was the radiation going to significantly increase the amount of furan that would be present in the total diet at that time.

However, as we got further into this project, we began to realize that there was a large number of foods for which furan was present and in substantial amounts, and it became clear that we needed to look at it further, as well as needed to know the quantitative numbers that were there, not just from a qualitative standpoint.

So, we modified our procedure. In order to do this, we were using static, headspace sampling with gas chromatograph determination with mass spec detection. Our quantitation was based on stable isotope dilution, as well as standard addition with known amounts of furan to each food product.

It is important to note that we were doing it on each food product because each food product had a different partitioning coefficient of the furan between the headspace and the sample.

This method has been peer verified within our lab group itself by three different scientists, as I mentioned earlier, and we are currently participating in a round robin study of the method.

Basically, what we did was we took for what I call liquid samples, we took 10 grams of the sample from the food container and placed it into a headspace vial. For solids and semi-solids, we took 5 grams of the sample, added 5 grams of water in the headspace vial. The headspace vial was then sealed and analyzed.

For some products, it was necessary to homogenize the sample, and for those products they were homogenized on ice either using a blender or a tissue homogenizer. After the samples were sealed upon the addition of either D4 furan or furan if necessary. They were vortexed to ensure adequate mixing of the samples.

It was important to make sure that we did have adequate mixing because we noted that when we did not, we retained rather spurious results, but upon proper control of our samples with proper

mixing and everything, we were able to obtain extremely good quantitation.

For our studies, we listed limits of quantitation on the data tables that were presented on the web. We used rather conservative estimates of those limits, and for liquid samples, we determined that was about 2 ng/g, and for solids, it was about 5 ng/g.

Like I said, these values are fairly conservative, however, we know that our limits of detection are much lower than that. For liquid samples, we estimate those to be about 0.7 parts per billion, and for the solid matrices, about 1.5 parts per billion.

As Dr. Beru mentioned earlier, we selected foods based on that initial survey that we were doing during our radiation studies, as well as from the literature reports of foods that were known to contain furan, and using the FDA database to determine which ones were higher consumption foods.

For each food analyzed, we analyzed from either two or three brands, and usually from two

different lots per brand. Using this data, we undertook a systematic manner to obtain quantitative data.

I am going to go through classes of some of the foods that we looked at. From the infant formulas, we looked at powders, concentrates, and what are called ready to feed foods. The concentrates and powders were prepared according to label directions, placed in the vials and analyzed. The ready to feed, of course, are already ready to feed, so they were just simply transferred into the vials.

You can see that we have a range for the powders of non-detected to 2 parts per billion, for concentrates of non-detected to 15, and for the ready to feed, non-detected to 13.

For the powder and concentrate, they are based on what would have been consumed.

The ranges I am listing here is because you still see in the next presentation on the exposure estimates, the range is what is used for doing that calculation.

For some of the baby foods that we have analyzed, you can see the apple juice range from 2 to 8, and you can go on down the list up to the sweet potatoes and garden vegetables, which were up to 100 part per billion. Again, you can see that we do have a fairly large range. Again, the garden vegetables, we are talking about three manufacturers and two lots per sample.

For some of the adult foods, we have done a lot more work. You can see that we range from bread, where it is non-detected to below our quantitation level. When we have less than 2 there, that means we can detect it, but it was below our quantitation level, and in the cases of the tuna and the canned meats, we listed as less than 5. That means it was within our detection limits, but below our quantitation level again.

Again, you can see the spread of the numbers that we are seeing and the various different types of products that we have been able to analyze so far. Just so you don't think it is all so bad, from our original survey, we do know

that many foods do not contain furan, some of those listed here, and you will notice that man of these foods are fairly high consumption products, such as milk and margarine and yogurt nowadays type of thing. We also included pasteurized eggs and potato chips in our original survey, as well.

I was asked the question about the heating the products. We haven't gotten to the point yet where we are actually cooking unprocessed foods to look at that, but it is something we do intend to do eventually, but what we did look at was what about the foods from the can and if you heat them.

For the foods we looked at here, a very limited preliminary study, we did chicken broth, two different pastas, and the infant food sweet potatoes. The pasta No. 2 and the sweet potatoes were only treated one way, that is why there is no second bar there, but you can see from the pasta sauces and the sweet potatoes, there is not what I call a significant change upon heating, whereas, with chicken broth where you basically have water, and not much lipids or proteins to be holding back

the furan, it does substantially decrease.

So, depending upon what the food would be, you would either lose the furan or not, and this gives us a little bit of idea that we may have less furan actually in the consumption than what would actually be in the food as we are opening up the jars.

For the heated samples, they were heated basically on a hot plate in an open environment until they boiled for about 10 minutes. In the microwave, they were heated to boiling, usually for about a minute for the chicken and pasta. The sweet potatoes, they were heated what I call until they were tepid, similar to what a consumer would have done.

What is ongoing? We are obviously analyzing more foods. This was set as just a preliminary survey so far, we are doing a lot more. We are now looking at foods based on using the USDA consumption database to say what are some of the other high use foods that we should go ahead and analyze that we haven't already done before.

Again, still looking at foods that have been reported in the literature that contain furan for which no quantitation is available in the literature. It should be noted that in most literature they would state that they found furan, but would not state what the amount was, they didn't quantitate the amount there.

Of course, we are going to continue to investigate the effects of heating on the concentrations of furan.

For those who would like to see the full tables, of course, the entire method that we used is available on the web site as was stated earlier, as well as all the foods that have been analyzed.

Thank you.

DR. MILLER: Questions?

DR. ARCHER: A question, just curiosity. What do you make of the potato chip data?

DR. MOREHOUSE: There was no furan in potato chips.

DR. ARCHER: Any hypotheses?

DR. MOREHOUSE: Nope. Again, you are hear

later on some of the mechanisms, and some foods that we saw high amounts of furan in, we look at some of the mechanisms that have been proposed for where furan is coming from, and they don't correlate with the products, so obviously, there is multiple mechanisms, multiple pathways, and potato chips was one of the things that we thought would contain furan, and did not.

DR. DOWNER: Thank you very much.

It seems to me that the higher fat foods tended not to have furan detected. I want to ask a little bit about the milk, though. Were you able to look at fat-free milk, 1 percent, 2 percent, regular milk to see if there were any detectable differences in those grades of fat content in the milk with respect to furan?

DR. MOREHOUSE: That was back from the survey work, and I believe all we did was whole milk, and we didn't see any furan in the whole milk, so we didn't bother with looking at any of the others. We figured if it wasn't in whole milk, why would it be in the others.

DR. MILLER: Dr. Waslien.

DR. WASLIEN: I was particularly concerned with the furan content of formula, maybe non-detectable, the 13 sounds low when you are looking at a gram quantity, but if an infant consumes a liter a day, you are up there in the levels.

I went and looked at the l.d., the least dose for mice or rats, and the calculated based on that, of course, we don't have any data for doses for humans, would indicate that the amount of furan taken in is 13, and the dose that is least detectable or least risk is something like 12, so you are getting close for some of those infant formulas.

Now, my calculation might be wrong, I just sat and did it right now, and we are encouraging infants to drink less than a liter of milk a day, but it is a concern, and that was my major worry.

DR. MILLER: That's true, but the issue that we are concerned with here is what work would we suggest to the agency in order to get enough

data in order to be able to come to that conclusion.

DR. WASLIEN: Well, partly I would think one of the things you might want to look at is age-related differences in metabolism since a newborn infant has all kinds of other metabolic differences.

DR. MILLER: Hold that thought.

DR. WASLIEN: Okay.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: I also have many, many thoughts as you do, but for the moment, I wondered if you could clarify the infant formula slide for me. I didn't quite understand non-detectable-2-15-13, and you also said based on consumed, and I may have heard wrong. I wanted to be sure I understood the slide.

DR. MOREHOUSE: The slide, that is the range that we found for the products that we have analyzed. From non-detectable to 2 for the powers, from non-detectable to 13 for the concentrates, I think it was, and the powders and concentrates are

based on as it would have been prepared by the consumer for consumption.

In other words, we took the powder and made up the solution was it was by label, so it is based on the prepared formula, not the powder itself.

DR. CHESNEY: I understand. Thank you.

DR. MILLER: Dr. Chin.

DR. CHIN: Going back to your table or figure that showed the effect of cooking on furan levels in various foods, there were I guess a couple of bars where either the value was zero or there were no values.

DR. MOREHOUSE: Those were because for the second pasta sauce and for the baby food, we did not do the second treatment, so the pasta sauce No. 2 was only heated, and the baby food was only microwaved.

DR. CHIN: Thank you.

DR. MILLER: Dr. Aller.

DR. ALLER: A question again on the infant formula. I know you mixed that. Was it heated

also?

DR. MOREHOUSE: No, just mixed.

DR. DWYER: Just a question. Could you explain the difference between limit of quantitation and limit of detection? It is just that you can't above the limit of detection, you can't quantify until you get to 2 parts per billion, is that right?

DR. MOREHOUSE: Right. Because of the mass spectroscopy's sensitivity, we can detect it or we put very stringent requirements on quantitation right now because the method has not been totally peer verified, we felt that we didn't want to say that we could do 1 part per billion, even though we can see it, but we don't want to take the quantitation level there yet.

DR. MILLER: Thank you.

The next speaker is Mr. Jeremy Mihalov, FDA, will talk about exposures.

Exposure

MR. MIHALOV: My name is Jeremy Mihalov, Office of Food Additive Safety. This also was done

with Dr. Michael DiNovi. I am going to give you an overview of our exposure assessment for furan from the consumption of adult and baby foods.

I will start off, give you an idea for the model that we used to estimate exposure, and this is fairly similar to most exposure assessments, simply that the total exposure for a person to furan is the sum of the exposures from each food, overall foods that contain furan, and exposure from each of those foods is simply the product of the intake of that food modified by the concentration of furan modified by the concentration of furan in that food.

We looked at adult foods, baby foods and also the infant formula, and they were considered separately.

The sources of our data. For intake data, we used the USDA 1994 to 1996 and 1998 USDA Continuing Survey of Food Intake by individuals. This was a two-day survey, two nonconsecutive days. For each of the years, there was about 5,000 people, so we have data for basically 15,000

individuals, and we know what they ate and how much for each of those days.

We then looked at the furan concentration data which you just heard about, and we looked at those lists of foods, and looked at the survey data, how much of those foods did those people eat multiplied by the concentrations, and you can get an exposure for each individual.

This may be somewhat of an iteration of what you have already heard. By looking at the infant foods, we group them into juices, fruit purees, vegetables, mixed chicken meals, had a separate for infant formula.

For the adult foods, we grouped them into brewed coffee, instant coffee, broths, soups that contain meats, spaghetti sauces, chili, pasta, ravioli--they were both canned--juices, pork and beans, canned string beans, canned tuna, canned corn.

Just to go over some of the levels again, within each food type, the ones I just listed, within the food types, there wasn't a lot of

variability. Overall, the range, looking at all the food types, went from limited detection up to about 125 mcg/kg.

Specifically, looking at the infant food groups, the highest were the sweet potatoes and the garden vegetables, juices were generally low, below 10 mcg/kg. The fruits and mixed meals were below 30. Other vegetables ranged between 30 and 60. With the formula samples, about half were below limit of detection, and we used the mean, which was about 7 mcg/kg.

With the adult foods, the coffee had the greatest variability, between limit of detection up to 80. The juices, tuna, broth, sauces were all generally low, below 15. The soups and the pork and beans had a fairly wide variation, the soups being the highest. The chili, beef ravioli, and spaghetti, the canned pastas were between 30 and 100.

Going back to discussing the model, generally, when you do an exposure assessment, there is a certain amount of uncertainty, and we

compensate the uncertainty with making certain assumptions. Whenever we make an assumption, we tend to make it conservative, and this is typical for agency exposure assessments.

The first assumption is that the concentration of furan and all the furan-containing foods will be at the mean within the food type, and as I said there is generally little variability within the food types, so we use the mean. When we are looking at chronic exposure, that is generally how we do it.

Second assumption, for all foods within a food type that are shown to contain furan, we assume that it does contain furan. In other words, they have seen it in canned chili, so when we did the exposure assessment, we assume anytime anybody eats chili, it also contains furan, and as there is more data collected in the future, those uncertainties could be reduced.

The last assumption is that the two-day survey intake data that we used reflects a lifetime exposure.

So, getting to the final numbers, we used the published April 20th concentration data that is on the internet. Using that, for the adult foods for people ages 2 and older, that ate those foods, the mean consumption was 0.3 mcg/kg-body weight/day.

The 90th percentile, which is what we consider to be the heavy consumer, on the upper end of the distribution, is at 0.6 mcg/kg/day.

When we looked at the infant foods, and these are age 1 or less, that ate those foods, the mean was 0.4 mcg/kg/body weight, and the 90th percentile was 1 mcg/kg.

We ran the exposure assessment looking at the individual foods just to get a sort of profile of how those different food types contribute to that overall mean, and this is just a table of how those foods contribute, coffee being the highest out of the groups that were tested, going down to broths being negligible.

For the infant formula, we took a slight different approach, a more simple approach. There

is sort of standard numbers for infant formula. In order for an infant to thrive, they need to consume between 100 and 120 kilocalories per kilogram per day, and infant formula is usually formulated to contain 0.8 Kcal/gram when it is prepared, and I used the mean furan concentration of 7 mcg/kg, and if you do the arithmetic, you can come out at a mean exposure of 0.9 mcg/kg/day for an infant consuming infant formula at the level needed to grow.

To sort of sum up overall, the variability of the furan levels within a food type is generally small, so we can pretty much assume that additional measurements within food types won't have much effect on the overall exposure, however, because the number of food types that have been tested is generally limited, additional measurements in other types of foods could have an overall effect on the exposure, especially with foods that are consumed in high quantities or also foods that have high concentrations could affect the exposure.

Thank you.

DR. MILLER: Dr. Waslien.

DR. WASLIEN: I did a quick recalculation of my numbers, and I am off by 1,000, so I skipped nanograms in there. I just wanted to make that correction.

But even so, I think when you look at infant formula, I hesitate to take the mean of values, because the likelihood of a person changing from one formula to another is not that high, so I think you are looking at the individual risk from formula, so the child who is consuming a formula with 13, if it is a ready to consume formula, is probably going to be consuming that reasonably every day.

DR. NELSON: I guess a similar question. On the other food products, did you use the mean in your conservative estimate, or did you use the highest value?

MR. MIHALOV: We used the mean of all the concentrations for all the food types. Generally, when you are looking at a lifetime exposure, you can pretty much assume that if there is a

distribution over time as you consume that food, one day you might consume the minimum, the next day you may consume the max, but over the course of time, you are going to consume at the mean.

Of course, if there is additional data to demonstrate that there is some reason to why there is a distribution, you know, that could change, but generally, for now we use the mean.

DR. RUSSELL: Just a question of information. With so many adult Americans eating out, particularly in fast food type restaurants, do you have any data on fast foods that have been prepared under high heat conditions?

MR. MIHALOV: Well, the survey data includes restaurants and home cooking. It is essentially the survey is given out and whatever was eaten by those individuals on those two days, that is what they report.

DR. RUSSELL: But in your analysis of foods that FDA has analyzed, how many foods come from that type of an environment that were analyzed actually? I noticed a lot of canned and jarred

things, very important for infants particularly, but I was just concerned about the adult exposure.

MR. MIHALOV: Just looking at the list, I would say a few of them are probably restaurant. Like I had said, if they found it in a food, we assume that it is in all foods of that type, so, for instance, the chili was a canned chili, but we assume that all chili contained furan when we did the exposure assessment, so if they had chili at a restaurant or if they made it at home, that was taken into account. If there is further data to show that canned is higher than home-cooked or restaurant, then, we can make that change.

DR. MILLER: Dr. Lee.

DR. LEE: To continue that thread, I assume that there is a fair amount of looking at canned and jarred foods because the furan is fairly volatile, so the packaging method itself keeps the furan present in the food, is that a fair assumption?

MR. MIHALOV: I couldn't say.

DR. MILLER: Dr. Nelson.

DR. NELSON: That would fit with the infant formula data because the powdered stuff is typically spray dried where you have a lot of opportunity for dissipation of furan as opposed to the canned concentrate or ready to drink formula.

DR. MILLER: Do you want to respond to that?

DR. LEE: I just want to continue along that line of thinking. Have you ever considered or does anyone have any data on animal exposure, particularly pets consumption, because you basically have a pretty monotonous diet, and there are pet foods that do come in cans, so one would expect that there would be a fairly good exposure there that you can model, is there any interest in looking at that?

MR. MIHALOV: That could probably be done if we had concentration data. I am sure that there is some information on how much food a typical animal eats per day, but it would be pretty much as simple as that, because a pet would consume one can or two cans, or something along those lines, but

that could be one.

DR. MILLER: Dr. Chin.

DR. CHIN: I just wanted to comment a little bit on the thought about foods purchased at restaurants. I think one of the other considerations in terms of foods that are purchased at restaurants is that not only do you consumer the food at the restaurant, but there are situations where you have takeout food and you take it home.

You might reheat it in the microwave. We have seen some limited data where you take a food from a restaurant, put it in the microwave, and under those circumstances, at home, you would produce some more furan.

DR. MILLER: Dr. Dwyer.

DR. DWYER: Just a question about the exposure assessment. I am a nutritionist and so when we use these kind of data, we use the Iowa State method for adjusting the nutrients to pull in the tails of the distribution.

Do you do that in exposure assessments, as well? In other words, you have two days worth of

data, and so you are able to get an estimate of usual intake from that, and I wondered if you adjust for that. The effect would be to change the exposure, i believe.

MR. MIHALOV: It doesn't sound familiar. Basically, we take the distribution of all the consumers and pull a mean in 90th percentile right from the distribution, but not adjusting it.

DR. MILLER: Dr. McBride.

DR. McBRIDE: In answer to Dr. Nelson's point, I looked at that data of the prepared formula and the powdered formula and thought maybe it was a difference in processing, might be heating it more when it is packaged in liquid form.

I also did the calculations for the worst case scenario because that is something I think you were getting at, and if you have a chubby 8-month-old who consumes a liter of formula and 5 jar of sweet potatoes a day, I assumed it had to be at least 8 kilos to do that, I got a worst case scenario of 8 mcg/kg.

DR. MILLER: How much?

DR. McBRIDE: Eight.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: Not why I am here, but the fast food issue is intriguing. I wonder if the packaging contains furan. Most fast food, you get plastic containers to put it in, and most people reheat it in the container. Just a thought.

DR. MILLER: Any more comments? If not, thank you.

The next speaker is Dr. Don Forsyth from Health Canada, who will take about the formation of furans.

Formation

DR. FORSYTH: First of all, I would like to thank the committee for the invitation to appear here today on behalf of Health Canada.

My name is Don Forsyth. I am a research scientist with the Food Research Division of the Bureau of Chemical Safety with Health Canada in Ottawa.

I would like to take you through the background as far as Health Canada is concerned on

this issue. In late March 2004, we became aware of U.S. FDA's investigation of furan in canned and bottle food commodities. Upon learning that furan has been shown to be carcinogenic in rodent models and has been classified as possibly carcinogenic to humans, we commenced method development as of April of this year for support of the study of mechanisms of formation, as well as a preliminary survey of Canadian food products.

Although furan is used in industrial processes, as has been discussed this afternoon, we considered that the likely source would be formation during food processing during the initial start-up of our investigations.

One thing we should mention about furans in foods, however, is that furan derivatives not only have been reported in a wide variety of foods previously, but they are also a significant flavor and odor component in coffee, cocoa, and various cooked meat products.

So, these are products or compounds, I should say, which arise naturally during the

processing and cooking of various food commodities.

Furan itself, the parent compound, has been previously isolated in coffee, canned beef, sodium caseinate, soy and rapeseed protein, as well as caramel.

Looking through the literature, you can find a variety of possibilities or comments from previous authors working in flavor and odor studies about how these compounds are formed.

The three that we chose to look are the thermal degradation of carbohydrates or the Maillard reaction, thermal oxidation of lipid, and decomposition of ascorbic acid and its derivatives.

Just to take a look at an older study conducted by Persson and von Sydow back in 1974, one of the first studies that you find where they are able to determine that certain components in a processed food could increase the levels of furan produced within that food commodity under typical canning conditions.

Using a beef, water, and sodium chloride formulation fairly typical of the day for canned

beef products, they found that even with just these basic components, there was fairly large levels of furan produced, however, with the addition of the fat, as you see in the second formulation shown here, the levels increased dramatically above the formulation without the fat.

Then, when they looked at the formulation with a small amount of carbohydrate added, they found essentially no increase over the basic formulation of beef, water, and sodium chloride, and then finally with the fourth different formulation shown here, with the fat and the carbohydrate added in addition to the other constituents, you get levels similar to the beef, fat, water, and sodium chloride formulation.

So, in this particular study, the authors determined that the fat was a precursor for the formation of the furan.

Briefly looking at our own analytical methods that we developed to support these studies, one was a headspace analysis which we used for the mechanisms of formation and for the food survey,

and also the microextraction technique, which is a SPME related method developed at Health Canada which we applied to the food survey results, which we will be showing later on in this presentation.

Both methods are based on isotope dilution using a d4 furan surrogate, measurement by gas chromatography/mass spectroscopy.

Formation studies. We took some of the test compound or precursor to a small vial containing 0.5 ml of water. The vials were then heated for 30 minutes at 118 degrees, conditions not too dissimilar to commercial canning procedures, allowed to cool, and then force cooled to 4 degrees when the D4 furan surrogate was added, so that we could analyze the resulting anilides which may have formed during this study.

The first table is on the level of furan which were formed with the addition of ascorbic acid and ascorbic acid derivatives. Virtually all of these compounds are commercial antioxidants which are used in foods, and you can see that the ascorbic acid with or without the iron present,

iron is a known promotor of oxidation and therefore, would be expected to, at least in some cases, increase the amount of furan which would be produced.

The sodium ascorbate, again relatively low levels. The dehydroascorbic acid, however, with either the iron present or absent gave higher levels, almost 10 times higher than the ascorbic acid.

Isoascorbic acid, again similar in this case to the dehydroascorbic acid, and the sodium isoascorbate in the presence of the ferric iron produced almost again 10 times as much as the sodium isoascorbate by itself, but again, both half the levels that we found with the addition of dehydroascorbic acid.

Finally, the ascorbyl palmitate compound produced fairly low levels of furan as well.

Then, when we looked at fatty acids and oils, we found that the degree of unsaturation in the compound had an effect with an increase in the levels of furan formed increasing as you go from

linoleic up to the linolenic with an increase of about 4 times in this case.

Now, in these two fatty acid series, we did see an increase in the production of furan with the addition of the ferric iron, and in the case of the oils, what you see in the last four rows of the table, again, a similar increase as you go from the trilinoleate up to the trilinolenate, approximately again roughly 4 times.

In this particular case, with the oils, the ferric iron had an increase in the production of the furan for the trilinoleate, but not for the trilinolenate.

Comparisons were made between the reaction products and the furan standard, and as you can see in this particular case, the comparison between the linolenic acid reaction mixture and the furan standard, you get a very similar pattern both for the total iron chromatogram as well as the fragmentation pattern for these two.

So, what we have determined so far is that at least in the model systems that we have tested

so far, we found that the polyunsaturated fatty acids, such as the linoleic and the linolenic, did produce furans likely through a free radical formation mechanism with ring closure resulting in the formation of the furan, and also decomposition of ascorbic acid derivatives particularly the dehydroascorbic acid and the isoascorbic acid also led to the formation of furan.

Some of our survey results in baby foods. Here, we have a comparison between our two methodologies, the microextraction technique in the first column, and the static headspace in the second.

Levels varied as low as 6 parts per billion, and went as high as approximately 154 parts per billion in the mixed vegetable. Each one of these values that you see is the average of two individual analytical determination for each method.

When you look at adult foods, we found that the chili products had the highest levels amongst those that we analyzed with levels ranging

up to as high 227, 236 depending upon the method value, as well as 152, soups there was a broader range ranging from as low as 35 ppb up to approximately 115, 117 ppb.

We have looked at one stew product so far with a value of approximately 80 parts per billion, one bean product with relatively low value, 14 parts per billion.

The luncheon meats that we looked, I believe were both beef or pork based, and they were all relatively low with levels down to 4 parts per billion, and no higher than approximately 30 parts per billion.

Fresh brewed coffee, as would be typically served, would range between 14 to approximately 50 parts per billion.

Next steps for our work at Health Canada include further studies on the mechanisms of formation using additional model systems, as well as precursor fortified food matrices.

Examining losses of furan during food processing and cooking operations, as well as

further examinations of canned and bottled products. We also have a round robin method validation study to complete, and that is ongoing as we speak, and we should be reporting back on that in just a few weeks.

Then, finally, to continue updating our health risk assessment as new data becomes available.

With that, I would just like to thank everyone for their kind attention.

DR. MILLER: Thank you.

Any comments or questions?

Questions of Clarification

DR. ACHOLONU: I was wondering, is it advisable to check the concentration of furan in mixed vegetables? Could you justify using that? Mixed vegetables, which has different kinds of vegetables put together, what do you do?

DR. FORSYTH: The premise of that, of course, is for health risk assessment, in which case we are interested in consumption of food commodities that are related to a typical diet, so

this is one particular food product that we happened to analyze, and that is essentially the extent of our interest in it at that point.

DR. ACHOLONU: But does it have any scientific basis?

DR. FORSYTH: It has a scientific basis in the sense that with that particular food matrix, those are the levels that you are reaching. It also brings to mind what is causing that formation, which is something that we are certainly interested in, because it doesn't fit into the existing models that we have pursued so far. So, yes, I think it has a lot of scientific interest.

DR. MILLER: Dr. Krinsky.

DR. KRINSKY: Could you just describe the conditions for generating the furan from the linolenic acid? Was this heated, cooked, baked, or was it just linolenic acid out of a jar?

DR. FORSYTH: I didn't actually conduct this study myself. My understanding is that the compound, which I believe was 10 mg of the precursor would have been added to the vial

containing 0.5 ml of water, and then that would have been heated to the 118 degrees for 30 minutes.

DR. KRINSKY: Thank you.

DR. MILLER: Dr. Lund.

DR. LUND: Looking at the Persson and von Sydow data, I wondered if you have had any comments with regard to the degradation of furan upon prolonged heating. Some of their data, at least on the surface, would suggest that upon prolonged heating, you probably get formation rates equal to degradation rates because the concentration is not changing.

DR. FORSYTH: First of all, I am not sure if that is what they were alluding to or not. I thought that data was to look at probably losses of furan due to revolatilization during heating and processing in the kitchen.

I know that there is some concern that you may actually be creating more furan with post-processing sample manipulation, but I don't know if anybody has actually really looked at that yet.

DR. MILLER: Dr. Callery.

DR. CALLERY: We addressed part of this already, but it's an impressive amount of work that you have done since March. I have been looking at this, and I admire you for being able to get so much data so rapidly. I have a couple of little questions, though.

The ascorbic acid one in particular, from what I remember the structure of ascorbic acid, it's a highly oxidized species and it gets even more oxidized readily, and that you are actually asking iron to participate in this reaction to facilitate an oxidation.

I think the point I am trying to make is that the furan is more like a reduction or elimination of water, a couple water molecules, and more a reduction. If you looked at the oxidation states of the various carbons, they are not at a higher oxidation state than ascorbic acid.

So, it may be something very different going on here that is involving the metal in the process of making furan, if that is what you are

actually doing. I think the question was also the yield that you are addressing here, maybe there is 10 mg of ascorbic acid or I am sure 10 mg of fat, but that nanograms per gram is incredibly small yield in the process of cooking, so I am wondering a little about that, too, if you aren't just making some furan this way out of this particular compound.

DR. FORSYTH: I have no doubt that the yields, particularly with the ascorbic acid tend to be quite low, but typically, levels used in food are reasonably high, and this wouldn't necessarily be the only way that furan would be formed, and it, of course, had been alluded to earlier by one of the other speakers, that we undoubtedly will find that there is multiple pathways contributing to the overall levels of furan present in the food.

These are, I can't stress strongly enough, preliminary investigations into possible means that furan could be formed. There had been previous work with some of the ascorbic acid related derivatives that had indicated that a variety of

furans were formed during thermal degradation, and this is what we were attempting to follow up on with this study.

DR. MILLER: Dr. Chin.

DR. CHIN: I would also like to compliment you on doing such an impressive amount of work in such a short period of time.

Just a question in terms of your thoughts on other possible precursors. Are you planning to look at the possibility that perhaps carotenoids and similar types of materials might be a precursor also?

DR. FORSYTH: Our immediate plans, and we are doing this as we speak, looking at Meyer type reactions at present.

DR. CHIN: Just a follow-up, and the reason I am asking is because in products like the sweet potatoes where there are amount of furan have been detected, I mean those materials are high in carotenoids, whereas, they are generally low in fat, and I don't think the ascorbic acid levels are particularly high, so just a possibility in terms

of another possible precursor.

DR. FORSYTH: It sounds like we will be following up with you shortly.

DR. MILLER: Dr. Dwyer.

DR. DWYER: Just a question more from ignorance than anything else. Are the methods that you are using in Health Canada and the Food and Drug Administration's methods the same? I just looked at chili, and it looked like the Canadian chili was much more potent than the American chili, and just wondered if there were some way if they are not the same method, if there is some way to get some comparable methods in both countries or to divide up the work and do some round robin studies, so that we are not overduplicating things that are basically the same trading area.

DR. FORSYTH: Round robin testing is underway. In our own case, it was set aside because we were concerned with what levels were present in the Canadian food supply, so that was our first priority. Now that that is completed, we do have the two methods which we wish to compare,

not only against FDA's method, but also any industry methods which are out there, and we will be doing that through the round robin study.

DR. MILLER: Dr. Russell.

DR. RUSSELL: Yes. Following up to Dr. Chin, I also had wondered about the sweet potato, but I was wondering if the soup, it says soups in both the FDA data and your data, I was wondering if there was any clues that could be gotten with the types of soups.

There was a three- or four-fold variation. What types of soups were looked at?

DR. FORSYTH: I believe there was--I hope there was a listing of the actual products that we tested included in your information package. In any case, we have tested 30 products so far. We hope to be testing more in the near future but for the time being, we will be participating in the round robin study first.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: Again, just for clarification, and this may seem like a very

simplistic question, but is it correct that the furan is created by oxidation of the ascorbic acid products and the polyunsaturated fatty acids, it's a product of oxidation of those entities, am I correct?

DR. FORSYTH: I think with the ascorbic acid, I would view it more as a thermal degradation as opposed to an oxidation per se, whereas, with the lipids, it is a radical-mediated oxidation mechanism, yes.

DR. MILLER: Dr. Nelson.

DR. NELSON: Following up on Dr. Dwyer's comment about the equivalence of method or recognition of each other's method, I guess, would the food supplies be considered equivalent enough for us to sort of accelerate the database by again sharing the activity? I don't know if we need the same trading area, we have to have a NAFTA database.

DR. FORSYTH: Is that related to me? Presumably, in Canada, we find that we have different branding as opposed to U.S. foods, but in

cases where you have the same manufacturer, I personally can't see any reason why the data couldn't be used.

DR. MILLER: Dr. Downer.

DR. DOWNER: I just wanted to respond to Johanna's question about the chili. I think in Canada, they may be using Spam from looking at the database here, so maybe that is where the difference is.

Thanks for a good presentation. I am just wondering about Dr. Morehouse's presentation when he looked at no furan detected in some of the different groups of foods, particularly foods from animal sources. I was thinking that perhaps because it was a bit lower in fat.

But on one of your slides, when you talked about the effect of canned beef formation and you added fats, it was really the opposite. Could you talk a little bit about perhaps the differences that were seen there?

DR. FORSYTH: Actually, in retrospect, when you look at their study versus our own results

on canned luncheon meats, I have concerns. It was a 1974 survey, well, different analytical capabilities than we have now, so I believe that that work does bear out the results that we were finding in terms of the presence of fat promoting the increase in furans.

However, I can't reconcile the findings that they reported in that publication with our own and also with FDA's current findings on luncheon meats, which I would have felt should be comparable.

DR. MILLER: Dr. Dwyer.

DR. DWYER: Just a question again about methods. Is there a standard method, or are you driving toward a standard method instead of everybody having their own method especially in North America, it seems like this might be something to agree about one way or another?

DR. FORSYTH: There has been a few factors, time being one of the largest. Essentially, with the time constraints that all of the organizations have had, you basically begin

with the people that you have who know how to do these types of analyses, and you ask them to come up with a working method, and I believe that is essentially what has happened here.

The next phase will be these organizations to have, and this is being done as we speak, a round robin study in which case we all examine the same food commodities and see if we get, hopefully, roughly the same answers. Depending upon the results of that study, there would then be either adjustments made or discussions amongst the various organizations to determine why, if there are indeed any, there are differences in our results on these particular food commodities.

DR. MILLER: Why don't we move on. Thank you very much.

The next speaker is Dr. Glenda Moser. You have got 25 minutes.

Scientific Overview of Furan in Foods

DR. MOSER: Thank you.

Well, I would like to begin by thanking the committee for inviting me today and say that my

talk is going to be a little different than those that have been presented up until this point, and, in particular, I am going to be talking about some in-life studies that we did to try and determine as best we could a mechanism for furan-induced liver tumors in mice.

In a two-year NTP bioassay, there were both non-neoplastic and neoplastic findings in rats and mice of both sexes. In particular, there were neoplasms, cancer, in the liver in the rats, and cholangiocarcinomas in the rats, as well as mononuclear cell leukemia.

The important thing here is that at 2 mg/kg, there was approximately a 90 percent incidence of cholangios. In mice, there was an increased incidence of benign tumors of the adrenal gland, as well as hepatocellular tumors at both 8 at 15 mg/kg.

Here, what you see is more of what I am going to be talking about today, is the incidence of your hepatocellular adenomas or your benign tumors, hepatocellular carcinomas, and then the

adenomas and the carcinomas together in female mice and in male mice, and you see a dose-dependent increase in both the males and the females.

Another important factor for the study that I am going to be talking about is the incidence of spontaneous liver tumors in both male and female mice. Historically, in your B6C3F1 mice, you will have somewhere between 20 and 60 percent incidence of liver tumors in control animals. Usually, it's much lower in your female mice. It is for that reason that we conducted our two-year study in female mice.

Sometimes it is difficult when you have 40 or 50 percent incidence of spontaneous liver tumors to find an increase in male mice.

Cancer, as we are well aware, is a highly complex, multistage process that is operationally divided into three stages, namely, initiation, promotion, and progression. Initiators or genotoxic agents directly damage DNA. They change the primary sequence of the DNA.

Genotoxic agents can be carcinogenic after

a single exposure, and, in general, it is found that genotoxic agents are better carcinogens if they also induce cell proliferation, so they can fix those mutations.

Metabolism, there are many carcinogens, not only liver carcinogens, but carcinogens in other systems, the parent compound is not carcinogenic, but it is metabolized or intoxicated to its toxic moiety.

In the case of furan, you have the cytochrome 2E1 in the liver that metabolizes the furan to its toxic moiety. There are a variety of mechanisms of genotoxicity which we are not going to talk about today.

What we have here is somewhat of a summary of assays for genotoxicity after furan exposure. We have those that are negative, those that are positive, those that are highlighted in mammalian systems, and those that aren't, are either in your Salmonella or your Drosophila.

In spite of the fact that there are some positive tests for genotoxicity, furan is generally

considered to be non-genotoxic. Non-genotoxic or epigenetic agents, they are generally believed to clonally expand those initiated cells.

They provide an environment in which those particular cells opportunistically grow and expand, that, in general, your non-genotoxic agents require multiple exposures, sometimes over the course of the entire life span of the animal, and generally, with your tumor-promoting agents or your non-genotoxic agents, it requires high doses.

In the early stages, tumor promotion is generally reversible, so that in a 13-week study with furan, the animals were dosed at 8 and 15 mg, then, they were held for either 6 months, 9 months, or 15 months, and 18 months, and evaluated.

In these particular studies, furan was not reversible, particularly the cholangiocarcinomas.

It is important for us that B6C3F1 mouse is the mouse used by the National Toxicology Program. Part of the reason that it is used is that it is sensitive to cancer, and that's both spontaneous cancers, as well as chemically induced.

In particular, the liver, of the 500 compounds that the NTP has evaluated, approximately 50 percent of them are carcinogenic in the mouse liver. Really, that was the reason that we conducted these studies was to try and make some association of what relevance are these mouse liver tumors to humans.

There are a whole variety of non-genotoxic mechanisms, we are not going to talk about them today. The one I do want to talk about is the cytotoxicity in cell proliferation, that furan, in short-term studies, is necrotic to liver cells, hepatocytes. It kills them.

After this, in order for the liver to try and maintain its homeostasis, we have regenerative or compensatory cell proliferation. There are certain hypotheses that believe that the mutations that you may find in the H-ras gene or some of the other genes are secondary to this cytotoxicity and cell proliferation, that the DNA is believed to be inordinately sensitive to mutation when it is dividing. It is kind of spread out there, kind of

opening itself up, if you will.

Liver cytotoxicity, how do we determine if a chemical is cytotoxic for the hepatocytes? Commonly, that is done by clinical chemistry, by evaluating serum ALT, alanine aminotransferase, or sometimes SDH levels, sorbitol dehydrogenase, and secondly, by histopathology, that liver sections are stained with hematoxylin and eosin, your H & E stained section, you will commonly find pycnotic nuclei that generally the nuclei of the hepatocytes are blue, and those cells that have been exposed to a cytotoxic agent, their chromatin and the nucleus is sometimes so blue that it is almost black.

You will find an inflammatory response. You have the recruitment of both your mononuclear and your polymorphonuclears to kind of clean up the debris as a result of this cytotoxicity, and thirdly are the degenerated hepatocytes or the cytoplasmic vacuolization.

We conducted a 13-week study in male B6C3F1 mice. These animals were exposed by gavage intergastrically 5 times a week. The dose levels

were 0.5, 2, 4, 8, and 15 mg/kg. We quantified cell proliferation by BrdU. In these particular studies, we used an osmotic pump, a 7-day. The advantage of that is that the liver--the life span of a hepatocyte in a mouse is generally about 200 days, so at any one point in time, you are only going to have 0.5 percent of your hepatocytes dividing.

So, if we go over the course of 7 days, then, we accumulate all the cells that are divided, all the cell replication that occurred in those 7 days.

So, this is an H & E stained liver section of the mouse, and what you see here, you see the inflammation that is common after exposures to cytotoxic agents. You have the influx of your morphonuclear, of your mononuclear and your polymorphonuclear neutrophils.

After you look at the incidence of liver cytotoxicity, after 1, 3, 6, and 13 weeks, you will see that the highest doses, you have a greatly increased incidence of cytotoxicity, also, at 8

mg/kg you have a significant increase, and at 4, you have an intermediate response.

Cell proliferation. One of the markers, the ways of quantifying cell proliferation is the labeling index that measures the S phase of the cell cycle. There are a variety of methodologies. You can look at mitotic figures, quantify those. You can look at KI67 gene, you can look at PCNA, a proliferating cellular nuclear antigen that is part of a quaternary complex.

For us, we used BrdU, bromodeoxyuridine. It's a thymidine analogue, so when the DNA is replicating, in place of the thymidine, a certain percentage of the BrdU will be incorporated.

Then, we have an antibody to the BrdU, so we immunohistochemically stain for cells that have incorporated this BrdU. There are a variety of routes of administration depending upon what the endogenous cell proliferative rate is. You can use a pulse. So, for instance, if you are looking at cell proliferation in the skin, you may inject IP an hour later euthanize the animals.

For us, there is the cumulative is the advantage, as I said earlier, because you can find out the cell proliferation that has occurred over a period of time.

Quantifying, how do we quantify this? Well, by light microscopy, we look at these immunohistochemically stained sections. [Off microphone.]

I am sorry, excuse me. At the 4 mg/kg, these are the cells that have incorporated the BrdU. Over here, at 8, you will see many more of them. So, what we do is we evaluate 2,000 hepatic nuclei and determine the percentage for those nuclei that have incorporated this stain.

In our 13-week study, you will see that we have a significant increase at the 15 mg/kg at all three time points, 1, 3, 6, and 13 weeks. At our 8 mg/kg, which I found to be interesting that your calculations were 8 mcg/kg, you have a significant increase at 1, 3, and 6 weeks. At 3 weeks, you also have an increase in 2 and 4.

We also conducted a study in female mice

in which they were exposed to 00.5, 1, 2, 4, and 8 mg/kg, and you will see that the highest dose of 8 mg/kg produced a significant increase in your ALT levels with an intermediate response in the 4.

The SDH was elevated in both the 4 and the 8 mg/kg. If you looked at the hepatic labeling index, again, you saw a significant increase in female mice at the highest dose of 8 mg/kg, and no other increases.

In light of this data, we conducted a carcinogenicity study, a two-year study. It was in female B6C3F1 mice. Our dose levels were 0, 0.5, 1, 2, 4, and 8. Eight, you will recall was the dose that produced both cytotoxicity and an increase in labeling index, that we had 50 to 100 animals per group, particularly in our lower groups we wanted to be able to detect the significant increase if there was one, so we increased the number of animals.

They were exposed for two years. They were exposed by gavage, and this was 5 times per week.

We conducted necropsies on these animals after two years, and what you will see here, on the left, is a normal mouse liver. This was an animal that was exposed to 8 mg/kg, and at gross necropsy, you will often find these masses.

We quantified the incidence of these masses at necropsy. There was a significant increase, 100 percent of the animals had liver masses at the final necropsy, and there was also a significant increase at 4 mg/kg.

We evaluated H & E stained liver sections for inflammation. As before, you will see that at your 8 mg/kg, that there was an increased incidence of livers with both moderate and marked subcapsular inflammation or cytotoxicity, and there was an intermediate response at 4 mg/kg.

This is an H & E stained liver section, and what we have here is a hepatocellular tumor. It is a metastatic one as it ended up being. You can see how there is loss, there is disruption of the normal liver architecture. So, we evaluated H & E stained sections for the presence of

hepatocellular adenomas, carcinomas, and foci.

You will see at 4 mg/kg, there was a significant increase in foci. Foci are believed to be pre-neoplastic liver lesions that have the ability to progress on to become benign or malignant liver tumors.

At your 8 mg/kg, you had a significant increase in the incidence of foci, adenomas, and carcinomas.

What we say here is that there is a very good correlation between cytotoxicity as measured by ALT or SDH, and labeling index, and the incidence of liver lesions. So, when those, at 8 mg/kg, where you had an increased incidence of cytotoxicity, you had an increase in labeling index, you also had an increase of masses at necropsy, and adenomas and carcinomas microscopically.

At 4 mg/kg, you had somewhat ambiguous intermediate responses in the short-term assays, and you had an increased incidence of lesions or masses at necropsy, and an increased incidence of

pre-neoplastic lesions by light microscopy.

So, in conclusion, what we can say is that this study demonstrated a dose-dependent increase in furan-induced liver tumors in female B6C3F1 mice, and a relationship between the dose, cytotoxicity, compensatory cell proliferation, and tumor induction.

An overview. In this particular study, we have reproduced the results of the NTP bioassay. In the NTP bioassay, they used 8 and 15 mg/kg. They had an increased incidence of liver tumors as did we.

What we noticed here is that there is a threshold that doses below 4 mg/kg did not increase the incidence of liver tumors or produce the short-term effects that would suggest that they would be hepatocarcinogenic.

At 13 weeks, I did not show the data, but at 13 weeks, we saw that there was an increase in cytotoxicity, there was an increase in labeling index. We have a stop group, so they were exposed for 13 weeks, then, they were held for an

additional 4 weeks. Both the labeling index and the cytotoxicity returned to normal in that group.

There are other chemicals with the same proposed mechanisms - chloroform, carbon tetrachloride, theocitamide [ph], a whole variety, that the mutations or the other events that we saw in these genotoxicity assays may be secondary to hepatocyte cytolethality or increased cell proliferation.

This is a biologically plausible mechanism. It makes sense that cells are killed, that new cells are produced, and that these particular cells may be more susceptible to DNA or genetic damage.

The furan-induced effects after short term exposure are inhibited by p450 inhibitors. We said that furan is metabolized by cytochrome p450-2E1 to its toxic metabolite, it's a dialdehyde. If you do studies in which you give the animals the furan and the p450 inhibitor, you do not get an increase in labeling index at these doses. You do not get an increase in cytotoxicity.

There are similar pharmacokinetics in the mouse and in the human in vitro, and in general, the rat metabolizes furan slower than does either the mouse or the human.

Future areas of interest. It would be interesting to know that if you gave your animals p450 inhibitors or maybe developed a transgenic mouse in which that particular gene was knocked out, would you get mouse liver tumors.

Are liver tumors due to the bolus dose? So, unlike food where you are taking a little bit in all the time, we gave them their furan in one big dose the first thing in the morning.

Are the positive genotoxic results, are they due to direct damage to the DNA, as some of the genotoxicity assays indicated, are they due to the high doses, or are they secondary to cell proliferation or other phenomenon?

What are the molecular or the gene expression changes in liver tumors? That is something that we have been looking at is to try and find out are there growth factors, are there

other things, surrogate markers that we could find in the blood that might help us identify those chemicals that are possibly carcinogenic, in particular, liver carcinogenesis, and, in particular, those that do a biocidal toxic mechanism.

Do these same mechanisms occur for cholangiocarcinomas? Is there a threshold for--and I am sorry, these say cholangiosarcomas, they should be cholangiocarcinomas--is there a threshold for cholangiosarcomas, similar to what we found with the mouse liver?

Is the mode of action of the cholangiocarcinomas similar to that of the mouse? Do biliary tract epithelial cells have pharmacokinetic parameters similar to that of hepatocytes? It is the biliary epithelial cells that are believed to be the precursors of the cholangiocarcinomas. What is the relevance of the mouse liver findings to cholangiocarcinomas and leukemia in humans?

This particularly has to do with the

pharmacokinetic parameters. What are the concentrations in the organ systems of interest?

Are there populations of humans that are susceptible to furan-induced effects and is age a factor, whether it be the baby food or the elderly? There is a lot of evidence that indicates that infants don't have the same intoxification or detoxification systems as adults, so does that make them more susceptible or less susceptible?

Finally, I would like to thank my colleagues, those who actually did the work, particularly, the--well, anyway, my colleagues--particularly the toxicology technicians and the animal care and the laboratory assistants who certainly did 99 percent of this work, the ILS Histology Department for the H & E stained liver sections, Dr. Robert Maronpot at NIEHS, who read the liver sections, Julie Foley at NIEHS, who helped with the cell proliferation studies, and Dr. Tom Goldsworthy.

Questions, please?

Questions of Clarification

DR. MILLER: Dr. McBride.

DR. McBRIDE: I have two questions. Firstly, is there any data in the mice that is age related? Secondly, if I am remembering right, you had the only slide that showed any changes at or below 2 mg/kg dose was the one slide on cytotoxicity, I forget how that was measured, and if I am understanding you right, that was reversible at least in time.

DR. MOSER: That was in the 13-week study, so let me see if we can find that data. Do you know, was that in the 13-week?

DR. McBRIDE: Yes, at the 13-week. Although it was reversible, it might still be of import because, of course, as we are looking for any change, we are looking for the lowest dose, especially in humans where there may be multiple factors that affect risk.

In other words, was this the only finding that you found at 2 or lower mg/kg?

DR. MOSER: I think that we had some cell proliferation at 3 weeks in this 13-week study, so

there was a significant increase at 2 weeks at 2 mg/kg at 3 weeks, and that is the only significant finding that we had.

DR. McBRIDE: But on your other slide, it was a 0.5 mg/kg, the one before that.

DR. MOSER: Okay, and I think what it was there, it's a statistical thing, we only had 10 animals per group, and we had 2 animals that did show some evidence of cytotoxicity. These slides were read blind. It is not a significant increase, but there is something.

DR. McBRIDE: And the question of age of mice?

DR. MOSER: Age of mice. All the short-term studies that we did, the mice were 6 to 8 weeks old when we started, and that is because the liver continues to divide and is really not mature until about 10 weeks of age, so we tried to make sure that all of our studies were done the same, be they the short term or the long term.

So, that our short-term studies and long-term studies, they all started exposure at the

same age. It is just that with your 2-year studies, of course, we went out to the end.

DR. MILLER: Dr. Gray.

DR. GRAY: I think something that we want to try and learn about here that is really important for thinking about this food situation is something you touched on in one of your last slides, and that is this question of dose rate.

Is there any other data to help us understand that, because as you mentioned, other compounds that are thought to act in this way show a strong dose rate effect. For example, chloroform gives you very similar mouse liver carcinogenesis by gavage.

Ninety percent, 100 percent response, you give the same dose in drinking water over the course of the day, no tumors at all.

DR. MOSER: Right.

DR. GRAY: And if dose rate is an important factor here, that is something we need to know because that is a big difference between our animal studies here and the way in which people are

likely to be exposed.

So, I mean I don't know if there is something in the literature you can help us understand, or if it is something that we need to think about as a study going forward is understanding whether there is a strong dose rate effect for liver tumors and the other tumors that are out there.

DR. MOSER: Let me say, and that is why I put it up there, chloroform has a mechanism of inducing liver tumors that appears to be very similar to that of furan. If you give chloroform by gavage, the same way we gave the furan, you will get liver tumors. You give them the very same dose in the water, you do not get liver or kidney tumors.

So, that indicates that maybe small amounts over the course of time doesn't have the same effect as just one huge dose, and particularly maybe first thing in the morning. They are nocturnal, you know, they move, they do things at night. So, who knows? But that is a very, very

important thing.

DR. GRAY: And at this point, there really isn't anything in the literature to help us on the furan front on this?

DR. MOSER: I think that Greg Kadaras [ph] has done a little bit of work, and I think it has been inhalation, and I will have to check on that, but he has done work that I believe has been by a mechanism other than gavage.

DR. MILLER: Dr. Krinsky.

DR. KRINSKY: Thank you for the nice cancer cell biology review. You used the term "threshold" and "dose dependent." Those are not identical, and I think that is important in terms of human consumption, because if, in fact, your data indicates that there is a threshold level prior to seeing toxicity, that may have very important implications as far as human consumption is concerned.

DR. MOSER: I would agree. The idea of the threshold is that there is, from my definition, okay, in this study, is there is a dose below which

you really don't see the cytotoxicity, you don't see the compensatory cell proliferation, and you don't see the liver tumors, as compared to dose response, which means, you know, a low dose you get a low response, medium dose, medium, high dose, high response.

DR. KRINSKY: And the mechanism for the threshold?

DR. MOSER: What we would have to say is that whether it's a matter of intoxication, you know, that there is just so many mixed oxidase function enzymes to produce the toxic metabolite, or there is a detoxification mechanism, you know, it is believed that glutathione is a way of detoxifying the metabolite, and there may well be, and we know that is the case, only so much glutathione, so there is only so much to help us cart that toxic metabolite out. Beyond that level, you may see toxicity.

But the truth of the matter is, when I look at the literature, glutathione is the only detoxification mechanism they have looked at.

There may be others.

DR. MILLER: Dr. Russell.

DR. RUSSELL: Again, thank you for that presentation. In thinking about populations of humans that might be susceptible to furan-induced effects, the one that comes to mind right away to me is alcohol users, because it is such a potent stimulator of p450-2E1.

DR. MOSER: Exactly.

DR. RUSSELL: I think that that ought to be looked at in your model, but in epidemiologic models if this gets carried on to humans, that this really may be a population that is much more susceptible.

DR. MOSER: I think there was a study done at CIIT, and it was a short-term study in which they induced the cytochrome p450-2E1 by exposing the animals to acetone, it was. I don't think it was the alcohol should have done the same thing.

DR. RUSSELL: Yes.

DR. MOSER: So, you have these higher levels of the enzyme that is producing the toxic

metabolite, and we also know that certainly alcohol consumption is a prerequisite to certain kinds of liver damage and ever certain kinds of liver cancer, so that is a very good point, and I wholeheartedly agree. Thank you.

DR. MILLER: Dr. Callery.

DR. CALLERY: Let me probe that 2E1 a little further. I want to know if you know that the 2E1 in mice is the same as the human 2E1 is one question, and then another is, I am probing my own memory here, do you know Carlson's work at Purdue on styrene?

DR. MOSER: No, I am sorry, I don't.

DR. CALLERY: I believe he has got a null 2E1 mouse.

DR. MOSER: Oh, does he?

DR. CALLERY: Where the styrene was still converted to styrene oxide and had the same viability for potential carcinogenicity.

DR. MOSER: I think that's interesting because it's like everything else, you know, we have looked at the 2E1. There may well be other

means of intoxication, we just haven't looked at them.

DR. CALLERY: Or detoxification.

DR. MOSER: Exactly.

DR. CALLERY: The other is I am trying to relate the mouse to the human, and especially in the glutathione concentrations and such, and the redox activity in the glutathione system. I don't know how that relates to the human.

DR. MOSER: I don't know either. Does anybody know if the glutathione levels are comparable in mice and in humans, or in the liver anyway? I am sorry, I don't know.

DR. CALLERY: Then, I guess the last one is you had mentioned that you were at 2 mg/kg and we have an estimation that the human exposure might be 1,000-fold less. Does that have any meaning to you?

DR. MOSER: Well, there is species extrapolation, as we all know, is extremely difficult. Not only that, we have got a tissue extrapolation here, you know, that we are not

necessarily talking about, a high incidence of liver cancer in this country, not to say that it is not important, and most of the liver cancer that we see in this country we believe to be more associated with hepatitis and alcohol consumption.

So, does that mean that I think that those levels are safer? Not absolutely at all. It does appear that the mouse is more sensitive to liver tumors than some of our other models. Actually, a better question is well, what about the cholangiocarcinomas. I mean they saw almost a 90 percent incidence at 2 mg/kg in your NTP bioassay. Is that relevant?

Well, here, we don't know, at what dose do you continue to get these cholangiocarcinomas. As I look at the literature, and I am not an expert on that by any means, it does appear that the rat is more sensitive to at least cholangiocarcinomas than are mice or humans, that in all the reading I have done in the last week or so trying to prepare for this, there is only one study in which there was an increased incidence of cholangiocarcinomas in mice,

and that was with PCBs, polychlorinated biphenyls, and they were on some sort of a restrictive diet, and the incidence was not as high as what you are seeing in your rat.

So, again, we go back to the species is extrapolation, is that data in rats relevant to humans at that dose. I wish I knew.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: You just mentioned hepatitis, and I was thinking that another human population that might be at risk for furan are patients with hepatitis B and hepatitis C, and there is an animal model for hepatitis B, and I am blocking on the species. I think it might be the prairie dog, but I am probably wrong about that.

DR. MOSER: Is it the woodchuck?

DR. CHESNEY: It's the woodchuck, that's right. Thank you.

DR. MOSER: Don't ask me how I knew that. That just came out of some deep recesses.

DR. MILLER: I am going to call for a break. Can we be back here in 10 minutes.

[Break.]

Public Comments

DR. MILLER: The FDA received only one request for public comment, that by the National Food Processors Association. It was to be delivered by Dr. Richard Jarman.

Dr. Jarman has said that in the interest of facilitating the discussion, he submitted a statement which he intended to read and which you have all received a copy of, and he is prepared to allow the statement to stand in lieu of having to make a presentation.

So, the statement will be incorporated in the record.

Summary and Charge to the Committee

DR. MILLER: Now we come to the nitty gritty. Before we begin our discussions, Dr. Terry Troxell of the FDA will summarize and re-present the charge and reading of the questions, and then we will proceed with our discussions.

DR. TROXELL: Thank you. My name is Terry Troxell, Director, Office of Plant and Dairy Foods.

I don't want to take much of your time. I just want to recapitulate a little bit to facilitate your discussion. The main points here are the actions we take and what are the data needs, the charge, and the questions.

As we have said, we have developed the method. The method was posted on the web site. We are going to be doing a round robin, so we should sort out any differences in levels being observed.

We did an exploratory survey, more than 160 foods were in our first round, and with 40 more since we published a notice in the Federal Register, and 30 from Canada. We are now at 230 foods, and we will be testing a broader range of foods.

The preliminary exposure assessment was done. We utilized the first 160 samples, obviously, because things are moving so fast, we were not able to incorporate all the data that we pulled recently.

We also obviously did our call for data and then we have established international

interactions. We provided Canada with our method, and they very quickly developed additional methods, similar, so that we could generate a lot of data together, and we also coordinated, so that we could minimize duplication of effort, so that we can maximize the results at this point.

The other thing you should be aware of is that the EU has a heat tox project, which is to look at investigating thermally processed induced toxins in foods, and they are going to incorporate furan in that process.

So, again, as with acrylamide, we are trying to maximize our collaborations to try to zero in on the problem as soon as possible.

Some future actions, of course, we still have another 30 days to go on our data call, and then we will evaluate that data, the whole group of data. We will take the tox studies to our Cancer Assessment Committee or CFSAN's internal expert group to have a look at the overall picture and to help us decide where to go from there.

We also want to develop an action plan to

provide guidance, not only for FDA, but also in our broader collaboration efforts, so that we can adequately assess the risk, and, of course, to serve as a basis for risk management.

Data needs. Or course, we need a broader range of foods in which furan occurs. We need to know the levels of furan in these foods, and then, of course, the formation and occurrence in home-prepared foods.

We have been working on a hypothesis that pretty much if you cook the food in a hermetically sealed container, like a can or jar, whatever is formed in there is trapped and can't escape, whereas, other foods, such as potato chips, which are produced in an open line, or cereal, or so on, that a significant amount of degassing would occur or volatilization would occur either in the processing environment or subsequent through diffusion.

So, then, it gets to the question of what happens in the home environment, what happens in the retail environment, where you might generate

some furan, and it does not have time for dissipation, so, yes, we do need to flesh out what is happening in the home environment, which also would be relevant to a retail environment.

The other consideration we need to bring into this is other environmental sources of exposure to furan, such as from smoking, there is a range of possible areas.

We have here possible mechanisms of furan formation. In addition to possible mechanisms, what we would be most interested is the principal mechanism of formation during standard process in home cooking.

We also have variables that enhance or mitigate furan formation, stability, or dissipation of furan in foods. Again, that goes to the dissipation over time, for example, and the stabilization with fats in the food, effects of post-production practice on furan levels.

Then, we have quite a few areas of toxicological needs, mechanism of furan and toxicity, mutagenicity, and carcinogenicity, the

reproductive and developmental toxicology of furan. We have basically zero on that to my knowledge. The metabolism of furan in vivo including characterization of reactive furan metabolites.

The diversity of furan pharmacokinetics in humans or the alteration of furan metabolism as a result of dietary, medical, or environmental interactions.

Data on whether sub-cytotoxic furan doses produce any adverse effects, such as a change in enzyme activities or ATP levels.

The effects of furan doses lower than those used in the NTP study: to establish a dose-response curve for various tox endpoints; to determine whether furan toxicity including carcinogenicity is a threshold dependent event; and to determine whether carcinogenic activity is secondary to hepatotoxic effects.

Still more. The mutagenicity of furan in the TA100 strain in the Ames test. It was weakly positive in one study, but negative in all Ames strains in the NTP study. The behavior of furan in

other in vivo assays for mutagenicity or toxicity.

Then, that brings us to the charge, which is in Tab 2 of your notebook. That is the Food Advisory Committee and Contaminants and Natural Toxicants Subcommittee are being asked to provide input on the data that would be helpful for further evaluation of potential risks posed by the presence of furan in food.

Then, the question which I will leave up here to help discussion. Taking into consideration the data needs already identified in the Federal Register notice requesting data on furan, and the presentations at this meeting, are there any additional data that are needed to fully assess the risk of furan in food?

The data needs we identified are fairly broad. Are there still gaps? What are the important missing data for assessing the human health impact from furan in food? Since there are studies available in some of the areas, such as mutagenicity, which areas need particular fleshing out and in what ways?

I guess this is what to me is encapsulated in the broad question. Finally, I want to thank you very much for your critical input here. We are at a very early stage in this process in trying to get our arms around this issue, and it is very important to have your thoughts to help us do the right set of work and develop the action plan, to guide our efforts for the future.

Thank you very much and I can try to answer questions that may be lingering and, if not, we can have our experts answer those I cannot before you start your discussion.

Questions of Clarification

DR. DWYER: Dr. Troxell, a couple of months ago, i think it was, or maybe longer, we discussed acrylamide in food, and I wonder if there is a sort of a large plan to look at all the heat-formed compounds that seem to be of concern, and is this part of it, or how is that being handled, the big picture?

DR. TROXELL: We have no comprehensive program to look at them. The EU has this heat tox

program that they put a fairly large sum of money in to work on, on thermally generated toxins. We are collaborating with them, I mean interacting and, as I said, we have contributed a new one to their effort.

DR. ACHOLONU: We are told that you have checked different kinds of foodstuffs so far, and you have plans to do more. Have you been checking these randomly, or do you have a special pattern that you are using to select the foodstuffs to check for furan?

DR. TROXELL: Well, again, the theory we used for several fold. Those foods we expected to contain furan, such as the canned and jarred foods, and also even if we expect at the levels to be on the low end, we also wanted to check the foods that were major contributors to the diet, such as the apple juice for the children.

So, yes, we have a strategy, and as with acrylamide, we will focus in on those foods which seem to be the major contributors and look at those intensely, but also expand more broadly to make

sure we haven't missed anything, and over time, we have picked up a few additional items that have contribute.

So, we have to start at the first base and we will keep digging in until we kind of flesh it out more completely, and with our collaboration with Canada and hopefully the EU, hopefully, we will get to fairly comprehensive understanding of the foods that contribute significantly to the exposure.

DR. MILLER: Dr. Waslien.

DR. WASLIEN: The total diet study analyzes now for toxicant suppression in food as well as vitamins, and I would think that it might be worth investigating to see about collaboration with that process, too, just to get a better mix of foods, say, go out and buy it, you know, a systemized fashion, and you can get an overall view of exposure in the U.S. diet, and it may identify at least for one shot, groups of foods, maybe that way more easily.

DR. MILLER: Dr. Lee.

DR. LEE: I was just wondering, I guess furan has been looked at by flavor chemists because it is part of the flavor, taste of food. Does it actually have a role in food acceptance or it is just an incidental compound that has no measurable positive impact on food?

DR. TROXELL: I can't answer that precisely. I know the class of foods, the class of chemicals is known as the furans of various derivatives. Some of those would have a role in flavor chemistry. I don't know that furan per se has a role in flavor chemistry. Certainly, once you start getting into that class and you get side reactions and decomposition, and so on, you are going to end up with tiny amounts of furan in foods as we have found, and with the new analytical technology, we are now able to tease out very precisely what couldn't easily be seen back in '74, for example.

DR. MILLER: Dr. Lund.

DR. LUND: Relative to occurrence and exposure, you mentioned specifically home-prepared

foods. Are you also going to be looking at food service preparations in restaurants?

DR. TROXELL: This is a good comment, and I think many of the approaches used in restaurants are the same approaches used in the home. I mean we have to look at microwaving, and so on, to see what generates food, what generates furan, you know, at levels that don't dissipate by the time the consumer ingests the food.

It is the same problem, of course, with the total diet study. We have worked on volatile organics and I don't know what protocols we used there, but we have to be careful that, you know, those foods are analyzed ready to eat, but by the time, you know, they are prepared by a church group, and if they are not specially processed after they are cooked, we could lose whatever furan was formed, and it will not be as consumed, so we need to be very careful that if we do such a study, we end up with something that mimics as consumed.

DR. MILLER: Dr. Nelson.

DR. NELSON: I just wanted to follow up on

Dr. Lee's question about the flavors. I don't believe furan is itself a flavor compound, but furanial is and furferal [ph] is, and I believe FEMA, the Flavor and Extract Manufacturers Association, has done a toxicological review, that they are pretty well clear.

DR. MILLER: Jean.

MS. HALLORAN: Earlier, you had a few cases where you heated the canned foods and had some interesting results. Are you going to do any more with the canned foods to attempt to mimic preparation, normal preparation situations?

DR. TROXELL: Yes, I think whether it be the canned foods, whether it be a soup that is put in the microwave and heated to see if that increases the levels or not, of other foods that are cooked that might generate from the get-go whether we might have furan in an oven-baked food, I think we probably have to check some of those things out just to cover the territory, so we understand the dimensions of the exposure.

DR. MILLER: Dr. McBride.

DR. McBRIDE: A couple of questions. Do we know anything in the animals about serum levels? I am not even sure that serum levels are valid because the boiling point is below body temperature, and probably first pass to the lungs, it is pretty well out of there, but do we know anything about serum levels in animals?

DR. MOSER: No.

DR. McBRIDE: I don't know whether that is worth pursuing or not. Also, do we know anything about furan exposure industrially in humans?

DR. TROXELL: I don't know anything about it. Certainly, there would be a component of interest, but it would probably be a small portion of the population, and if you are thinking of using such a group, for example, to look at epidemiology to see if there is an increase of tumors that way, such as was done with acrylamide, that certainly is a question that could be asked.

I do believe on your other question that there is some comparative metabolism studies in different animals on furan, although I don't know

that there is serum levels, but there certainly is as compared to metabolism, I believe.

DR. McBRIDE: But it would probably be the two issues, you know, whatever wasn't metabolized in the liver may or may not stick around in serum, probably wouldn't by the boiling point, but I am not sure.

DR. TROXELL: I think it is cleared fairly fast, I think the half-life is fairly short.

DR. McBRIDE: Another, at-risk population might be smokers.

DR. TROXELL: Absolutely.

DR. McBRIDE: Houses with lots of smokers, that sort of thing.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: I have one specific question and then I have a whole list of suggestions. Is it appropriate to do that now or did you want to wait?

DR. MILLER: Why don't we wait until we finish with Dr. Troxell.

DR. CHESNEY: The more specific suggestion, people are mentioning other things to

test. I wondered if it would be worth testing intravenous and parenteral preparations that are given to humans because a lot of them have lipid in them, are given continuously for long periods of time, and they are often warmed or heated before they are given.

DR. TROXELL: You mean intravenous or tube feeding?

DR. CHESNEY: Both. The tube fed ones do come in cans, and the intravenous ones obviously--

DR. TROXELL: There is also a time factor. To put it in context, there is a time factor here in that we are talking about generally chronic effects, so if you are talking about a short-term exposure, even though we may be particularly concerned because it is higher or a worse case exposure, the effects we are talking about generally are chronic exposures, so we should put them in our equation what we look at.

DR. CHESNEY: I understand, but we have children, for example, who are on parenteral preparations for years because they have had their

GI tracts removed, or many people are fed enterally for other reasons continuously for years and years.

DR. TROXELL: It would be good to check.

DR. MILLER: Dr. Downer.

DR. DOWNER: I am just going back to Dr. McBride's question about environmental exposure. We did mention cigarettes, but also I read in one of the studies that exposure or people who are bakers, who are expose to baked bread, that was also an environmental exposure risk, so perhaps we may also want to look at individuals who are in the baking industry to see if perhaps there is some consideration there.

DR. MILLER: Dr. Chin.

DR. CHIN: I just want to actually go back to something that Dr. Beru mentioned. Just for point of clarification, I thought that he said that when FDA did the initial assessment of the risk, that you took the work that was done by the National Academy of Sciences and NASA, and you came up with a NOEL of 80 mcg/kg per day, I believe.

DR. TROXELL: National Academy came up

with number.

Committee Discussion and Recommendations

DR. MILLER: If there are no more direct questions, he will be here in case the issues come up again.

So, now the time comes for us to start making some recommendations for studies that ought to be done, that have not been thought of by the agency.

The question is what can we add to their action plan that would be useful. I am sure you all have your favorite lists. A number of them have already come up in our discussion. The impact of development on metabolism of furans, I think was one of the earlier ones, and I think that is a very important issue, particularly given the fact that there is a substantial part of the exposure is in infants, and so we need to know better whether or not there is a difference in the sensitivity of infants or elderly, for that matter.

The question of dose rate, all the experiments that have been done so far have been

done with daily bolus and there is some suggestion that there may be a difference between bolus exposure and continuous exposure, and that becomes very important from a methodological point of view, as well as from the point of view of trying to interpret the potential hazards associated with furan exposure.

Another interesting one that came up in the first part of the discussion is the question of glutathione, is glutathione the biological detoxification step, the principal biological detoxification step for furans, and if it is, can you associate things such as the cytotoxicity of furans with the regulation of the glutathione. That it seems to me needs to be done.

The question of food service has come up quite a bit. I am not so sure it's true, Terry, that the processes used in food service, preparation of food are identical to those used in the home. They are often cooked at higher temperatures and maybe for shorter times, so I think it would be worthwhile to take that into

account in determining your sampling program.

The other thing that occurred to me, and I may have missed it, but I didn't see anything on mother's milk, how much of mother's milk, how much of furan or of the metabolites, potentially toxic metabolites of furan show up in mother's milk.

I think, Dr. Chesney, you said you had a long list.

DR. CHESNEY: Is it my turn?

DR. MILLER: It's your turn.

DR. CHESNEY: Thank you very much.

Let me just start with a general overview. As you know, we have a very yuppified generation of young parents out there, and I think as soon as they hear more about this, they are going to be quite active and involved, and I know you have anticipated that, but the first thing I wanted to mention is very simple, and that is that it might be interesting to look at levels of furan in home-prepared baby food.

This was very much in 20 or 25 years ago, and it was a lot of work, and so I didn't last very

long with that, but that is certainly something that you would be able to give a very quick answer to people if they had questions about if I made my baby food at home, and didn't heat it, would my infant be better off.

So, my other issues, obviously, as pediatricians, we think very much developmentally. We think about the mother-fetus diad, and then the newborn who is very immature in many respects, and then the infant and ultimately the child.

So, in terms of we wonder about tissue penetration, tissue concentration, and impaired metabolism and excretion depending on the age. So, starting with the maternal fetal model, I think maybe using animal models of pregnant mice or rats, and then newly delivered mice or rats to look at issues of transmission, is there enhanced transmission across the placenta, is the placenta particularly concentrated in terms of furan or none at all.

And then I also had breast milk on my list, is a very important issue, and somehow it may

be even clinically in humans ultimately tying levels in breast milk to levels that the mother ingested in the recent past.

And then turning to the newborn, we have seen the levels in formula and infant food, but the newborn GI tract is a relatively porous organ, and i don't remember enough of my GI pathophysiology to remember how long that goes on, but is there enhanced furan uptake in the first few weeks or month of life, and does it concentrate in other tissues and/or pass into other tissues.

We have talked about the liver and the background materials we were given, talked about the kidney, but obviously, again in terms of development, as pediatricians, we are always very worried about the brain. Could studies be done in fetal and infant animals to show whether the furan is penetrating the brain because that is a question that will come up very quickly when more and more people learn about this.

Questions about infant PK studies of furan, again maybe before and after ingestion. The

issue of home preparations and finally sick infants. We have a lot of children who come in for fairly acute self-limited illnesses that do develop liver damage, transient liver damage, and then we have large populations of premature infants and ill infants who spend prolonged periods of time in the hospital, who often are fed with these intravenous or nasogastric preparations, and they often also have underlying liver disease in part because they get the intravenous parenteral preparations.

So, if there were any question about significant furan levels and what we are giving them on top of the fact that they already have cytotoxic liver damage in many cases depending on the underlying disease, I think that would be important to know.

That is my list for the moment.

DR. MILLER: Good. Thank you very much.

DR. KRINSKY: I just want to point out that once you move from the food into the animal or the human, that furan is not the only thing that should be analyzed because furan is not the active

component, furan is metabolized, and I think we have to have information about what the metabolites are, what the active metabolites is, and whether, in fact, this can be assayed in tissues, in fluids, or anything of that nature, but not just the furan.

DR. MILLER: Dr. McBride.

DR. McBRIDE: I think it is a good suggestion to look at it in breast milk, and again, one might want to look at it in the at-risk group, smokers, for instance, versus non-smokers or something like that.

The second suggestion is in looking for mutagenicity, one might want to do second-generation studies in the rats especially in the females.

DR. MILLER: That is a good idea.

Dr. Callery.

DR. CALLERY: About the metabolism and follow-up on what we just heard about the metabolic activation, I am wondering if there isn't the use of the Ames test, not just for furan, but also in the presence of human liver microsomes, such that

it would have a bioactivation potential to screen for mutagenicity.

The comment about glutathione as a detoxification pathway, the compound that is in the literature that is the potential mutagen is a sis-buten-dialdehyde [ph], which I believe has been shown to be mutagenic in some system.

But the way that that compound may also be detoxified in addition to glutathione or other sulfhydryl compounds is by oxidation of the aldehyde and further conjugation of those products. So, beyond the cytochrome p450s are the soluble enzymes, probably the aldehyde dehydrogenase or aldehyde oxidase that would certainly want to be looked at.

The comment about the infant and aged, in their metabolism, if this is a metabolically activated compound, it might be advantageous to the newborn not to have 2E1 and another p450 isoforms might be to their benefit.

So, that might beg the question of whether you want to do any even--I don't know if there is

genotyping, pharmacogenomic issues about whether 2E1 is available and in different population sets or if you wanted to actually consider relating 2E1 concentration to potential for mutagenicity.

I had another comment that was about the broader collaborations that we have been hearing about and especially since one of the issue here is the mechanistic aspect of how this substance might be bioactivated, is the possibility of working with NIH to have the National Institutes of Health consider an RFA on the mechanisms of this, and then when you look to the comments of the workplace exposure to furan might also involve a collaborative association with the EPA.

DR. MILLER: Dr. Gray.

DR. GRAY: Thank you. I have just got two areas that I would like to comment on. I think that there is really no way that we can continue in a situation where we haven't done reproductive and developmental tox on this material, and it just seems to me that that is one of the things that should be in an action plan pretty quickly, at

least getting some feel for this.

Some other compounds that act like it, it is probably not something where there is a serious concern there, but it is just the kind of information I think that needs to be developed in a fairly short time.

The other thing I wanted to do is to encourage the work on the mechanisms of formation. I think that is a really important thing, and I think it is important for a reason that was actually raised by Jim Coglin in his letter that he submitted.

This already looks like it is in. I think the estimate that we just saw it's in about 20 percent of the food supply now, and that's not consumption weighted, but 20 percent of the food supply, it may end up in 30 percent or 40 percent. We are not going to be able to avoid it.

Then, we are going to have to start thinking about ways in which we will try to manage it, and I think it will be important about understanding the mechanism is by in managing that,

we want to make sure that we are not making risks worse, that some of the things we have talked about, like pasteurization, have their own real benefits that we have to understand.

So, if we can understand the mechanisms, perhaps we can design ways to control it if that is necessary, but we can at least keep in mind the tradeoffs that we might face. So, this is just I think a plea to continue working the great work that has been done in Canada, work that is being done in FDA, to try and move along the mechanisms, so we don't find ourselves making things worse by trying to address this problem.

DR. MILLER: Thank you.

I think ideas like that were incorporated in the plan. I say that only because it is important to reiterate it because I think it is a basic thing. The issue for these adventitious contaminants that are natural products, the only thing you can do is mitigate them. You can't bann the food.

DR. GRAY: I don't want to give up my

coffee.

DR. MILLER: That's always a personal thing.

Dr. Downer.

DR. DOWNER: I agree with what my colleagues have said, I just wanted to add a little bit by saying we really need to do some more comprehensive data on the furan that is found in foods and beverages, and perhaps using the U.S. data tapes or some of the NHANES data or some other database, so that we can get a better perspective of the different kinds of foods and groups of foods in particular and the furan levels in them.

Also, I think it would be good if we found that we should establish an acceptable level. If furan level is low, then, we might not need to implement a risk management protocol, but if it is higher than the level that was established, then, we will want to do some interventions then.

I go back to what you said, Dr. Miller. I concur because we really don't prepare foods at home as we do in a restaurant. We haven't look at

condiments, for example. We don't know the impact of that. It may be small, but it may be significant.

So, I think it would be prudent for us if we look to see if there are any risks in food preparation and packaging. We may want to look at home prepared, restaurant prepared, as well as pre-packaged foods to see what levels may be there, as well.

DR. MILLER: Dr. Lund.

DR. LUND: Just to follow up with regard to the types of processes that ought to be looked at. It would be good to look at a couple of the newer processes. You might not expect to see much furan formation in high pressure processing, for example, but it is certainly an alternative for thermal processing in some cases, and at some point needs to be looked at among other processes, as well.

DR. MILLER: Dr. Dwyer.

DR. DWYER: I think I share other people's concern that a wide variety of possible suspects

among foods be look at, not just the ones that have been looked at. I am still puzzle by this business of heat formed compounds occurring together. Last year, we heard that potato chips were very high in acrylamide.

So, is it because all the acrylamide is taking up the main line of reaction, who knows.

The second thing I am concerned about is the whole issue of risk and the sensitivities that have been mentioned. I believe Dr. Russell mentioned alcohol. Dr. Krinsky mentioned I think hepatitis B and C, smokers have been mentioned. Coffee has been mentioned, but only in a positive context, it might have some effects that would be worth looking at, and then I wonder about common OTC, particularly OTC, but also perhaps prescribed drugs that might affect those same systems as being important to at least take a look at.

The final area has to do with mechanisms and metabolism, the issue of dose rate of compounds, is there a threshold versus the dose response business, the issue of getting common

methodologies so we don't overduplicate all seem to be important.

DR. MILLER: Thank you.

Dr. Lee.

DR. LEE: I suppose if the FDA could wave its magic regulatory wand and eliminate all of this compound in food effective tomorrow, but the question needs to be asked, is the human condition going to be substantially improved, and I just don't know if you have got there yet.

Particularly, I would like to see what the relative exposure is to humans from other sources of furan. I don't have a good feel for that, is food really the problem, or is it just that we are sensitive to it because it is showing up in foods and things that we think ought to be absolutely pure.

I think that is really a very subjective judgment whether or not the human condition is improved by elimination of the compound, but we need the data on where the problems lie outside of the food system.

DR. MILLER: That's a good point, but the problem is that you can't answer that question until you know all this other stuff, and I think in the end, that is what the goal ought to be. Or course, there are others, it is going to depend upon the agency's decisionmaking, but that should be the primary goal.

Dr. Chin.

DR. CHIN: I guess one area that I guess gets back to the basics in terms of measuring furan, and I know that a lot of work has been done already in terms of validating the methodology, but as we got further into this and especially as people make decisions or make assessments, I think we need to probably do more work on the methodology, you know, more collaborative study, maybe develop some type of an official or standardized methodology.

We also probably need to make absolutely sure that we are neither producing the furan during the analysis, nor are we missing the furan in analysis. So, I think those issues still need to

be looked at as we go forward.

DR. MILLER: Dr. Acholonu.

DR. ACHOLONU: We are told that FDA has found measurable furan in jarred baby foods, in canned infant foods, in canned adult foods, emphasizing can and jar. It may be necessary to find out if the jars and the cans have anything to do with the concentration of furan in the food.

That is a suggestion I would like to make.

DR. MILLER: The situation that has to be looked at, and the situation like this is a risk-risk situation, what are the risks of doing something versus the risks of not doing something. In other words, if you give up reprocessing of foods, what is the hazard associated with that, too? I think that is the question that you were asking.

DR. ACHOLONU: The can itself, the can, the jar. Does the can have anything to do with the content?

DR. MILLER: Packaging.

DR. ACHOLONU: Yes.

DR. MILLER: Your question is the packaging.

DR. ACHOLONU: And this you can do by comparing, as they test different kinds of foods, get the ones in jars and cans, and the ones that are not in jars and cans, and compare them, and find out if there is any difference, and then use that to make some extrapolation.

DR. MILLER: That issue has arisen several times concerning the packaging. It is not the only thing that you have to worry about, there are other kinds of packaging that might be a problem.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: I haven't heard anything about serum levels of furan or its metabolites. Do we know if it is normally detectable in normal humans, or has anyone looked for furan levels in serum, or the metabolites?

DR. MOSER: I think that there has been a PD/PK model, pharmacodynamic, pharmacokinetically based model, in which they predicted serum levels, but I am not sure that they actually quantified

them.

DR. CHESNEY: So, they have never actually drawn blood and tested it in humans.

DR. MOSER: Not that I know of.

DR. CHESNEY: I think Dr. Lee's point is very important, that this may be nothing, this may be a nothing issue and we have to be very careful not to overreact, but certainly, as I mentioned, when the yuppie population hears that it is in infant formula, they are going to become very alarmed, and I think it might be nice to have some sort of very--if the methodology is out there for measuring normal furan levels and serum, and just doing a very simple PK/PD study based on ingestion of high furan foods and low furan foods. That seems like it would be fairy straightforward.

DR. MILLER: I would only modify that by saying that whatever the principal toxic metabolites are may be more important than looking for furans.

DR. CHESNEY: I agree. I keep saying furans, but furan or its metabolites or just its

metabolites.

DR. MILLER: Good. Dr. Krinsky.

DR. KRINSKY: I just want to emphasize the potential nothing aspect of what we are dealing with, and since we don't know, we don't know what the levels are that might be toxic, we don't know whether we can achieve that level with the diet. There is so much that we don't know about this that that is why we have a series of questions where there are things that we want to have done.

What concerns me is that the FDA will treat this in an appropriate fashion. I must say that I am very concerned about the people sitting behind us. I don't know how many of them are from the press, but I am really concerned about how the press is going to interpret what we are raising here in terms of furan in foods.

We don't know if what we are looking at is potentially harmful. We don't know if it is harmful. We don't know if it is potentially harmful, and yet I think what we have been talking about can be misinterpreted so easily that I would

like to censor any press release that comes out from behind us.

DR. DWYER: Go the pentagon, this is the wrong agency.

DR. MILLER: That temptation is never fulfilled.

You are right, of course, I mean this committee was not put together to consider that issue, but nevertheless, I don't see how you can come to any conclusions unless you do the experiments to get the data to see whether it's a problem of not, and it may turn out to be nothing, but you have got to do the experiments to find that out. You just can't ignore it.

Dr. McBride.

DR. McBRIDE: Along with that, we don't want to spend a lot of the agency's money looking at something that may turn out to be a non-entity.

Again, back to the serum levels. It seems to me the first place to look is those rats that got those high doses, because if it isn't there, chances of our finding it in the human are small,

and, of course, looking at the brain, a high lipid organ, as well.

I was in my wildest dreams trying to figure out what groups of kids would we go, and how would we even approach the mom, but there are a group of kids who are actually orange from eating so many sweet potatoes out of the jar, so if we feel a need to go to humans, that might be one.

DR. MILLER: Dr. Chesney.

DR. CHESNEY: In response to Dr. Krinsky's comment, I think, as we said, this may turn out to be nothing, but I really would like to commend the FDA for making this public so quickly in your deliberations.

I mean you keep coming back to the May 7th on the web, and most of us go to the Federal Register on a regular basis, but you had it out there immediately, and I don't know how you could do otherwise, and I think that that is the most important message. We don't know what this means, but we are letting you know that we are looking at it, and so I just wanted to make that comment.

DR. MILLER: Thank you.

Any other comments? Any other suggestions?

DR. CHIN: I guess just to reiterate, it seems to me that the most important piece of information that we need is to come to some closure in terms of whether or not a threshold exists and what that threshold level might be, and so either the continuation of the work that was reported by Dr. Moser or something along those lines, I think it is very important because that is really going to guide us in terms of how much more resources to put into this issue.

DR. MILLER: To answer the question, I think some kind of risk assessment is going to have to be made. It is not going to be a very good risk assessment because they don't have all of the data that they are going to need, but they need to have some ballpark figure.

If I had to personally mark a suggestion concerning this, I would make that my highest priority, develop that data that allows me to make

a risk assessment.

Also, I think from a regulatory point of view, I think if you do this risk assessment, you also should be doing risk assessment about what would happen if you weren't able to process food according to the technologies that we have available.

Dr. Waslien.

DR. WASLIEN: That would eliminate me for the Atkins Diet, wouldn't it?

[Laughter.]

DR. WASLIEN: As part of that risk, I think you said that once mice or the rats were off of the diet, that you saw the disappearance of any carcinogenic effect, so it is not cumulative, so it's purely a sort of short duration or ongoing exposure that has to be present, or was that documented enough?

DR. MOSER: There is also data that demonstrates that one high dose was able to produce tumors two years down the road. There was also a stop group that got higher doses than we gave them.

They got that for 13 weeks. Then, that exposure was stopped, and they found increased lesions as compared to the group that was continuously exposed.

DR. WASLIEN: Then, concentration effects are part of the metabolism you have to look at, too. Thank you.

DR. MILLER: Any other comments? If not, that's very good. I am not going to even try to summarize all of the very good suggestions that have been made. I wanted to keep them for the end of the meeting, so that in the preparation of the report of the committee, summary of the meeting, from the transcript, all of these would be in one place and make it easier to identify these recommendations to the agency. For that, I thank you.

The next step is to develop a verbatim transcript of the deliberations of the committee, and that will be made available on the internet, and then a summary document of the committee's deliberations will also be prepared, and we will

share that with you directly.

I have nothing more to add except to thank you all very much. I appreciate you remaining focused and disciplined generally.

Terry, do you have something to say?

DR. TROXELL: I just would like to say for the Center for Food, Safety, and Applied Nutrition, and FDA, thank you for your two days of close attention and careful and well thought out input, and we know it is a tremendous effort out of your busy schedules to do this for us, but it is invaluable and thank you for myself, Dr. Laura Tarentino, and the rest of FDA.

DR. MILLER: Thank you, Terry.

Hope you all make your planes and I want to thank you personally very much for being a very good committee.

We are adjourned.

[Whereupon, at 5:00 p.m., the hearing adjourned.]

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