ajh

TCB

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

PUBLIC MEETING

TO REVIEW THE CURRENT SCIENCE RELATING TO SPROUTS AND NEEDED CONTROL MEASURES

Volume II

Tuesday, September 29, 1998

8:00 a.m.

Crowne Plaza Washington Hospital
Sphinx Club Ballroom
1375 K Street, N.W.
Washington, D.C.

MILLER REPORTING COMPANY, INC.
507 C Street, N.E.
Washington, D.C. 20002

C O N T E N T S

P R O C E E D I N G S

Interventions and Regulatory Review

MS. OLIVER: Good morning. I am glad to see our panel and working group were able to make it here on time. Not everyone is here, but most.

I just want to take a minute to remind you of some of the things I said yesterday about what we wanted the Fresh Produce Working Group to consider, and that was the information that was presented here, and to provide to FDA some recommended controls or interventions that you believe we should consider in the near term to enhance the safety of sprouts.

As I said yesterday, we have various avenues available including voluntary guidance, performance standards, good manufacturing practices or HACCP, and one of the questions we had is do we have the science available to support any or all of these and, if not all, which options, if any, and the other thing is to look and see are there any additional specific research areas that we should consider.

Yesterday, we heard from a number of groups including the consumers. We heard a number of people talk about what controls we should put into place, about separating the seeds for agriculture from seeds for human production. We also heard from the industry and individuals how difficult that actually would be.

There were a number of other questions that came up. We heard a little about the morphology of the seed and the fact that the way it is harvested, you are probably going to get cracks in your seeds and have problems, all the different types of seeds no matter whether it be an alfalfa seed, a clover seed or whatever, even though all of the seeds are a bit different.

A lot of other questions came up yesterday about interventions and interventions in the seed process, as well as interventions during the manufacturing process. In talking about some of those in the seed process, we talked about irradiation, we talked about chlorine, sodium hypochlorite, calcium hypochlorite, ozone, and others.

A lot of these come either under EPA's regulations or under FDA's regulations from our Office of Premarket Approval. Some even require food additive petitions. We wanted to take a few minutes this morning to have both EPA and FDA give a presentation from that standpoint, so that the panel could understand this and the working group in the background.

The two individuals from EPA who will be making presentations first can only stay a short time because they have another meeting to go to, so after their presentations I would like them to stay and take questions. We can have three or four questions from the panel and working group if

you have any specifically for them, and there will be somebody available that will stay around later from EPA if there are questions later in the morning in the general session.

With that, I would like to introduce Dr. Frank Sanders and Dr. Connie Welch from EPA, who will talk about EPA considerations.

EPA Considerations

DR. SANDERS: Good morning. Again, my name is Frank Sanders, and I am the Director of Antimicrobial Division of the Office of Pesticide Programs in EPA. We are responsible for the registration of pesticide products including germicides and other antimicrobial products.

I have with me, as was pointed out, Connie Welch, Chief of the Regulatory Branch II, and Michelle Wingfield, Chief of the Science Support Branch. Michelle is in the audience, but she will remain behind in the event that you have additional questions. Both Connie and Michelle are critical to our registration process, and they will provide more details on alfalfa sprout and seed issues in a few minutes.

Let me say we at EPA are prepared to work with the states and industry to rapidly approve the use of products that will control E. coli on alfalfa sprout and seeds. Products that make public health claims, such as kills E.

coli or strep, require registration under the provisions of FIFRA. This process can often be difficult and can also require several months to complete, and there are basically three ways in which products can be approved for use by EPA. They are Section 18, Emergency Use, Section 24(c), Special Local Needs, and Section 3, Registration.

Currently, there are no applications pending with us for the use of any products for the control of E. coli on alfalfa sprouts or seeds, however, it is my understanding that the State of California had issued a special local needs registration for the use of calcium hypochlorite on alfalfa seeds. This is a prewash soak in five pounds of seed and one gallon of 2 percent calcium hypochlorite solution.

We at EPA have not yet received this application for review. With respect to 24(c), we have disapproval authority, but the states have the authority to issue that for 90 days.

California has the only 24(c) that I understood, that I know about, that can be used for control of E. coli on alfalfa sprouts. Other states may also wish to take similar actions.

Let me say that there are chemicals, such as calcium hypochlorite, sodium hypochlorite, and hydrogen peroxide that we will likely be able to reach registration

decisions on rather quickly because tolerance exemptions or tolerances are already established for those chemicals.

For chemicals where there is no tolerance or exemption from tolerance, it would take much longer for us to reach a registration decision.

Connie will briefly outline the process required to obtain registration under the three categories of Section 18, Special Local Needs, Section 3, and, if necessary, Michelle will briefly talk about the methodology that will be needed to determine the product efficacy.

If you wish to contact me in the future on any of these issues, I can be reached on Area Code (703) 308-6435.

Connie.

DR. WELCH: As Frank mentioned, there are three options right now for registration. One would be under a Section 18, the other one is 24(c), which is a Special Local Need, and what would be a new registration or new use.

Under the 24(c), the calcium hypochlorite already had a tolerance exemption, so that was pretty easy, and as he mentioned, the only state that we have worked with so far is the State of California.

We have not seen the 24(c) officially. We have gotten like a fax copy and made a few comments, but we have not seen it officially. We do have 90 days to approve or disapprove. We do not foresee disapproving this because it

is calcium hypochlorite, we do have a database, we feel familiar with calcium hypochlorite, that it will be efficacious.

As I mentioned, under all three areas, the tolerance or tolerance exemption would be required if one does not already exist, and usually, in our data packages for tolerances or tolerance exemptions, you are looking at such data as residue chemistry data and toxicity data to establish a tolerance or tolerance exemption.

Many times the tolerance exemption is based on the toxicity of the chemical or if there are no residues.

As Frank mentioned, time frames vary with each registration option. A Section 18 obviously will be put through rather quickly especially if the tolerance already exists. As far as the 24 (c), like I said, we have a 90-day approval time to approve or disapprove.

Under our FQPA, our Food Quality Protection Act, we have certain time frames that we must abide by, because this is a food use, it does not come under that time frame, however, given the situation, we would certainly give it a priority as far as moving it through the registration process.

Of course, in all areas, efficacy data would be needed. This is a public health claim, and so we would need to see some form of efficacy data. At this point, I would

say just in seeking a registration, it would be very helpful upfront to contact us, so that we can go over the data requirements.

There may be some that may be waived given the circumstances or the use involved that, you know, it may be applicable to waive some of the data requirements, so it would be very helpful in the beginning to contact us, that we can talk with you and work with you over each of the data requirements, and, you know, go a little bit more into detail what would be required for the use that you are seeking to register.

We can take questions right now.

DR. BUCHANAN: Do you have an outline of the efficacy requirements that you need for testing that we can get our hands on?

DR. SANDERS: We do have an outline for most efficacy testing. Michelle Wingfield will be available to talk about that in more detail.

MS. WINGFIELD: As far as the efficacy data requirements are concerned, this is a relatively new area for us, looking at public health related uses on fresh foods and produce. Given that it is a new area, last September 1997, we presented before our Scientific Advisory Panel the question on the state of the science for efficacy evaluation on fresh fruit and produce.

We are currently working with groups who are establishing methodologies for evaluating the efficacy of fresh fruit and produce. According to the Scientific Advisory Panel, these methodologies should be simulated in use, actually using the subject fruit and vegetables as carriers, if you will, to determine the efficacy of antimicrobial products.

Again, this is a new area. We will be looking at peer review of these new methodologies and techniques, and validation processes before we approve any applications in these areas.

Any other questions? Yes.

DR. FARRAR: We just want to thank you for your assistance with Cal/EPA in speeding through our 24(c). One of the points that we sort of had to compromise on to get our request pushed through was the 12-hour re-entry issue that I think you folks had some input on, as well as Cal/EPA.

I would ask you, when the petition comes forward to you for both our 24(c) and the national application, to look at that issue again. A 12-hour re-entry is just not feasible in many of these sprout production facilities. We think there are other alternatives that we can work out, but 12-hour entry puts kind of a real burden on a lot of these facilities not designed for a separate area with separate

ventilation, and so forth. We just ask that you take a look at that.

MS. WINGFIELD: Agree. In our comments back to I believe Dr. Jerry Campbell, those were some of our exact comments. We can work with you on that and possibly lower that. We don't foresee that as a show-stopper in this application.

DR. KVENBERG: I have a question relative to your protocols on efficacy and the advice you have received.

Do you have any information since pathogens are quite a rare event and can't be used in actual tests within food facilities, any information on surrogate pathogens or indicator organisms that would demonstrate efficacy that you could give us information on?

MS. WINGFIELD: It was the recommendation of the panel that we focus mainly on the two pathogens most affecting at this time food safety, which would be E. coli 0157:H7, and the Listeria monocytogenes as at least the basis for establishing efficacy.

If an applicant wishes to list other pathogens on their label, they would have to test against those other pathogens also, but as far as the basic baseline, we are looking at the Listeria and the E. coli 0157:H7.

DR. KVENBERG: Let me restate my question. I understand that efficacy has to be demonstrated of the

chemical against the actual pathogens of concern. My question went to efficacy testing and actual implant operations.

Is there any ancillary data of non-pathogen bacteria that have the same characteristics that would be useful to EPA's application for efficacy?

MS. WINGFIELD: Not to my knowledge at this time although we would be willing to talk with several, in fact, we need to talk with several experts in this area for answering questions such as this.

DR. KVENBERG: Thank you.

DR. WELCH: I don't believe I left my phone number. It is (703) 308-8218. So far, like I say, we have only worked with one state, and the calcium hypochlorites and sodium hypochlorites fall within the jurisdiction of my branch.

If there are other chemicals, you can still give me a call, and if they don't fall within my jurisdiction, we can direct you to the right person.

MR. BERNARD: An additional clarification, if I could.

What are the categories of data that you need? You mentioned efficacy, you mentioned toxicology.

DR. WELCH: Toxicology was the chemistry.

MR. BERNARD: Environmental?

DR. WELCH: Some environmental depending on the use, if it would come under an indoor or an outdoor use.

MR. BERNARD: So, somebody seeking approval for a new compound would have to provide data in all three of those categories.

DR. WELCH: Yes. For the tolerance exemption, we mainly look at the residue data and the toxicology data. For purposes of a registration, that is where maybe eco or environmental fate data may or may not come into play.

MR. BERNARD: But for efficacy, you still don't have the protocol that you expect to be followed in place?

MS. WINGFIELD: Right. As I said before, this is a new area for us. Really only one perspective applicant has come forward thus far seeking a registration, and we are working with them in development of their efficacy protocol.

MR. BERNARD: How long do you think it might be before you have decided on a protocol, because I think most of the people here feel some urgency that if there is a compound that shows good efficacy, that we would like to get it on line and using to deal with a, "real public health issue" here.

MS. WINGFIELD: Agreed. In fact, one of the people that we are working with is Dr. Larry Beuchat, and I believe he will be addressing some of the efficacy issues in his presentation later this morning.

DR. SANDERS: Keep in mind that this is an unusual situation. To the degree that we can provide some assistance, we want to do that. We may have to be creative in some approaches. As long as we maintain good science, I think we can make some decisions to allow certain uses to occur, but we will have to keep in mind that we have to have quality science in reaching these decisions.

DR. FARRAR: One more question. There was some confusion, there is some confusion still regarding the tolerance exemption. You mentioned that you would still like to look at residue data even though there is a tolerance exemption established already. That seems a bit confusing to a lot of people.

DR. WELCH: Okay. I am sorry. Maybe I didn't state it clearly, but the residue data would only be needed if there is no tolerance currently on the books. So, it is only required in seeking a new tolerance or in proposing a tolerance exemption.

MS. OLIVER: Fine. Thank you very much, Dr. Sanders and Dr. Welch and Michelle. Michelle will be around later and can take questions during the group questioning at 10:50, correct?

MS. WINGFIELD: Yes.

MS. OLIVER: Fine.

The next presentation will be from FDA, our Office

of Premarket Approval, and Laura Tarantino will discuss FDA considerations. Laura.

FDA Considerations

DR. TARANTINO: I will come up here and try not to stand behind the podium but where you can see me, but I do have a couple of overheads, so it might be easier if I came up.

I think, very much like what Frank and Connie just did, I just want to take a couple minutes to probably just give you an idea of what the regulatory framework is that people would need to deal with if they were going to come in to say, okay, I have got an intervention, it looks pretty good, what do I need to do to make sure it is lawful.

So, just a very quick sort of Food Additives 101, and there will be a test, but there is really only one question, and the answer to that is come in early. As Connie said, I think the bottom line take home is if it looks like there is something that looks effective and useful, it would be very helpful if people came in and told us about it as early as possible in the process. No matter how good an intervention might be, it is not going to be of use to anyone if it is not legal.

[Slide.]

Why would you need to come to us? Of course, if what you are dealing with turns out to be a food additive,

the Food, Drug, and Cosmetic Act does require premarket approval for new food additives. It also defines what food additive is, and that is on the next overhead.

[Slide.]

Food additive, any substance the intended use of which results or may reasonably be expected to result directly or indirectly in its becoming a component or otherwise affecting the characteristics of any food.

This is very, very broad. It is applied not only to ingredients intentionally added, it is applied to anything that may affect the characteristics, so that given the definition on its own, it would cover virtually everything, but on the next overhead, Congress also provided us with a bunch of exemptions from the definition of food additive.

[Slide.]

One of the classes of exemptions were things that are authorized by other laws, and one of those is pesticides, so if you have something that falls under EPA jurisdiction, then, you go to those folks.

Color additives, new animal drugs, one that could go on there now, which wasn't there when we first made this up, was dietary ingredients and dietary supplements, so that if it is covered by other laws or other parts of this law, it is exempt from the food additive definition.

Prior sanctioned substances, kind of a very narrow group. Those are substances that before 1958, when this whole part of the Act was passed, had gotten a written specific approval from either the FDA or the USDA for a particular use of an additive.

Then, of course, the last one there, generally recognized as safe, is a very significant exemption, important and widely misunderstood. In brief, I think what is most important about this is a substance is not a food additive and is exempt from the need for premarket approval if experts and qualified experts -- and these experts are not reserved to be necessarily the government -- determine that a particular use of a substance is safe based on either a history of wide use and safe use before 1958, or evidence that is widely available and widely available to experts that the use is safe, and that widely available evidence is such that experts can generally agree that the evidence supports the safety. It is actually a fairly vigorous burden, but a very important exemption, and one that we might come back to.

So, all of these are things that do not fall under the food additive definition, but if we go back to the definition on the next overhead, earlier, I ended the definition right about here, and then had three dots.

[Slide.]

Well, more of this definition talks about including any substance intended for use, producing, manufacturing, packing, processing, et cetera, and relevant to this discussion, any source of radiation intended for any such use.

So, this is, I think, where I know ionizing radiation is one of a number of techniques that is under active investigation for this, and this is where, as a matter of law, ionizing radiation and treatment radiation would be defined as a food additive and thus would be subject to premarket approval.

[Slide.]

So, let's say we have determined it is a food additive, it is not a pesticide, you come to FDA. It is subject to premarket approval. What then?

I just want to mention a couple of characteristics of the approval process under the food additive and some things that are perhaps different from EPAs. What is not different is the burden for establishing the safety is on the sponsor, and one does that by collecting the data and submitting it to the Agency in the form of a petition, and then FDA has the responsibility to review what is I think worth noting is then we issue a regulation.

What is sort of unique about the food additive process, not quite, EPA has this for registrations, but we

do rulemaking, and there are two things about it. The rulemaking is generic. That is, if we approve an additive, anyone can use it, not just the petitioner, under the conditions of use for which it has been approved.

The other is I think the sad fact that rulemaking takes time. It takes time, it's hard, and even if we were spectacularly efficient -- and sometimes we are not -- it still take time. I think this comes back to my earlier stricture that if you have something that looks promising, the earlier you can come and talk to us about it, and get us looking at it, the better off folks are going to be.

FDA is not permitted to consider possible benefits in making the safety decision. We can't change the safety standard, we can't change the standard for review. What we can do, what we will do, what we do do is make sure that if there is a thought that something has a potential public health benefit, to make sure it gets prompt attention, but that is different from saying that we have different standards.

The other thing we don't have is anything that is particularly analogous to either the exemption or the local use. We don't have any codified way of allowing a food additive on the market, if it is a food additive, prior to the issuance of a regulation.

[Slide.]

This just lists, I think, the same kind of question that Dane had asked. This is the standard information that is required in a petition in law and regulations, and, of course, it is very, very broad and much of it is common sense.

What is it? What do you want to use it for? How much are you going to use? Does it do what it is supposed to? Sometimes you need method, sometimes you don't. Safety studies, that is the tox studies. Proposed tolerances if needed. Most of the time you may not. Environmental information maybe, largely having to do with sites of production or ways that the additive itself would be disposed of and such.

These are very generic, and certainly don't give much help in terms of what would be needed in a particular case. Once again, I think it does behoove folks to talk to us because, in particular, for example, in the case of irradiation, in the case of petitions for irradiation, obviously, some of these don't really apply. They are very different beasts indeed.

[Slide.]

The kinds of things that are on here are just generic safety issues that we would consider in any petition. Obviously, toxicity in the case of irradiation, this might be chemistry really, radiation chemistry data.

Nutritional adequacy in the case of radiation petitions really has to do with are you irradiating and changing the levels of nutrients in a way that would affect a major source of a nutrient in the diet. This is possibly not terribly relevant for sprouts, but would be something.

Potential microbiological risk I think is where we would just look to make sure that you haven't done anything that would make the food less safe. For example, if you had very, very sensitive to whatever treatment it was, chemical, radiation, spoilage organisms, and a very resistant pathogen, might you be doing it, the process, in a way that would kill off spoilage organisms and leave the pathogen without competition.

I think when people were talking about efficacy earlier, and I talked about one of the things we need in the petition is evidence that it does its intended use, I think it would be helpful if we tried to separate out the safety review from efficacy per se, the standard for the food additives is for it to be safe. It is not like the drug standard where it is safe and efficacious.

If you do have performance standards, it is something that you are going to want to think about, and you are going to want to make sure that the conditions of use that you apply for cover the conditions which will allow you to reach a performance standard, but on our side in terms of

the safety review, we are less concerned about performance standards, and would need to have you talk to other folks, but I think that is something we would very much want to talk about as to how you frame an application and how you position it in terms of what the technical effect is in any potential rulemaking.

On that, I am going to stop except to reiterate that we do recognize the importance of some of these things. We can, we do, we will work with folks on anything that you might want to come forward, and I guess one thing that I will go back to is a regulation needs to cover a particular use of a substance, so quite analogous to some of the things that EPA said, in some cases we may have food additive approvals for a substance for some uses, but they may not cover the particular use you want.

That is all I have to say, so, questions.

MS. OLIVER: I think I would like to save all the questions for later, and we will do the questions after the next panel.

The next panel is going to deal with science and Dr. Peter Feng from FDA, Center for Food Safety and Applied Nutrition, is going to moderate that.

Dr. Feng.

State of the Science

DR. FENG: Thank you, Janice, and good morning.

The next session, we are going to be addressing the state of science of two main aspects. One, of course, is detection methodology for these pathogens in seeds and sprouts. The other one is on preventive measures.

We are very fortunate to have a very international group of experts to address both of these topics. On the aspect of detection, one thing I want to mention is there is two aspects that are deficient in detection of sprouts. The first was mentioned yesterday, and that is our inability to consistently isolate pathogens from the seeds.

The other aspect that nobody has brought up is the absence of standardized detection methods for Salmonella and 0157:H7 in sprouts and seed products. Hopefully, our experts will be able to provide answers for those questions.

My first speaker today is Greg Inami from the California State Department of Health. He is a microbiologist who has been actively involved in all the outbreaks of pathogens in sprouts in California. He has been analyzing most of the samples, and on occasion he has been able to isolate Salmonella from the seeds.

Greg is going to share with us his experiences in analyzing these products.

Greg.

Methodology/Salmonella

MR. INAMI: Thank you, Peter.

[Slide.]

Today, I am going to cover some of the work that we have done over the last two years in the isolation and detection of Salmonella in naturally contaminated alfalfa seeds.

There have been a number of outbreaks of Salmonella gastroenteritis connected to consumption of sprouts. These are some of the more notable outbreaks, and I am not going to cover these because a lot of these were covered yesterday, but what I do like to emphasize here are some of the lab results some of these investigators got in isolating Salmonella from sprouts and seeds.

The first two outbreaks that we have here, the investigators were actually able to isolate it from both the sprouted product and also from the seed. In the latter outbreak, the Bovismorbificans outbreak, the investigator was only able to isolate it from the seed after sprouting and growth, and I am going to be referring to this outbreak a little bit later.

In contrast, in 1995 -- and this has been mentioned many times yesterday -- the Salmonella Stanley outbreak. In this case, no Salmonella was isolated from the sprout or the seed, and the outbreak was determined by epi data and also by molecular comparison of the patient's isolates.

I want to skip around now. We worked on the Montevideo, Meleagridis outbreak, and that is a very interesting case. We did not isolate Montevideo from any of the sprout product or from the seed, however, with the Meleagridis, we were able to isolate from several packages of sprouts, but not the seed.

As you can see, in some cases, it seems like it is really easy to isolate these organisms out of the sprouts and seeds, and in other cases, as in the Stanley outbreak and the Montevideo, epi data and molecular testing of the patient's isolates were required.

The distribution of Salmonella and the numbers on the seeds may vary from lot to lot, and what we have seen, they may vary from bag to bag.

As I said, I am going to discuss some of the work that we have done over the past couple of years.

Our story begins in 1996, when public health officials in Oregon and British Columbia independently noted an increase of Salmonella serotype Newport, and this was in January of 1996. After an epi investigation, alfalfa sprouts were found to be associated with the illness.

Further traceback found a single lot of seeds to be the common source of these alfalfa sprouts. These seeds were imported from the Netherlands by a U.S. company, and they were distributed to sprout growers in California,

Oregon, and British Columbia, and actually, 90 percent of the seeds were shipped to Oregon and British Columbia, and some of them were shipped to California.

As part of our investigation, alfalfa seeds from the suspect lot were embargoed from a California sprouting company by the California Department of Health Services, Food and Drug Branch, and they were submitted to our laboratory, which is the microbial diseases laboratory of the California Department of Health Services.

They initially submitted 10 sample units to our laboratory for testing, and each of these sample units consisted of one bag of seeds weighing approximately 500 grams. Now, we were looking for the best method that would give us the best recovery of Salmonella, and we searched in the literature and we found this paper in the Lancet by Ponka, and others, and they reported an outbreak of Salmonella Bovismorbificans connected with the consumption of alfalfa sprouts, and in this report, they were able to isolate the Bovismorbificans from both the sprout and the seeds.

However, they were only able to isolate it from the seeds after sprouting and growth, and not from the germinated seeds, and they have the statement here that our experience shows the Salmonella cannot be cultured from the seeds, but only after sprouting.

So, we followed their lead and decided to cultivate the seeds for four days before enriching and testing for Salmonella.

[Slide.]

This is our procedure for our sprouting procedure, and the procedure was done under aseptic conditions. All material, glassware, and media were properly sterilized before use. We examined 100 grams of seeds from each sample unit, and the seeds were soaked in two times sterile distilled water for 18 to 20 hours at room temperature.

The soaked seeds were subsequently planted and spread out onto moistened sterile gauze in a beaker, and they were covered loosely with an aluminum foil. These seeds were incubated for four days at room temperature under fluorescent lights and these seeds were not watered over the four-day period.

At four days, the seeds had germinated and grew to about 2 to 3 centimeters, and we tested 100 grams of the sprouted seed using a modification of the U.S. Food and Drug's procedure for Salmonella, and this comes out of their bacteriological analytical manual.

This procedure includes a pre-enrichment and a nonselective lactose broth, and then it goes into two selective enrichment broth, and we use a selenite and tetrathionate, and these are added to a post-enrichment M-

broth, which isn't labeled here.

The modifications of this method is the use of an enzyme immunoassay for detecting Salmonella, culturing from the M-broth to hecto and an XLD, and the exclusion of bismuth sulfite agar due to lack of resources at the time we were doing this exam.

Of the 10 sampled units examined, we were able to detect 4 using the EIA. Salmonella serotype Newport was isolated from 4 of the sample units, and salmonella serotype Albany and Schwarzengrun were isolated from a fifth.

One interesting note to point out is sample unit No. 9, we observed this negative EIA, and we had a positive culture, and we have observed these previously in our lab with other EIA's and testing with other food products, and this may suggest that the low numbers of culturable organisms may not be detected by EIA.

So, we were successful in detecting and isolating Salmonella from the alfalfa seed after germinating and growth. Now, as you could see, this procedure was very time-consuming and labor-intensive. It took over four days before we can start testing. Testing usually takes an additional three to four days before we get isolates on plates.

So, what we did is we did further testing using these five culture-positive sample units in a limited study

examining alternative seed processing methods that were faster and less time-consuming and less labor-intensive.

These alternative seed processing methods included seed shredding, seed soaking, and a washing method before culturing. The seed shredding was investigated since we believed that the Salmonella may be contained within the seed.

Shredding was thought to release the Salmonella into the enrichment broth, and the method involves taking 100 grams of the dried alfalfa seeds, placing them in a blender jar, and shredding the seeds to a coarse powder, after which a 1 to 10 or a 900 ml of lactose broth was added, and the shredded seed was incubated accordingly.

We also explored a soaking and washing method to determine if we could isolate the organisms from the outside of the seed. The seed soaking method, 900 ml of lactose broth was added to 100 grams of seeds. These were gently mixed and incubated accordingly.

For the washing method, 100 grams of seeds were mixed with 200 ml of lactose broth, and these were mixed over an hour period of time. The lactose broth was decanted and incubated.

The enrichment detection and isolation method that we used for these alternative seed processing methods is the same procedure that we used for the sprouting method, and

that was the modified FDA-BAM Method. To rapidly identify any positive sample units, we used the EIA as initial screen before culturing. However, because one of the sample units was EIA-negative, culture-positive in our sample unit No. 9, we cultured sample unit 9 and 10 as a control regardless of EIA results.

[Slide.]

Our results shown here show that of the five culture-positive sample units that we tested, three were positive by EIA using the shredding method, two were positive by the soaking, and none was positive by the washing method.

Salmonella serotype Newport was isolated from all of the EIA-positive samples and also from one EIA-negative sample unit. The shredding method seems to have given the best detection out of this limited study that we did, however, overall, it looks like the sprouting method may show slightly higher detection with the EIA.

Interpreting the culturing, results are difficult since we had to sample unit No. 10, which showed a negative EIA, positive culture for Salmonella. Again, I want to stress that using an EIA to screen out negative samples may actually underestimate how many sample units contain Salmonella in low numbers.

So of the four methods that we looked at, the two

methods that seemed to be the most promising were the sprouting growth method and the shredding method, and recently, within the last month, we did some more work, and we did a little bit better controlled study in which we did a comparison of sprouting versus shredding for the isolation of Salmonella from these naturally contaminated seeds, and in this study we examined 30 sampling units of alfalfa seeds, and each of these sampling units were about a one-pound bag or 500 grams of seeds.

These were reserve sample unit of seeds from the 1996 Oregon and British Columbia outbreak, and these were never tested before. The method we followed, that was the FDA-BAM method for the isolation of Salmonella.

We excluded the EIA because of the EIA-negative culture-positive units we had seen previously, and we tried to streamline the procedure, and how we did this is we tested previously positive bags of seeds, and we sprouted them for three days, and we found we were actually able to isolate Salmonella, so we shortened our incubation period from four days to three days, and we tested 100 grams of seeds from each of the sample units for each of the methods.

This is just a diagram of the BAM method that is listed in the BAM.

Our results are very interesting. With the sprouting method, we were able to isolate 3 out of 30 sample

units. With the shredding method, we were able to isolate 2 of the 30 sample units. There is not much difference there, but it is interesting to note that the two positives that we got out of the shredding were different than the ones that we got out of the sprouting.

So, in total, we were able to isolate with both of these methods 5 positive sample units out of the 30.

This data may suggest that sprouting may be slightly better in detection than the shredding, but the jury is still out on that, but there are two other items that I want to mention that sort of support the sprouting and growth of these seeds for detection of Salmonella, and both of these come out of a paper that Jaquette and Dr. Beuchat published in 1996, and I think this was mentioned yesterday.

What they did is they seeded Salmonella Stanley onto the alfalfa seeds and they were able to show a 3 to 4 log increase during a 54-hour germination sprouting period, so we actually are getting amplification as you are growing the sprout.

They also reported phenol compounds present in the seed coat which could possibly be toxic to Salmonella, so this kind of goes against shredding the seeds, these phenol compounds may be released, may be toxic to the Salmonella. Results from this study, our study, and the results from

Ponka may support sprouting and growth of the alfalfa seeds before culturing to increase the level of the pathogen for isolation, however, I think further studies need to be done to determine the most sensitive method.

[Slide.]

Very quickly, we did another limited study using these naturally contaminated seeds and looking at sodium hypochlorite solutions efficacy in killing Salmonella from the seeds. Again, this work was prompted by the paper from Jaquette and Dr. Beuchat.

There are just two items I want to point out on this slide here. One is that we were able to isolate Salmonella Newport from these seeds after we treated them with 700 parts per million of a sodium hypochlorite solution for one minute, and also treating with 270 parts per million for one minute and five minutes.

The other item I would like to point out has to do with sample size. On the bottom here there are two distilled water controls that we tested, and when we tested 50 grams of these controls, we were not able to isolate the Salmonella, however, when we went back to the same lot and tested 100 grams, we did get isolation of the Salmonella.

We have observed this phenomenon twice in our laboratory again, however, because the number of bags we tested were limited, further work is needed to determine a

proper sample size.

[Slide.]

So, in summary, the distribution of Salmonella may vary from lot to lot, and actually may vary from bag to bag and within a given sample unit. The numbers appear to be low on these seeds, making isolation very difficult.

We are currently testing 100 grams from each sample unit, and then we follow the FDA sampling plan for Salmonella. When we can, we routinely test about 60 sample units per lot.

Sprouting gives us better recovery of Salmonella from the seeds versus the alternative seed processing methods, and there is a question mark there. I think the jury is still out, but among the two methods used in tandem, the sprouting and the shredding method, those two methods gave us a higher positivity rate from those 30 sample units that we tested.

We are currently sprouting and growing the seeds for three days before following the FDA BAM method of Salmonella. We are no longer using an enzyme immunoassay for detection because using an EIA may be not sensitive enough in detecting low numbers even after an enrichment period.

Our experience with sodium hypochlorite solution shows that it does not eliminate Salmonella from the seed,

and Dr. Beuchat, I am sure, will be discussing this in more detail.

[Slide.]

The last item that is very interesting, these seeds that we tested are two years old and actually they are probably even older than that, and we found that Salmonella is viable in these alfalfa seeds for up to two years or longer when stored at room temperature in the dark.

With that, I would like to thank you for your attention.

DR. FENG: Thank you, Greg.

Our next speaker is Mr. Steve Weagant from our FDA lab in Bothell, Washington. Steve has been very active in 0157:H7 methodology. He is mainly responsible for developing most of the assays that we use in our FDA BAM manual. He was involved in the FDA sprout survey that was done several years ago, and he is also very active currently in the compliance program for sprouts that is in place right now.

Steve.

Methodology/E. Coli 0157:H7

MR. WEAGANT: Thank you, Peter.

As Peter said, I am Steve Weagant from FDA/Seattle laboratory, and I am going to talk to you about the development and evaluation of methodology for use in FDA

labs for the enrichment and isolation of E. coli 0157 in foods and particularly in sprouts.

Our attempts to isolate E. coli 0157:H7 from hamburger during the large outbreak in Washington State and other western states in 1993 was frustratingly unproductive. We felt that the methods that were currently recommended at that time needed improvement.

Our efforts led to an improved method for isolation of E. coli 0157:H7 in foods which was published in the Journal of Food Protection in January of 1995. This method was adopted in FDA's Eighth Edition of Bacteriological Analytical Manual.

Since then, this method has been used to provide food isolates from several outbreaks of E. coli 0157:H7 infections. These include an outbreak of home-prepared hamburgers in 1994 in Washington and Oregon, an outbreak in several western states associated with salami produced in California and they produced the isolate for the Odwalla outbreak recently.

Just an overview of the method that we had developed using enrichment in what we call EEB or EHEC enrichment broth, which is based on the modified TSB developed by Dr. Doyle's group, and we did not add the novobiocin supplement for inhibition of competitors, but instead used vancomycin, cefsulodin, and cefixime.

I will just kind of walk you through this method. Here is a photo of adding the 25 grams of sprouts to the enrichment broth, and this homogenized and then incubated at 37 degrees for 6 and 24 hours with agitation, which is extremely important.

Then, after the enrichment period, the enrichment was diluted and spread plated to sorbitol MacConkey's agar supplemented with tellurite and cefixime, and that is referred to as TCSMAC. Some authors refer to it as CTSMAC. It's the same formulation.

These plates were incubated overnight and typical colonies were picked for purity and screened through spot indole test, and then typical growth on EMB agar and HCMUG agar. Then, the presumptive positives were tested for the 0157 and the H7 antigen by latex agglutination kit, and then subsequently tested for the Shiga-like toxin genes with PCR, and they are also confirmed as E. coli using either Vitek or API20E.

We also used an alternate technique in this work using immunomagnetic separation, or IMS, and this is a step between enrichment and the isolation which involves placing a ml of the enrichment broth with magnetizable plastic beads that are coated with the 0157 antigen, and these trap the target bacteria, and we go through this procedure of adding the enrichment to the beads in a small tube, microcentrifuge

tube, and then tumbling them for about half an hour, and then they are rinsed twice and then they are trapped to the side of the tube. We remove the enrichment, and then they are rinsed twice, and the beads are then plated onto the TCSMAC agar.

This is the appearance of a TCSMAC plate through the enrichment and isolation without IMS. As you can see, the vast majority of the colonies are the pink colonies, which are not the target organism.

This a typical colony for the E. coli 0157 on this agar, and with IMS, we can see that the majority of the colonies present are the target colonies. And here are a few of the competitors, as well.

This methodology was validated in five different food types. In this study, the results showed overall with five food types that the direct isolation was about equivalent in results to the use of the IMS.

We had heard from other labs and had observed ourselves that in pure culture, the EHEC enrichment broth, the EEB with the cefixime at 0.05 mg/liter was somewhat inhibitory to some strains of 0157, and we investigated this and found indeed it was a problem.

This is a look at the EEB broth in pure culture with E. coli 0157 strains, and we found that at the 0.05 mg/liter, all the strains seemed to be at least somewhat

inhibited. As we lowered the cefixime level, we found that the inhibition finally ceased at between 0.025 mg/liter and 0.0125 mg/liter.

As a result, we thought it advisable to lower the level of cefixime in the EEB.

Because of the rapidly advancing problems with E. coli 0157 in sprouts and the upcoming FDA nationwide sprout assignment, we turned our attention to devising a workable method for isolating E. coli 0157 in sprouts.

I had recalled that from our previous work that in sprouts particularly, we did have a very good success using the IMS method, and this is a summary of our data from 1995 that shows as we were using artificially contaminated sprouts with E. coli 0157, and as we lowered the levels of contamination down to 0.1 organisms or CFU/gram, in 16 trials, we had 3 positives with straight technique and 10 using the IMS, so it was pretty helpful. That is about 60 percent positive with that method.

We began a series of experiments to compare six different enrichments for the recovery of E. coli 0157 from artificially contaminated sprouts using TCSMAC agar and with and without the IMS procedure after 6 and 24 hours of enrichment.

We also looked at detection by immunoprecipitin tests called VIP from BioControl. As we progressed, we

gradually reduced the level of contamination from 1 CFU/gram in sprouts to 0.12 CFU/gram.

We just look at the methods that we used. Three of our enrichment methods were done at 37 degrees with shaking. This included the original EEB formulation, EEB with one-quarter of the cefixime and then the modified EC broth that is the recommended enrichment for USDA.

We also looked at modified TSB with novobiocin, the original formulation by Doyle, but at 37 degrees without shaking, which was recommended in the procedure by BioControl.

We also looked at modified buffered peptone water, which is a formulation that has been proposed by Larry Ristano in Illinois and also buffered peptone water which we modified with acriflavine, cefsulodin, and vancomycin.

As you can see, the direct plating at six hours was fairly unproductive. We had some better results with IMS, but as we increased the enrichment time to 24 hours, we began to see much more positives, and as we added the IMS procedure at 24 hours, those positives increased the modified TSB at static was not as productive.

As we lowered the inoculation level from 0.42 to 0.35, we began to see that the six-hour enrichment was fairly unproductive, and continuing the trends at 24 hours, I failed to mention the VIP detection was fairly successful

at above this level, but began to fail at this level.

Then, we got down to 0.12 CFU/gram, again, our six-hour procedures were fairly unproductive, 24 hours much better, the static 37 degrees was not productive, and VIP had failed us on these completely.

Just a look at the overall data from four different experiments, and tried to draw some conclusions from that.

One to six-hour enrichment was not as productive or as sensitive for sprouts, and we have not extended these into other food types which we are planning to do. Shaking seemed to be essential to the enrichment procedure.

Several enrichments were fairly comparable, so our overall results, looking at the EEB and the EEB with one-quarter cefixime modified BPW, and then with the additives, and then also the modified EC broth seemed to produce fairly comparable results.

One interesting note is that the reduction of cefixime in the inoculated food samples was not nearly as important as in pure cultures. There may be some modifying effects of the food matrix or the competitive microflora that seemed to modify the harsh effects of cefixime.

We feel that IMS is particularly valuable. It was the most productive method at 24 hours with TCSMAC, and we also feel that the VIP at least for this product was not

very productive.

So, out of this we developed a recommended procedure that is being used by FDA in its sprout assignment, and that is, to use the 25 grams of product with 225 ml of the EEB, and we are lowering the cefixime to a quarter down to 0.0125 mg/liter, homogenized, and then incubate at 37 degrees C, 24 hours, with shaking, and then go through the immunomagnetic separation technique, and then plating onto the TCSMAC agar and overnight incubation of TCSMAC.

Typical colonies were then picked and streaked onto TSAYE and then screened through EMB, HCMUG, spot indole test, the Remel 0157:H7 latex agglutination kit identified as E. coli by Vitek or API20E, and then the STX1 and 2, the Shiga-like toxin genes were confirmed with PCR.

I would like to acknowledge some of the others involved in the work that has been done. Jim Bryant and Andy Ballen from the CL District Lab, Karen Geneman from the Seafood Products Research Center in Seattle, and Dr. Feng at FDA CFSAN for his guidance and support.

DR. FENG: Thank you, Steve.

Our next speaker is Dr. Larry Beuchat from the University of Georgia. Dr. Beuchat has already been quoted by many speakers on his work in preservatives and disinfectants, and that is the topic he is going to address.

Dr. Beuchat, please.

Disinfectants

DR. BEUCHAT: Thank you, Peter, quoted and misquoted, but thank you very much for inviting me to come in to present some of the data, some of the results that we have generated, some on Salmonella, as well as on E. coli 0157:H7, with, in the case of Salmonella, our observations on seeds, as well as sprouts, and on E. coli, mostly just seeds to date.

I should also acknowledge before I begin to review and to share with really a summary of the observations that we have made, but the Salmonella work was done for the most part by Cindy Jaquette, who was a Master's degree student with me in 1996, 1995-1996. The work with E. coli 0157:H7 was done by another Master's degree student, Peter Taramena. So, those two individuals deserve the credit really for the data that I will be presenting today.

So, I will get started here on the first slide.

The first section that I will present is on the Salmonella work, and I will be a little more brief on these data because, as a number of people have mentioned, the data have actually been published and are available for your observations and whatever you want to draw from them.

But the work that we did in the 1995-1996 years were actually done as a response to the outbreak that was

noted earlier or one of the outbreaks with Stanley, Salmonella Stanley.

These are the objectives of that study, and actually the objectives of this study were very much similar to the study that we have more recently done with E. coli 0157:H7, and very basically was to test the efficacy of chlorine at that time, and we did try some other chemicals subsequently on the behavior of really the inactivation of Salmonella Stanley on alfalfa seeds to determine the survival characteristics of this particular Salmonella isolate on dry seeds as they may be stored in-house at a processing sprout operation or perhaps in a warehouse, a seed warehouse; and thirdly, to determine the behavior of the organism on seeds subjected to conditions that might be used commercially to grow and market seeds, and there is such a wide range of techniques that are used to produce sprouts we used a tray method, not having the drum system available then or now actually.

I am going directly to the data, the observations that we did make on a number of different chemicals. Of course, at that time, we were interested largely in the sodium hypochlorite, the chlorine source, that is, the hypochlorous acid and its activity on Salmonella.

We tried up to, in this particular set of data, up to 2,000 parts per million. Now, this is active chlorine.

We ran the tests at room temperature, about 70 degrees Fahrenheit, and I don't recall the time, 10 minutes in this case, but were able to reduce from about 8,000/gram down to less than 1.

On these particular tests, we did not run enrichment, so we do not know if, in fact, there were any viable cells, but the limit of detection was 1 CFU/gram, and with 2,000 ppm, we were not able to measure any viable counts.

We also did germination studies along with all of these chemical treatment studies, and in no case, in this particular table, did we find any reduction, at least significant reduction in terms of statistical significance with any of the treatments.

We also looked at calcium hypochlorite a little less in terms of concentration, but essentially, the same type of reduction. I should also mention these letters here, when you see a different letter -- let me back up -- if a number, a count is not followed by the same letter, then, they are significantly different at 95 percent confidence level. In other words, a reduction of 7,900 to 331 is a significant reduction, another significant reduction, and a third here with the 2,000 ppm.

We got the same scenario essentially with the calcium form of the hypochlorite. Hydrogen peroxide up to 6

percent, we got reductions, although in this case, we were able to count or at least detect a very low number of colonies. So, hydrogen peroxide also looked promising for the reduction of Salmonella on seeds.

Ethanol at 80 percent, we got a tremendous reduction. I will get to this later, but with the E. coli, we also lost germination; with the Salmonella studies we didn't, at least with this particular lot of seeds, observe any reduction in germination.

Now, we did follow up. I think Dr. Tompkin questioned yesterday had any work been done on temperature, that is, heating, and perhaps even heating in conjunction with chemicals.

This is a summary of some information, data that we did generate. We looked at several different temperatures in three-degree increments at higher temperatures. This would be our control of the room temperature, but also 54 and 57. We also looked at 60, 63, and somehow we chose three-degree increments.

We dipped, that is, submerged the seeds for either five or 10 minutes in each instance, then, we measured the population, in this case, of the Salmonella both in the dip solution, that is, what might be washed off during the dip process but remained viable, and also in the seeds.

We, of course, can pick up viable cells in the dip

solution. We also, in this case, reduced populations at 54 degrees, and we did not detect the Salmonella at 57 or above. The same general trend holds true for the counts on the seeds, however, I must also mention that we did not do enrichment on the seeds, nor in the water, so we don't know quite whether we actually eliminated in terms of any viable cells the Salmonella from the seeds.

This bar graph shows you percent germination versus various temperatures of treatment, either in the green for five minutes, or the red for 10 minutes. Room temperature, not too surprisingly, we didn't reduce the viability, the germination at all even out to about 60 degrees for five minutes we did not lose germination, however, for 10 minutes you get a significant reduction certainly at 60 degrees, and you see it beginning to fall off even at 57 degrees.

So, we have, as somebody mentioned yesterday, a fairly narrow window here in terms of the temperature that might be used, an elevated heating process that could be administered to seeds for the sole purpose of reducing populations of Salmonella.

There is some promise here. There are through strict controlled conditions the possibility that a temperature treatment could be, at least in conjunction perhaps with the chemical treatment, a possible step forward

in reducing, eliminating, not only Salmonella, but maybe E. coli and Listeria and other pathogens from alfalfa seeds.

This graph shows you the population of -- again, this is Salmonella Stanley versus time, and what we tried to do here was to grow the inoculated -- well, we inoculated alfalfa seeds, then, we grew sprouts using a system at least we thought would be within the boundaries of what might be used in the commercial setting, a six-hour soak time followed by a 24-hour time in which the seeds would be germinated, and then a three-day period from 30 hours to 102 for the actual sprout growth and maturation, and then we put the sprouts into refrigerated storage. This was about 9 to 10 degrees for 10 days, 102 to 342 hours.

So, as I believe Peter mentioned, or Gregory mentioned, we do get amplification during the germination process. Now, admittedly, the initial inoculum was high, but still we do get a tremendous increase during that period, and then an increase to maybe a high 10^6, 10^7, which holds incidently through 10 days of storage at refrigeration temperature.

I do not have a graph showing this kind of data from the E. coli studies. We did that, and it looks very similar. I don't have a figure or a slide showing that, but 0157 behaves very similar to this during the same treatment, the same system that we used there for the Salmonella.

Now, we did work also with dry seeds. We didn't go out to the year or two years that was mentioned earlier, but we did inoculate seeds and then we stored them at 8 degrees Celsius or 21. The 8 degree for nine weeks, population was reduced by about 5-fold, and this was on dry seeds.

We also did studies at 8 degrees for one week and then 21 degrees for eight weeks. Now, you might ask why did you do 8 degrees for one week and 21 for eight weeks. Well, this was an afterthought. We had all the seeds in 8 degrees. After about one week, we decided, well, maybe we should store some of these at room temperature, so we pulled some of them out and actually did the rest of the study storing them at 21 degrees.

But after eight weeks, we had a reduction of about 40-fold at room temperature. So, we don't get a tremendous reduction at room temperature.

Our conclusions from this are that the concentrations of chlorine at that time that were traditionally used to sanitize alfalfa seeds were not effective, and still are not effective in eliminating Salmonella Stanley. Hydrogen peroxide and ethanol likewise were not certainly totally effective. Heating at maybe 54 degrees for 10 minutes reduces populations by 100-fold. Heating at 57 at 135 degrees Fahrenheit for 10 minutes

reduces the population by about 300-fold, but does cause some loss of germinability.

The organism survives longer in seeds at 8 than 21. Not too surprisingly, the organism can grow, does grow on alfalfa seeds during the production of sprouts, and does not lose viability during subsequent storage at refrigeration temperatures, 5 degrees for 10 days.

Now, to go on to the E. coli work, and this is more recent work that Peter Taramena has done, and I am going to go into a little more depth here on the objectives and the methods that we used, and methods of preparing seeds and analyzing, as a number of individuals have brought up, are a dilemma, a challenge, not only for seeds, but in produce in general.

But our objectives here were similar to those that we had when we began the Salmonella studies, that is, to determine the efficacy of several chemicals on their activity to kill E. coli on alfalfa seeds destined for sprouting and to determine the viability of the organism on dry seeds during storage.

We chose for this particular set of experiments, five strains. I have listed them here, one of which came from the Michigan/Virginia outbreak. It is the same strain. We actually got the one from the Virginia outbreak, and also one that had been isolated from a patient that was

associated with a lettuce outbreak. So, those are the five strains that we used of E. coli 0157:H7.

We would grow the strains under fairly traditional techniques, tryptic soy broth, 24 hours, 37 degrees, and then we would actually mix those strains. We, through a lot of experience, have been able to -- well, we have observed that we get about the same numbers. If we don't, we add little different volumes, but we wanted to have about the same number of each strain in this cocktail of E. coli that we would actually use to inoculate the seeds.

We did inoculate about 1 kilogram of seed at a time. We mixed for only one minute. We did not want the seed to take up too much of the moisture of the water from the dip, and then we would dry those seeds for 48 hours under a laminar flow hood. The temperature ranged from 22 to 23 degrees, and the final moisture content after about 48 hours was about 5.1 to 5.4 percent.

Then, what we would do, we have learned also in anticipating that some of the cells that might remain viable at that period of time could die off, those in various stages of debilitation and stress could die off within the first week or two, we always store our seeds for several days before we actually begin these tests.

We store them at refrigeration temperature, and I will show you a slide later that will indicate that we do

have die-off during the first week or so at refrigeration temperature and then it levels off.

Well, here are the chemicals listed in no particular order and the concentrations of these chemicals that we have evaluated. All of the results that I will show you are from at least triplicate experiments, triplicate trials, and then plated in duplicate on the enumeration media.

The pH of the solutions that were used are given here. We have done a little testing to determine carryover of residuals. In the case of the two acidified products, the pH, surface ph of sorbitol MacConkey agar is around 5.7, maybe a little stressful for already stressed cells, but not necessarily probably are going to inhibit the recovery resuscitation and colony formation by most.

In the case of the trisodium phosphate, the pH of the surface of the sorbitol MacConkey agar is about 8, 7.9 to 8.0, so again, not really out of line with what would be reasonable to expect the colonies to form.

Now, the treatment of the seeds. Our system was to use 10 grams of seed, 40 milliliters of the treatment solution. If I don't mention otherwise, this was all done at room temperature.

We would have usually two exposure times, and they may differ, and that will be shown later. We would decant

the chemical, we would add 20 milliliters of a 10 percent peptone water, and then dilute that and plate out on sorbitol MacConkey agar. We would also remove some seeds for germination using a standard procedure for germination.

This slide and the ones that follow all use the same format, so I would like to spend a little time just setting you up on how we are going to show these data.

On this axis are the populations, that is, the number of viable cells of 0157 that we detected using the system just described. The concentration of the chemical on this axis and the time of treatment here, the blue in this case 3 minutes, and the red, 10.

All that data that we present on individual slides were compared for significant differences, statistical differences. So, we compared time, as well as concentration in this grid. Again, if you see a bar that does not have the same letter, then, there is significant difference, however, in this case, for example, 10-minute treatment down from zero, that is, the control down to 1,000 parts per million, well, we did reductions in counts, we got no significant reduction. Going to 2,000 parts per million, we did get a significant reduction. The same for three minutes.

Now, you might ask why, if you treat for three minutes, you get a lower number than you do for 10 minutes.

We have seen this on several occasions even with the Salmonella data.

Our thought is that in the case of those chemicals that are neutralized or in some way inactivated, reduced in their lethality upon contact with organic material, that whatever happened in terms of lethality happens early on in the process.

If, in this case, you leave the seeds in the 2,000 ppm chlorinated water for another seven minutes, what we think is happening is that the cells that had adhered to the seeds are being removed or at least being loosened and we then add the peptone water and we vigorously shake that seed-peptone mixture, that we are actually separating cells from one another and separating more easily the cells from the seed in the 10-minute treatment test versus the three.

So what we are doing is we are seeing less colonies formed here at three minutes on our enumeration medium, but, in fact, there are probably -- well, one would think -- as many as 10 minutes and maybe even more. But this is real, this is something we have seen over and over again, not just for chlorine, but with some of the other chemicals that we have evaluated, as well.

That was the sodium hypochlorite. Here is the calcium hypochlorite giving you the same setup in terms of presentation of the data, and similar results in terms of

the effectiveness of the two different products. One is not too surprised because the basic active component is hypochlorous acid whether it comes from calcium or sodium.

Now, I don't have on slides data that we have generated on 20,000 parts per million. It was mentioned yesterday and indeed we did observe that at 20,000 parts per million, that is 2 percent calcium hypochlorite, that 3 out of 3 replicate trials that we were not able to detect E. coli 0157 by enrichment.

Now, knowing that this is as promising as any really of the chemicals that we had evaluated, we wanted to actually follow up with more replications, which we did, and I don't have those data on slides, but I have them on a table here, and it is important that I give you the proper information.

We did the test actually six more times, six additional replicates. There of these replicates were the very same as we did when we generated the data indicating that we could not recover through enrichment in three replications the organism from seeds that had been treated with 20,000 parts per million of calcium hypochlorite.

The second set of three replicates, we didn't get the same results. In fact, out of the three replicates, we could isolate in each of the replications the organism through enrichment, we could detect E. coli 0157:H7 on the

treated seeds.

A third series of three replicates, this time at 21 degrees and also at 55 degrees, thinking that the heat may have some synergistic effect, came a little closer to eliminating the organism, but in one out of the three sets, three replications, we would even with a 55-degrees treatment, 20,000 ppm calcium hypochlorite, isolate the organism, detect the organism through enrichment.

We did a double treatment, that is, treated with 20,000 parts per million for three minutes. This was all three-minute data now. Then, we analyzed through enrichment, and then we went back, we treated the very same seeds again, 55 degrees, 20,000 parts per million, and could still isolate the organism.

So, while we have made I think certainly progress and have I think through all the chemical treatments that we have evaluated, this one does look the most promising. We still cannot say that we can eliminate about -- this, in the latter case, was a little over 2 log from the initial population the organism from alfalfa seeds.

Now, to go on to the other chemicals that we have evaluated, acidified -- this is actually chlorine dioxide acidified product -- we are seeing fairly large and significant reductions at 500 parts per million. Even at 100 parts per million of chlorine dioxide, acidified

chlorine dioxide, we are seeing tremendous reductions even at three minutes, certainly at five minutes.

The other product is essentially I think -- let's see, chlorine dioxide product -- this is a product from [Alside] Corporation. It gives us similar results. The formulations that we were provided and that we used started with a 500 and then there is 850 and 1,200, there is actually a process in which you mix two containers of solution to get the concentrations that are used, in this case presented here, 500, 850, and 1,200.

MS. DeROEVER: Dr. Beuchat, two minutes, please.

DR. BEUCHAT: Yes. We got tremendous reductions at the 500, and this I think follows the observations that we saw with the other chlorine dioxide product, ethanol does a pretty good job, unfortunately, ethanol reduces the viability of this particular lot of seeds.

Hydrogen peroxide, I mentioned earlier with Salmonella, did a fairly good job in reduction. We also saw that here even at less concentration, 1 percent, we got a reduction. However, even at 8 percent, through enrichment, we could isolate 0157 from seeds, treatment for 10 or for 3 minutes, and that was starting out with just a little over 3 log of E. coli 0157:H7.

Trisodium phosphate, up to 15 percent, even at 4 percent, a very low count, not being able to detect it by

direct to plating, but even at 15 percent, picking it up by enrichment, 3 out of 3 reps in every case.

Two products that are formulated and commercially marketed by Ecolab, Tsunami, and Vortex -- I don't believe Vortex is authorized certainly at this point for the use on produce or seeds, it is another product, but we wanted to try it -- again, with 80 parts per million, this is a peroxyacetic acid-based product, as is this one. We did get large reductions in populations.

A product called Vegi-Clean out of Microside in Troy, Michigan, we got reductions even with, well, 1 percent, 2 percent.

These are the data for the storage study population versus time of storage. This is at 20 weeks. This is the 5 degree data and the 25 degree and 37. We have additional data. This is a little less than five months. We had 8-month data in which we are still picking up by enrichment the organism at 37, the 5 degrees flattening off.

MS. DeROEVER: Time, please.

DR. BEUCHAT: Conclusion. Low concentrations of those chemicals that you see, either peroxide, trisodium phosphate, or acidified sodium chlorite, chlorine dioxide do cause significant reductions of the organism on seeds with little effect on germination, but all the chemical treatments reduced the numbers of cells recovered on the

seeds, but none really eliminated the pathogen in our hands. An elevated storage reduces the population, but does not eliminate it. Those are alfalfa seeds up close.

Thank you.

DR. FENG: Thank you, Dr. Beuchat, for a very comprehensive, thorough review of disinfectants.

Our next speakers are Dr. William Fett and Dr. Kathleen Rajkowski from ARS, USDA, in Wyndmoor, Pennsylvania, and they will detail to us their work on disinfection and controlling pathogens on Sprouts.

Control of Human Pathogens on Sprouts

DR. FETT: I am Bill Fett, and Kathleen and I are going to share our 15-minute slot here, so we are going to have to move very quickly, 7 minutes apiece.

I wanted to mention one thing. The ARS information staff has put together a news background on what we are going to be talking about, and this has been provided to the panel members here on the stage, and there are some other copies in the back.

If I could have the slides, please.

I also want to mention that some of the work on irradiation that we will be talking about today was also done by Don Thayer, who is a research leader at the Eastern Regional Research Center.

We are firm believers that to have the best chance

of producing a safe sprout that we are going to have to employ multiple hurdles, multiple techniques along the way to give us the best change of having safe sprouts.

So, the first set of data that I will show you is some very recent data. I won't go into too much detail on the methodology because I don't have much time here. I will just say that Dr. Buchanan has been provided with a detailed report on these experiments.

This is the result of two experiments that were done fairly recently looking at the effect of calcium hypochlorite at various high concentrations on the effect of the viability of E. coli 0157:H7 that was artificially inoculated onto the seed.

We used a cocktail of four strains. One was the sprout-related strain from 1977, two were cider related strains, and one was the hamburger strain from a 1993 outbreak. We started out with seed inoculated at about 10^6 cells, CFU/gram of seed.

The treatment was for 10 minutes at room temperature, and it was preceded by two rinses of the inoculated seed with sterile tap water, and also there were two rinses with sterile tap water following the treatment with calcium hypochlorite.

I should say that the seed that we used for these experiments was sterile seed produced by very high-dose

gamma irradiation 25 kilogray. After treatment and rinsing, the seeds were pummeled in a sterile peptone water and plated either onto trypticase soy agar or E. coli/coliform Petrifilms produced by 3M. The Petrifilm media is selective and the TSA is not.

If we look at the TSA data, we can see that with the buffer control about 10^6 CFU/gram, we look at calcium hypochlorite, now, this is weight per volume. I think there is still some confusion when we are talking about calcium hypochlorite on what the actual concentrations are. In conversations with the people in California, it is my understanding that the registration is for 20,000 ppm of available chlorine.

Now, to get 20,000 ppm of available chlorine on a calculated basis, one has to use about 3 percent on a weight per volume basis because the commercial calcium hypochlorite is about 65 to 68 percent available chlorine. So, initially, we were going to start with just a look at 2 percent and below, but after those conversations, we upped it to 3 percent to give us about 20,000 ppm of available chlorine.

You can see in our experiments that it did make a big difference going from 2 to 3 percent even though statistically with two replications there was no significance, but I am sure when we replicate this some

more, we will probably see a statistically significant difference between 2 and 3 percent.

The other thing I would like to point out is that on the Petrifilms, we didn't detect any E. coli 0157:H7 survivors at the two highest levels of calcium hypochlorite. We believe that this is due to injury of population of E. coli 0157 that will not grow on the selective media, but will grow on the non-selective media.

We also have been looking at various chemicals as additions to the irrigation water. We use a tray system shown here, usually employing one tray with smaller trays on it. You can see a carboy here. That is where we put our solutions and they are pumped up, and the growing sprouts are sprayed through a spray nozzle up here.

This work was done with alfalfa again, and this just shows you some control values for total aerobes, coliforms in use in molds. These experiments were not done with human pathogens. Here, we have the seed treatments, sodium hypochlorite, calcium hypochlorite. This is on a weight per volume basis here.

Then, no addition to the irrigation water, and these are the kind of levels that we are seeing, and you can notice that at least with these two seed treatments, no reduction in the final microbial loads on the sprouts.

This just lists some of the chemicals that we have

tried so far. Unfortunately, we have not come up with a chemical that we can add to the irrigation water that will give us even a consistent 1 or 2 log reduction in the microbial loads.

Hydrogen peroxide, when we got up to 1,000 ppm, caused injury. We had some injury with the sodium hypochlorite. We have taken EDTA up to I believe 400 ppm with no effect. Right now we are trying some commercial sodium chlorite preparations.

I think we know the reason that it is very difficult to reduce the microbial loads on alfalfa sprouts, and that is due to much of the natural population being present in the form of byofilms. It is important because bacterial cells and biofilms are well known to be very resistant to antimicrobial compounds over free living bacterial cells, a difference of 1,000- to 5,000-fold.

We did some SEM studies on four different types of sprouts obtained at the retail level, and found that indeed natural biofilms are very abundant on all four of the types of sprouts that we looked at on all different plant parts.

Also, alfalfa sprouts that we grew up in our own lab for four days harbored extensive number of biofilms.

MS. DeROEVER: You have got two minutes, please.

DR. FETT: Okay. The last intervention that I would like to talk about -- and this is something we are

kind of just getting into -- is the area of competitive exclusion, and that is the use of innocuous or non-harmful microbes to exclude human pathogens should be from nutrients and/or colonization sites.

I am not going to go over this, but if you can read quickly, this is our protocol that we used. Here, we are looking at Salmonella. We had a cocktail of four Salmonella strains that we inoculated the seed with, and then we put the potential antagonist into the soak water. So, we think this could be a very easy technique for growers to do at the grower's site.

This just continues on with our protocol, a seven-day incubation period. This just shows you the vials that we use for the bioassay. These are about seven-day-old alfalfa sprouts and about seven-day old broccoli sprouts. I can tell you that of the 70 or so plant-associated bacteria that we have tested so far, that we have come across one strain that, in two experiments, reduced Salmonella by 5 logs, and actually in the first experiment, it was undetectable compared to our control which had about 10^7 CFU/gram of Salmonella.

So, I think this is a very interesting technique that might be very useful for the growers at the growers' site.

I am going to turn over the microphone now to Dr.

Rajkowski.

DR. RAJKOWSKI: In order for the irradiation process to be considered for use in the reduction or elimination of pathogens on sprouts or seeds, some points must be considered. On the onset of the work, the first consideration was would the sprout be a viable product.

What I did here is I obtained commercially grown sprouts and irradiated them at 2 kilogray and kept the control and the irradiated product in the similar circumstance, which was in a refrigerated room approximately 8 degrees Celsius for a week, and then we photographed the results.

These were the alfalfa sprouts. As you can see on the control, there is browning and deterioration particularly in the root area where the 2 kilogray sprout looks just like as obtained a week before.

The next procedure was to take the sprouts, artificially inoculate them with both Salmonella and E. coli 0157:H7 to determine a D-radiation value of these pathogens on the sprouts.

Most of the data previously reported in the literature are from meat, and I used both the meat cocktail for E. coli and for Salmonella. The strains are located over here, which are ATCC 35150, 34889, 43894, and two that were isolated from meat outbreaks, and for the isolates from

a sprout isolate, which was F4546.

For the Salmonella, they were Dublin Enteritidis, Newport Seftenberg and typhimurium. These are the ones that are reported by Dr. Thayer in all of his works. The strains that we used for the sprout isolates were Anatum Stanley, which is what was reported today and yesterday, Newport, and infantalis.

The results showed that compared to the meat strains on sprouts, which is a moist product, there is no difference in the D-values.

Using a similar procedure that Dr. Beuchat mentioned, we inoculated alfalfa seeds. We inoculated them to a level of approximately 10^8 to 10^9 to obtain our D-radiation values. The seeds being a dry product would have a slightly higher D-radiation value than any moist product.

Using the same strains as was used for the sprouts, these are the ones that are the meat isolate strains, we obtained a D-value about 0.6 kilograys. It is reported in percent inactivation as opposed to log reduction, so at a 99.99 percent reduction at about 2.5 kilogray, we have reduced the population of 0157:H7.

The D-radiation value of the alfalfa seed using the sprout isolates were similar to those found for the meat.

The D-radiation values for the Salmonella, the

meat isolates that we used was slightly higher than had been anticipated, but again, these were done on a dry product, and the percent moisture, as you do your radiation, must be taken into consideration.

So, for the Salmonella of the meat isolates, we were able to obtain a 99.9 percent reduction at about a 2.8 kilogray, with a D-value of close to 1.96 plus or minus 0.05. Using the sprout isolates, we obtained approximately the same amount or the same D-value of 1.02.

Statistical analysis of the D-values has shown that there is no statistical differences between the meat isolate or the sprout isolates on the seeds, but they are much higher than those ever reported for the meat.

To show that the seeds are still viable, this is an experiment that I ran where I controlled all the seeds received 5 kilogray, and these are alfalfa seeds, of irradiation. All I varied was the temperature, and the temperatures ranged from 30 degrees down to zero degrees Celsius, which is about 32 to about 94 degrees Fahrenheit.

The control over has approximately the same mass weight as those that were irradiated at 5 kilogray. So, even at 5 kilogray, which would be much higher than used or would be proposed used the seeds were still viable.

In conclusion, competitive exclusion was an effective hurdle in controlling the growth of Salmonella on

sprouts. The shelf life of sprouts is increased at the irradiation of 2 kilogray. What we would like to do is go back, repeat this experiment and continuing it longer than just one week. The irradiation D-value of E. coli and Salmonella on sprouts are similar to the D-values for meat products being that these are moist products.

Reduction of microbial growth on growing sprouts will be difficult due to biofilm formation that Dr. Bill Fett mentioned. The radiation D-value for E. coli and Salmonella on alfalfa seeds appeared to be higher than the D-value for meat products, which means that a combination of irradiation with the chemical treatment is a viable alternative to the reduction or elimination of the pathogens on the seeds.

Thank you.

DR. FENG: Thank you, Dr. Fett and Dr. Rajkowski.

We have been going about two hours now, and we are running a little bit behind, so I have been informed that we are going to take a break now for about 15 minutes, and we will pick it up again at 10:05. Thank you.

[Recess.]

DR. FENG: We are going to continue with the last two speakers for this session entitled "State of the Science." We have two speakers from Japan. The first speaker is Dr. Kenji Isshiki from the National Food Research

Institute. He has been working a lot with control measures, and he is going to be discussing with us some of this work done using oyster shell calcium in preventing growth of 0157:H7.

Dr. Isshiki.

Control Measures

DR. ISSHIKI: Thank you Dr. Feng.

Good morning, everybody. I am Kenji Isshiki. I work for the Japanese Ministry of Agriculture, Forestry, and Fisheries. Actually, I work in National Food Research Institute.

First of all, I would like to express my sincere thanks to all of you. After we had the severe E. coli 0157 outbreaks in 1996, we thank you for your kind help. I am poor in English communication, so please be patient to my broken English.

This photograph shows radish sprouts growing in Japan. In Japan, we have various kinds of grow foods in fish and vegetables. Radish sprouts are eaten and grown, and after the outbreaks of 1996, radish sprouts also eaten after cooking.

Next slide, please.

In Japan, we have some kind of sprouts. One is radish sprouts, and the other is alfalfa, and traditionally eaten mung bean sprouts, and the new type of sprout is pea

sprout. This type of pea sprout and mung bean sprout cooked and then eaten.

Next slide, please.

We take quick look at the radish sprout production. This is a seed. These seeds came from the U.S.A., and this is making chlorinated water, and this is washing of the seed.

Next slide, please.

This is preparing the mat under the seed. It is put into the corner of the seed.

Next slide, please.

This is humidifier, hold the sprout for germinations in dark rooms for about two days. After, sprout moves to the greenhouse, and about five days after, are harvested and packaged.

Next slide, please.

In Japan, Ministry of the Health and Welfare announced that radish sprout was suspected epidemiologically as most likely food that cause outbreaks in Japan in 1996, but no E. coli 0157:H7 was detected from the radish and others.

Ministry of Agriculture, Forestry, and Fisheries decided to make this guidelines for the growing radish sprout to improve the safety of the product. A committee was organized and discussion on hygienic matters related to

radish sprouts production was carried out.

We make some experiment for these committees. This photograph shows the surface of the stem of the radish sprout. You can see some bacteriums. This is a normal flora of the radish.

Next slide, please.

This is also the normal flora of the radish, maybe to make biofilms. If the E. coli 0157 attaches to these stems or leafs, we could not wash out all of the E. coli 0157. We could only 99 percent of E. coli by the washings.

Next slide, please.

The seed and the E. coli and the water is very important. If the seed were polluted by E. coli 0157, water is added, the E. coli was very increased. This seed intentionally contaminated with E. coli 0157. Chlorinated water was used for the disinfection of the seed, but small time is observed, but after then the growth if very rapid.

We carried out some experiment. We make polluted seed and mixed with non-polluted seed. If we mixed one polluted seed with 1,000 non-polluted seed, the initial counts of E. coli is less than 300, but one day after adding water, the E. coli was increased to 12,000. It is very important. One seed were polluted, and non-polluted 10,000 seed, they were also polluted.

Next slide, please.

So, we have done various kind of disinfection experiment for getting the clean seed, but most of them not successful. Some take care of the 0157, but actually we cannot use this method because we have food sanitation laws and other laws.

This is one example of disinfection of seed. This is dry air heating, polluted, intentionally polluted seed dry air at 75 degrees C. First day, 0157 was detected, six days or seven days after, 0157 was not detected, but the seed were put into the water and 20 hours after we took the E. coli 0157, the product is polluted by the 0157, which is very important.

Next slide, please.

This is my laboratory. These are oyster shells, oyster shells heated by more than 800 degrees Centigrade. The main component, calcium -- calcium oxide, so it makes a powder, and then added to the radish sprout, at this point 0157 is added, and results calcinated calcium the 0157 is rapidly increased, but the addition of the 0.4 percent calcinated calcium, 0157 is killed and then 0157 is not detected. Radish sprout is not affected by the calcinated calcium.

Next slide, please.

We recommend two-day disinfection method. This is hot water treatment. The first step, it is dipped in hot

water for 55 degrees C. for six minutes, 131 degrees.

The second step is cool down with water and dip into a 2 percent calcinated calcium for five minutes. We can disinfect. Final product is negative, but heavy pollution of the E. coli, all of the E. coli was killed, and final product is positive. It is very important to check the seed and also check the final product, because the E. coli 0157 can recover during the cultivation of the radish sprout.

Next slide, please.

This is another recommendation of the seed treatment, hot water and sodium hypochlorite. The first step is dipped in hot water at 60 degrees C. for 90 seconds, and cooled down with water, and dip in 200 ppm chlorine water, and this is needed twice. Heavy pollution, cannot get final product negative, but slight pollution we can get the final product negative for E. coli 0157.

Next slide, please.

So, our committee recommended three treatments. First, wash with chlorinated water using water containing more than 0.1 ppm chlorinated water. The second step is sanitize with hot water and chemicals. The third step is keep water changing during the germination step. If the water is not changed, the concentration of the E. coli 0157 is very high.

So, the outline of the clean sprout production we recommend is, first, examination of the seed microbiologically. We need E. coli is not detected. E. coli is indicator of bacteriums. If the E. coli is detected, we need second step, examination of the E. coli 0157. Next step is wash seed well with clean water. The next step is sanitize seed, hot water and chemical treatment.

The fourth step is keep water changing during the germination. Fifth step is grow sprouts cleanly. Sixth step is distribute them under 10 degrees C.

Next slide, please.

So, our committee make final recommendations, sanitary guidelines for growth radish sprout. In October 1996, a procedural manual was put forth by the Ministry of Agriculture. It has been revised in 1998.

It is pointed that the water and seed are very important to obtain the clean radish sprout. It is recommended to use tap water or disinfected chlorine water. Every lot of the seed must be checked as free from E. coli. If the E. coli was detected, it should be necessary for use the seed to contain free from the Shiga toxin producing the E. coli and Salmonella.

It is recommended that the seed should be sanitized with hot water. Other issues, such as the

sanitation facilities and employees are discussed in manuals.

This photograph shows one product of the radish sprout. This shows this product was produced under these guidelines.

Next slide, please.

So, we need more research. Actually, we have small amount of the information about the E. coli, so in our ministry, we start research, making the compost for other items under research.

Next slide, please.

Next year, we will make guidelines for clean production for the hydroponics or hydroculture of vegetables. We need more research to make the guidelines.

This is my presentation. Thank you very much for your attention.

[Applause.]

DR. FENG: Thank you, Dr. Isshiki.

Our final speaker for this session is Dr. Susumu Kumagai from the National Institute of Infectious Diseases in Japan. Dr. Kumagai has recently published a few papers on artificial contamination of radish sprouts using 0157:H7, and that is the work he is going to be sharing with us.

Dr. Kumagai.

Control Measures

DR. KUMAGAI: Thank you, Dr. Feng, for the kind introduction and for also inviting me to this very important meeting.

Slide, please.

My talk today is our study of experimental contamination of radish sprouts with E. coli 0157:H7.

In 1996, there were several large outbreaks in Japan, and the largest one among them took place is Sakai City with more than 6,000 patients and hydroponically grown radish sprouts were implicated in this outbreak.

Radish sprouts are sold in such containers, and people usually eat them after removing their roots, and usually eat raw as salad, sushi, and other dishes as was shown by Dr. Isshiki.

A question we had at this time was how can radish sprouts be contaminated with E. coli 0157:H7, and in order to see it, we performed a series of experiments of contamination of radish sprouts with E. coli 0157:H7. Those were the experiments, contamination of edible parts of radish sprouts by soaking their roots in E. coli 0157:H7 containing water, and through activation of the organism during growth of radish sprouts from artificially contaminated seeds.

The location of the organism in artificially contaminated radish sprouts by immunofluorescence and

immunoscanning electron microscopy, and the effects of this infection and heat treatment on survival of the organism in artificially contaminated radish sprouts.

At first, we started the contamination of edible parts of retailed packed radish sprouts after contamination of their roots. Retailed packed radish sprouts, they have a height of 12 to 14 centimeter, and we made holes at the bottom of packs, and then these packs were dipped in the water containing E. coli 0157:H7 at the level of 4 to 7 log CFU/ml, so that their roots were dipped in the same water.

Eighteen hours later, we cut the edible parts off the sprouts, 4.5 to 5.0 centimeter long, and then determined the presence of E. coli 0157:H7.

The results were expressed as the number of contaminated packs per quarter number of packs used in this experiment. In either contamination level, the edible parts were contaminated with E. coli 0157:H7 for the two strains used in this experiment.

From this result, we thought of the possibility that E. coli 0157:H7 can be absorbed from the root to the edible parts of radish sprouts, and in order to see whether it is true or not, we did the next experiment as shown here.

The seeds were placed on the net in the beaker, and sterile water was given to the seeds, and they were grown for eight days, and then they were separated to four

groups. The first two groups, the roots remained intact, and for the third group, the roots were cut in half, and for the last group, the roots were completely cut away, removed.

Then, for the first group, the remaining roots were dipped, half of the remaining roots were dipped in E. coli 0157:H7-inoculated water, and for the second group, the whole roots and the lower part or hypocotyl were dipped in the same water, and for the third group, the roots were also dipped in this water.

For the last group, the robust part of hypocotyls were dipped in the same water. Eighteen hours later, the edible parts of sprouts were analyzed for E. coli 0157:H7. This shows the seeds on the net in the beaker, and this shows the grown radish sprouts. You can see sprouts were not so crowded as retailed packed sprouts.

This shows the result of this experiment. For the first two groups in which their roots remained intact, no edible parts were contaminated with E. coli 0157:H7, however, for the third group, and last group, the edible parts were contaminated with the organism.

The former experiment using retailed packed radish sprouts showed the edible parts of them became contaminated. If the roots were dipped in contaminated water, and this experiment showed however contamination doesn't spread from intact roots to the edible part, if sprouts were not so

crowded and spaces between sprouts are wide enough, and also this result showed that the contaminations spreads to the edible parts if the roots were hypocotyl or injured, and from all these results we thought that the observed contamination of the edible parts of the retailed packed radish sprouts may have been due to possible injury of their roots were action caused by a crowded condition of sprouts.

The next subject was contamination of edible parts of radish sprouts after contamination of seeds with E. coli 0157:H7. In order to clarify it, we did the following experiments.

The seeds were on the net and dipped in the water containing 0157:H7 at a level of 3.5 log CFU/ml, and eight hours later, radish seeds were took away from the water and analyzed for E. coli 0157:H7, and the other seeds were transferred into sterile water, and 24 hours later, also germinating seeds were analyzed for the presence of the organism.

In the other experiments, also, the seeds were contaminated with the organism in the same manner as these experiments, and then they were grown for about one week, and then the edible part were analyzed also for the presence of E. coli 0157.

This shows the seeds in the water containing E. coli 0157, and this shows the grown radish sprouts. This

shows the results of these experiments. At eight hours, the seeds contained 4 log cells of 0157:H7, and then 24 hours later, germinating seeds contain more than 8 log cells, and then at seven days, the edible parts contained nearly about 6 log cells.

This result indicates clearly that E. coli 0157:H7 increases very rapidly during the early stages of growth of sprouts including germination stage, and then after grew, they contain very high level of 0157:H7 in the edible parts.

In order to see the location of E. coli 0157:H7 in artificially contaminated sprouts, we did microscopic observations. This shows the method. We prepared contaminated sprouts as mentioned before from contaminated seeds, artificially contaminated seeds, and then after grew are cut a part of hypocotyl was removed and fixed in formalin, and then treated with anti-E. coli 0157:H7 antibody, and then are treated with fluorescein conjugated secondary antibody, and observed on the fluorescent microscopy.

The other group was treated for scanning electron microscopy including treatment with anti-E. coli 0157:H7 antibody, and then with gold-labeled secondary antibody.

This shows horizontal cut of hypocotyl of artificially contaminated sprouts. There were many strong fluorescence in and on the vessels of the sprouts, and also

near to the epidermis of hypocotyl. This shows uncontaminated control of hypocotyl, showing background fluorescence.

This shows the outer surface of contaminated hypocotyl by scanning microscopy. There were many bacteria on the outer surface of hypocotyl, and they were stained, they were labeled with gold, and this shows the inside hypocotyl. This is vessel and although far fewer than the outer surface, there were bacteria, and some of them were labeled with gold.

This shows stroma of hypocotyl, and around the stroma there were also many bacteria, and they were labeled with gold.

This is the same, but inside of the stroma also there were some gold-labeled bacteria, and this bacteria is just dividing to two cells. This is also the same.

These are on the outer surface of hypocotyl, and these were gold-labeled bacteria, and these are not gold labeled, perhaps the other bacteria than E. coli 0157:H7.

Next, our interest was whether the bacteria inside tissue are viable or not, and in order to see it, we prepared contaminated sprouts from artificially contaminated seeds, and then cotyledons and hypocotyl were dipped in mercury chloride solution for 0.5 to 10 minutes, and mercury chloride is often used for surface infectant in the field of

plant pathology as very strong disinfectant, and sprouts were dipped in this manner, and then a part of cotyledon was removed and cut half, longitudinally cut half, and this cut surface and the outer surface were placed on the others for detection of E. coli 0157:H7, and then incubated overnight at 37 degree for colony formation.

The results were expressed as positive slices, a number of positive slices as seen in yellow. E. coli 0157:H7 on the outer surface was killed completely by mercury chloride treatment for 10 minutes, however, the same treatment could not completely kill the bacteria inside tissues.

The former experiment indicated that E. coli 0157:H7 exists inside sprouts where mercury chloride cannot reach in 10 minutes. Next, we examined the heat treatment of the edible part of artificially contaminated radish sprouts. As mentioned before, we prepared contaminated radish sprouts from artificial contamination of seeds, and then after grew, we took the edible part, 1 to 2 grams, and then heated in boiling water for 5, 10, or 60 seconds, and then they were analyzed for E. coli 0157:H7.

This shows the result, and the 5-second heating is very effective to kill the bacteria. The reduction was more than six orders of magnitude.

These are seeds of experiments. We could find

contamination of intact roots doesn't spread to the edible parts if the sprouts are not so crowded and spaces between sprouts is wide enough, and the edible parts can be heavily contaminated when they are grown hydroponically from contaminated seeds.

In the experiment, heavily contaminated sprouts, a large number of the organism exists on the surface of the plant, but a small number is also inside the plant where mercury chloride cannot reach in 10 minutes. However, five-second heating in boiling water is very effective in reducing viable number of the organism in the plant.

Thank you very much for your interest.

DR. FENG: Thank you, Dr. Kumagai.

This concludes the session on the State of the Science. I would like to thank all the speakers for presenting, and I now turn the session back to the panel.

DR. KVENBERG: Thank you, Dr. Feng. I am standing in for Janice Oliver for the moment again on the panel. In a moment, I would like to bring this morning's speakers up for questions and answers, but the Chair has received a request from Mr. Bob Rust who would like to make a very brief statement.

If you could come up to the podium for a few moments, and then we will ask the speakers for this morning to come up.

MR. RUST: I am Bob Rust with International Specialty Supply, and we have been selling sprouting seed for about 18 of the 20 years that we have been in business. There is a few things to me that are starting to add up.

We look at a lot of seed, and I have looked at a lot of seed at 30 times magnification. I don't recall ever seeing any chipped seed. I have seen broken seed, and I have seen cracked seed, but I don't recall ever seeing chipped seed. Indeed, seed tests, when you purchase seed, it has the amount of cracked and broken seed listed on the seed test report. It is generally under 1 percent.

I believe -- I could be wrong -- but I very strongly believe that the slides that we saw from Dr. Wick today was of scarified seed. Hard seed is seed that won't readily imbibe water, it has a hard seed coat, and if you scratch the seed coat, which is called scarifying, it will imbibe water because the water can get in.

Now, we used to buy seed from Italy. In fact, I contracted about a million pounds from Italy, and this was maybe 10 years ago. The first about a quarter, maybe 250,000 pounds was good seed, it had a low hard seed count. All of the rest of it had a high hard seed count, and we ended up rejecting it.

In that period of time, we have got in many samples from Italy, because it is not very expensive seed,

and if we could buy it from Italy, we would, but the hard seed count has been very high, and there has not been any acceptable seed to me, and Idaho is another place where there is a very high hard seed count in the seed. In fact, we have never bought seed from Idaho.

If you scarify that seed, it is then sellable to sprout growers. Now, some of the contaminated seeds, contaminated sprouts came from Italy and Idaho, and I also know the grower in Denmark where the Danish outbreak came from, and they bought their seed from a company in Italy. That seed almost certainly was scarified, as well.

Seed that is not scarified, which is nearly 100 percent of the seed in a seed lot, is just as smooth as silk. It is very, very smooth. You look under a microscope and it looks very smooth. It looks like the portions of the seed that were not cracked.

Now, Dr. Beuchat, when he was doing his research, he found out that there was one study that he did that killed all of the Salmonella and E. coli, and another study that he did, it didn't, even though he did the same study.

Now, I spoke to him, and he said that those two studies were done using two different lots of seed. Now, they did come from a supplier who does scarify seed, so it is possible that one of the lots was scarified, and a lot of bacteria could get in the seed coat, and that the other lot

was smooth as silk and basically, when he spiked it, there was just Salmonella on the outside.

I have a strong hunch that intact seed can be surface sterilized, so what I am proposing is that we take a very serious look at the problem of scarification and that for the time being none of the seed companies scarify their seed and that sprout growers surface sterilize their seed, and I believe that we will seriously reduce the incidence of pathogen problems that we have got.

DR. KVENBERG: Thank you, Mr. Rust.

At this time, could I ask the speakers from this morning's panel to please come up and find a place where you will have access to a microphone. We will begin the phase of this morning's program where we go into questions and clarification. My watch may be running a little slow. I have about five after the hour. The panel, please, and Produce Working Group, take the time you need.

Before we break for lunch, which we plan to do at 12:30, I would like to allow sufficient time for public comment before that period, but let the questions begin and take the time you need.

Dr. Doyle.

Questions of Clarification

DR. DOYLE: Could I ask Mr. Rust a question?

MR. RUST: Certainly.

DR. DOYLE: How would you perceive the pathogens contaminating the seeds during this scarification process?

MR. RUST: The process of scarification, you take the seed and you scratch the seed. You put it into a machine that is designed to scratch the seed. As it scratches the seed, in the process, it could basically be -- and I am not saying it is -- but it could possibly be injecting Salmonella into it.

If there was feces or something in the scarifier, and as it scratched it during the process of scratching, it could basically be injecting it with Salmonella.

DR. BUCHANAN: Could I ask a follow-up question?

Is there typically any cleaning steps of the seeds after scarification, or is scarification always post-cleaning of the seeds?

MR. RUST: No, that would be the last step.

DR. BUCHANAN: It would be the last step.

MR. RUST: Basically, what you have done is you have taken seed and you have sent it off for germination tests, and the germination tests shows that is has a high hard seed count, and then it is not sellable to sprout growers, it is generally sold into the farm industry.

Now, I am not saying, I am not intending to say this is an unscrupulous practice or anything like that, I am just saying, I am trying to put some things together, and it

is possible that this is what could be the problem in the industry.

DR. KVENBERG: Questions, please. Dr. Troxell.

DR. TROXELL: I would like to go to the issue of the surfactants and Dr. Beuchat's and Dr. Fett's work. With the treatments you used, were they straight up sodium hypochlorite and calcium hypochlorite, or did you make any attempt to use surfactive agents to optimize the wetting and get into those little grand canyons in the seeds?

DR. BEUCHAT: In most of the treatments that we evaluated, we did not use surfactant, however, in a few we did, and in those cases we used between Tween 80. Just to give you some numbers on the effectiveness of Tween 80, we used 1 percent and 2 percent and a control. We used treatments of 3 and 10 minutes, and this was at room temperature, 22 degrees Celsius.

We were able to reduce populations of E. coli 0157:H7 by less than 1 log even with 2 percent of just Tween 80, so there is some impact of just surfactant without the chlorine.

We did some other studies in which we pretreated the seed with Tween 80 for three minutes both at 1 percent and 2 percent, followed by treatment with calcium hypochlorite -- this is in parts per million -- up to 2,000.

In the case where we would pretreat the seeds with

1 percent of Tween 80 for three minutes, we were able to reduce again populations of E. coli 0157, but not by more than 1.5 logs for 10 minutes.

Pretreatment with 2 percent Tween 80 resulted in a little higher reduction with subsequent treatment with 2,000 parts per million, but in any case, not more than 1.4 logs. So, the treatment with the Tween 80, then, subsequent treatment with the calcium hypochlorite had some beneficial effect, but at least with those relatively few experiments that we did carry out, was not as beneficial as we had hoped it would be. But in all of the other experiments, we did not do pretreatment followed by any of the other chemicals, just the hypochlorous acid in the form of the calcium hypochlorite.

DR. FETT: No, we did not try surfactants. That is something that we would like to do, but from a regulatory standpoint, I don't know how they would view the use of surfactants, but those are experiments that we plan on doing in the future.

DR. KVENBERG: Dr. Goosby.

DR. GOOSBY: Regarding germination and the likely impact of different chemical treatments, heat treatments, the use of radiation, is it reasonable to conclude that the health of the seed and its resistance to those factors might be influenced by the age and storage conditions of the seed,

and has anyone looked at that closely to see that, in fact, a younger seed in managed storage conditions might be a factor and still contribute positively to the germination percentage and its resistance to those kind of treatments?

DR. BEUCHAT: In the two series of experiments that we conducted in which we used in both instances the 20,000 ppm of calcium hypochlorite, the only difference, as was pointed out by Bob Rust, was the lot of seeds that we used.

In the first instance in which we were not able to detect through enrichment after the 20,000 ppm treatment E. coli, we had seeds that were at about a 96, 97 percent germination level.

In the second series of experiments, the germination level was in the range of 80, 81 percent. Now, I don't know what the degree of physical damage was in either case, but I would guess, have to assume that there was more damage or at least certainly the level of viability was much lower in the second case, and perhaps the degree of physical damage through scarification, through whatever means may have enabled through our inoculation process these cells to enter the cracks, the crevices, the tissue, and therefore, be less accessible to the treatment with the calcium hypochlorite.

I do not know. That is speculation, that is

theory, but certainly the only difference that occurred, the only change in these two sets of experiments was the lot of seeds that we used, the two lots.

DR. RAJKOWSKI: We did inoculate Salmonella on two different seed lots, and statistically, there was no difference between the seed lots and the D-radiation values found. So, the seed lots did not make any difference.

DR. GOOSBY: Do you have any information about the age of the different seed lots or their storage conditions that may impact on the health of the seed?

DR. RAJKOWSKI: When those seeds were received in our building, they were stored in the same room, under the same conditions, and handled identically. I do have the information from the seed companies, but I do not have that with us. That would be a consideration, but I was told that these were both 96 percent germinable seeds.

DR. BUCHANAN: In order to evaluate the differences between Larry's study on 2,000 parts per million chlorine and Bill, yours, can I ask two questions, one of each of you.

Larry, in your evaluation methods, your detection methods, did you go right to a selective medium?

DR. BEUCHAT: On the direct plating, yes, we used sorbitol MacConkey.

DR. BUCHANAN: So, you wouldn't detect any injured

cells?

DR. BEUCHAT: Most likely not.

DR. BUCHANAN: Bill, did you have any background data to indicate whether or not the behavior of seeds subjected to 25 kilogray of irradiation behaved the same as seeds that are non-irradiated to such a high dose?

DR. FETT: No, I don't have any information on that. Initially, I was planning on using seed that was not sterilized, but even with selective media, you run into a lot of problems, at least we have, with background, natural microflora on seeds and especially on sprouts, but also with seeds.

We looked at a few different selective media, and I guess at this point I would have picked rainbow agar with novobiocin and tellurite if I had to do the experiments with non-sterile seed or go to antibiotic resistant strains, like we did with the competitive exclusion experiments, but we would have to do those experiments to see if there was a difference between sterile seed and non-sterile seed, but I kind of doubt there would be, but I am not sure.

DR. BUCHANAN: But your data would be consistent with direct plating on a selective medium overstates the efficacy of the disinfectant?

DR. FETT: That is correct.

DR. NEILL: I have a question for Dr. Isshiki. It

has to do with the calcinated oyster shell. Two things.

I am not all that familiar with this, and I am not sure everyone else is, so if you could give us a little information about exactly what that is, and then the second question that I have is you had a graph, and as I remember this, the 0.4 percent calcinated experiment has a red line that is at the bottom of the graph that shows from 0 to 1 for time, there is a marked decrease, and then staying level in comparison to the controls.

This almost looks as though this is directly bactericidal. Can you tell us about that aspect of this compound for 0157 and/or any of the other microflora or whether you think this simply inhibited outgrowth and then there was die-off?

DR. ISSHIKI: Calcinated calcium means heated well calciums. We use this type of calcium as food additives for a long period because Japanese people in shortage of calcium intakes. It is a traditional, new, traditional supplement.

For example, the oyster shells, the main component is calcium carbonate, but after heating it turn to calcium oxide and mixed with water, it turn to the calcium hydroxide so the pH is very high, 0.2 or 0.3 in suspension, pH is 12 or 13, so E. coli or Salmonella and other bacterium is killed by the 0.1 or 0.2 suspension of the calcium.

It is not easy to dissolve in water. The maximum

point is 0.05 percent. So, we use in suspension state, 0.1 or 0.2. We make examination of the seed treatment. We attach intentionally E. coli 0157 to the radish seed, and put into the suspension of 0.2 or 0.3 percent calcinated calcium, the E. coli was killed. Other type of gram-negative bacterium was also killed.

DR. NEILL: Thank you.

DR. KVENBERG: I believe the order of questions is Dr. Swaminathan and Mr. Bernard. I note Mr. Bernard has got his placard on end. That is very helpful to the Chair to maybe expedite time if I can understand, if you have a question, put it on end, that will cue me, and I will know how to put it through.

DR. SWAMINATHAN: I had a question for Mr. Inami, but I would like to follow up on that question on the oyster calcium. If this is simply a pH effect, could one use sodium hydroxide, and I think with the oyster shell, you also pointed out that the effect on germination is pretty minimal. Do you have any data on sodium hydroxide?

DR. ISSHIKI: Yes. It is effective to kill the microorganisms with sodium hydroxide or potassium hydroxide, but more effectively killed by the calcium hydroxide, much more killed by the calcinated calciums even though the eggshell-treated calcium also kills Salmonella and E. coli.

DR. SWAMINATHAN: Now, for Mr. Inami, the question

was regarding the surfactants. We have had a lot of discussion of surfactants for its inactivating E. coli 0157 and Salmonella, but I was wondering if a surfactant might be helpful in allowing your enrichment broth to reach those organisms that may be trapped inside the seed.

Did you try Tergitol 7 or Tween 80 in any of your experiments?

MR. INAMI: Actually, that is an excellent idea. They do use surfactants for ground beef samples to release the organisms from the fatty material. We have not used surfactants on any of our seeds in any of these studies, and that is something we can take a look at later, though.

DR. KVENBERG: Mr. Bernard.

MR. BERNARD: Thank you, John. I would like to go back to the questions I was asking earlier. As my colleagues on the panel know, I am easily confused and I think the discussion on approval of additives, while I am aware of what the law says in terms of certain requirements, with the passage of the Food Quality and Protection Act and the changing of responsibilities, I am not sure I understood from the discussions this morning who makes the decision nor exactly how that decision is going to be made.

So, I guess I, in fact, have two things that I would like. One is a clarification on how the process works in terms of if somebody wanted to gain approval for a seed

treatment, who should they talk to, and how do we make that decision, and also, I would like to go back. I did ask a question that didn't get completely answered, and I understand we were under some time constraints earlier, but in terms of the APA protocol, I did ask when we might expect to have something and to make sure that you understood the feeling of urgency that many have here.

I, quite frankly, find that a bit surprising because the approval, while it may be new to EPA, is not a new process. There has to have been some protocol in place somewhere that has served us up until at least the passage of FQPA. So, with that, I would like Michelle to maybe help clarify some of those points.

DR. TARANTINO: I will begin and happily pass it on to Michelle, and gratefully. I think the jurisdictional issue is a complicated one, there is no question, because of the changes that are taking place almost as we speak, but let me give you sort of the easy answers.

One, I think, and I will go back and sort of like a broken record, if you are planning to do something, you probably ought to call because we are in constant contact these days about just this issue, and we certainly were at the time of the calcium hypochlorite, to make sure that we are both on the same page as to who is doing what.

The perhaps straightforward answer at right this

moment with the passage of FQPA, is FDA would have approval for something that was used on a processed food or on wash water that would contact a processed food in a processing plant. Just about everything else is EPA's, so that the calcium hypochlorite and the analogous treatments that were being talked about by Dr. Beuchat and Dr. Fett right now are EPA's.

I will, and I will regret it immediately, say that there were some things that FQPA passed, there were some things that happened kind of accidently, and there are some activities now to try to make some adjustments in the jurisdiction of some things. At least some of those proposals would imply that some of these kinds of things could come to FDA in the future.

For example, a raw agricultural product in a processing plant, that this would come back, and I think probably sprout seeds would be there, might come to FDA. I shouldn't say come back to FDA, would come to FDA, but right now if it is not a processed food and if it is not water contacting a processed food in a processing plant, water contacting food in the field would be EPA's, water in a processing plant would be FDA's.

Having said that, given that the calcium hypochlorite and the analogous chemicals are EPA's at the moment, I will let you talk about the process.

MS. WINGFIELD: Thank you.

My comments before pertaining specifically to the efficacy evaluation in this line of products and my statement that this is a new area for us, traditionally, fruit and vegetable rinses or washes, as Dr. Tarantino has stated before, have been evaluated by FDA as indirect food additives, and some of that information basically funneled over to EPA without any direct scientific evaluation on the tolerance or a tolerance exemption level for our particular office.

Where EPA comes into play is that antimicrobial products used against what we call public health related pathogens, such as the E. coli 0157:H7, Listeria, Salmonella, and the like, fall into the jurisdictional area of the antimicrobials division. Fruit and vegetables sanitizing rinses, particularly for home owner use, are a new area for us. It is my understanding that there are currently no products registered for this particular use pattern.

In that light, we are now evaluating protocols going through a peer review and a peer validation of new methods. To give a specific time frame, as Mr. Bernard is looking for, as far as when we will have a product out on the market, is somewhat difficult to say at this time, because the state of the science, we are not really sure

that it is such that you can evaluate these products totally. We did go before the Scientific Advisory Panel last year. They gave us some recommendations on perhaps relaxing our current performance standards, so that a level of public health benefit will be provided to the consumers.

We are working with the Scientific Advisory Panel for any additional updates. We will also be seeking the counsel of noted food experts, such as some of the panel members here, or some of the members in the audience, but this is really new territory for us and we need to take a good, strong look at the science itself before we make a regulatory decision.

MR. BERNARD: A follow-up, if I may. Just as clarification, then, in terms of jurisdiction, there may be some change in this, I recognize, but if one were to want to wash a head of lettuce before it is chopped, is that an EPA approved sin, but once it becomes a salad, then, it's an FDA item?

MS. WINGFIELD: Yes.

MR. BERNARD: Okay. I just want to get that clear.

MS. WINGFIELD: That is the state of the jurisdiction right now.

MR. BERNARD: As a further follow-up, I recognize that benefit can't be considered in the decision, only

looking at safety, which seems to run counter to the current consideration of risk analysis, risk assessment in regulatory decisionmaking, in other words, if we have a significant risk reduction, but only an insignificant increase in risk, you can't consider that, I take it.

MS. WINGFIELD: That is not true from an EPA standpoint. That was an FDA. EPA is a risk-benefit statute that we operate under FIFRA. If the benefit outweighs the risk, and there are no unreasonable adverse effects either to humans or the environment, then, a particular product would be licensed and registered.

MR. BERNARD: I guess the tag line to that question that I was looking for is there some legislative change that is needed in either of your opinion, to help us streamline this process? I know Congress is occupied with other things these days, but there is still a considerable focus on food safety on the Hill.

DR. TARANTINO: I guess since you are referring to the Federal Food, Drug, and Cosmetic Act, which is the food part is not a risk-benefit statute, I am not sure that, you know, the explicit consideration of benefit is what we would want, where we would want to go.

One of the things that is very different in our statute, then, the FIFRA, is we have nothing analogous to the emergency exemption or the local use. That is something

that, you know, gives us a little bit of problem in the sense of if we are going to try to something on an emergency basis, it is difficult for us to do it.

MR. BERNARD: Thank you.

DR. TROXELL: I have some other questions, but I think I would like some more follow-up on what we have just been talking about. As I understand it -- and I would like our FDA and EPA people to clarify -- I mean we have two components you have to deal with. One is the registration and the other is the additive or tolerance component.

So, even if we have a food additive situation, the sanitizer still needs to have a FIFRA registration, so they have two applications to deal with, and the second thing -- and if we could clarify that to the extent necessary, please -- but the second thing is please comment on the role of the chemical companies that manufactured these sanitizers to get the registration, it is not necessarily simply a matter of, say, California/EPA getting their registration, the emergency registration, but you have to have the buy-in of a chemical company to carry this.

MS. WINGFIELD: For food sanitizers, currently, the tolerance exemption and registration process basically go hand in hand. Establishment of a tolerance or exemption from a tolerance is part of the registration process for food sanitizers.

As far as a Section 24(c), the special local needs, which is what the State of California instituted, I am not sure what level of direct buy-in they got from a particular registrant company or not. Perhaps you could answer it a little better.

DR. KVENBERG: Before we go on with the discussion, please, can I ask the speakers to identify themselves for the recording.

MS. WINGFIELD: Michelle Wingfield speaking.

DR. KVENBERG: Thank you, Michelle.

DR. FARRAR: Jeff Farrar. In response to that, we did get the buy-in from I believe two or three specific chemical companies. Olan was one of those, I can't remember the other two.

DR. TARANTINO: Let me follow up a little bit on Terry's question. I think his question in terms of the two separate hurdles is one of the things that was kind of fixed by FQPA in the sense that both those processes are now at EPA and can go hand in hand.

I think one of the struggles that the grower community is having perhaps is finding folks who have the wherewithal to come in with the data package for registration or for a food additive application, and I think there, you know, the companies that make the chemicals that are used for agricultural purposes or for processed food are

probably in the best position to accumulate that data package to some extent, but that is probably something that needs to be worked out because in both cases, for EPA and for us, it is the sponsor's responsibility to pull together the data.

DR. KVENBERG: Thank you. Another follow-up?

DR. TROXELL: Laura, you are saying that if it is a food additive situation, that is under FDA's jurisdiction, there is no registration needed, is that what you said?

DR. TARANTINO: No, no, no. What I was saying is the current situation where they are talking about sanitizers on foods for the seeds and sprouts. This would all be at EPA and the situation as Michelle had talked about. Frankly, in fact, now that I think about it, probably most of the cases where there was an FDA approval and followed by a registration have been fixed by FQPA. There may be some out there somewhere, but I don't think so. I think most of them have been fixed.

By the way, I might add, just to go back, although I said that the calcium hypochlorite and the other chemicals are all going to be under EPA jurisdiction, at least traditionally, and I think still, we would hold because of the way it is written in the statute, that the irradiation would come to FDA.

DR. KVENBERG: Thank you. The questions I have in

order that I saw the placards go up, Dr. Sperber, you are next.

DR. SPERBER: I have a few questions for Greg Inami. As I understood your data, it looks like you had several positive seed samples that you tested and found positive two or more times with the different methods you tested. Did you make any effort to quantify the Salmonella in those seeds?

MR. INAMI: No, all the testing was done under that two-step enrichment of pre-enrichment and selective enrichment, so we did not quantify. I must also say when we did do the enrichments, we would end up on the low end of about two colonies per a couple plates, and the high end maybe about 10 or 12, so the numbers on the seeds, to start with, are very low.

DR. SPERBER: Even when you are doing the pre-enrichment and enrichment, you could do an MPN procedure to get an estimate of the total count present, and that could be helpful. I think especially in cases like this if you are dealing with incriminated lots of seed or any other food in an outbreak like this, it would be good to get a fix on the initial number of organisms there. It could be helpful in a risk assessment for establishing guidelines here or in even other food categories.

So, I would advise all microbiologists, when they

run across materials like this, to do two things. One is to get a fix on the numbers, doing MPN's if you have to, and then, two, save as much of that material as possible for your research on disinfecting the seeds and running it through other protocols to see how these organisms will grow, how you can keep them from growing.

I think it is much more meaningful to do this work with naturally contaminated materials than it is with artificially inoculated materials. We have seen a lot of information presented here in two days, most of it with artificially inoculated materials, and it might not be that realistic,

MR. INAMI: The one comment I want to make about the naturally contaminated materials, there is so much variation in the contamination level. There are points where we would be sampling 100 grams from a bag where you get a positive out. We go back, take another 100 grams, and we get a negative out, go back, take another 100 grams, get a positive. So, with natural contaminate, you are not going to have the consistency in your work that you do with the inoculated seeds.

DR. SPERBER: One more point on your work. I think that -- well, yesterday, many people alluded to the fact that it is really hard to find Salmonella or E. coli in seeds, and I was thinking then that perhaps you needed to do

something to break the seed coat or liberate the organisms, and you might have hit upon something there by soaking the seeds and going through a little sprouting.

In your first experiment, you sprouted the seeds for four days and then later you found out if you sprouted them for three days, it was just as good. How about sprouting them for one or two days?

MR. INAMI: Actually, that has been brought up before and we haven't had time to really examine that. The outbreak of Salmonella Seftenberg, when the San Francisco District Office isolated that, they isolated out of a one-day-old sprout sample, it was an in-line sample at the company, so that has been proposed and that is one thing we may look into.

DR. SPERBER: That may be one area for further research where if we need to go through some protocol like this for analyzing seed, that we would want to have the most efficient method possible.

While I have got the microphone, I would like to make just one comment, and perhaps nobody wants to respond to it, but something has been bugging for about a year, and that is the use of 200,000 ppm chlorine for treating seed. That is just not on this planet in my experience when it comes to disinfecting process equipment or, you know, treating water or things like that. To me, that is like

deer hunting with a cruise missile. Somehow it just doesn't connect logically. I don't know how you would need such a large quantity of material to kill bacteria. Is it simply because you are overcoming dust in the seed and you are inactivating so much of the chlorine?

DR. KVENBERG: Response to the question from anyone?

DR. BEUCHAT: All I know is that 20,000 parts per million calcium hypochlorite does not reduce populations of 0157 by more than 2.5 to 3 logs. So, I think we are not getting the active component to the site of the cell in these instances.

DR. TROXELL: Maybe that is what we have been dancing around with these questions on surfactants, and so on, and then I think Dr. Fett had pointed out biofilm problems, and maybe the issue is not just a concentration, but optimizing the surfactants and I don't know if it's a crazy idea to think about other mechanical approaches to optimize the contact, such as, I don't know, if sonication at some level would kill the seed or whether it might actually facilitate breaking up some of the biofilm. Somebody needs to start thinking about other ways to get better contact if that is the problem, so, we don't need to use the cruise missile.

DR. KVENBERG: The next placard I have is Dr.

Goosby.

DR. GOOSBY: A question and a few comments to Dr. Kumagai. You presented some very interesting work that talks in terms of the potential for contaminating a product even if we began with a sterile seed, and again, the concern for GMPs and some follow up.

I am wondering, though, on the artificial contamination and the 3.5 log CFU/ml was your standard for the artificial contamination of the radish sprouts, how much that relates to natural sprouting, growing conditions, and if other work may be underway that you are aware of, unpublished or otherwise, that may be looking at some lower levels that perhaps would relate more to the natural growing condition to see what our lower end of natural contamination might be that we need some intervention strategies for.

DR. KUMAGAI: We don't have any data on natural contamination level, and at least as far as we know, we have done 100 cells per ml. If we use the water containing 100 per ml of E. coli 0157:H7, then, when sprouts grew, then, the edible parts contained about 7 log cells per gram, and we don't know the level of natural contamination level of seeds, we don't know, so perhaps in future, the study using very low level of contamination of water will be needed, I think. Is it right to your question?

DR. GOOSBY: That is correct, that is what I was

asking for. Just to see if, in fact, there is some understanding that you may have far greater than I on what the natural sprouted conditions for radish sprouts, to relate the artificial contamination you have done with the natural conditions, and as you just indicated, maybe other levels, other approaches to research in trying to more narrowly define the question and the answer.

Thank you.

DR. KVENBERG: Dr. Tompkin.

DR. TOMPKIN: With regard to the calcium hypochlorite, my recollection is that the higher the pH, the more stable the product, but it is the hypochlorous acid that is active, and you have to have a neutral or lower pH for it to be effective, so we are working at the high pH range where it would probably be least effective. That might be why it is taking so much.

But other than that, I was questioning, would like to know how much naturally contaminated seed is available for research, and does anyone know where it is, how much there is, can it be used. I know the tendency is to bury it as soon as you can get it or whatever, but I would really like to see some of that seed subjected to the irradiation at a 2 kilogray or whatever different levels to see whether or not irradiation will, in effect, be effective in naturally contaminated seed.

MR. INAMI: Well, we actually have some of the seed and as Jeff said before, it is going to be auctioned off after the end of this conference, but I think the research on looking at irradiation is very important.

I don't know if I have the authorization to say yes, you can have seeds, no, you can't, I mean that really goes to the chief of our laboratory.

DR. FARRAR: A follow-up on that, John. I think this is a very precious commodity at the moment and perhaps with the help of the National Advisory Committee, we could begin to establish some priorities for that remaining small amount of seed.

In addition, we are also convinced we have some naturally contaminated seed, probably over a ton of it in storage from our latest Seftenberg and E. coli outbreak that we have tested repeatedly in different labs across the country, and been unable to isolate the product, but we are firmly convinced that it is there.

DR. KVENBERG: Thank you. Is this a follow-up on this, Larry?

DR. BEUCHAT: Yes, follow-up to Bruce's point on the pH. The data that I have shared with you here on the high levels of calcium hypochlorite, the 2,000 to 20,000 ppm, the pH there was 6.8 to 6.9. We used a mild 0.05 molar phosphate buffer to keep that hypochlorous acid in at least

a range in which it would be active, active component.

DR. FETT: We used 500 millimolar of potassium phosphate buffer, pH 6.8, so our final pH was about 7.0.

DR. KVENBERG: Dr. Doyle.

DR. DOYLE: I have a labeling question. Perhaps, Laura, you could answer this. If seeds were irradiated, would the sprouts resulting from those irradiated seeds have to be labeled as having been irradiated?

DR. TARANTINO: You know, that is a good question. Probably. The way the reg reads now is a food that has itself been irradiated needs to be labeled. It would be hard to me to think of how we would decide that the sprout itself, because the point of that regulation was that you don't need to label if it is an ingredient in a multi-ingredient food where it has been processed and it is obvious that it has been processed.

The point of the labeling of a food that has itself been irradiated has mainly been applied to produce to say yeah, this has been processed, something has been done to it, so consumers can know that, and it would be hard to see how we could separate the sprouts, but it is a good question actually. I hadn't thought about it.

DR. KVENBERG: Dr. Buchanan.

DR. BUCHANAN: I want also to go back to the regulation a little bit because I have to lead the

discussion later this afternoon, and I thought I had a grasp of it, but then a couple of comments you made during the end confused me, so let me ask you real two straightforward questions.

My impression was that the seeds' disinfection would be regulated by EPA, and the sprouts would be regulated by FDA. That is part 1. The second question is of all of the treatments that you heard discussed this morning, are any of them currently approved by both FDA and EPA for use or whoever?

DR. TARANTINO: Your first question was? Try again. Oh, the seeds and the sprouts.

DR. BUCHANAN: The sprouts versus seeds for regulation.

DR. TARANTINO: The sprouts themselves I don't think we have discussed in the post-FQPA era. My impression would be it probably would be us, but that is one that I would want to get on the telephone with those folks, too, and talk about it. It is the seeds that we have talked about explicitly, but that would be my guess, as well.

Let me say that I think except for irradiation, I think the things that we have talked about in terms of the chemical treatments on seeds would be EPA, so take it away.

MS. WINGFIELD: Actually, I think some of the sprout would be EPA also, particularly in home owner. It is

very strange at this point particularly for homeowner use. If an applicant decided to market a product for homeowners to use on their sprouts once they had been bought from the store, that would be in EPA.

I am sorry, your second question?

DR. BUCHANAN: Were any of the technologies that you heard discussed today, would any of them be approved for use, are they approved for use, any of them that you heard discussed today?

MS. WINGFIELD: As I said before, we do not currently have any products specifically registered against pathogens for fresh fruits and vegetables. The calcium hypochlorite has an existing tolerance exemption on the books, one of the reasons why it had not been disapproved for the 24(c) special local needs, but as far as any of the other technologies that were discussed, it is my understanding there is nothing specifically registered.

DR. BUCHANAN: And the calcium hypochlorite, at what level is it approved?

MS. WINGFIELD: Both the calcium and sodium hypochlorite for post-harvest washing, I believe is approved at 25 ppm.

DR. FARRAR: A follow-up, John.

DR. KVENBERG: A follow-up from where?

DR. FARRAR: This raises another issue I think for

the committee to take under advisement, that we are approaching a very blurry line here, a division.

What about pea sprouts grown in a greenhouse or pea sprouts grown outside, for that matter? We have come upon the line between agriculture, raw agricultural commodities, and processed foods, and it is staring us in th face, and we need to begin making some decisions on those areas.

DR. KVENBERG: I guess it is a prerogative of the Chair to interject at this point. Perhaps I am not making the working group's task any easier, but I see what I might call a parking lot issue for you to come back with a recommendation to the agencies to settle the matter.

I think it is clear from the discussion we have had up to this particular point in time, further discussions between agencies are going to be necessary on these points. My observation -- it is not in the form of a question -- but just put forth is if you can give us some clarification for what the tasking for the discussions might be between the agencies that might be useful, if you can identify the issues.

The next placard I saw up was Dr. Troxell.

DR. TROXELL: I will just make one actually follow up on this, and I have another question, but it is not necessary. You know, if we view, as we do, the sprouting

process, the growing, as being a food manufacturer, it is not necessary for EPA, with respect to the sanitizers, to view that as a food. It could still be a raw agricultural commodity, and we would consider it a food process, so the lines don't have to be exactly the same for regulating the sanitizers as they are for our view on jurisdiction of the setting up the GMPs, and so on.

My question was to Dr. Kamagai, who talked about a pretty dramatic reduction in the levels of E. coli using five seconds of boiling water, and I assume -- well, I assume there the quality of that product is not usable for commercial sale after that. So, would you clarify that, and secondly, what about internal contamination after that process? You had described two separate kinds of contamination, external, and then some internal contamination. Would that process deal with the internal contamination also?

DR. KUMAGAI: Five-second heating in boiling water was enough for killing the bacteria even inside of the tissues. In Japan, Dr. Isshiki showed some people using radish sprouts by adding to very hot soup, a kind of soup, but not boiling, but nearly to boiling.

At the moment, many facilities preparing school lunch don't use raw vegetables for their dish, instead, they are now using the vegetables boiled briefly and perhaps

until the safety of raw vegetables can be confirmed, they continue to use boiling the vegetables.

DR. TROXELL: So, the utility here would be just prior to consumption rather than as a final process step?

DR. KUMAGAI: Yes, immediately before serving.

DR. KVENBERG: Just so you have an indication of time and questions, I have four people that have questions. In order, they are Dr. Swaminathan, Mr. Bernard, Dr. Slutsker, and Dr. Buchanan. I have received one request for public comment so far, so we will continue the question period. We certainly have time allotted to do that.

Dr. Swaminathan.

DR. SWAMINATHAN: I have a question for Dr. Tarantino and Mr. Weagant. Can I ask them at the same time?

DR. KVENBERG: Certainly.

DR. SWAMINATHAN: Dr. Tarantino, I have two questions. EPA indicated that they usually decide in 90 days whether they approve or disapprove a petition. Can you give us a time line for petitions if you fast-track them?

The second question is a hypothetical situation. I am just curious. Can a federal public health agency submit a food additive petition if it deems that it is in the public interest?

DR. TARANTINO: I will answer the second one first because it is by far the easiest. Absolutely. We would

welcome a petition from a sister agency without any problem. The time line is harder. Obviously it is not fast. I can see from filing to a petition to publishing something in the Federal Register including the rulemaking realistically is unlikely to take less than a year. It could, but it would be, you know, I would be fooling anyone if I said it would be a whole lot less than a year, it might be, but that would truly have to be fast-tracked on, it would have to be a really good petition coming in.

DR. SWAMINATHAN: Thanks.

MS. WINGFIELD: Excuse me. I just want to add a note of clarification to your question. The 90 days is not for approval of a petition. The 90-day approval or disapproval is for the 24(c), special local needs. A petition approval takes much longer than 90 days.

DR. TARANTINO: Thank you.

DR. SWAMINATHAN: Steve, I have two or three clarifications from your presentation, two or three questions. First of all, how were the alfalfa seeds or sprouts contaminated in your experiments, the artificially contaminated ones?

The second, could you confirm that 37 degrees with shaking and 42 degrees with shaking gave equivalent results?

Third, you mentioned that two of 40 strains of E. coli 0157 were not inhibited by cefixime at 0.0125 mg/liter,

but in your later experiments using these various conditions of enrichment and shaking and immunomagnetic beads, you didn't specify as to which strains you used in the experiments, whether these were cefixime resistant or cefixime sensitive at that concentration.

MR. WEAGANT: First, on how the sprouts were contaminated, we added the sprouts to the enrichment broth and immediately added a contaminating liquid to them, and then proceeded with the blending.

As to your question to the efficacy of 37 degrees and 42 degrees, those were different enrichment matrices or broths, so a direct comparisons of two temperatures would take some more experimentation, specifically, just varying the temperature, and not the composition of the broth.

It appears to us that both are effective. Previous experiments have shown us that 42 degrees is not inhibitory to the E. coli 0157 and some of the other, STEC, as well.

Your third question, we did in our inoculation experiments in sprouts use two of the most sensitive strains that we had encountered in our pure culture work, so that these strains were two of the most sensitive of the four that we used, and we did see that effect, that in the inoculation experiments, the inhibitory effect of the cefixime seemed to be modified.

DR. KVENBERG: We had a follow-up, I think from Dr. Slutsker.

DR. SLUTSKER: Yes, just on the issue of naturally contaminated seed, there actually have been two outbreaks that were seed, yielded the pathogen responsible. One was the Newport, which Greg has described quite well. The other one is the Infantis/Anatum outbreak in Kansas in 1997. Those seeds were cultured at the FDA lab in Denver. I don't know whether any of that seed remains or not, but that could be another source of naturally contaminated seed.

DR. KVENBERG: Dr. Buchanan.

DR. BUCHANAN: I actually have two quick questions. One is for the methods people, and then the second one for Dr. Isshiki.

Methods people, to put this real simply, your primary limiting factor now in methods is sampling problems, not detection systems? It is finding the occasional sporadic contaminant within a lot.

MR. WEAGANT: I guess I will start with this. As far as 0157 in seeds for sprouting, no one that I am aware of has detected naturally occurring 0157 in seeds for sprouting. We have not pursued that in our experiments so far. It is something that needs to be looked at.

MR. INAMI: For the Salmonella work, the Salmonella procedure has been around for a number of years,

and it is an AOAC-approved procedure, however, there are a couple items that you can attach to them.

One is the use of a Rappaport vasalis as a selective enrichment instead of selenite, and growth at 42 and 43 degrees can also be looked at, and we haven't looked at either of those two methods yet, but the question about distribution is a big question and in some cases when we have tested seeds and sprouts from other outbreaks, we are testing upwards of 150 to 160 bags of seeds, 200 or so packages of sprouts, and in some cases we don't isolate the organism, so distribution is a really big problem.

DR. BUCHANAN: My second question was for Dr. Isshiki. In the use of your calcinated oyster shell, I needed some clarification. Is this used as a treatment of the seeds only or do you continue to use the calcinated water as the sprouts germinated, so you have a residual effect throughout the sprouting process?

DR. ISSHIKI: In my laboratory, university laboratory, it could be used during all stage of growing the sprout, but actually, we bring to the grower's farm or factory, it is very difficult to use sprout growing steps, so we recommend only using the seed treatment..

DR. BUCHANAN: What is the limiting factor, why is it difficult to use in the growing factories while you can use it in the laboratory?

DR. ISSHIKI: It is very alkaline, so training people to use high alkaline solutions, but training the people is dangerous for the alkaline solutions.

DR. KVENBERG: Dr. Neill, do you have a question?

DR. NEILL: Yes, I have another question for Dr. Isshiki, if I might. If I took this down correctly, this is from the revised manual, and it was suggesting that seeds be tested for the presence of E. coli, which I understood would probably be generic E. coli, and then if a positive were found, to check to see if it were an STEC.

My questions are is such surveillance work being done, and what do you have at this point in terms of finding STEC in seeds, and what do you do with those seeds?

DR. ISSHIKI: We recommend to discard the seeds. Firstly, check the E. coli as an indicator. Second step is detection of the E. coli 0157 STEC and Salmonella. If the STEC were Salmonella detected, we recommend not to use this seed, and inform our government and the seed suppliers.

DR. NEILL: I think I might be wondering whether you would have any naturally contaminated seed if something like that could be discussed perhaps with any of the authorities, then, if one had found any contaminated seed, it would probably be something that would be very useful to the community at large.

DR. ISSHIKI: We go direct to ask the seed test

people if they find polluted seed, inform the government. The government or seed supplier inform the sprout growers, so all sprout growers informed which is very dangerous for the sproutings, so they discard that seed. So, we recommend to inform to the government and seed suppliers about seed.

DR. KVENBERG: Dr. Swanson, do you have a question?

DR. SWANSON: Actually, I have a question for perhaps the seed suppliers or sprout growers that may be out in the audience related to sampling. They previously indicated that before they purchase seed, they frequently will test the germination and other properties of those seed lots, and I am just wondering what size of sample they look at when they are doing it, is it perhaps greater than 100 grams. I heard 50 pounds. If they sprout 50 pounds, perhaps we could look at culturing the water that is used to grow those 50 pounds as a means of enhancing.

DR. KVENBERG: One of the ways we may be handle this question, Dr. Swanson, is when we switch over, in a moment we will have public comment period, and then maybe we can get them to come forward and address that at that point. I have got several people who would like to speak, and I think the seed suppliers are here.

DR. SWANSON: Thank you.

DR. KVENBERG: Dr. Nagle.

DR. NAGLE: I just wanted to follow up again with Dr. Isshiki. I guess we are hearing different things. We are hearing from Dr. Weagant that we can't detect E. coli in seeds, and yet Dr. Isshiki is saying that they are testing for E. coli in seeds, and I guess my question is do you find it, have you found positive E. coli in seeds or especially E. coli 0157, and if you did, then, could you tell us how you did that, so that we could find that in other supplies of seeds?

DR. KUMAGAI: E. coli 0157 have never been detected from sprout seeds in Japan as far as in the form of culturable living bacteria. Other E. coli, we have found from Oregon seeds. Is it right?

DR. NAGLE: Yes.

DR. KVENBERG: Mr. Bernard.

MR. BERNARD: Thanks. Just an observation, and our regulatory colleagues can correct me if I am wrong, but back in our discussion of cruise missiles and other such things, there was mention of buffers used to poise pH, so that calcium hypochlorite was in the most active range, and I am assuming that anything used as an efficacy enhancer, as an adjuvant to the process, would also require regulatory approval. So, if we are using certain buffers, that would have to also go through a similar review process.

So, if one were to petition, for example, for

calcium hypochlorite, it would have to be done in the context of everything that was used to achieve those results.

MS. WINGFIELD: That is correct. My statement before about the current acceptance for calcium hypochlorite was calcium hypochlorite in meat or just dissolved in water without any added surfactants or anything of that nature, but certainly we would need to evaluate the surfactants in a tolerance petition, again, their exemption from tolerance would have to be established in those, as well.

Public Comment

DR. KVENBERG: If there are no other questions from the working group or the panel, I have received a request in order from several people to provide public comment. The first is Frank Shaller, and the second is Robert Sanderson. Others are also invited to speak at this point the program if they desire.

I would ask for public commenters to please limited themselves to a five-minute period. Also, we have a peculiar arrangement here with our microphones, so I would invite you to the dock, as you would have in an English court of law in the front, so you can be recorded.

Is Frank Shaller available, would you like to make a comment?

MR. SHALLER: First, I would like to point out

that if I make any statements about experts or government agencies, please, don't take it personally. I do not understand half of what I heard today or yesterday and today, but what I would like to do is just take for the next four and a half minutes a step back from all this kind of information and why are we here.

We are here because we are concerned about the health of the people of this country, and the health of the people of this country depends on our food supply. The big picture. Jeffrey Blanda, biochemist, I have heard give an explanation, a description that the most important thing in our life is food to our health because we consume about 2,000 pounds a year. That is a lot if you think in terms of it all in one place at one time, 2,000 pounds a year. In five years, that is 10,000 pounds. You can do the numbers.

Food is important. It has got to be good, clean, living. It has got to nourish us and our children. The term quality science was used earlier. There may be some quality science that we have seen today and yesterday, but remember, quality science has given us DES, DDT, Olestra. It has given us the confusion over when to get a mammogram or whether or not to do a PSA test. 41- 42,000 men are dying of prostate cancer. Four years ago, 38,000 men were dying. Quality science, $30 billion in 30 years, and we are losing the war with prostate cancer. The numbers are going

up, and that is just one example.

Where does this quality science come from? The experts, the people that work with data. Step back from the data and realize experts can be quoted and misquoted. The data can be interpreted and misinterpreted. Step back from the data and say why am I here, why am I on this committee, why am I working for this government agency, and my analysis is you are here to do a job of providing good quality food.

Now, what scares me, what really concerns me is when I hear cruise missiles. Chlorine, another expert says chlorine is a synthetic biocide, halogen-based, that degrades into carcinogens and depletes ozone layers. I don't want any more carcinogens being promoted by my government agencies.

Irradiation. We are talking about irradiating food. Children in grade school are being learned to use a microwave and get a line of plants, and microwave some water and don't microwave some water, and then grow these plants. The obvious conclusion is which is going to grow better. Do the experiment at home. Have your children do the experiment. Microwave, yeah, it is great, it is fast, it satisfies, but there is something missing.

What is a molecular effect, and be very careful if you move in the direction of irradiating our food. It is really scary. We don't need any more carcinogens. We are

losing the war on cancer. You have relatives, I have relatives, we all have relatives who have already lost the war on cancer.

Disinfectants. They have been around for hundreds of years, oil of lemon, orange skin, pine oils. I am not an expert, I don't know what can be done or where to go with this. I beg of you, look to nature. The pathogens are our enemy, but they are also our teachers. Learn from the pathogens. Don't do head to head battle because we will never win. Just one issue of the antibiotics. We are losing the war with antibiotics. It is in the press regularly. The bugs are winning. We have got to learn to cooperate with nature, learn to cooperate and understand the pathogens, and not try to blow them away with cruise missiles.

The bottom line is the immune system. We have got to help support the immune system of the people of this nation. The standard American diet is not doing it, and the Food and Drug Administration, the other agencies are responsible for lowering the quality of our food and for dumping more carcinogens into our environment.

Thank you. Nobody said time up. Thank you very much for this opportunity, and I ask you, please, step back, look at the total picture, keep the kids in mind that we need to work with nature and nourish the body.

Thank you.

DR. KVENBERG: The next individual who has asked for comment is Robert Sanderson of Jonathan Sprouts. I promise I won't start your five minutes until you are physically at a microphone.

MR. SANDERSON: Hi. I am Bob Sanderson. This is not a comment, it is just a question. I had a confusion with some slide of Dr. Isshiki. You did some work with dry heat, and my question is it looked as though perhaps the seed was held at 75 Centigrade for up to 7 days. Did you test the germination, the viability of that seed? Thank you.

DR. ISSHIKI: My presentation data is all checked for the germinations. Germination is not so bad. It is useful for the commercial sprouting, 99 or 98 percent germinations.

MR. SANDERSON: I think we have been told that with the hot water soak, temperatures in the same range have to be within a very, vary small window, and I find that a little confusing, if you can hold seed dry at 170 degrees Fahrenheit for a week, and it is still grows, but if you soak it in hot water for 20 seconds, it won't grow.

DR. ISSHIKI: One is dry heating, other is in hot water. Both, germination is good, more than 99 percent.

DR. KVENBERG: Another request I have is from

Sally Stanfield from Ellridge Farms, also bearing in mind Dr. Swanson's request, if I could impose on Bob Rust, if you are here, and Dan Caudill, she had a question, as well, going back to the question of what do you do on the seeds, but let me go first to Sally Stanfield.

MS. STANFIELD: Thank you all very much for this brief opportunity. What I would like to present is the thought. Obviously, it is a watershed time in terms of creating regulations, creating a structure that works as we look at how to keep the food supply safe, specifically looking at it from the sprout standpoint.

We are located in Randallstown, outside of Baltimore. We have been farmers for years, now we grow broccoli sprouts. The issue that we have seen, which I now see coming yet again, is we all have the common goal of a safe food supply, we have the common goal of the public health.

We also must make very sure that we don't regulate our producers in such a fashion that they can't produce, because people, when they go to the grocery stores, they have an abundant supply of choices in this country, but they don't understand what goes into the gallon of milk, they don't understand what comes behind the nice-looking vegetable, and now, too, they don't know what must come when one produces a good, healthful sprout.

So, what I invite the panel and anyone who is involved with all the decisions that are now here, the opportunities that are here, and I know time is precious. I am a lawyer by trade, I have changed hats and become a farmer now. Time is real precious, but come see us at our sites, come learn what we do. Come see us on the job.

We want to work with you. We all have the same goals. We clearly want our consumers to be safe. We live where we work. We eat the food that we produce. But it is a practical standpoint that goes with the science, and that is what I invite you, and all of you collectively, to think about. It is a time that it is simply we look at it as a group project, as a common goal, and I think in the long run, yes, it's hard work, but we end up with a much better result.

Thank you.

DR. KVENBERG: We have another commenter. If I could, I would like to go to the seed question that was asked. Our next commenter is Rob Carver from Carver Research.

MR. CARVER: Good afternoon. Yesterday, I spoke about a journey that I took with an indoor farmer who became a food manufacturer. I would challenge you to closely examine the semantics of that statement.

What we must look at is just by the sheer act of

placing a food into a labeled container, use that as a symbol that this is no longer a raw agricultural product or that it should be handled as a raw agricultural product

I believe that sprout manufacturers can benefit from the years of practical experience of a lot of common food manufacturing entities. The milk industry, the meat industry. Most of the sanitation processes and information already exists. All we must do is make a conscious choice to say when it comes to regulating under FIFRA and EPA for antimicrobials, yes, they are agricultural, but when we germinate seeds for a finished product that is packaged, let us say this is food manufacturing, let's apply 21 CFR Part 110, existing current good manufacturing procedures to this industry, and quit dancing around the idea of I am a farmer.

You are not a farmer, you are food manufacturer.

Thank you.

DR. KVENBERG: I received one final comment before we close off for lunch from Dr. Wick, who would like to ask a question, and I also have got a proposal or suggestion to get to the question that Dr. Swanson asked. Due to the lateness of the hour, maybe we could, at the chairmanship of the working group, ask for comment perhaps from the seed manufacturers or other comments you would want to draw from at that time. If that would be appropriate, then, I think we could handle it that way.

Dr. Wick, would you like to have the last word before lunch?

DR. WICK: The first one is for you, Dr. Kvenberg. I want clarification on whether or not your office has initiated or will initiate -- I don't know if we call it a Section 18 or not -- but this chlorine issue, and also I gather that it would take at least a year if it started today.

DR. KVENBERG: My office will not initiate a request for a Title 18, but we have made contact with or will make contact with the U.S. Department of Agriculture, who is dealing with the area of application in support for the industry. I think we have yet to do that, but I think on a national basis, it is possible for USDA to work in that regard, and that is our plan.

DR. WICK: My second question is that I am not sure about calcium hypochlorite, but sodium hypochlorite is an EPA-registered pesticide, and I wonder if that exempts it as a food additive.

MS. WINGFIELD: I am not sure. I believe there is, but I am not sure that there is a current tolerance exemption for sodium hypochlorite. I believe that there is, but we will have to check on that.

DR. KVENBERG: With that, my watch may be somewhat slower than others, but mine says 12:30 at this point in

time, which brings us to the close of this session.

Lunch is scheduled for this point in time, and a reconvening of the Working Group on Fresh Produce is scheduled for beginning of discussions at 1:30 this afternoon in this room.

[Luncheon recess taken at 12:30 p.m.]

AFTERNOON SESSION

[1:35 p.m.]

DR. BUCHANAN: We are going to try and get started. We are going to first call on John. You had some unfinished business from your session this morning. Would you like to take care of that now?

DR. KVENBERG: Thank you. I guess you are referring to when we were going through the questions of clarification section, Dr. Swanson asked if we could get some comment and input from the seed suppliers.

DR. SWANSON: The question was when sprouters obtain samples to determine whether or not they will accept a lot of seed, how much seed do they obtain and run germination tests on, for example, as a routine basis. We heard 50 pounds and 8 pounds.

MR. FABRE: Fred Fabre with Cal West Seeds. I can only generalize and give you the benefit of my experience. The general understanding as a seedsman is the larger the sample, the better, so, you know, we always encourage people to take as large a working sample as they find workable, almost always a sample large enough to actually do a production run, so generally, from 20 to 50 pounds from a carefully composited sample. That is generally the size that most of our customers require or that we ask them to test.

MR. RUST: Bob Rust with International Specialty Supply. A lot of growers ask for different things. There is no standard thing. Some of them will just want enough to look at, some will want 500 pounds, enough to grow out. Probably a standard is somewhere around 20 pounds, but there is a lot of people that don't even ask for any.

DR. SWANSON: Thank you.

DR. KVENBERG: That concludes any business we had from this morning.

Fresh Produce Working Group Discussion

DR. BUCHANAN: This afternoon has been devoted to the Fresh Produce Working Group discussing the information that we have heard today along with its invited technical experts and also the members of the panel.

We would like to divide the discussion into three basic parts. I will give you just a time frame, so we can sort of keep focused on this.

Between now and approximately 2:40 to 2:45, we would like to focus on the questions of currently available interventions and related activities that could be used to enhance the safety of sprouted seeds, particularly alfalfa seeds. The focus here will be evaluating the scientific information that we heard in the last day and a half.

The second half will be from approximately 3 o'clock to 4 o'clock or 3:45, where we would like to

identify both the research needs associated with sprouted seeds and the priority of those research needs.

In the last segment, we would like to focus on other issues associated with the safety of sprouted seeds, in particular recommendations in relation to food safety systems, education, and other items that might have an impact on the safety of sprouted seeds.

My job here today as the Chair of the Working Group is to primarily act as a referee and a timekeeper, and to try and keep the discussions in an orderly fashion. To do that, what I would like to do is currently divide the discussions on the efficacy of potential interventions, first, on seed treatment, and then subsequently on other steps within the sprouting process.

Kathy, I assume that we have reserved some time at the end of the day for any additional public comments?

MS. DeROEVER: No, there will be no more public comments.

DR. BUCHANAN: Then, we will have the opportunity of continuing this up until 5 o'clock. I will take a break at about 2:45 to change subjects. It is a good breaking point, and to give you 15 minutes to get up and stretch.

Let's start focusing on the interventions that were described here for the treatment of seeds. They included everything from disinfectants to irradiation. We

also have any interventions that might be useful at the seed mills themselves and at least one recommendation coming from our speakers on treatment of seeds for scarification that we also should discuss.

Comments, recommendations, and areas of potential use? Yes.

DR. SWAMINATHAN: I actually have a question. Yesterday, I think in the public comment period, somebody pointed out that Australia requires treatment of seeds that are imported into the country, but it was never specified as to what that treatment is.

Could someone amplify on that, please?

DR. BUCHANAN: Does anyone know what the treatment requirements are for the exportation of seeds from the United States to Australia? It looks like we have a volunteer.

MR. RUST: I am Bob Rust, and I heard that comment also, and I am not sure whether that was a misunderstanding or what, but a lot of times they ask the seed to be [foxtoxined] before it comes to the United States to kill insects.

DR. BUCHANAN: So, it appears that the treatments required by Australia are not in relation to a foodborne disease transmission.

Yes, Bruce?

DR. TOMPKIN: May I comment on that particular question that you are asking for?

DR. BUCHANAN: Yes, please.

DR. TOMPKIN: From what I was hearing -- and you folks correct me if I am wrong -- that the outbreaks that have occurred in the U.S. have occurred independent of GMP in a sprouting operation. So, that would suggest that GMP, important as it is, it alone is not adequate to control this issue.

The available evidence indicates that the seed is the primary source, thus, in this particular case, elimination and not reduction is the ultimate long-term goal for controlling this particular issue or hazard.

It appears from what I was hearing that some of the pathogen is under the seed or in cracks or wherever you wish, of the seed coat, and there is some issue of that, a less than 1 percent on cracks and defects of that sort can naturally occur, and I am sure that must vary, but it seemed as though some pathogen contamination below the seed coat may be occurring.

It is evident that the pathogen survives for at least two years at ambient temperature, and that is not surprising. So, it seems to me that it comes right back down to some method of decontaminating the seed is what is needed, and we might consider a short-term and a long-term

goal in that regard.

Along those lines, it may be possible to actually come up with some guidance with regard to a performance criterion for each of those goals, but then relative to the methods of decontamination, which could fit into a short-term application, the immersion in a disinfectant certain does show considerable death, but it in itself is unreliable, at least based on inoculated product seeds, whether it would be effective in naturally contaminated is unknown.

The data on irradiation, limited as it was, certainly would address whatever is under the seed coat, if that is the problem, so it has an appealing from that standpoint, it's an appealing means to address the question.

Certainly, immersion in hot water is an alternative to disinfectants, and there is microbiologists, heat is certainly a preferred method to disinfectants. If you can come up with the right time temperatures and, in fact, there were slides or a video showing that a heating process can be done under commercial conditions. It is a question of expense, and so on, whether or not that could be made to be a suitable approach to smaller operations.

One area that I didn't hear enough of was decontamination through dry heat. There was one short comment actually on that, but there are a lot of examples

over the years of needs to eliminate Salmonella, for example, in gelatin, gums, spices, powdered egg white, and even now, fresh whole eggs, and so that you still have the egg with a liquid egg white and yolk, which has now been demonstrated to be possible.

With the switch from ethylene and propylene oxide for spices, the spice industry is using irradiation, but they have gone to other means, time, temperature, it is essentially a dry heating process with control of humidity. There is quite a bit in the literature, as well as in the patent literature, of systems designed to reduce Salmonella in particular in gelatin, rendered animal byproducts, bone meal, flour, and so on.

I haven't heard anyone having considered those approaches as a means to treat seed. So, it is an area that should be explored.

So, just with that kind of thinking as a background, there are really those four approaches - liquid immersion with a disinfectant, hot water, irradiation, and then this other that I have just described.

DR. BUCHANAN: Does anyone have any additional technologies that they think we ought be considering in this, that were presented today or which they know is effective now? I want to keep this away from research. At this point, we are currently looking at the state of

technology now and what could be implemented.

Dane.

MR. BERNARD: Thanks. There was mention in one of the presentations -- and it may have been Dr. Davis -- about vapor phase hydrogen peroxide, but there was only a mention that it didn't show good results.

I am wondering if there has been a significant amount of work. In our past experience, we have found it to be a very good and very penetrating means of inactivating microorganisms, a little bit exotic, but shows some potential. I am wondering if there has been sufficient exploration of that technology.

DR. BUCHANAN: Anyone on the committee aware of any research in that area? Yes, John.

DR. KVENBERG: Just to clarify your remarks, I think hydrogen peroxide flushing and aseptic processing has probably got databases that you were referring to.

MR. BERNARD: Our experience with it was in the aseptic area. There is one piece of packaging equipment that employs that. Hot hydrogen peroxide is much more effective than cold peroxide. It needs to be inactivated or, excuse me, the molecule needs to be activated to get the hydroxyl radical, so experiments that might use 4, 6, 8 percent at ambient temperature, those are not optimal conditions for hydrogen peroxide's effectiveness.

So, there are a number of things that can be used to activate it. The vapor phase uses heat to essentially evaporate a hydrogen peroxide solution, and the vapor phase is very effective.

Other things that could be tried with hydrogen peroxide, as I said, it needs to be activated to be effective, the technology has been used for some years in biotechnology research as a random agent for breaking apart DNA, and that is typically done by using a metal catalyst in direct contact with hydrogen peroxide, which causes it to activate, specifically iron and/or copper can bring a pretty immediate dissociation of the molecule.

I am wondering if anybody has really explored these other ways of more effectively using hydrogen peroxide.

DR. BUCHANAN: I think if we don't have anyone that is focusing on some data that they know in this area already, then, I think we will need to relegate this to research areas. Bill, I know you made earlier comments about things that work on food contact surfaces appearing not to work on sprouts. I wonder if this is another example of that. Would you care to comment?

DR. SPERBER: Well, I don't know about the peroxide directly, but when to comes to chlorine compounds like hypochlorite, in the food industry we traditionally use

1 to 200 ppm for sanitizing surfaces, and that may be the key here in the seed discussion is that you can only sanitize a clean surface. We don't go in and use 2,000 ppm chlorine to sanitize dirty equipment. First, you have to clean it. So, there may be an issue here of using the liquid disinfectants for seeds of the disinfecting material is being inactivated by the seed itself or by dust or soil in the seed sample.

So, if we are to use -- my comment about deer hunting with a cruise missile was serious. I think that is what 20,000 ppm chlorine is like, and if we are going to use chlorine as a disinfectant for seeds, we ought to figure out how to first clean the seeds, so that we can use a more judicious amount of chlorine.

DR. BUCHANAN: Bruce has provided us a framework on how to deal with these questions as we go through at least in this first phase on seed treatment, and he talked about an ultimate goal of eliminating foodborne pathogens from the seed.

He talked about then being able to compare the efficacy of the technologies that he has identified against that standard, and then identified the need to establish short-term and long-term goals in terms of their efficacies. I would like to thank Bruce for providing that framework, and I think it is a good one for us to deal with.

I would like to thank Bruce for providing that framework, and I think it is a good one for us to deal with. I would like to give an opportunity to add to this list of four technologies.

Are there any others that we heard during the last day and a half that should be included in evaluating them in terms of their effectiveness for eliminating foodborne pathogens from seeds? To remind you what they are, they are a liquid immersion with a disinfectant, radiation, hot water and dry heat.

Are there any others that are currently available that we should add to that list as we go through our consideration of this aspect of seed decontamination?

Larry, as a person that has been doing this for a while, are there any others that we should be adding to that list that we could take off the shelf?

DR. BEUCHAT: The subject of surfactant and properties that would be associated with delivery of a potentially lethal chemical or treatment to the site of the cell, I think deserves attention.

It may be in combination with the liquid immersion or perhaps one of the other approaches that have been enumerated. Also, other potentially lethal volatile materials, gases, these could be from natural sources. We have some preliminary information and data to indicate, for

example, that iso-L-thiocyanate will, in a peer system, eliminate, kill 0157 rather quickly. This is a component of a number of plant mustard family, but there are others that we know are antimicrobial bactericidal, bacteriostatic that perhaps should deserve attention in terms of a battery of approaches, and maybe a combination of approaches, treatments, hurdle approaches that should be looked at.

DR. BUCHANAN: I gather at this stage, though, most of these gases that should be considered are really at this point at the research stage.

Likewise, we have a hot water here. Is anyone aware of anyone that is doing any work with steam as a decontaminant? Yes, Bruce.

DR. TOMPKIN: Actually, I think some of the systems or processes that I had in mind do involve controlled humidity, so that, in fact, you don't have the product becoming wet, but by controlling the humidity, along with temperature, you can get a faster kill.

DR. BUCHANAN: Yes, John.

DR. KVENBERG: Just to point out, I think the body of literature that is available on inactivation of microbes with wet heat and dry heat is probably largely known and can be found in various texts, such as [Elmer Marse] a big compendium. People are nodding their heads. So, we have a lot of knowledge about the time/temperature requirements for

inactivation are relative to moist heat, at the various degrees, be in live steam or other elevated temperatures with moist heat, and I think it is also known that dry heat is a lot harder to accomplish at kill level temperatures.

Obviously, you have to consider the viability of the sprout. That is one of the things that is different about this than other foods is you have to maintain a viable seed in order to have a sprout. That is probably a research piece.

If I could, one of the things that we heard in this presentation, a remark that has been made is that pasteurization may not be -- it is nice, but it costs so much. I am not really sure that has been explored to the extent. If I heard the presenter correctly, basically, every particle has to be able to see this, and to me that is a combination of temperature in immersed hot water and agitation in order for the seeds to get a uniform application on a time process that may or may not be a high cost issue. The question was can the seeds be "pasteurized," I think that is the simple model.

DR. BUCHANAN: What I would like to do now is to go through each of the four technologies that have been identified and get some kind of an assessment from the committee about what expectation do we have in terms of their efficacy, what kinds of risk reductions we can expect,

and are they capable of meeting the overall goal, which would be the elimination of foodborne pathogens from the seeds.

I would like to start off with liquid immersion, and I think I am going to direct a real direct question to Larry, since he did double duty as a member of the panel and also as a person that has been doing this kind of work.

What is our expectation currently in terms of coming up with disinfectant treatments, water immersion-based systems that would be effective, and what is the level of effectiveness we can expect from them?

DR. BEUCHAT: The difficulty in part is testing whatever treatment it may be and expecting a straight line reduction in counts. We all know, in working in the area of food microbiology, we get sometimes a shoulder effect, oftentimes the tail effect. This is the tail effect to the highest extreme in terms of being able to eliminate a given population of either Salmonella or 0157 from alfalfa seed.

So, I think we should not come up with a certain log reduction as a goal to obtain, say, whatever it might be, to zero. We could come up with a target, but with the expectation of certain percentage of risk associated with the probability that a viable cell would remain or enough that through the germination and therefore amplification process it would have grow and reach fairly large numbers in

the sprouting process.

The combination of temperature, just heat and moisture would be one of the most practical ways, approaches, if that probably fairly delicate balance between time, temperature, and moisture content can be demonstrated, can be identified, and if systems can be, and I am sure they can be, devised to actually process the seeds in a fashion that upon immediate exit from that system could be contained in a way that they wouldn't subsequently be contaminated. That would seem to me to be an area that should deserve research attention, the balance between moisture, temperature, time, viability, and also including among all these experiments different ages of seeds, different varieties of seeds, if scarified seeds need to be included, so be it.

DR. BUCHANAN: Larry, you had a working assumption there, and I would like to go back to it, and we will revisit it again later in another section when I talk about interventions, is you had the assumption that you would, after treatment of the seeds, be able to then move them into a germination and sprouting system that then assured that they weren't recontaminated.

Based on your experience, how likely is that?

DR. BEUCHAT: Not likely.

DR. BUCHANAN: Because I think that is a very

important point that we are going to have to wrestle with when I come back to this area, is what level of assurances, if we provide assurances on seeds, what is the likelihood that they will be recontaminated and you will get the grow-out problem that we have seen.

DR. BEUCHAT: On that point, if, however, a higher degree of assurance could be made in terms of the seed as it arrives at the sprouting facility, and then the GMPs and the other practices and regulatory aspects that would hopefully improve and maintain a higher level of standard overall in the processing of sprouts, the preparation and processing of sprouts, at least there would be perhaps a better chance of reducing the risk of the end product overall in the industry of having Salmonella or 0157 as it exited the processing facility.

DR. BUCHANAN: Can I read into this, then, that you are suggesting that no matter what solution comes forward, it will have to be a multiple hurdle type of a system?

DR. BEUCHAT: Yes.

DR. BUCHANAN: And that implies that no one system currently available supplies you in your mind with a level of assurance that you consider needed?

DR. BEUCHAT: Correct.

DR. BUCHANAN: I throw this out because these are

some pretty strong statements, and I would like to bounce it off the rest of the members.

DR. SWANSON: I would like to go back to a point that Bruce made earlier and tie that with what Larry said. Bruce pointed out that the source of contamination that seems to be the primary problem in all the outbreaks that we have seen to date seems to point to seed, and not necessarily a GMP issue.

I would agree with Larry that there is ample opportunity for seed to become recontaminated after disinfection treatment is applied, but with the potential for amplification that certainly exists in sprouts, if it was highly likely for that contamination to occur, I would suggest we would see many more outbreaks than we currently do.

The source of contamination in these processing facilities, I don't think is inherently there or we would see many, many sprout outbreaks, and we are not. So, I agree with the fact that we need a multi-hurdle approach at each one of the stages, and there isn't one simple solution, but I think we need to keep in mind that it isn't a high probability that there is going to be contamination in these sprouting environments.

DR. GOOSBY: Two points that you are working on, Bob, presently. I would suggest strongly that we look at

combinations in addition to the single use of any of these four, that perhaps some of those used in combination with another may offer a closer goal of zero of sanitary seed.

It has my experience, however, regarding GMPs, in working with food processing and production facilities, to pay close attention to the human factors, shall we say, and the entire environment associated with food production operations, and not to single California, they are probably one, if not several, steps ahead of some of the other areas of the country in that industry, but as I recall, there have been numerous actions and opportunities taken on the part of the people in the production area to diminish the likelihood of the safe product.

So, my point is I would encourage consideration of GMPs in the production area, and in agreeing with the other concerns and comments made, as well, because we haven't heard of particular outbreaks to an excessive level in the minds of some regarding sprouts as a vehicle for foodborne illness, because they are not recognized or reported or identified, it may be less than an accurate portrayal of what is really going on.

DR. BUCHANAN: Jeff, in your presentation, you made some rather strong recommendations in relation to the GMPs. Would you like to reiterate your position?

DR. FARRAR: I think it has been summarized pretty

effectively by everyone that we share the belief that GMPs have to be a vital ingredient in these solutions. They have to be a given. There can't be any compromise on GMPs, but to depend on GMPs solely, I am concerned that from what we have seen in our outbreaks, that may not be sufficient. Multiple hurdles are needed.

Seed disinfection is one step that is available now, that a lot of sprouters are already doing. A lot of these other technologies we are talking about, and even alluding to as readily available, really haven't been that thoroughly tested yet, dry heat, hot water are two that come to mind.

So, I think we really need to focus our attentions on what can we do in the next 30, 60 days here to intervene.

DR. BUCHANAN: And what do you think we can do in the next 30 to 60 days?

DR. FARRAR: I think we can put forward a recommendation that USDA and/or FDA submit a petition to EPA for approval of calcium hypochlorite at the 20,000 ppm level for disinfection of seeds, and whether we go forward with other interventions that we have discussed here today, that is open for discussion, but that is one move we can make along with strict enforcement and more frequent inspections for GMPs.

DR. BUCHANAN: Discussion on that specific

recommendation?

DR. SWAMINATHAN: At this point, I am talking about the 30 to 60-day recommendations. Instead of immediately the FDA saying that calcium hypochlorite needs to be approved or petitioning for the approval of calcium hypochlorite, perhaps the better thing would be to come up criteria that four process controls that we can achieve at this point, and this was mentioned actually by Bruce when we were coming back from lunch, and at this point, there are three places where one could intervene, at the seed stage, the sprouting stage, and after the sprouting process.

At the disinfection stage, there are at least two parameters on which criteria need to be established. One is the degree of kill that one needs to achieve, and I think it is almost unanimous that we cannot accomplish complete inactivation of E. coli 0157 and Salmonella, so what is the best possible control that we can achieve, is it going to be a 3D or a 2D or a 4D process, if we can come up with criteria for that, and the second parameter would be germination, what is acceptable for that, is it over 90 percent, over 95 percent, and so on.

I think we need to talk about those a little bit more and establish the criteria first and then see which of the treatments we have discussed is practical and also meets those criteria.

DR. BUCHANAN: Additional comments? Terry.

DR. TROXELL: I just want to clarify that we have already been in discussions with USDA to move forward with an authorization of the 20,000 parts per million calcium hypochlorite with EPA. I don't know what hurdles there might be, but it is envisioned that we are going to get something quick, you know, hopefully, a Section 18 or some other sort of temporary clearance.

I think we all need to look at what the other treatment, the efficacy is, you know, of the other treatments, and let's assume for the time being anyway that calcium hypochlorite is fairly shortly down the road.

DR. BUCHANAN: Swami, can I ask a point of clarification? You came up with three stages - the seed stage, sprouting stage, and post-sprouting stage.

DR. SWAMINATHAN: Yes.

DR. BUCHANAN: You eliminated from that list any consideration of pre-seed stage, that is, seeds before they got to the sprouter. Are there any interventions that should be considered pre- the seeds arriving to the sprouter?

DR. SWAMINATHAN: The only one I could think of was the scarification, which was pointed out by different people, the hard seed counts and scarification apparently. What I was hearing was some requirements and standards could

be set up in that area. We are talking the short term now, not research. That is the only thing I could think of.

DR. BUCHANAN: Was there sufficient data to make a case for scarification? I am bouncing around a little bit on purpose. Yes, John.

DR. KVENBERG: Just going back to the conversation that we heard, if I can go to the pre-seed discussion for a moment, it seems I recall that climatic conditions and adaptations of the seed made it a good hot weather sprout or not a hot weather sprout, and there was also an indication of origin of where the seed came from, so if you make a somewhat tenuous connection between what may or may not be true, the seed that is subject to scarification has a thicker seed coat and acclimated to a northern climate.

Maybe there is some -- I don't know if we have heard enough to actually make a recommendation, but it seems that there is some linkage between the origin of the seed and its need for scarification for sprouting purposes. That, I think I heard here at the meeting.

So, I don't know if there is enough here for the working group to make a considered opinion of that, but that is kind of the gist of what I had heard through various pieces of this was that seed selection source and the sprouting capability of a hot weather seed made it more applicable to sprouting without scarification. That is what

I heard.

DR. BUCHANAN: Bruce, do you no longer have a comment?

DR. TOMPKIN: No, I do.

DR. BUCHANAN: Okay. You were next, then Dane.

DR. TOMPKIN: I am sure that we wanted to, we could come up with some good agricultural practices at the farm level. I would suspect that what you have in place for growing lettuce, for example, might apply to this, but it is just a matter of going through that list and sorting out those that would be appropriate for seeds.

DR. BUCHANAN: Can we take that as a recommendation, that we should emphasize the needs for good agricultural practices in the production of seeds for sprouting?

DR. TOMPKIN: Yes. However, even with those good agricultural practices, I think it must be assumed that certain lots will contain enteric pathogens, because we cannot control wildlife nor our environment, and so I think it comes down to a means to decontaminate the seed at the seed handler level, and essentially, we are thinking of a pasteurization system. It is just like collecting milk from a bunch of farmers and then you bring it into a centralized place and pasteurize it. We are all familiar with that.

To move down to that next step, however, we heard

that there are 350 approximately sprouters, so in a short term, what we have been discussing is to use a liquid disinfection system for use at the sprouter level, and that would be the calcium hypochlorite or if someone could move on it, perhaps with hot water or something of that sort, that would be done at the sprouter level.

You have got 350 operators to actually manage and hope that you can provide them with the tools and the means to decontaminate the seed to the best that is possible with that technology, but there is still a question, and so I could see where we could come up with a short-term goal to apply a decontamination system at the sprouter level of whether it is a 4 or 5 log kill, whatever that particular requirement may be that we could come up with.

But then beyond that, I think that a longer term goal, based on seeds, at the seed distributor level, would be to come up with a means to provide a pathogen-negative, that is an enteric pathogen-negative seeds, that perhaps we could come up with a guidance of, say, start with the seeds that are inoculated with 10^4 per gram, or something of that sort, and the system must provide a pathogen-negative result after sprouting, so that you take advantage of the growth that may occur during sprouting and increase the sensitivity, and so as you work in that direction as a longer term goal, to come up with a pathogen-negative seed

and then, where possible, verify that or validate that with naturally contaminated seeds, and maybe we would have more confidence. Essentially, we are just trying to build greater confidence into the kill step.

DR. BUCHANAN: I get the implication here that a pathogen-negative seed, is this a seed that is supplied to the sprouter that is pathogen-negative?

DR. TOMPKIN: Yes. If I were a sprouter, in anything that we would do, we would have to have a negative product because it is something over which a sprouter has no control.

DR. BUCHANAN: Based on the discussions that took place at this meeting, are there any steps that the sprouter could do short term, right now, to provide a pathogen-negative seed?

DR. TOMPKIN: Yes.

DR. BUCHANAN: What?

DR. TOMPKIN: The individuals providing seeds are, from what I can see, would have to -- it is going to take time to do the research, irradiation certainly looks like it would work, but it is long term in terms of getting approval.

DR. BUCHANAN: Putting approval aside, are there any barriers here, I mean do we need the research on irradiation or is there sufficient data to say that it is

effective?

DR. TOMPKIN: It looks pretty good to me, but I would like to see it on some naturally contaminated seeds. So, I think that irradiation is high on the list from my perspective, but I would not rule out others that are also possible.

Commercially, we used to put powdered egg white into rooms, large rooms, for a week to 10 days, 130 degrees is what comes to mind. We also did that with gelatin. When we had problems with gelatin, it would go into a hot room. We have done that. We came up with a time, temperature, humidity controlled system with drums, and as I recall, that was for gums and stabilizers. There are ways of approaching a decontamination of dried materials.

That really didn't impact on functionality. Whether it will impact on germination is a question, but from what I could hear, it looked like you had a pretty good shot at coming up with something that would work, and not jeopardize your germination rate.

DR. BUCHANAN: Larry, I know you did a little work on dry heat storage. Did you go up to any temperatures where you could assure a pathogen-free seed?

DR. BEUCHAT: No. The highest temperature in terms of alfalfa seeds that we have stored is 37 Celsius, so we really haven't done any temperatures higher than that.

DR. BUCHANAN: I was going to say that is a cool afternoon down in the Imperial Valley.

DR. BEUCHAT: But I think it is a bit beyond, well, certainly, the germination rate is important, but I have learned a little bit about other things besides just germination. There is something called vigor, and sprouters in the audience can help me on this. It is not just germinating, but it is the rate of growth after germination.

When we get into dry or moist heat in terms of a system to kill pathogens, I know a little bit about the behavior of other legumes in terms of something called hard to cook, the problem in the bean industry, and what happens is, it is accelerated by temperature, elevated temperature, and moisture, and it comes down to a gelatinization of carbohydrate, polysaccharide layer, and the difficulty of taking up water and perhaps in that case difficulty to cook, but in this case, difficulty to take up water by the seed to germinate and then carry through processes necessary to be very vigorous during the sprout growing and maturization process.

So, it is going to be a balancing act here. I think that definitely we need to know more about it, but it is not just germination rate in the end, but rather how quickly it gets to that point where the sprouters can control spoilage and other factors that impact on sensory

quality.

DR. BUCHANAN: So far I have heard a lot of discussion to this point recommending some kind of an active intervention with the seeds. Do I have anyone that wants to take the opposite position, that such a step is not necessary?

DR. NAGLE: I am not sure that this is appropriate now, but I do want to talk about this GAPs that we have just put down there for the field. Given the discussions that we have had about the lack of being able to identify these pathogens in seed, isn't this a bit of an overkill of going out and trying to tell farmers how to farm when we wouldn't be able to measure whether they were succeeding or not.

I think this is a little bit beyond. I think we do need to do something with the seed, but I think we have heard repeatedly over the last two days that this is probably an event type of contamination, not a gross contamination caused by processes or by ways of doing business.

It is a deer walking through a field or a bird flying over a field, it is not just a gross contamination of the way of agriculture being conducted, and I think we might be going down the wrong path if we focus too much on this GAPs for this for alfalfa. I am not talking about lettuce, I think that was thrown out before, but to put it out for

alfalfa now, I think is way premature until we can find something. I just don't think that this is going to bring us any increased level of satisfaction that we have done something about the problem.

DR. BUCHANAN: Comments?

DR. KVENBERG: Can I just go back to comments I heard during the meeting, and that had to do with percentage of damaged seeds, not to harp on the scarification question, but my earlier point was selection of seed source may have a bearing on the condition of the seed, whether it comes from a hot source or not may have a bearing, we just don't know. Maybe this is a research question, but, you know, suppositions have been put out that seed source as opposed to growing condition or good agricultural practices is a pre-seed condition because it is selection of source that may reduce the likelihood of occurrence. It was a point I was trying to make earlier.

Maybe that is a research or needs to be delved into, but the question of the likely contamination of the seed before it gets to the seed house is a point that has been discussed at the meeting, it hasn't been discussed here.

DR. BUCHANAN: Additional comments on GAPs? David.

DR. GOOSBY: I am really kind of drawn both ways.

The complication here is, as I recall from the seed producers and distributors is that they are producing primarily seed for agricultural purposes and secondarily for the sprout industry. Therefore, we are asking a lot of an agricultural business to entertain GAPs for this small segment of their work.

On the other hand, if there were to be producers of seeds destined for the sprout industry, and that could be identified early on, I really think there is value in trying to create these additional hurdles of protecting the public health by addressing such concerns as the use of manure, water, pesticides, those kinds of things at the farm level to be included in the broad category of GAPs.

MR. BERNARD: I have to agree with Nancy on this one. We have heard repeated testimony to the fact that you can take lots, even lots which have been implicated in outbreaks, and test them. Very rarely do you find it, which tells me this is not a broad contaminant, as something that might result from an irrigation practice, a fertilization practice, or a spraying practice. Then, we would expect more general contamination. We don't see that.

These people behind us here know a good deal more about how seeds are produced, selected for sprouting, et cetera, and I haven't heard any consensus from them as to the implications of any of those practices on the resultant

problems.

To say that there is something, obviously that, you know, seed for human consumption should be handled such that it is certainly acceptable for human consumption, but beyond that, to say that we can come up with any fix on the good ag practices side that has an implication on the potential for elimination or prevention of outbreaks is speculation.

There is a long-term research agenda. I would agree that this is worth looking at, but if we are looking at some short-term things, I think we have got to go back to interventions that can be applied either after you have the seed or in the sprouting and grow-out, and we have got some priorities that we can talk about there.

DR. BUCHANAN: Bill.

DR. SPERBER: I think perhaps the seed distributors do have a bigger role in here than we suspect, because several times the point was made that only 10 or 15 percent of the alfalfa seed is of sprouting quality, so somehow the seed distributors are needing to segregate the sprouting quality seed from the normal agricultural seed, however that is being done.

Another seed person yesterday said that the grew alfalfa seed by contract, and if you are growing anything by contract, you can specify where it is grown or you can

specify that it not be next to a field lot or places like that, so you could have small degree of control there.

DR. BUCHANAN: I am looking for a consensus to develop here, and so far I am seeing a consensus not to recommend anything related to GAPs at this point, but to regulate it to research. Do I have anyone arguing the opposite side? Bruce.

DR. TOMPKIN: I wouldn't want to tie people's hands raising alfalfa in the field . I think there is some very basic things that the application of fresh manure to a field for fertilizer for this particular crop, I think would be inappropriate, and you might not have to go any farther than that.

So, when you asked would I agree that GAP should be on the list, certainly, but to go beyond that, I think may be inappropriate.

DR. BUCHANAN: Why don't we at this point circle that one with a star or square brackets, cross it out.

Let's go back through these real quick on a couple of the interventions. We had some discussions about scarification. Do we know enough to make a recommendation on that now, or do we need to relegate that to research?

DR. SWANSON: Research.

DR. BUCHANAN: Okay. That gets relegated to research.

DR. BEUCHAT: I don't know of any studies that have been done to compare, for example, a given lot of alfalfa seeds, half of which is scarified, the other half not, inoculate it, and then test disinfectants. So, to support the point that research needs to be done, yes, I would do that.

On the other hand, I would be very surprised if we don't see from that type of research that it is much more difficult to eliminate, to reduce pathogens from the scarified versus the non-scarified. We will have more difficulty removing it from the scarified product, but that the hard data, the scientific data, to my knowledge, has not been developed.

DR. BUCHANAN: I see a consensus there to recommend scarification as a research area.

The other consensus that I have seen to point, and I haven't yet had anyone argue against it, was that there should be some positive interventions in decontaminating the seeds, reducing the risk associated with foodborne pathogens, that is, some positive intervention step.

Do I hear anything from the committee that surfaces as less than a consensus?

[No response.]

DR. BUCHANAN: There being none, that there should be a positive intervention step to reduce the level of

microbial contamination of seeds. Lee Ann, you want to put that up as a recommendation.

DR. DOYLE: Mike Doyle. I think when you say a positive step, that is fairly broad. Maybe if we narrowed that down and say X number of log reduction or something in that range.

DR. BUCHANAN: That is where we are going now. I get consensus where I can. If I can get you all to agree that something needs to be done, then, we can talk about how much.

Let's open the floor. Mike, how much? Then, after we get how much, then, we will talk about how.

DR. DOYLE: I guess I would suggest we might start out with 4 logs.

DR. BUCHANAN: And on what basis do you provide that estimate of how much we should achieve?

DR. DOYLE: Because I think we have a practical approach that can achieve that with calcium hypochlorite.

DR. BUCHANAN: You are talking about the 20,000 parts per million hypochlorite.

DR. DOYLE: Right.

DR. BUCHANAN: Larry.

DR. BEUCHAT: If I could get enough E. coli on the seed in the first place to get 5 or 6 logs, I think I could get the 4 log reduction now. It's that very small

percentage that lingers behind. If I could get 10^6 per gram, I could probably reduce it to less than 10^1, but it is that small percentage that remains behind is amplified during the sprouting process.

DR. BUCHANAN: That was a question I asked during the session this morning, because obviously -- Bill, are you here in the audience somewhere? I guess Bill Fett left. They got fairly high numbers. Was that an artifact or was that -- how did they do it?

DR. FARRAR: This whole question, though, gets back to the point that we don't know at what level these seeds are naturally contaminated.

DR. BUCHANAN: Correct.

DR. FARRAR: To assume that they are contaminated at the 10^6 range, I think would be faulty logic at this point.

DR. BUCHANAN: No, that wasn't the logic. It was whether he would be able to measure whatever he started with because he starts with such a low level, and I guess the next question would be is there any indication at all that the contamination at low levels is different than the contamination in artificially higher levels.

MR. BERNARD: I think the advice given to me ages ago by one of my mentors when we were talking about how many logs we needed to achieve a certain target in a product, and

he said I don't care how many logs you get it, just kill the last 10 of the buggers. That is exactly what Larry is talking about here.

You know, what we are seeing with tailing and the chemical methods is very classic. Somebody comes in to me and says I have got this stuff, and I can give you a 5 log kill, I yawn, because that to me means nothing. If you start out with 10^7 artificially inoculated, and do a count o it, you are down to usually 10^5, 10^6 just because you can't recover them.

So, you know, getting down to 10 is fairly easy with most chemical treatments, but because of masking, because attached bacteria are embedded, bacteria are much hard to kill than the planktonic type. It is exactly what we went through with biofilms, so it depends on the state of the organisms.

I think the natural contaminants would probably be very hard to kill even though they are there in very low numbers with any chemical treatment. That is the problem I think we are running into.

Tailing is a classic problem. It is less of a problem with heat, even though clumping and other ways of masking give you some tailing with heat, it is much less of a problem there. Irradiation is really the only treatment that gives you pretty much a flat, straight line regardless

of whether you artificially inoculate or whether it is a natural contaminant, because of the hit theory, and so I think it depends on what intervention you are planning on as to the importance of natural contaminants versus artificially inoculate contaminants.

I think obviously, if you use high concentrations of calcium hypochlorite, it is going to reduce your risk, but as Larry's data has shown, we can't get to a point where we can have absolute surety with that kind of a system.

DR. BUCHANAN: Let me ask you -- we turn the question around on you -- assuming that you were using a chemical disinfectant, how effective should that chemical disinfectant be?

MR. BERNARD: As good as you can get it, but you are still -- it is what do you expect. You know, we are reducing risk with whatever chemical you can get approved through the process.

DR. BUCHANAN: What should we expect, Dane?

MR. BERNARD: What should you expect?

DR. BUCHANAN: Right.

MR. BERNARD: Mike's number was a good one, but are you going to get the last 10. No.

DR. BUCHANAN: Mike.

DR. DOYLE: I think we are looking at the short term fix, and I think that you hit it on the head, that

irradiation would be the most likely long-term fix, but I haven't seen anything else that looks reasonably practical.

DR. BUCHANAN: Jeff did point out that we have a short-term mount on this one, but 20,000 parts per million chlorine is not approved yet other than in the State of California.

MS. DeROEVER: You are coming up on quarter of 3:00.

DR. BUCHANAN: Okay. Let's talk about very quickly did we see any interventions that were associated with the sprouts themselves, the sprouting process, or after germination that would appear to be effective short term or long term?

DR. TOMPKIN: That calcinated water I thought was pretty intriguing, and certainly having more information on it or to understand what was behind it, is it a pH effect, can something else be used, and if we could take advantage of that, that would be helpful, because what is unique about this product is that you will have growth as a result of sprouting.

DR. BUCHANAN: As far as I could tell, I only heard to technologies. One was the oyster shell technique, and the other one was radiation of the sprouts after sprouting.

DR. TOMPKIN: But irradiating the sprout when you

have 350 sprouters, that is a matter of working that part out. If you come up with something else that could be used in-house, it would probably be better.

DR. BUCHANAN: What is the importance of coming up with some kind of intervention either during sprouting or post-sprouting in terms of the overall safety of this product?

DR. DOYLE: I think it's darn important because we have a condition which will promote the growth of pathogens if they should be present.

DR. TOMPKIN: There is the possibility of a competitive exclusion or some sort of prebiotic that we talked about. There was a little bit about it, and it is a research idea still, but that would be a third one.

DR. BUCHANAN: Additional comments on this section?

MR. BERNARD: Bob, where did we end up with talking about heat treating either the seeds before sprout, well, the seeds just before sprouting, is that on the table at this point in time or not?

DR. BUCHANAN: Yes, it is. Do you have some additional comments on it? I had it identified as a viable short-term solution.

MR. BERNARD: I think that is where I would come out. Obviously, the death of microorganisms begins about

five degrees above their maximum growth temperature, which opens the door to some low temperature, long-time treatments. Then, it becomes a race between viability, and -- what was the other word you used -- vigor of seeds versus death of microorganisms.

If we could be fairly certain that most of the contaminants were either surface contaminants or very near surface contaminants, then, that opens the door to high temperature short time as being probably the most favorable to the seeds and the most effective in killing, but the further up in terms of control of time and temperature on that scale you move, in other words, if you are talking about 150 degrees for just a few seconds, for example, that gets a bit on the expensive side in terms of the equipment to make that kind of thing happen.

On the other hand, if you have a possibility of 140 for 10 minutes, that is something that could be done in a kettle with good temperature control and hand-operated thermometer. So, those are kind of the range of options, and I guess there needs to be some research done to kind of fill in the data gaps as to what we are talking about in terms of time, temperature profile of seed response versus the thermal death time data that I am sure the literature can help us out with.

Also, Bruce's discussion earlier about dry heat,

not really dry heat, but not adding liquid moisture, but in a controlled atmosphere, I think is something that could be explored, as well. Our experience years ago with roast beef could be utilized. A lot of that data could be used for that.

DR. BUCHANAN: Before we take a quick break, let me reiterate what I have down in my notes, so that I understand and we have for the record the consensus that I think has been developed, that there were four possible short-term interventions or there is four possible interventions, two of them that are short term in terms of they could be applied quickly in a 60- or 90-day period, would be liquid immersion in a disinfectant, and/or hot water treatment. The reason I put in "and/or" is that there was some indication that you could use them in combination.

A longer term solution -- and I gather this was more from the fact that there is some regulatory hurdles that have to be reached -- was irradiation of the seeds, and then finally, dry heat, there seems to be not much data available while it could be applied, there wasn't a lot of data confirming its efficacy.

Have I reached the consensus correctly on those four technologies? Yes, Bruce.

DR. TOMPKIN: With regard to that last one, actually, there are commercial systems in place, and it

should be possible to actually go around and talk to people who do these things now, such as the spice industry, and so on, find out what the conditions are, what equipment is available, and so on.

DR. BUCHANAN: You just need to do some validation studies.

DR. TOMPKIN: Right. Textbook information is helpful, but there is actually ways of going around and getting such information in a shorter term. I agree with the four approaches.

DR. TROXELL: I just would like to know where the group came out now with respect to these four on elimination versus minimized levels, levels of the microbes.

DR. BUCHANAN: Unless I am wrong, there was nothing on eliminating. All of these technologies would reduce, but not eliminate. Yes, Bruce.

DR. TOMPKIN: I would envision that the two short term, the hot water and the disinfectant, would be to reduce the risk, but it will not, I don't think we could guarantee that the disinfectant from what we saw could be relied upon to eliminate it.

Hot water, if you come up with the right system, and as it was shown on the video, I would have a pretty high level of confidence in that one as eliminating the pathogen, but how that would fit in to 350 sprouters is another issue

that has got to be dealt with. The one for seeds should be to eliminate.

DR. BUCHANAN: Let's get clarification. I don't know if Tom Farrar is still here or Kathleen, I didn't see of the data that they presented today that they were going for an elimination, they were going for a reduction. They were going for about a 4 log reduction.

DR. SWANSON: 99.9 is what they said.

DR. BUCHANAN: 99.9. We will get clarification on that.

DR. DOYLE: I think the goal is to eliminate, but we just don't have the technology to do that, and that long term with irradiation.

DR. BUCHANAN: Any additional comments on this section? When you come back, come back with your research ideas and your priorities and justification for those priorities.

[Recess.]

Because of time constraints, and the fact that some of our board members or panel members have flights to catch, I would like to change the order that we will be discussing things.

I would like to take things out of order. We are going to save research needs and priorities for the end, and I would ask any panel member that has to disappear to please

submit their comments on research needs and priorities to us in writing during the next week, so if you don't have any opportunity to be here for those discussions, I would ask you to get that stuff in to us and we will incorporate it.

A procedural matter. We are going to take the draft recommendations we get here, put them together for the working group members, and then get them back out to you during the next week, let you review them, see if you are in agreement, and if not, we will get further discussions on points of clarification.

I would like to now focus on additional approaches for assuring or improving the safety of sprouted seeds, particularly alfalfa, but before doing that, I would like to revisit the good agricultural practices for a moment. Where is Nagle? I did want Nancy to be here for those discussions.

Do we know enough about good agricultural practices to make any kind of a recommendation about the use of manure or the positioning of a field associated with alfalfa production particularly, but for that matter, seeds for sprouting across the board, and there has been some discussions that, that at the end of our discussion that we had enough information for a general recommendation about the uses of manure. Comments?

DR. FARRAR: Bob, the good agricultural practices

are general recommendations in each of the areas including water, manure, proximity to livestock, and so forth. I have a little difficulty seeing why they wouldn't apply in this situation.

DR. BUCHANAN: Very honestly, so do I. That is why it is coming up again. I have a hard time not being able to figure why you would want to be putting uncomposted manure on a product that was going to be food.

DR. SWANSON: I think that the point was there is no -- the farmers that are growing the seeds don't necessarily know if the seeds they are growing are destined for human consumption or the vast majority of the agricultural practices that are currently being carried on, so to suggest that you have to use GAPs is almost fooling yourself to think that they are going to know that these are for human consumption.

DR. BUCHANAN: Let me turn this around and say would you as a sprout producer, want to knowingly buys seeds that had been treated with uncomposted manure?

DR. SWANSON: No.

DR. BUCHANAN: Why are you, as a sprout producer, willing to say that at this point? I mean you must be making an assumption here.

DR. NAGLE: Bob, can I comment?

DR. BUCHANAN: Please, Nancy.

DR. NAGLE: I think we have to look at this, too, saying okay, when -- remember when we were at the sprouters, these alfalfa plants are out there for three years, now, are you going to tell me that it is going to matter to you whether uncomposted manure was put on there three years ago if this is the fifth cutting or a sixth cutting of this stuff?

I think we have to be realistic and look at that and say, you know he may not know three years ago that he was going to be making sprouts now, but is it even germane?

DR. BUCHANAN: I think the current recommendations from the Organic Board -- and the representatives, if they are still here, can verify this -- I think the recommendation is that you shouldn't be using manure with less than 60 days between its application and harvest.

So, those are the good agricultural practices' recommendation. I see Michelle back there.

DR. SMITH: [Off mike]

DR. KVENBERG: Bob, they can't hear that, nor was it recorded, so that is going to get lost.

DR. BUCHANAN: Michelle, would you please come up to a microphone and give us your comment. This isn't an comment period. This is a person that has supplied technical information on this issue to the committee.

DR. SMITH: The reference to good agricultural

practices and the 60-day minimum that the Organic Standards Board puts in place for the use of raw manure on edible food crops, that 60-day minimum is not linked to any guarantee of food safety, so the good agricultural practices that we are working on right now are focusing more on just maximizing to the extent possible the time between application of raw manure and harvest of fresh produce in the case of the broad-scope document.

DR. BUCHANAN: I guess that sounds very reasonable to me, and I would like to question. I have some concerns about not even being able to make a statement about the application of manure.

DR. NAGLE: I think we have to be careful what we are saying. The way the question was posed is would you accept it if it had had uncomposted manure. I hate to get into what do you mean by that, is it ever, you know, or whatever.

I mean as long as we have certain reasonable differences, but to just say not ever treated with composted manure or uncomposted manure, that raises the question of what do you mean by ever, you know, in the last two years, five years, you know, next 10 days, and I think we all expect to be reasonable in the 60-day number or some reasonable number is okay.

DR. BUCHANAN: I am going to try and draft up

something for the final report that comes up with this reasonable estimate about manure application, because that was identified as something during the discussion that there was some concern about. Yes, David.

DR. GOOSBY: A couple other comments. In my interpretation or appreciation of GAPs, they really are quite general and broad, and we can have them as narrowly or as broadly defined as might be reasonable, based on common sense, and dare I say but I think it is appropriate to do so, that in working with Michelle and others and the GAPs and GMPs, that we did for other segments of the produce industry, in most situations, and the concerns we addressed there were not science based necessarily, but were derived from good common sense with the cooperation and support to be embraced by the industry.

Because they don't have a regulatory impact, GAPs that we might recommend here would have that same bearing, meaning that there would be something in the recommendation or suggestion, a level of encouragement to be embraced and adopted by the industry whether we make recommendation only regarding manure and/or water, and/or pesticides, some of those main categories that may impact the safety of the product, and yet are fairly readily controlled by the farm operations, and this kind of approach be done in balance, so that we don't impede the farmer's ability to sustain his

business operation.

DR. BUCHANAN: I am going to switch gears a little bit and I would like to move into other areas associated with food safety systems that should be considered and possibly encouraged by the working group.

These can be any of a number of areas. For example, we heard some discussions about education, we have heard discussions about HACCP systems, et cetera. So, I would like to throw this open to the working group concerning recommendations on other safety assurance systems and related topics.

DR. DOYLE: I would like to raise the question of testing. I think Katie brought out an interesting point regarding the water treatment that is used to water sprouts and testing that water for pathogens.

DR. BUCHANAN: I personally found that intriguing. I have some concern about the capabilities across the board in terms of sprouters being able to do that technology, but I think that seems to be a very viable approach to increasing the safety of the product.

Certainly, if I was not wearing my current hat, but if I was a consultant, I would be recommending that as certainly a technology that somebody should be looking into. Do we have additional comments on that?

MR. BERNARD: Just a clarification. We are

talking about the water that would be used by the sprouter, applied to the seeds, and continually through the sprouting process?

DR. BUCHANAN: Right, the water that drains off the sprouts and analyzing that.

MR. BERNARD: Oh, okay.

DR. BUCHANAN: The water that is drained off of the sprouts during the germination process, then analyzed to determine whether or not you are pathogen free for whatever your indicator organism was.

MR. BERNARD: Spent water.

DR. BUCHANAN: Spent water.

DR. NAGLE: I guess the one thing to think about, though, we are talking about 350 sprouters as we said. What would we expect them to look for in this testing, and then what would the cost of that be, because that would be a cost to every batch that they make as far as I could tell, the way we are talking about it now, right?

DR. BUCHANAN: Yes.

DR. NAGLE: So, we would need, you know, it would need to be something that wasn't an unreasonable cost to add to every batch, because that does kind of go in a different direction. It is almost a hold and release testing that we are recommending here. Is that what I am hearing?

DR. BUCHANAN: I think what we have to consider in

any kind of recommendation, are we recommending that we think every batch should be tested or are we recommending that that technology be encouraged within the sprout industry.

DR. NAGLE: That is what I am asking because if we make a recommendation, it may carry more weight than we thought it was going to have or it may become de facto regulation almost, so we want to make sure that we have looked at all of the potential ramifications of this, and then the windfall that laboratories would be getting.

DR. BUCHANAN: Mike and then Bruce.

DR. DOYLE: I think there are ways in which you can composite samples, so that there is not an unusually large number of samples that would have to be tested on a daily basis. I would be interested in looking for specific indicator organisms and/or specific pathogens like Listeria and Salmonella.

DR. BUCHANAN: Bruce.

DR. TOMPKIN: Since 1995, from what I understand from the data that were presented yesterday, there have been eight outbreaks, recognized outbreaks attributed to sprouts. There were the six Salmonella and two from 0157. You consider how many batches or lots that have been produced of sprouts. I don't think -- yes, the risk to consumer is high if it does contain a pathogen, however, it would appear that

the prevalence or the frequency with which this occurs is pretty low, and to try and to impose pathogen testing on this particular industry as a hold and test program, I wouldn't recommend that, however, it would be very desirable for the processors to understand their process, know where the weaknesses are, and then build in some control measures to address those weaknesses.

Just as a beginning, if they could test perhaps for E. coli as an indicator, which could be done at relatively lower cost and certainly 350, probably certainly the larger ones could be using a Petrifilm, it could be done in-house, you get the results rather quickly, it is low cost, it provides information that would enable the processor to understand what the risk is, and to adjust the process.

I don't think that considering what we heard to test for 0157 or Salmonella in each production lot, the frequency is just too low to justify that.

DR. BUCHANAN: Peggy.

DR. NEILL: I just want to mention something almost I think as a sidebar to Bruce's comment, because it has come up a couple of times. It has to do with this concept of how many outbreaks we have seen and what that means.

I think in the past 10 years, there is an

increasing appreciation that foodborne outbreaks are more often low level, sporadic contamination, and widely dispersed. Our current infectious disease surveillance networks do not pick those up very well unless the pathogen is something extremely unusual.

We could be having frequent Salmonella typhimurium, for example, outbreaks associated with sprouts, and unless they have unusual additional attributes for them to be caught in the surveillance networks, there is no other way that you are going to see these.

So, I think that we need to probably be somewhat cautious. We are new even at finding sprout-associated outbreaks, and I think we should just work forward from saying that they are occurring and then take it from there. I am in agreement, however, with Bruce's other comments regarding the use of testing for other purposes.

DR. BUCHANAN: Bruce, i would like to restate my own personal opinion, which is in agreement with Peggy's, is that the only reason or one of the major reasons why these outbreaks were identified is that they were all unusual serotypes, at least on the Salmonella side.

If they weren't unusual serotypes, we probably would have never identified them except maybe in California where they do a really good job.

Additional comments on testing? Yes, Katie.

DR. SWANSON: There is a couple of angles you can play on this testing scenario. One could say that rather than testing every batch, you could run a test on every lot of seed, so you would fill a drum or your trays, or whatever, just to verify that that particular lot was okay and once you have done that, you would be fine, because the amount of seed that they are running through in those drums are a lot greater than the 100 grams that microbiologists will sample and run, so it is likely that you would be able to pick something up that way. So, that is one angle to consider.

Another one is there were comments from the sprouters saying, well, epidemiological data may implicate sprouts, but you have had a hard time testing it. They could view this as an insurance policy if they are testing their product as it goes out the door, and someone says, hey, you have had an outbreak, they could say, look, I have done this testing, and it is not fair, it might be something else.

So, you know there is numerous considerations that can be given around this testing.

DR. BUCHANAN: Bruce, can I ask for one clarification? Your proposal for testing is to focus on generic E. coli, and to use this in approximately the same way that E. coli is used for validation of HACCP systems.

DR. TOMPKIN: That is correct. It is to generate background information to allow the processor to design a controlled system that would address enteric pathogens.

DR. BUCHANAN: This brings me up to the next question I had is that we have had several comments related to that this is not a GMP question. We have had some discussions on whether we have interventions that can be used in a positive fashion, and we have identified that they are.

The next question I guess is does this need to be structured in a HACCP system. I would like to bring that up as the next subject. Mike.

DR. DOYLE: Before we get onto that, I would like to go back to the testing the organisms of interest. I asked the question do we have enough data to show that E. coli is a reasonably good indicator of the presence of Salmonella and other potential pathogens that we are finding in sprouts.

I don't know. I don't think we do, but based on what we have seen with E. coli as an indicator in poultry, from what I read it is not necessarily a good indicator of the presence of pathogens, and so I would suggest even though the incidence may be low, i would be more interested in the results of Salmonella testing than I would be E. coli testing.

Secondly, I think it would also be important to look at the environment in which the sprouts are growing, and there we tend to look for Listeria as the indicator of environmental contamination.

DR. BUCHANAN: You have brought another dimension into this, the testing of environmental samples. So, before we go to environmental samples, let's focus on any kind of a recommendation we have about potential testing as a possible intervention tool or possible aid to working in a sprout operation. Do we know enough about which organism to test for or is that the recommendation, that we encourage this type of testing and that would include identifying an appropriate indicator organism?

[No response.]

DR. BUCHANAN: Okay. We are working towards a recommendation that testing of the spent water be evaluated as a potential intervention. That is basically what it is, it is intervention be it an indicator organism of process control or, if you wanted to use it as a batch-by-batch clearance, it is in essence an intervention we are going to be using.

Do we know enough to make a recommendation about what should be the organism of choice or does this need to be evaluated further? Yes, Bruce.

DR. TOMPKIN: I think probably we need more to

understand its value. The assumption is that we would only test the water. If I were a processor, I would actually test some finished product, too, because there are a few things that happen between the sprouting and the package.

DR. SPERBER: Just one quick sidebar. I have missed some of the previous meetings on this topic, but Mike opened up another topic, too, by mentioning Listeria testing in environmental samples. Do we know that LM is not a hazard in sprouts? It is a refrigerated, perishable food, and all of the discussion on food safety issues has been 0157.

DR. BUCHANAN: I am going to step back because I am going to give you FDA's policy.

MR. BERNARD: I am not sure that we know that it is not, but in our experience in trying to find it in various types of establishments, this is a kind of an environment we wouldn't expect to find it, because it is not a good competitor. One thing we know is that we are getting a lot of microorganisms growing. As an example, if you try to find Listeria, as we have, in fermented meat plants where you are working with starters and things like that, it is difficult to find in most of those environments.

I wouldn't expect it to be a high yield microanalysis here, but I don't know enough to say that it is not a problem.

DR. GOOSBY: To underscore what Dane said, in my opinion we know too little to declare that it is not a problem, and I recall when Listeria raised its ugly head in a major way in the food industry, and it was a post-pasteurization contaminant of dairy products hardly a decade ago, and I can draw some analogies between the environment in a dairy processing plant related to the environment in a sprout producing plant, temperature, humidity, those kinds of things, and the LM is so ubiquitous, I really think there is a potential threat there that needs a real close look.

DR. NAGLE: Can I ask a question -- back to our original question --

DR. BUCHANAN: I am trying to get closure on one before I go on to the next.

DR. NAGLE: Okay, I will wait.

DR. BUCHANAN: I would like to get closure on a recommendation about testing --

DR. NAGLE: That is what I want to ask. In any of the data that we have, that Jeff has or anyone has, about outbreak of sprouts, do we have any microdata about the other organisms that might be there in those foods, were any of the processors running routine, just total plate counts or anything, do we know any of the data from there? That might help us understand this.

DR. FARRAR: I can report on a couple outbreaks in

California. Unfortunately, the first one is tragic and all too obvious, our 1996 Montevideo and Meleagridis outbreak when we finally found the sprout planter and told them why we were there, the sprouter said, oh, I am not surprised to see you. Ultimately, he told us that he had been testing his product recently after Stanley and Newport, he had been testing his product for Salmonella, and got a positive Salmonella isolate about two or three weeks before the outbreak started, and continue to ship his product.

Another recent outbreak in 1997, the sprouter was doing periodic test of his product, total plate counts, Salmonella and E. coli along with some environmental monitoring, and that outbreak, we did see some positive generic E. coli in the environmental monitoring preceding the outbreak.

There were no positives in that product, however.

DR. BUCHANAN: Bruce.

DR. TOMPKIN: Didn't we hear that FDA is going to do a survey of sprouts?

DR. BUCHANAN: Yes.

DR. TOMPKIN: Why not just include E. coli as an analysis and see what we find.

DR. BUCHANAN: I heard a second recommendation related to microbiological testing that focused on environmentals. Mike, do you want to further discuss this

as a recommendation, and then we will focus on it and then we will let the rest of the panel respond to it?

DR. DOYLE: Well, as Bruce has indicated, we ought to be looking at the entire process within the plant, and do testing that would be relevant to get baseline information as to how well the plant is operating, and the environment is one of those conditions in which you need to address, and Listeria has in the past shown to be a good indicator of how well processes are operating within the plant from an environmental perspective.

DR. BUCHANAN: Does anyone know any available information on environmentals in sprout operations?

DR. FARRAR: There are a couple private labs in California that have a fair amount of data from several sprouters. They could be asked if they would be willing to share the data by not identifying the sprouter, but there is substantial data there.

DR. BUCHANAN: Since we didn't focus this during the day and a half, we are probably going to have to come back to it because as far as I know, we don't have any data. We would have to go out and acquire it.

DR. SWANSON: Wasn't environmental sampling part of the ISGA recommendation, and perhaps that organization could poll their members and provide some data?

DR. BUCHANAN: That is a good idea. We will

approach them.

Let's move on to HACCP. We have heard comments that this is not a GMP problem.

DR. TOMPKIN: Not true.

DR. BUCHANAN: That this is not just a GMP problem. That, of course, then begs the question do we need a system on top of GMPs or would that be helpful. I would like to throw that around to the panel to get their feel on the importance of HACCP, are we ready for HACCP, do we have the scientific data, and what should we be moving towards. Yes, Mike.

DR. DOYLE: I question do we have the critical control points that are needed to have a reasonably effective HACCP plant.

DR. BUCHANAN: I assume you are using our committee's definition of a critical control point.

DR. DOYLE: As you wish.

DR. BUCHANAN: Which is a step or steps that reduce the level of risk, reduce, eliminate, or control the level of risk to an acceptable level.

DR. SWANSON: And are essential to reduce.

DR. BUCHANAN: And are essential to doing it. Okay. Do we have critical control points, Mike?

DR. DOYLE: I am not sure that we have enough. We may have some, but I am not an expert in the area of sprout

growing, so I am not a good one to ask.

DR. BUCHANAN: Larry.

DR. BEUCHAT: Neither am I an expert in sprout growing, but from my experiences recently, I would say that we probably don't know as much as we should about critical control points or don't have enough of them that could be identified at this point.

DR. BUCHANAN: Wait a second. I want Bruce --

DR. TOMPKIN: Maybe I will pull you out of the hole?

DR. BUCHANAN: Right.

DR. TOMPKIN: Well, HACCP is a good food safety management system, and this particular industry should look at its value and apply the principles of HACCP which may lead to the development of a HACCP plan. So, going through the advisory committee's nine principles, and so on, they may result in a HACCP plan, and I think that we should leave that process to that group to determine how best to apply it.

On the other hand, GMP, of course, is essential as the foundation of HACCP, and those principles of GMP should be applied also. Those should be applied across the board. HACCP may be built on top of it.

DR. NAGLE: I think as we have discussed a little earlier today, you know, we are talking about a widely

disparate industry, we are talking about a group. I think we need to get to the base level. We all believe that, you know, GMPs and SSOPs are all important bases for HACCP, and that you can't really build a good HACCP plan unless you have all of those things in place.

I think we need to keep encouraging the development of those building blocks for the industry, so that then we can use -- having those in place, can then go in and truly identify what appropriate critical control points can be developed.

DR. BUCHANAN: Let me rephrase this another way. The ISGA recommends in their documentation, encourages the development of HACCP systems for all sprout growers. Should we encourage the rest of the sprout growers that are not members of ISGA to take the lead of this segment of their industry?

DR. NAGLE: I will comment sure, but the other part is, the other thing we have to do is we heard people over the last two days reminding them that they are not just agricultural growers, that they are food processors, and we need to make sure that they understand CFR Title 21 first before they develop a HACCP plan, because otherwise it's like trying to teach somebody how to climb a mountain before they learn how to walk.

DR. GOOSBY: I would comment in concurrence with

what Nancy just said. It is an evolutionary process to get from no real evaluation or agency inspection programs all the way to a HACCP based system, however, by encouraging the support of ideas and recommendations by ISGA to be more broadly embraced by the entire industry toward the ultimate goal of looking toward a HACCP size based system.

DR. BUCHANAN: Nicely put.

DR. NAGLE: One more comment. I think we could also encourage the sprout growing industry to look at the pre-cut salad industry as an example. Ten years ago, they were in a similar situation as this, that they were a widely disparate industry that really didn't have a lot of things in place, and I think as what we have seen is that the pre-cut salad industry is doing a lot on their own to make sure that they haven't had any problems, which if we look at the data, we notice that pre-cut lettuce salads are not -- pre-cut, pre-packaged lettuce salads are not on the list of things that have had significant outbreaks.

So, you know, they could take an example from there because those guys all thought they were just farmers, too, when they were first cutting lettuce. Well, I am just chopping it, I am not doing anything else, it's the same old stuff that it was before. So, I think that is a good way for them to go.

DR. SWANSON: While I fully believe that HACCP

must be established on solid GMPs, there is one particular part about HACCP that is different than implementing GMP programs, and that is the fact that the producer needs to understand what their hazards are.

If sprout producers understand that they do have this amplification process that is in there, just the exercise of going through that, so they fully understand what is at risk, I think is helpful, and for that matter, having them address HACCP does seem to make some sense, the principles of HACCP as Bruce indicated.

DR. BUCHANAN: Bruce.

DR. TOMPKIN: I am kind of stepping back and looking at what is going on. I have been through a lot of issues over the years with trade associations as problems were identified, whether it was Salmonella and fermented sausage or cooked meat patties, roast beef, and so on, and in each of those instances, the industry developed a guideline that was distributed to all the processors or members of the trade associations as an educational tool to bring up the industry.

I don't know, I haven't received anything in this material that has a rough draft, for example, from the Sprout Growers Association that suggests that they are essentially developing their own guidelines based on their knowledge because they know what they are doing.

I would hope they are not relying on the Federal Government and this panel to tell them what to do, because they will really be in trouble.

DR. BUCHANAN: Bruce, the ISGA has provided us -- and I am sorry you didn't get a copy of it with some of the historical information since we have been at this for a little longer than you -- they have several documents they have provided us.

But that does bring us up to the next subject, Terry, I want to make sure we cover before we take a five-minute break, and that is education. I would like to assess and then get any recommendations you have about education, and in this, I am looking for education in its broadest sense. This is education of consumers, producers, regulators, whoever.

Do we have educational needs and what are they, and who should they be addressed to? Comments.

DR. FARRAR: We certainly have educational needs in the sprout-growing population, at least in California. We have severe deficiencies in knowledge and awareness of SOPs and GMPs, so that is a critical missing link right now that we hope to remedy in the short term, sprout specific training, not generic food safety training, but sprout specific training.

DR. BUCHANAN: Lee Ann, do you want to scribble

that down on your pad as one of the recommendations. Unless I hear something to the opposite effect, that sounds like good solid advice. I see everybody nodding their heads.

How about other than the producers? I haven't heard anything for consumers. Do I gather that you don't consider that as high a priority or as efficacious, of if you feel that if you train the sprout producers, you will largely eliminate the problem?

DR. DOYLE: We have already put out an advisory to the high risk populations. What more would you see needs to be done in the area of educating consumers?

DR. BUCHANAN: Was that sufficient? Do you feel that that type of a notification was sufficient to meet the needs for educating the public?

DR. NAGLE: We know Caroline does not think it was sufficient.

DR. FARRAR: In the way of consumer notification, would the committee entertain a recommendation for a minimum of "keep refrigerated" marking on packaging?

DR. BUCHANAN: Is there any indication scientifically that "keep refrigerated" will help?

[No response.]

DR. BUCHANAN: I am getting no overwhelming response. Everyone is looking up at the ceiling.

Larry.

DR. BEUCHAT: Should we go back in terms of education to seed handlers, seedsmen, even to growers in terms of bringing them into this process, the total system in terms of education, not just beginning with the sprouter?

DR. BUCHANAN: That is a good point. Would you agree with that, Jeff, is the need similar there?

DR. FARRAR: We are back to lack of science based understanding of good agricultural practices, but yes, a general education in those broad policies wouldn't hurt. Our situation in California is we have basically one seed supplier in the entire state. It would be a small audience, but it is important nonetheless.

DR. BUCHANAN: It's really high efficiency, high attention. That is the kind of student-teacher ratio you always dreamt of. I guess at that point it is actually mentoring.

I am going to have you take a five-minute break and then we are going to come back and do research.

[Recess.]

DR. BUCHANAN: We are going to start the last section which is going to be the identification of research needs and then also trying to give some relative priority to when these things should be done.

I just want to re-verify what we are going to be doing here as the Produce Working Group of the Advisory

Committee. Lee Ann and I will go back, take your comments, and we are going to draft up a working group series of recommendations which we will submit to both the full committee in time for its next meeting and we will be also submitting it as part of the public comment coming from this meeting to FDA right now. This is just in keeping with the procedures that we have used before in these kind of public meetings.

So, you will be seeing quickly, after this meeting, the comments, the draft. We would appreciate that you get it back to us immediately and then we will be providing it to the FDA as part of the record of this meeting.

Research. I think we all have come to the conclusion that there are a number of things associated with the microbiological safety of sprouted seeds that require additional research. I would like to get at least some of the broad areas identified and then determine in which order we should be addressing them with the resources that are available, and if you know where research is currently taking place that we have not identified here, we would also appreciate you letting us know where this is taking place, so that we can attempt to obtain any information from them and also to help coordinate the information, so that we are getting the most, at least on the federal side, from the

public investment into research. That is one of our objectives is also to make sure that we are doing research cost efficiently.

Research needs. I throw it open to all of you researchers out there or you people that need researchers, and maybe that is the place to start. We have got some people that need research -- I can see Jeff just waiting to get at that microphone. Jeff.

DR. FARRAR: We certainly fall into that category of people needing research. I think this item falls both into the future research and thing that can be done short term, Bob. That is the potential development of a sprout outbreak investigation questionnaire that all states could collect similar bits of information in these outbreaks, information on all aspects of sprouting - sprout facilities that may be implicated in outbreaks, seeds used, a variety of seeds, type of seeds, disinfection methods, a generic template that each state could perhaps add onto with additional questions, but a minimum amount of information collected across all states, possibly even going back to past outbreaks, as well.

DR. BUCHANAN: One of the areas that we heard about today, we had the state of the science on detection methods, and one of the lessons that I have taken home when dealing with E. coli 0157 in sprouted seeds or any commodity

for that matter, is that it occurs at very low levels and very sporadically, and current systems for taking samples tend to focus more and more on smaller and smaller samples, and I think that we can take a larger sample or to be able to remove a sample and concentrate it even more than we now have with the immunomagnetic bead capture systems would be extremely helpful.

I would like to make a note here, and that was identified as a FDA priority need this past year in our solicitations for proposals. It was also solicited as a need by CREES in their proposals, and neither organization found the submissions really to the point, that we need to encourage people to be exploring how to concentrate samples microbiologically or to be able to look at larger and larger samples, so that we can get better data.

Certainly, the sampling techniques for sprouted seeds and the methods we do have some concerns that they are not up to the needs we have unless we take them and literally sprout the seeds at this point.

DR. NAGLE: I was going to say, to speak for Katie, you know, what she was saying before is that that method of using 50 pounds of seed, you know, whatever, growing out a whole thing of seed, we may need some work in that area of trying to find what is the best way to do something like that, so that you can have a really broad

sample.

DR. BUCHANAN: Which I might note if you want to go all the way to sort of the Buck Rogers type approach, theoretically, the runoff from a sprout operation in conjunction with a biosensor is like an ideal application where you could just literally continue to monitor all of the water all of the time. So, certainly this is an area that has a lot of potential.

Additional areas. Short-term needs. Let's talk about short-term needs. Bruce.

DR. TOMPKIN: It seems like the short term could be the disinfection of seeds at the sprouter level, and essentially come up with a procedure, and there is one already, and that would be helpful. There was concern expressed yesterday in particular over the discontinuation of research on chlorine dioxide, I think it was -- or ozone, excuse me, in Beltsville, and if that really has merit, and the problem of this nature, it seems to me that it should be supported rather than -- they should be doing more rather than less. I don't know who is going to pick that up.

DR. BUCHANAN: I might note the immediacy of your input on this, your list that you come up with will also be taken out to the meeting that will be held at the National Center for Food Safety and Technology on the 5th to discuss research needs. This list is going to go out there. So,

there will be some immediacy in your recommendations.

DR. TOMPKIN: I think the priority should be given to a means to decontaminate the seeds where we would have a high level of confidence that upon sprouting, they would be negative, that is, the seeds per se would not be a source of these pathogens.

DR. DOYLE: Can I add to that? It is more of a long-term approach, but it fits with this. I think we need to better understand how these seeds become contaminated by pathogens, is it an internal contamination or an external contamination, learn more about how they locate within these areas on the seed, and define what surfactants, if at all, might be best for removal.

DR. GOOSBY: Talking about the seed, too, and the interventions to sanitize it, maybe it is obvious, but to clarify, the consideration of research for combination interventions, if, in fact, the liquid sanitizers in conjunction with radiation or any groups thereof, offer greater potential for having a sanitary seed than any single treatment by itself.

DR. KVENBERG: I think one area as far as applied work that ought to be examined further is the quality of the seed itself. It is being assumed that all seed is equal. It may not be. We probably ought to look at the seeds themselves and see if there if variability in addition to

the organisms.

DR. BUCHANAN: The other one that we had identified earlier was the effect of scarification.

DR. KVENBERG: That is where I was going.

DR. BUCHANAN: Right.

DR. KVENBERG: Or any other variable differences that might be adjunct to that.

DR. GOOSBY: Along the line John is saying, the viability of the seed, considerations of such factors as storage conditions and age of the seed, perhaps attend somewhat to what it will stand up under as far as treatment procedures.

DR. KVENBERG: That may be a mid- to long-term. We may have to get into survival. Storage conditions of the seed came up, cold storage, warm storage, dry heat, all those things which I think are time studies. Survival is the issue I guess here.

DR. TROXELL: I was going to add on the dry heat is certainly something that looks like will be done in a relatively short time to find out what the log reduction and viability would be.

DR. KVENBERG: Just on a practical matter on the premise on heat, as long as we are on the subject here, also, is pasteurization, the question came up on the feasibility and cost. I think the question is what are the

operating parameters that really you need to get to irrespective of current systems that may be out there, what do you need to do to accomplish it, and then you worry about cost applications.

If you have got either steam applications or high heat, plus agitation, in a water system, that could explored probably in the short term, non-chemical treatments.

MR. BERNARD: I would agree. I think the short term application of lower temperature, longer time, which to me is a relatively inexpensive way of applying a pasteurization treatment to the seeds.

A literature search could look into D-values, times and temperatures to give us an idea whether it is feasible or not. You know, we don't need specific data, but we need general data to see if we are in the ballpark.

What I think needs to be done, although in conjunction with that, and relatively immediately, is the time and temperature effects on the viability of the seeds themselves, and if those two don't match, then, you begin to look at high temperature, short time alternatives, a more expensive way to do it, but certainly effective.

DR. BUCHANAN: So far we have focused on the seeds themselves. I know Bill is not here. He had to head for the airport. But I know one of his points was that you have your primary control at the beginning of the process, is it

worthwhile to be exploring technologies for actually treating the sprouts themselves.

For the purposes of the transcript, Nancy Nagle, who is standing in the back of the room said yes, that it is very important. Thank you, Nancy. Have a good trip home.

DR. DOYLE: Also, in between, if we could develop approaches where we could control the growth of pathogens during the sprouting process.

DR. BUCHANAN: Right, reduce the amplification.

Are there other research areas that are unique to sprouts that we need to be considering?

DR. KVENBERG: Maybe it is a subset of what Mike just said, but it would be a review of the process themselves and equipment and design as it contributes.

DR. BUCHANAN: Do you have some specific aspect of equipment design that you thing we should be focusing on?

DR. KVENBERG: Well, there is the two processes I am aware of, and that is the nested tray operations and the tumblers, which is the only real processing that is going on is the outgrowth of the sprouts. See if there is an amplification step or reservoir step, blind spots, or recontamination, whatever, that would be a subset of reducing the likelihood of amplification or an intervention step that could be imposed on existing equipment.

DR. BUCHANAN: You say you are looking for better

sanitization of the equipment, easier to clean.

DR. KVENBERG: Right. Above all, do no harm, but hopefully there will be something that may be evident in the equipment that can actually improve the situation that hasn't been applied.

DR. BUCHANAN: Janice just reminded me of calcinated oyster shells, and to take a look at that under conditions that are more typical of alfalfa sprout operations here in the United States and its potential.

DR. TOMPKIN: Along those lines, we did have a lot of questions as to whether or not it would be possible to control the conditions of sprouting in such a way that we would essentially control the growth. We talked in terms of pH, temperature, temperature of sprouting, and so on, essentially build in some hurdles to limit the growth of enteric pathogens, and still have an acceptable sprouting process and product.

DR. BUCHANAN: I am losing my panel. I am trying to do this quickly. Talk about GAPs. I am just going to start at one end and move my way through in terms of research needs.

Is there any additional identifiable research needs at the production end of the seed itself, that is, out on the farm?

[No response.]

DR. BUCHANAN: In the transport and mill operations of the seed as they are being cleaned?

DR. TOMPKIN: I think they have been expressed. We have talked about it.

DR. BUCHANAN: Anything in how seeds or after they have come from the mill, are transported, stored, handled, et cetera?

[No response.]

DR. BUCHANAN: Anything additional on the treatment of seeds after they have come into the sprout operation?

DR. GOOSBY: We have identified that as a good research area.

DR. BUCHANAN: Right. We have identified several issues related to the germination and outgrowth of the sprouts themselves. Is there anything additional on the packaging and distribution of the sprouts after they have been produced?

DR. TROXELL: The one thing on the spent water to identify what good indicator organisms, cheap indicator organisms, I know we have talked about that, but we haven't said it again here.

DR. BUCHANAN: We have identified that as a research need and also as a result of our survey.

DR. TROXELL: Right.

DR. BUCHANAN: Anything that has to deal with the general environment within the sprout operation? Any additional needs associated with the distribution and marketing of sprouts?

DR. GOOSBY: We just need a package that continuously liberates the bactericidal gas all the way from the final packaging to the consumer.

DR. BUCHANAN: David's wish list.

DR. GOOSBY: In an idea world.

DR. BUCHANAN: Right. Finally, anything additional that we should talk about on the ability of the consumer to use these or anything that would help the consumer use them more effectively?

DR. TOMPKIN: I haven't thought it through, but I think that in terms of your understanding from a risk assessment standpoint, you would need to know as much as possible about the consumers, how it is used, so that you can come up with a better grasp for the exposure assessment. Isn't that would be what you would go after? What impact those factors may have.

DR. BUCHANAN: We haven't had any discussion at all that I know of on interventions that can be used in the home other than the five-second boiling of radish sprouts.

Is there any additional information needed to these types of interventions?

DR. TOMPKIN: Is the agency going to undertake a risk assessment for this product?

DR. BUCHANAN: Certainly at this point we are attempting to gather the scientific information that we would need to do a risk assessment. That is one the primary needs that we have in terms of identifying the research.

Seeing no additional comments, I am about ready to turn the meeting back over to Janice Oliver. Do I have any additional comments or recommendations related to research or overall recommendations by the panel on this area in light of the meeting that has taken place here? Terry.

DR. TROXELL: Would we benefit from some research on washington of the sprouts by consumer? We heard a lot about the relative numbers of bacteria that the Japanese had in their studies on the external versus internal, but most of them seem to be external, so if consumers wash them, they might knock the levels down some and could knock them down to a level that wasn't infective for some people anyway. Would that be something to look into?

I am posing the question pretty much to the leftover panel.

MR. BERNARD: As a leftover panelist, I think it is worth a look at what strategies might be able to be used at that level.

DR. BUCHANAN: Certainly anything that empowers

the consumer we think is worth the research investment.

DR. TOMPKIN: Could we position that in with other fresh vegetables, and so on, such as lettuce, and so on? Sprouts have been used for quite some time, and to come up with a specific recommendation to consumers to treat sprouts in such and such a way, actually doing the research is worth doing, what can be accomplished by rinsing, is it of value and how best to do it, but you might keep it in mind that this could be applicable to other ready-to-eat materials and position it in that way so that it has broader application.

DR. BUCHANAN: Excellent point.

MS. OLIVER: It is also applicable to the retail industry.

DR. BUCHANAN: David.

DR. GOOSBY: Memory fails me on this question. I recall that some concern was raised about labeling "for refrigeration only," and we I think responded that we weren't sure what the science says on that.

Did we ever reach an answer to that and in the retail environment, as well as in transit, if that question was answered and I wasn't listening, good, or if not, I would suggest that is a research agenda.

DR. BUCHANAN: Larry has had his hand up.

DR. BEUCHAT: Just to continue along the discussion of treatment near the end of the system in terms

of time between treatment and consumption, either in a food service setting or in the home, those interventions aren't necessarily dependent upon the success of those, storage for any period of time between that treatment and consumption. That is, if quality is going to be affected, say, in three or four days, that is not at that point as large a factor to consider. I guess what I am saying is those interventions I think should be part of this total system from the seed from the field to the point of consumption, food service setting or in the home.

DR. BUCHANAN: Dane.

MR. BERNARD: As you progress with what ever risk assessment, whether an extensive one or one to just get through, that you go through here as part of risk management strategies that you consider once you get to that point, I think some research on the effectiveness of messages and on specifically whether information provided through health care givers, dietary advice, so to speak, might be an effective management strategy or part of an overall management strategy.

DR. BUCHANAN: As my panel just started to get even smaller, I am going to turn the meeting back over the Janice Oliver.

MS. OLIVER: I think the meeting is about over and the panel has about dissolved, but I would like to thank you

all, but what I basically heard was that multiple interventions are needed and that not one intervention will do, and that the panel is recommending interventions at three different steps - pre-seed, seed, and at the sprout, or in the pre-seed the only thing you are recommending in good agricultural practices is looking at fresh manure, and Bob will work on writing up something there, but at the seed, you are looking at multiple interventions.

You are looking at irradiation, but it is not approved right now, and you are looking at use of calcium hypochlorite, some type of liquid intervention at the processor, 20,000 parts per million is what I hear the recommendation, and applying the principles of HACCP is important especially so that the sprouters know what is happening during the sprouting operation, that the microorganisms are growing at a rapid rate.

Education is important for the sprout growers and for the seed producers, and that GMPs, everything is based on GMPs and SSOPs, that is a basic assumption that you are going with, besides the research areas which go from -- I think the beginning through the end, they are multiple. I think most of them you have cited here, and some others I think were cited even during the meeting.

I would like to thank all of you for your time. Bob will draft up the recommendations and pass it by all of

you to make sure you agree, and I would also like to thank all of others who stayed and gave us their advice, their presentations, and talked to us in between breaks, and all.

Thank you.

[Whereupon, at 4:42 p.m., the meeting was concluded.]

CERTIFICATE

I, **THOMAS C. BITSKO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

THOMAS C. BITSKO