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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING
OCTOBER 13 - 15, 2004
ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF PLANT-INCORPORATD PROTECTANT (PIP), SPECIFICALLY THOSE BASED ON PLANT VIRAL COAT PROTEINS (PVCP-PIPS)

THURSDAY, OCTOBER 14, 2004

VOLUME IV OF IV

(Afternoon session)

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C O N T E N T S

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A F T E R N O O N S E S S I O N

MR. ROBERTS: Let's reconvene and where we left off at lunch we're going to give Dr. Kramer the opportunity to see whether over lunch she had another way, another follow-up question or another way to phrase the question on 16, so let's check with Dr. Kramer.

DR. KRAMER: Thank you. I just wanted to

start out and address a concern of Dr. Tepfer's and just to say that we are not asking the panel to judge what an acceptable level of risk is, we're really trying to focus our questions to get an idea of what the level of risk is both the frequency and the hazard, and then that really brings me to the question of maybe what we've been doing is trying to draw too narrow of a comparison and that is that we were asking a comparison between a PVCP-PIP transgenic plant in some cases in a non transgenic counterpart or a PVCP transgenic plant in a mixed infection.

And when we're talking about the hazard that might result from a viral interaction in that circumstance what we're thinking of is the creation of

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a new virus, the creation of a virus is some kind of ultra transmission property that could be a concern. And really when we're trying to draw a very narrow comparison I sense that there's some disagreement on how much that level of risk rises above that narrow comparator, but what I'm hearing is that there actually is a very broad circumstance under which we can produce that same hazard that can be reduced in a lot of different ways, not just through viral infections.

And when we take this question in that broad context in the situation in the world today in which we're producing lots of new viruses, viruses with new transmission properties in different ways if we take that as our baseline how does the level of risk associated with these products rise above that. I guess I'd start off is that maybe is that a more appropriate comparator, is that something that you're more comfortable with or would you prefer to stick with the comparison to a natural mixed infection first off and then could you maybe try to address somehow qualitatively how the risk changes.

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MR. ROBERTS: Dr. Kramer do we want to tackle that in the context of this question or do we want to do that at the end?

DR. KRAMER: I think it's really in the context of this question because it is related strictly to the viral interaction discussions, not the gene flow concerns or the later ones.

MR. ROBERTS: Fine, let's see if somebody wants to step up to the microphone and respond to that, Dr. Falk.

DR. FALK: If I understood your last couple of sentences what you said there is are we more likely to get new different viruses as opposed to the viruses that we're generating already that are occurring already.

DR. KRAMER: Right. But the question is I mean this whole discussion has been framed in terms of the comparison to what happens in a natural mixed infection but of course new viruses are introduced in

the areas maybe not created new but carrying viruses around the world in many different types of ways and maybe in that context how does this level of risk

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compare to that, is that why I sense there's some -- I'm wondering that's why there seems to be disagreement among the panel members where some members are sticking to the very narrow comparison of a natural mix infection or a non transgenic counterpart where others are really looking at more broadly in terms of the types this hazard that places us through many different avenues.

DR. FALK: Well in that context I would say that it is not, does not have more potential to create different or potentially more damaging viruses, that's what you're asking.

DR. KRAMER: Right.

DR. TEPFER: When I think that if you're looking at facing new viral problems and the arrival of plumb pox virus (ph) in North America is an excellent example of a big problem. So I find it a little bit difficult to make sort of comparisons as to what is the magnitude, is that worse than or not than another type of new virus problems that could rise.

One of the sort -- I think we have two very distinct categories of new virus problems, ones that

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are introduced and ones that emerge more or less de novo, I mean there's sort of a graze on in between as well. So in the case plumb pox I think that it was clear that this is something that was unfortunately likely to occur because it is a well-known virus, widely distributed in Europe, absent in North America, international commerce is such that it was going to arrive one of these days.

But in terms what are the cases of truly emergent viruses that have come about by new combination events or new interactions with vectors or new other sorts of biological properties, the information data base is relatively small. I mean I think that it is very difficult to demonstrate that a virus is truly new, it may be the first time that you have noticed it or there may be some subtle change in condition so that it suddenly starts to infect a commercial crop and suddenly it's a big problem, they may not have been new at all, so I think that in a way I think it is a much more difficult comparator to deal with because of the narrowness of the data that we have on new viruses and new virus problems.

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MR. ROBERTS: Other viewpoints, Dr. Sherwood.

DR. SHERWOOD: I would say that I don't see that this technology is going to result in anymore new or altered viruses anymore than deployment of resistance genes through normal breeding practices, and certainly there are lots of examples in the literature where resistant genes have been short lived

in regards to providing plant protection.
DR. MELCHER: I can agree with the rest of them that it is very, very, very unlikely that there would be something absolutely strange to come out of using coat protein transgenes, however I am reluctant to say absolutely nothing is going to happen, that there is -- I don't know it could happen, but not very likely.
MR. ROBERTS: Dr. Hammond.
DR. HAMMOND: We have seen the emergence of new viruses from wild vegetation as we have planted new areas in many, many instances. Over the past 20 years we have become aware of a number of whole new virus groups that we did not see before, as far as I can recall the cronie (ph) viruses we were not aware

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of 20 years ago and pelo (ph) viruses, offeo (ph) viruses, there are a number of virus groups that we were not aware of 20 years ago, in retrospect these may have been responsible for some virus outbreaks that were observed and unexplained and certainly there's a probability of new viruses being discovered especially in woody part species which have been relatively little examined and where there are still virus or virus-like diseases that have not been adequately identified.
We still have a lot of viruses to find that we don't know about. I think that many of those are of much greater concern than the probability of new viruses arising from recombination with transgenes and there will certainly be new viruses arising from recombination and mixed infections, so I think this is a minimal issue, I'm not concerned about it.
DR. SHERWOOD: Just to add to Dr. Hammond's list that the tospo (ph) virus isn't until 20 years ago or even less than that was limited to one species, tomato spotted wilt virus and now we have some 12 to 14 recognized species which obviously is an artificial

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system but at least differentiated based on some standard set by virologists, so there continues to be as we've said an emergence or at least finding of these new viruses.
DR. ALLISON: Finding new viruses is often times just a matter of going into an unexplored area and looking for them, there are plenty there and the direct comparison between a new virus being discovered that way and a new virus forming is that they're not comparable really because the viruses that are already tried and true in some host are probably much more likely to be responsible for some sort of damage than one that has to go through a complete evolutionary passage as a recominant (ph) in order to become a good pathogen.
DR. HAMMOND: Let me counter that with the example of the gemini viruses and the tosvo (ph) viruses where there has been enormous diversity

arising largely as the result of movement of vectors and movement of viruses in crops that have resulted in the emergence of re assortance and evolution of new isolets either through re assortment or through

recombination followed by evolution. In both of these virus groups tosvo viruses were essentially extremely limited distribution in the United States, there are now eight present here, more present in other parts of the world.

The increase in number of known gemini viruses and the increased diversity has been phenomenal over the last 15 years and this is primarily due to their ability to move into different crops as a result of the spread of the vectors into the regions where they were not present before and the new biotype of the white fly for the gemini viruses.

DR. MELCHER: Part of the increase in the number of gemini viruses is also due to the fact that people have been looking hard trying to delineate how many there are, so I agree with Dr. Hammond but there is also the fact that we are looking for them.

MR. ROBERTS: So not to words in the panel's mouth, but it sounds like what the panel is saying is that deployment of this technology was not impossible that it would result in the appearance of a new problem plant pathogen, panel considers it to be

unlikely or quite unlikely, however you want to qualify unlikely, is that correct?

I mean we're not saying that it's impossible based on the previous technical discussions, but at the same time but the panel considers it to be extremely unlikely, quite unlikely. I mean you can qualify it however you want, but unlikely that practice will result in the appearance of a new problem pathogen, is that correct, did I get it right or close? Dr. Allision you want to --

DR. ALLISON: I would say the unassisted, that is non PVCP whatever evolution of viruses the unassisted okay, that will probably develop far more viruses that are important to us than this particular geamo (ph) related approach.

MR. ROBERTS: So it's unlikely to result in a significantly greater risk of appearance, so the baseline is sort of the natural processes by which these viruses appear.

DR. ALLISON: Yes.

MR. ROBERTS: Is there any other discussion, does that seem to be the general opinion of the panel,

are there other opinions, differing opinions?

Dr. Kramer is that reasonably clear, do we need to phrase it another way or is there other information related to this that you need to get from the panel?

DR. KRAMER: I mean I guess if I were just to

translate that back to question 16 the answer would be that none of those conditions would be necessary, not to guarantee that nothing would happen but such that the level of risk would not rise above what's already there.

MR. ROBERTS: Does the panel agree with that statement? I would say many nodding heads indicate that the panel agrees with that statement.

Should we go onto the next question, let's go onto 17 then.

DR. KRAMER: To what degree and in what ways might a PVCP gene be modified for example through truncations, deletions, insertions, or point mutations while still retaining scientific support for the idea that humans have consumed the products of such genes for generations and that such products therefore

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present no new dietary exposures.

MR. ROBERTS: Dr. Gendel, this is going to be a very interesting question, I can't wait to hear your response.

DR. GENDEL: I'm sort of tempted to expedite the opportunity for the virologist to get back to talking about how many genes can dance on a capsid (ph) by saying if the question is to what degree the answer is not too much, but somehow I have this feeling you guys want a little more detail than that, so let me go ahead with what I've got written down but before I get into the heart of the question I want to make two points of context.

The first one is something which has been more or less implicit explicit in everything we've been doing but I would like to make it explicit which is to say we're only going to consider PVCPs used to control diseases in food plants, the PVCPs that are modified for the sake of what is known as biofarming with a PH are not being considered here and that's a whole separate issue.

Second, from the point of view of human

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health concerns it's only expressed protein products that are of concern, situations only involve neuclayic (ph) acids are not of concern in human health issues. So the question is posed by the Agency is based on the generic assumption that PVCPs are safe because there's a history of safe consumption. The Agency in the original form of the question sited some literature examples of that in expert consultations in support of the assumption.

As a non virologist and somebody who is not really intimately familiar with the field my major concern with this assumption is in regard to whether or not the data that exists can be generalized from the specific example to all virus families, is there enough evidence for a wide variety or viruses that we might be exposed to to allow that assumption to be generalized or are the published data really to only

serve the specific families. I don't know the answer, it may be that some of my colleagues do, but that was a concern that occurred to me especially because history has shown that occasionally the widespread consumption of food types that previously have been

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rare have resulted in unexpected consequences and one of the classic examples of course was the spread of allergy in kiwi which was unknown in this country and I guess in some parts of Europe until economic and transportation conditions resulted in the widespread occurrence of kiwis in grocery stores and a few years later widespread occurrence of allergies that no one had heard of before, so it's just something to thing about in terms of exposure, how exposure affects the validity of your assumption.

So the question is posed by the Agency asks how much change can be introduced before the safe history assumption is no longer valid. In this context only changes that affect an express protein are of concern, changes to regulatory are non translated regions of a gene are not relevant. In my opinion the answer to this question needs to be considered in relation to the natural variation of the individual virus, how much variation occurs in the natural population, what are the relative frequencies of point mutations insertions and deletions, are there hot spots for these types of changes in the sequences.

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In other words the correct question may not be how much an individual protein has been changed, but how the change that you see relates to the range of different aleels (ph) that are seen in the virus population, changes that can be considered to be within the bounds normally found in the viral population can probably be considered to be as safe as the initial viral coat protein.

For changes that fall outside this range however you would define, there are as far as I can see two potential health effects that might be of concern, one is the generation of direct protein toxicity and the other is allergies. I'm not aware of any examples where plant viral coat protein is known to be a human toxin and again I defer to the experts in virology if I'm wrong on that. It's also difficult to see how the kinds of changes we're talking about here could result in toxicity in an unknowing matter since most forms of protein toxicity actually involve some extremely specific interactions, so it seems unlikely that this is going to be an accidental consequence of a change that is being made for other

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purposes because you need to target the specificity for that to happen.

So that leaves allergenicity and allergenicity is the most difficult issue because it is not yet clear why only a few of the thousands of

food proteins that are consumed each day become allergens. Searches of several allergen data bases including my own failed to find any viral proteins that have been identified as allergens. I didn't have time to do the inverse search which is to compare each member of the allergen data base to the set of know PVCP sequences, but doing so might provide some further assurance of safety and that if there's no apparent sequence relationship there is not much possibility of there being allergens.

Regardless it seems to me it would be a simple task to as a developer to apply the same allergen assessment procedures that are used for other viral engineered foods and other PIPs to highly modified PVCPs to provide some assurance that there's no potential cross reactive sequences. The procedures are straight forward in a lot of ways, in the last few

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years they have become much more standardized. It would not be a very serious burden to ask the same analysis to be done for these. And in the future structural analysis sequence based by informatic structural analysis might also be carried out as the data base on allergen sequence structures improves.

Further, a point relative to this, it's generally accepted in the field that the level of exposure plays a critical roll in allergenic sensitization, that is not in how people react to an allergen but how they actually develop the allergy in the first place, and it seems to be a pretty common thing that you become sensitized only to proteins that you're exposed in large amounts. So as long as bioengineered PVCPs are being expressed at levels below those naturally found in plants sensitization does not seem to be very likely because the exposure level is so low.

Finally I would like to suggest that another reason to catalog the degree of variability found in natural virus populations is get an estimate in how much a PVCP can be modified and still be functional in

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the context of virus disease reduction. Given the various kinds of structural interactions that are involved in all of the functions that PVCPs play it's likely, although this is just a guess, that a modified protein would prove to be ineffective for its role disease control long before those changes are likely to result in concerns in human health, and that's it.

MR. ROBERTS: Thank you. Dr. Hammond do you have comments to add?

DR. HAMMOND: Yes, I do. Firstly I believe that there is little point in making extensive efforts to ameliorate perceived problems of a wild-type coat protein. In many cases resistance obtained by our (inaudible) resistant mechanisms primarily post translation or gene silencing is superior to that observed with protein mediated resistance and in many

instances the resistance conferred by expression of coat proteins genes have been shown to result from post translation or gene silencing rather than a protein mediated mechanism, although protein mediated mechanisms do appear more effective at conferring limited resistance to related viruses or virus isolets

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whereas post translation or gene silencing may be isolets specific in some instances.

Removal of the varian (ph) surface exposed amino and coboxi (ph) termini from coat protein to (inaudible) viruses does not affect the overall structural integrity of the coat protein, but can still confer significant resistance and that's documented in work especially from the Dougherty Lab. Untranslatable or antisense coat protein constructs also confer highly effective resistance but confer aren't mediated rather than coat protein mediated resistance and that's not relevant in this context .

The use of coat proteins from natural deletion mutants lacking the amino residues involved in aphid or other vector transmission or laboratory mutations of other residues is recommended. Oblation of RNA binding sites as demonstrated for plumb pox virus by varalm (ph) in mice will effectively mitigate the possibility of heterologist (ph) encapsidation and also reduce the probability of a viable recombinant being transmitted out of transgenic plants.

In some other virus groups such as lutea (ph)

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viruses coat protein constructs lacking the read through domain will lack competency to affect transmission if heterologists encapsidation or recombination occurs. Alteration of amino acid residues at subunit subunit interfaces may interfere with particle assemble but may also have the potential to induce a hypersensitive response in some hosts as is the case with some tobacco mozaic coat protein mutants and that's worked from Jim Colver's (ph) lab.

Coat protein constructs should avoid inclusion of subgenomic RNA promoters which might increase the possibility of recombination. Now there is documented a high degree of variability in amino sequence between the coat proteins of different isolets of the same virus. In the case of podee (ph) viruses especially within the amino terminus as seen and documented with for example from plumb pox virus, papaya ring spot virus and turnip mozaic virus, and that's been fairly well documented in the book, the Podee Variety edited by Shukler, Ward, and Brunt (ph).

Anything less than a major mutation is thus unlikely to differ significantly from the variability

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extent in viral populations. Now we consume plant viral coat proteins and all of the other viral proteins in many of the foods we eat. A large number of viruses are easily isolated from (inaudible)

purchased in the supermarket. I have myself isolated viruses from store bought tomatoes, peppers, cucurbit, and I know of viruses have been isolated from potatoes, asparagus, celery, and other store bought (inaudible).

A number of virologists at the Rotham State Experimental Station Bazel Costanis and Borden (ph) and others who had handled and mouth pipetted (ph) many purified virus preparations over many years collected their own blood and assayed it for antibodies against any of the common viruses which they had used. They had found no virus specific antibodies against any of these common viruses. This suggest that there's no significant probability of harmful response for any normal food consumption of transgenic plants especially as the levels of transgene coat protein will typically be lower than in an active virus infection.

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And while Dr. Gendel was talking I had an additional thought in that there are several plant viruses that are currently being used in clinical trials for vaccine productions expressing epitopes or fusion proteins of vaccine significance. And among these viruses the capee (ph) mozaic virus, tobacco mozaic virus, alfalfa mozaic virus, zucchini yellow mozaic virus, potato virus X and possibly tomato bushy stamp virus, so these are representatives of at least five different virus groups and there have been no problems with these.

They make good epitope presentation systems because they present the vaccine related epitope in a semi-regular array which stimulates the immune system to a higher degree than free sub unit. And also for vaccine purposes it's been shown that it's possible to reuse the same virus coat protein as carrier expressing a different epitope and to get a response to the second epitope that is displayed upon the same carrier molecule, so it does not appear to be a tolerance against the carrier coat protein, so again this indicates that it's not likely to be a problem

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and not likely to be an allergic and allergen and there have been no problems with normal intake of plants viruses.

The only instance that I'm aware of allergenicity of a plant virus is a case in which an isometric plant virus was spray inoculated on many occasions and the individual who did that did develop an inhalation allergenicity to that virus and ended up with something close to enophilatic (ph) shock, but that is an isolated incident and is certainly not related to food consumption of plant viruses.

DR. MELCHER: While I agree with Dr. Gendel on allergens and toxins and Dr. Hammond on allergens I think perhaps my perspective is enough different that I can present my prepared remarks. I will also have a

third concern to raise which I will deal with at the end, so what I had to say was that the possible harmful effects of human health that could be generated by modifying PVCP genes fall into three categories, increase potential for the modified protein to serve as a potent allergen, possibility that the modifications could lead to a toxic protein,

and the possibility that the modified protein will modify the network of regulation of metabolism to lead the plant to produce substances harmful to human health and I will look at each of these separately before looking at the predictability of harm from these modification, so the parts of a protein that are most likely to be antigenic as Dr. Hammond's pointed out at the parts that are exposed on the surface of the protein and especially on the surface of the verium (ph).

These are also the parts of a protein that are most likely to be variable among isolets of the viruses as Dr. Hammond pointed out. There are fewer constraints on variation of surface exposed residues that's evolutionary constraints than on residues that must interact with others to form core structures or with other sub units or the neuclayic (ph) acid gene formed on the varyon (ph) particles. Thus it's not likely that point mutations or even small insertions or deletions will be changes that have not been explored during the course of virus evolution because of the variation, including during the period of virus

evolution that includes the human consumption of plant materials that may contain the virus in question.

On the other hand it's not outrageous to suggest that some modifications may lead to potent allergens for some individuals, immune systems of humans vary in their ability to respond to particular epitopes both because of genetics and because of histories of exposure or non exposure to the epitopes whether approaching as an allergen or not depends not only on its collection of epitopes but also in the concentration that is encountered on the immune system that Dr. Gendel has pointed out.

Low concentrations and high concentrations induce tolerance, the modification of the PVCP gene is on that results in higher levels of PVCP and the consumed material and it's typical for natural infected material which I don't think is the case, the possibility exists that the rise in concentration moves the material from the tolerated zone to the allergenic zone, so that's a comment on allergens.

Modifying a protein to be toxin generally requires a large input of intelligent design on the

part of technologists, because it seems highly unlikely that modifications of the PVCP intended for ecological safety reasons might accidently lead to a

toxin. On the other hand many PVCPs have domains on their external surfaces that appear to not play direct roles in encapsidation, indeed a variety of additional functions have been attributed to PVCPs, we've heard about these already including intracellular, intracellular, and long distance targeting.

Some of these functions may be enzymotic. Alteration of the PVCP sequence may modify such enzymotic functions yet to be discovered so they would perform toxic functions as they were entering the body cells, others have discusses the potential for coat proteins to resist or will discuss the potential for coat proteins to resist digestion in the elementary tract. Beyond such survival attachment and are entering the body cells as necessary for an enzyme to perform as a toxic catalyst, some but not all of the PVCPs have domains that are responsible either by themselves or with other proteins for attachment to animal cells, specifically insect cells and it serves

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in transmission but it could possibly conceivable lead to introduction but I think this like most of the other scenarios that we've looked at are pretty rare ones.

Now the third on, the one that is novel in this discussion is that the modified PVCP whatever will interact with regulatory machinery of the plant altering it in such a way that the plant now produces substances harmful to human health. Plant viruses already interact with the plant regulatory machinery, alterations are often pretty profound lead to what we observe as the symptoms of disease. I'm not aware of any hazards to human health that have been associated with the consumption of plants with obvious symptoms of viral disease, but regulatory networks are just now beginning to be unraveled, experiments to date have revealed many unexpected associations, there will likely be more, so we can't rule out that changes may lead to production of harmful substances.

The probability of such changes is probably greatest for those plants for which human experience has already taught that consumption of some parts of

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the plant or the plant at certain stages of growth is dangerous. We regard stems and not leaves, some plants like to potatoes can under certain circumstances produce toxic substances like celenene (ph) there's also caster beans and so forth, so that's the third one and whether we can predict whether these are changes that will occur without doing experimental tests I'm not sure, but I think that like Dr. Gendel said that it should be fairly easy to assess whether there are such problems and there is no reason that they wouldn't be done by the company that's producing them to limit their liabilities. Thanks.

MR. ROBERTS: Dr. Cooper.

DR. COOPER: Well there's very little to add

really, it's certainly not an area of my special expertise, but I would say that harm might result from the virus derived protein other chemicals that present solicit in plants, and there's a consequence absolute safety in the judgements that modified proteins do not present new dietary hazards to humans or wildlife does not exist. There is very little knowledge on which to base the prediction except in perhaps point mutations

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are going to be very minor and undoubtedly occur naturally anyway. The two sorts of allergy, one of them is particularly well understood but neither of them are well understood. One of the IGE binding systems are perhaps with histamine released better understood than the cell mediated systems, but we know very little about either of those.

The shape of proteins are likely to be modified by the sorts of changes that are proposed and there currently is no single predictive test to define which proteins are allergenic before or after that sort of treatment. It is expected that the proteins ingested by animals will be altered by the digestive process and it's possible this can reveal new confirmations that were not present in the (inaudible) protein, but I'm not aware of any new data on structures of viral coat proteins that have been investigated after passage through the human digestive system, although it has been done, I know of at least one case that's published and I told Dr. Gendel about it.

Furthermore that I believe in the one

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deliberate test of passage which was a virus that was infectious for plants, those experiences were not particularly useful when fundamentally they don't tell you anything about the proportion of virus proteins were modified by the process and if so in what ways.

I think the only contribution I would make is to the fact that pollen is an item of food eaten in honey, it is likely to be contaminated by the (inaudible) of the cells in which it was made and that the presence of the virus or the modified in the way it has been proposed could potentially modify the allergenicity of a known allergen namely the pollen. Allergen is namely the pollen. And it seems to me that the substantial grants for assuming that there may be some possibility here at least it has to be investigated, but technology undoubtedly exists but it is not a very easy science to predict from it seems to me.

MR. ROBERTS: Other comments, Dr. Zaitlin then Dr. Nagy.

DR. ZAITLIN: People who look for the characteristics of proteins that make them allergenic

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have characterized certain amino acid sequences that these proteins seem to have. The sequences make the

protein a little more stable and less likely to be digested, and last year at a meeting I heard a presentation where someone looked at the coat protein of papaya ring spot virus and they claim that these sequences re there, I don't remember the details of the data they gave or how homogola (ph) sequences were.

But I think that the point that Dr. Gendel made that even in fact if it has potential to be an allergen the concentration in those plants and the lack of a prolonged exposure would probably not make them a thing of concern.

MR. ROBERTS: Okay, any other points, Dr. Allison.

DR. ALLISON: Maybe I'm off base again and not being an immunologist it's not my area, but it may be possible to conclude from what we're talking about that plant viruses are not allergenic, and that's from the point of view of the mucosal route that may be true, but the basis of a lot of the plant virus

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identification is based on their allergenicity because it's based on antibodies, so while they're not allergenic through the mucosal route they are allergens in that they stimulate the antibody productions.

DR. GENDEL: That's an issue which comes up a lot in the whole field and generally there's a distinction made between aminogenicity and allergenicity. There are a lot of proteins that are aminogens (ph) if you inject them into mice or whatever and treat them properly, all proteins are aminogens, which ones are allergens through a mucosal exposure is a much more limited group, so it is a distinction that's been made.

DR. TEPFER: I just want to come back to something the Steve Gendel said at the very beginning, I think that even though there's no evidence that viral coat proteins are known allergens there is also a finite possibility that there could be a motif, an amino acid motif that could be identical to a known allergen just within the variability of the viral coat protein sequences and I point this out because a case

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of this type has recently been sort of put forward as a possible allergen cross reaction system, it's not published yet, but there is some evidence that this can occur.

So I think that what Steve was suggesting is relatively simple, that is to say to routinely use the biointomatic (ph) screens that exist for looking for amino acid sequences that are identical to known epitopes of allergens and if any of these are identified then you can go to the vesera (ph) from patients who are allergic to the allergen and see if there's cross reactivity. It's a relatively simple thing to do and it is just a bit of a safeguard that

might be put into place.
DR. ZAITLIN: I just want to make one comment about a point that Dr. Hammond made about studies where cow pea mozaic virus was used to incorporate into the genome of its coat protein were sequences of animal viruses and other foreign proteins in order to stimulate antibody production an interesting thing about it was that the plant virus itself, the cow pea mozaic virus carrier acted as a very effective

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(inaudible) in that situation.
MR. ROBERTS: Are there any other comments in response to this question? Dr. Kramer.
DR. KRAMER: I would guess I would push to try to get a little more of a succinct answer. Is there -- I guess I would step back a minute, is there agreement that if we have an unmodified coat protein that this sort of allergenicity screens that have been mentioned would be unnecessary?
DR. MELCHER: For me, yes.
DR. KRAMER: So if we are in agreement at that point I am hearing that point mutations would not be a trigger, I'm trying to understand where the trigger would be, at which time it would be prudent to actually do the types of screens that you're mentioning and I was hearing that perhaps point mutations would be okay, that they would not trigger such concerns.
DR. HAMMOND: There is considerable variability especially in the rocheck (ph) in the amino and coboxee (ph) terminal extensions to the coat protein, but there's also considerable variability

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within the core region of the coat protein between different virus isolets, and so I think it would require something considerably more than a point mutation to have an effect and it would probably require an insertion or deletion that would change the structure of the plant virus coat protein significantly and there is a high probability that such a change would reduce the protective effect of the coat protein against plant virus disease induction, so it would probably not be worth doing in the first place and point mutations to oblate RNA binding or to oblate insect or vector transmission are likely to be effective to ablate those potential risks but not to make significant changes in allergenicity.
DR. STEWART: So what little I know about allergenicity and food safety at least trying to assess that from a known protein sequence is that you take known allergens and then you compare amino acid sequences so maybe you're looking for six amino acids sequences in a row, something like that, I mean is that right, you could use the informatic approach as a rough cut if there were going to any amino acid

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changes.

DR. HAMMOND: Basically yes that's true, as Dr. Tepfer said it's very simple to do a screen even on the natural sequences against these data bases, that's what we do with all of the other kinds of PIPs that the Agency has looked at, the exact criteria that are used are somewhat controversial but there's enough different ones known that it's not hard to do.

DR. STEWART: So you can get a point mutation changing amino acid and that might change your similarity index, so you can't rule out a point mutation if you're changing an amino acid.

DR. HAMMOND: But I think that in the field when you do these assays they're not taking a simple binary yes, no, it's a problem or it isn't situation, it's considered in the context of a variety of other data like digestibility and exposure and so on and so forth, in the similar sense if a sequence similarity was found but there was an argument that this is a naturally occurring one that people have been exposed to a lot that would probably sufficient to suggest that it's not a problem because people have been

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exposed to it.

If on the other hand you were looking at a virus family where -- to take a random example, I don't even know if such a thing could occur, there's place on the protein that is absolutely a hundred percent co served in every member of this worldwide virus family and you're making a change in it and that change creates a similarity to an allergen then you might want to consider that differently than you would a change that's similar to in regions that change naturally a lot, so to answer the question as being asked like with the other PIPs there probably is not an absolute measure that could say a single point mutation is going to be safe in every instance it has to be judged in context, but the context is previous exposure and what's known about the variation in the population and how that relates to other allergens.

DR. KRAMER: Can I ask directly then about deletions?

DR. HAMMOND: I think that what was just said a couple of minutes ago that certainly large deletions that are going to affect the structure of a coat

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protein are more likely to be an issue but the chances are greater that they're also likely to be ineffective for the purposes that you want to use them, but that was part of my point is that again deferring to the virologist, it would be worthwhile to know to what extent indells (ph) occur in the natural population, are they never seen, are they common, are there certain regions which are subject to them, and again that's the comparator that you want to use, it's not absolutely. I have my original protein and I made a change to it, how does that change compare to what's seen in the natural population is the comparative that

you want to look at.
DR. KRAMER: I guess if I could just try to put this in a little bit more context, we're trying to judge really whether there's under any circumstances where we would not need to do a case-by-case review, and I'm hearing, you're hesitant to say that even in a case there's the unmodified proteins that appear to be okay but any modification at all including a point mutation would require a case-by-case review for it's safety.

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DR. STEWART: The way I would put it is any changes which are within the range of changes which are seen in the natural population would be okay because the people are exposed to that population. I don't have an a strict definition of what that means because I'm not a virologist, but you see I've heard from others here that there is a third degree of variability in the population of viruses, so I would say that that whole range would be considered natural exposure to which would be acceptable.
MR. ROBERTS: Dr. Hammond, Dr. Tepfer, and Dr. Melcher.
DR. HAMMOND: There are viruses in which there is a considerable range of coat protein variability within the podee (ph) viruses in particular the amino terminus is very flexible in size, there are deletion and insertion mutants, some that have duplications, some that have altered sequences, some that have deletions with respect to other isolets, and those have little effect on the virus structure, some of them have effects on aphid transmissibility. One of the best known cases is an

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isolet of plumb pox virus which is non aphid transmissible as a result of a deletion, though I think of 15 amino acids that includes the DAG sequence that is recognized by aphid transmission.
Removal of the entire amino terminal segment and carboxee (ph) terminal segment leads you with a coat protein structure, the core sequence, which is very stable, that can be done proteilitically (ph) if you treat virus particles with prodeasis (ph) under mild conditions, you removed the amino and carboxee termini, but you are left with a stable the virus particle, the virus is still infectious when they have been treated in that manner and coat protein with those deletions expressed in transgenic plants still confers resistance, this is work from Dougherty's group.
With cow pea mozaic there have been in the course of making vaccine derivatives from cow pea mozaic from infectious virus clones there have been insertions and deletions of various sizes in the coat protein and some of those act similarly to the wild type virus and have no effect or little effect on the

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virus symptoms, some of them deleterious to the virus and so the level of virus replication is significantly reduced, some of them effect the virus symptoms, some of them make the virus symptoms worse, but there is certainly information available on the size of insertions in the external loop of cow mozaic virus that can be tolerated. That work comes from George Lominosoff's (ph) lab and WDO Hamilton's lab, so there's a considerable body of information is present on that.

With some other plant virus coat proteins it's very difficult to make mutations and retain a either a viable structure or a virus that is viable able and will reproduce. So there's good information, you can make insertions and deletions in some virus coat proteins and there is good data on some of that.

MR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I just wanted to make a brief comment to suggest we could place this into sort of the in the context of how much work for how much benefit. I think that considering how simple it is to do the bioinformatic analysis to see whether any of

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these modifications have changed the amino acid structure and created similarity to known allergens if I were a developer of a transgenic plant that would certainly be rather high up on the list of things I would want to do, it probably take as few hours.

And I think that it provides a certain degree of safeguard, whether that means it's just something that EPA should consider mandating is a whole other questions, but in any case if I were a developer I would certainly do it, it seems like a pretty simple decision to make.

DR. ZAITLIN: Getting back to allergens, a few years ago we had the incident with star link corn which I'm sure members of the EPA here are very familiar with, the issue there was that a BT constrict which had not been thoroughly tested at the time for its allergenic properties was released to be used only as animal feed and it got into the food chain, that's another issue, but I think as a consequence of that there are now rather stringent requirements for allergenicity tests before any new product would be introduced. So any petitioner would have to

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demonstrate the data, the product, the gene that their inserting was not allergenic.

MR. ROBERTS: Do you want to comment on that, Dr. Kramer?

DR. KRAMER: I think that would not be a fair assumption in this case, that would not be a fair assumption in this case that the issue up for the question is actually whether there could be a blanket tolerance exemption, that's the context for the question.

DR. MELCHER: I would feel very comfortable

with the point mutation being not a condition for requiring further, tests but the insertions and deletions I think there is a reasonable chance they should be tested.

DR. ISOM: To follow-up on that, there is a lot of information available on bemailant (ph) allergenicity to viruses, rhino viruses, I have some colleges that have done a lot of structural work on the agnogenic determinants on the protein coat. And in the case of mamillion (ph) viruses it is very difficult for the human immune system to recognize

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agnogenic determinants on that virus and that is why we have problems in developing certain types of tasks and treatments of viral infections like the common cold. And there are two problems that exist at least in the case of the rhino virus that creates problems for the human immune system.

First of all delivery to the immune system and then secondly being able to recognize those allergenic determinants and that protein coat and there are really embedded down deep in the protein coat in a kind of a canyon that prevents the human immune system from recognizing those allergenic determinants. Now how does that relate to the plant I would say the point mutations on any virus coat probably is not going to change the confirmation enough to create amniogenicity to the human immune system or be recognized that way, I would agree with what you just said, it would probably be more deletions and major changes, that's extrapolating from a long ways from amelon (ph) viruses to the plant virus, but I would assume that the three dimensional structures are similar.

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DR. GENDEL: Just to extend this discussion I have to agree that if you were to rank relative risks point mutations are obviously very low, I would also say that deletions that are terminal deletions are probably of relatively low concern of the only indels that occur in the middle that are likely to affect the structure of the protein in such a way to make them of a greater concern, I think he might be able to make a point at least in some virus families that deleting one end of the other is still going to leave intact the protein as you already know it.

My perhaps more overriding concern is it's not clear to me whether you can generalize that the principles that are the same are the same for every family of viruses, it may depend upon the structure of the virus itself.

DR. NAGY: One issue that we have not yet discussed is that I would like to see the companies actually demonstrating that that is not a significant (inaudible) of the translation of (inaudible) because when I engineer new coat protein genes it's possible that translation (inaudible) sometimes of the coat

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protein -- I think it is very important for allergenicity to demonstrate that 99.9 percent of the proteins are going to be just the coat protein because it (inaudible) actually.

DR. ALLISON: I'm going to agree with Mark, in that Dr. Tepfer, that these tests are cheap enough and the amount of time involved is very reasonable and I think that even for point mutations these should at least be run through some sort of computer check for amnionicity or for allergenicity.

The monoclonal (ph) antibodies have been used to distinguish variance in viruses and often times the variations are due to very subtle changes in the virus itself, so I think even point mutations if it is a probably point can change the way an individual could interact with a virus.

DR. NAGY: The other factor is that the virus is I believe as a quasi species so very likely lots of those point mutations will be naturally you know existing in infected plants.

MR. ROBERTS: Dr. Kramer it sounds like everyone is comfortable with an altered viral coat

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protein not being a problem, some panel members feel that a point mutation would trigger the need for an additional analysis along the lines outline by Dr. Gendel, other panel members felt that a point mutation would probably not be significant, but deletions and additions beyond that would trigger analysis.

Is that a fair summary, Dr. Allison?

DR. ALLISON: Let me just clarify that, I think point mutations is beyond what is known in the natural variation of the virus.

MR. ROBERTS: Good correct.

DR. KRAMER: Thank you.

MR. ROBERTS: Yes, Dr. Nagy.

DR. NAGY: Actually I would like to return to this question about the read through because I think it is a significant question and then you randomly need (inaudible) something to the plant genome you know you really can create a situation where you have a significant read through and that's a large insertion you know to -- it can be a large insertion so I think that is important for allergenicity to test.

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MR. ROBERTS: So part of the panel's feedback is the caveat about read through has to be addressed.

DR. KRAMER: And that would be even for unmodified coat protein, is that correct?

DR. NAGY: Yes.

DR. ZAITLIN: I have one question there, under the rules, I think it's the food and drug administration would they not require this as new submission to go through these tests irrespective of what we decide here.

DR. KRAMER: The EPA would be responsible for either establishing the tolerance or granting the exemption from the requirement of a tolerance and if we were to grant an exemption for the requirements of tolerance there would be no FDA requirements.

DR. ZAITLIN: Putting on a slightly different hat, as I understand it for pesticides the EPA is the lead regulatory agency --

DR. STEWART: But in terms of this allergenicity question in foods it's --

DR. ZAITLIN: That would be the case, it's like the cry proteins and so on that are pesticides,

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the EPA was the lead Agency considering these issues, the FDA considered whether there are other food safety issues involved. If it was a protein which was not a pesticide but was a food additive, then the FDA would have the basic requirement, but I would say that the standards which are used, the tests and the considerations are pretty much the same between the agencies, the same considerations are used, the sources for suggestions on how to go about this comes from groups like th FAO WHO LCIF BC and other agencies, some government some not, so it's pretty uniform in terms of what's considered it's just the matter of -- like in the discussion we had yesterday about the relative rules of USDA and EPA in this case the way the laws are written anything which is a pesticide the EPA takes the lead on as the major agency.

MR. ROBERTS: So as I understand the answer then not necessarily.

DR. STEWART: Well I guess the answer is both not necessarily but I don't think the results would be any different, the standards that are applied and the

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way they are analyzed are the same.

DR. HAMMOND: With respect to the read through proteins there are relatively few plant viruses that have a coat proteins that ends in a read through and most of them in a group such as the lutea viruses where we have already discussed the fact that it is undesirable to express the read through because that contributes to vector transmission, and so I think in most cases the construct will lack read through domain and will have a deliberately engineered stop coat on if it does not naturally have one.

The other cases those viruses that process their coat proteins by proteolytic cleavage from a polyprotein and in those cases one has to provide them either or both and engineered start and stop coat on, and I can't think of anybody who would take a construct and put it into a plant without sequencing it first and making sure that they knew what they had, that certainly, the sequence is typically part of the submission for regulation and so I don't think that that is as much of an issue as Dr. Nagy.

DR. NAGY: I agree that part -- this was not coming from the virus this read through, but because we have random insert your gene into the plant genome for expression and read through depends on the context, actually people who are very careful doing this they knew subsequent the three indifferent -- (inaudible) stop coat because this is that significant problem in the plants, so this is not coming from the virus, it's coming from the fact how you engineer these coat protein into the plants and their efficiency of plant to read through if the context is right.

So what I would suggest is that a company would test not a (inaudible) but at the protein level that indeed the protein being produced in plants is the authentic protein with the correct stop and not a read through.

MR. ROBERTS: Any other comments on this question? Dr. Kramer.

DR. KRAMER: That answer is fine, thank you.

MR. ROBERTS: Let's go ahead and take the next one.

DR. KRAMER: What are the potential adverse effects, if any, of such modifications that would be truncations, deletions, insertions, or point mutations, for example on nontarget species for example wildlife and insects that consume PVCP-PIPs.

DR. STEWART: I'm going to read what I've written so far and then if anyone wants to add or change things let me know. Potential adverse effects in wildlife can be manifested as both direct and indirect effects. Direct effects are effects that occur in the organism exposed directly with a potential toxicant whereas indirect effects are effects on organisms that have not been directly exposed in a food web but have not been exposed directly but have been exposed in a food web due to changes in populations of the exposed organism.

Direct effects can further be subdivided into lethal and sublethal effects. Mortality, the lethal effect may occur rapidly or may be delayed. Sublethal effects include but are not restricted to reductions in life span, reductions in a number of vital proginy (ph), failure to reach optimal weight, delays in the time of the first reproduction, janet (ph) mutation of gametes (ph), tumors including cancers, changes in behavior resulting in less competitiveness for food made in the ability to avoid predators, and multiple sublethal effects may be manifested in organisms after exposure to a toxicant, that's sort of a background.

Lethal effects in animal life after feeding on a PVCP-PIP plant is highly unlikely because plant viruses are not know to have deleterious effects on animal life. Additional animals routinely feed on non

engineered virus infected plants and do not die. If animals did die after ingestion of virus infected plants then these viruses would be developed as insecticides or denicides, mulescocides (ph) et cetera.

Production of other toxic substances such as an increase in secondary plant metabolites in response to this PVCP-PIP may be a possibility in result in toxicity, however this scenario is also improbable. Sublethal effects after feeding on PVCP-PIP plants may occur if for example nutritional changes within the plant occurs due to a trade-off for having additional viral genes, they may be also be some subtle

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mechanisms of toxicity that have not been defined to date such as toxicity to specific viral proteins as well as production of other toxic substances in response to the insertion of these genes.

Nevertheless sublethal effects are not expected to be manifested in animal life because again because wildlife and insects regularly feed on non engineered virus infected plants with no apparent sublethal damage. Indirect effect, those were our direct effects, indirect effects are very unpredictable and cannot be entirely ruled out. Examples of indirect effects in other types of genetically modified crops have been reported such as in BT engineered and herbicide resistant crops and I'll leave it at that.

DR. HAMMOND: I essentially agree with that. Induction at the hypersensitive response as a consequence of coat protein modification to disrupt subunit subunit interactions would probably result in loss of plant productivity and adverse effects for the producer as well as possibly limitation of food resources for wildlife species and other target

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species and such lines would rapidly be withdrawn or never presented for use in the first place, otherwise I see no obvious adverse effects of coat protein modifications.

DR. COOPER: I know of no obvious effects that there would be unless there were incidental ones attributable to the sterility that they were using to contain the transgenic gene which might effect the bird population and the amount of seed in their diet. The other thing that's worth remembering it seems to me is that the wildlife is not a particularly well researched group of subject for this sort of treatment. We know very little about the human, certainly not enough and about the wildlife we know very little even more because they react to immunogens and allergens in slightly ways, they get itchy skin as opposed to the aspire something similar when they inhale allergens I really feel at the moment I don't know enough about the risks to do anymore than simply support what has been proposed before.

MR. ROBERTS: Are there other opinions by panel members. Dr. Tepfer.

DR. TEPFER: When we talk about indirect effects we tend to talk in effect indirect effects on desirable organisms and it just occurred to me that there is evidence that certain plant vectors such as aphids can be effected. The pathological state of the host organisms that certain aphids are distinctly attracted to infected plants, so if you drastically change the number of virus infected plants in an ecosystem this could have an effect on the feeding behavior of the aphids.

This may sound completely off the wall but there is some evidence for this type of thing occurring. And I would say in conclusion say that I don't believe that this would have a significant impact on the ecosystem that we need to worry about.

DR. SHERWOOD: What evidence is that, you said there was evidence that existed to support this.

DR. SHERWOOD: There are two papers by Busco Perez (ph) that just came out recently, she presented these results also in a symposium last month in (inaudible) I can give you the -- I don't have the proceedings with me, but I'll send you a copy if you want.

DR. FALK: Aren't you referring to these papers that are sort of suggesting that this is advantageous to the virus, I mean the virus itself is modifying the plant therefore attracting its vector to come there so they can disperse the virus itself, I mean you did say it is sort of off the wall.

DR. HAMMOND: It's been established for a good number of years that many aphid species are more attracted to plants that have a yellowish cast as a result of the mozaic infection and then to healthy green plants, and so you would -- this effect is well documented.

DR. STARK: Increases in aphid numbers due to a virus could have a change in the ecosystem or in a located area in that it might attract additionally more lady bird beetles and parasutoids (ph) and things like this. I think that the question here though is we're dealing with transient ecosystems within agriculture and if the overall implications on a larger scale are probably pretty minimal.

MR. ROBERTS: Good point. Any other comments?

DR. KRAMER: That answer is fine, thank you.

MR. ROBERTS: Let's go ahead and take the next one.

DR. KRAMER: Number 19. To what degree and in what ways might a PVCP gene be modified for example through truncations, deletions, insertions, or point mutations before it would be a concern that novel

viral interactions due to the modifications could occur because the PVCP gene would be significantly different from any existing in nature.

MR. ROBERTS: Is there background on this one or do you think it's pretty straight forward?

DR. KRAMER: I think it's straight forward.

DR. TEPFER: This is going to get repetitive perhaps because we have gone over some of these similar sorts of questions regarding modifications for other types of features, but I just want read into the record a brief text.

It should be pointed out that arnie (ph) viruses are thought to lack (inaudible) by the replicases and so population of viral genomes within

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an infected individual is structured in a quasi species i.e. a cloud of variance of a consensus master sequence. Concretely when one simply sequences a number of c d aclones (ph) with infected individual a rich assortment of modifications is observed including ones that are certainly nonviable but are replicated in trans. These include deletions, insertions, and point mutations.

When comparing related viruses the array of possible variances is also quite broad. If the modifications made in a PVCP gene go beyond what is naturally occurring (inaudible) that these modified proteins would be that much less likely to be involved in viral interactions and that much less a concern for a reason for concern.

DR. ALLISON: If I can just read what I have here, modifications to make a transgene less virus like would make it less useful to a challenging virus through recombination. Recombination is lot limited to the transcript of the viral gene but rather heterologist recombinations may involve any host generated RNA such as a messenger RNA, TRNA, et

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cetera.

If the transgene is to be modified the challenges to distinguish it from viral RNAs while ensuring that it provides resistance. Truncations, deletions, insertions, and/or point mutations are all well accepted methods.

DR. NAGY: The concern I have if the modification would include a making a primary coat proteins to for example make resistance against several different virus and in this situation I think that can nearly change the (inaudible) combination of what kind of new viruses are generic, so if the companies producing coat protein you know this PVCP-PIP resistance if they use (inaudible) sequences for this I think we should ask them to do more careful examination about its possible effect on a recombination.

DR. MELCHER: I think anyway that you modify a PVCP will reduce the possibility of a novel viral

interactions because of the significantly different from what it might be interacting with, so I think the answer is no there's no degree that it might do that.

Does that make sense or did I misunderstand something?

DR. KRAMER: That makes sense, thank you.

DR. HAMMOND: Mutations that might increase the probability of recombinations with other viruses such as inclusion of a three prime non coating region from a heterologist virus should be avoided because it might increase the probability of recombination. However in general removal or mutation of vector transmission motifs, the DAG for podee (ph) viruses, the read through domain for ludio (ph) viruses et cetera, or RNA binding sites would immoderate perceived risks otherwise the significant variability between isolets of any particular virus that is reflected in antigenic variability and for podee viruses in considerable variation in length and sequence of the menaterminal (ph) domain in the coat protein. Excessive introduced variability intended to ameliorate perceived risks might have a greater probability of ablating resistance than of resulting in novel virus interactions.

MR. ROBERTS: Other comments? Dr. Kramer.

DR. KRAMER: I would just ask is there some disagreement with what Dr. Nagy was saying and the other respondents or am I misinterpreting that?

MR. ROBERTS: Can the panel clarify that? Dr. Nagy, could you repeat your points?

DR. NAGY: There is no example for this, but in the near future I can imagine that companies would create primary coat proteins which be for example two different related viruses or too close related strains and this way they might be able to engineer you know even broader resistance, but in this they would create a coat protein which if through recombination it can really change the features of that virus in nature of conditions.

So if they create -- so this is not in -- it is kind of in the insertion category if you look at it, but it's a special type of insertion that they would use two different coat protein sequences from different viruses. And in this situation I think you know it's very important that gene to be utilized much more carefully than in a natural viral coat protein genes.

DR. HAMMOND: This is not actually hypothetical. Several years ago I created two podee virus coat proteins with the immune internal domain from one virus and the coboxee terminal domain in; three prime non coating region from another virus. They conferred fairly effective resistance to being yellow mozaic virus and somewhat less resistance to a number of other podee viruses, but I would not

consider releasing those as for crop protection, they were done for the purposes of dissecting mechanisms and I would -- they do have the possibility of increasing the probability of recombination between different viruses and I think that they would be unwise to use, so agree essentially with what Dr. Nagy said.

MR. ROBERTS: Does anyone else disagree?

DR. KRAMER: Okay, thank you.

MR. ROBERTS: Anything else, any other follow-ups on this one?

DR. KRAMER: No.

MR. ROBERTS: Let's go ahead and do number 20 and then take a break.

DR. KRAMER: Would any additional

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requirements related to PVCP-PIP identity and composition for example demonstration that the transgene has been stably inserted be needed for significant reduction of risks associated with PVCP-PIPs.

DR. ZAITLIN: I found this to be a tough one, but I think the example given about stable insertion is a nonstarter. I think because of the way the transformations are done and selected large numbers of plants are transformed and then in the process after transformation the plants are selected for the, selected for the trait that one is looking for and tested and the only saves those plants which display the selected trait and that it is stable over a number of generations.

Now I had two other things that I was going to mention but they have been discussed here in some detail and that was related to allergenicity and I think that for new coat protein constructs I think we still have to have tests for allergenicity, I think that's a given, and the other point that I was going to make has been stated here many times that we're

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dealing here with a technology that's probably going to be superceded in the future that there are many ways of making virus resistant plants so they don't have to involve creating and transforming with a functional coat protein.

DR. HAMMOND: I'll amend to that. If the coat protein transgene is not stay be inserted it won't persist and therefore pose no risk at all. I do not think there are significant risks from coat protein expression in general, the hypothetical risks are of minimal consequence comparable to risks from mixed infections which are a frequent natural occurrence.

DR. MELCHER: Any additional requirements related to the identity and composition of these things be needed for significant reduction of risks additional to what we've already discussed not to my knowledge.

MR. ROBERTS: Comments from other members of the panel? Dr. Kramer.
DR. KRAMER: That was clear, thank you.
MR. ROBERTS: Let's take a 15 minute break,

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we only have one more to go, but it's more general, I want everybody fresh for that one, we're also going to take up number 3, we're going to finish off that and then I was going to give you the opportunity to if there are some points that need to be made relevant to these issues that aren't covered in the questions the opportunity to make those points, so we will do all three of those things when we reconvene in 15 minutes.
(Break.)
MR. ROBERTS: As I indicated before the break I would like to go ahead and take the last question posed by the Agency, number 21, and then I would like to revisit number 3 which we left open previously and come to closure on that question and then we can discuss come general points. So let's go ahead and take question 21
DR. KRAMER: Are there any considerations beyond gene flow, recombination, and heterologous encapsidation as posed in the preceding questions that the Agency should consider in evaluating the risk potential of PVCP-PIPs for example synergy.
MR. ROBERTS: Dr. Hammond, could you lead our

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discussion on this question.
DR. HAMMOND: Expression of viral proteins that contribute directly to synergy should be avoided. If such plants were to be produced and released they would almost certainly be voided by producers because of the potential for adverse effects from mixed infection or infection by heterogenous virus which would have a more dolitarious effect due to synergy.
The use of alternative constructs effective through PTGS or other mechanisms or non viral mechanisms effective against multiple viruses is probably favored. The pyramiding of genes to confer additional resistances or multiple mechanisms against a single virus is also preferred because of the reduced probability of any viral mutant overcoming multiple mechanisms, and this was discussed in Hammond et al chapter in 1999, epidemiological risks for mixed virus infections and transgenic plants expressing viral genes.
Another issue to consider is effectively the lack of difference between virus coat protein transgenic plants and the use of cross protection to

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get higher yield using a mild virus isolet to protect against the effects of a severe isolet. This has been deployed in several places around the world in a number of crops and is currently being used at least informally by tomato growers in the United States against papino (ph) mozaic virus. The reason being

that if you infect tomato plants with papino mozaic virus early you get essentially no viral symptoms on the fruit, whereas if the plants become infected at the time when fruiting is initiated then you get significant symptoms on the fruit and some of it is unmarketable.

I think another thing that we should bring up is the fact that the coat protein mediated protection against papaya ring spot in papaya has essentially saved the papaya industry in Hawaii, this is extremely beneficial use of this technology and should certainly not be compromised. I believe however that there are no compelling reasons to prevent the large scale usage of PVCP-PIPs nor to restrict their usage to any significant extent. And a quote that I had from the AFIS AIB Workshop in college Park from `95 because

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transgenic plants resist infection the frequency of recombination events between viral transgene RNA and viral RNA from homologous or closely related viruses might actually decrease with use of transgenic plants compared with non transgenic plants, and this should lower the probability of sufficient RNA RNA interactions to generate a viable new virus.

Now however Dr. Tepfer has produced data earlier today that indicated that that's not universally true, but I don't see any significant problems other than the potential of synergy if inappropriate virus genes are used and that should certainly be avoided.

DR. COOPER: Synergy could be a potential hazard but probably more of a commercial safeguard than a real environmental hazard except when pollen transmission of the synergy into a wild species has potentially harmful effect or consequence. So if the transgenic gene can be carried to a wild relative in pollen then the possibility of environmental harm at least locally cannot be discounted it seems to me.

DR. TEPFER: I don't have very much to add to

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the two previous speakers, I just would like to say that I think there's a point where there needs to be not only the risk but also the question the balance between risk and benefit and I think this is part of what John Hammond was alluding to, he mentioned. I think at the end of the day that has to be taken into account and I think that this is also going to involve a consideration of what is acceptable risk because as we all know particularly in these complex biological systems zero risk does not exist, cannot exist, and so we always must face what is acceptable and this can only really be addressed if you also look at benefits.

So that's all I wanted to add.

MR. ROBERTS: Are comments from other panel members, other concerns other than the ones that have been discussed up until now that the Agency should be aware of? Dr. Melcher.

DR. MELCHER: I'm wondering if I can argue against one of the concerns that was raised that are moving a transgene to a wilder weedy relative and then expect synergy to occur in the wild and weedy relative when it does not occur in the crop plant, it doesn't

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seem to make sense to me, it seems that if there was synergy in the close relative cross crop plant there also would be synergy in the wild and weedy relative and has been pointed out if it's synergy in the crop plant it would have never come to EPA regulation anyway because it wouldn't be commercially viable.

MR. ROBERTS: Other viewpoints, Dr. Cooper.

DR. COOPER: I don't have a lot to say, but it seems to me possible that the wild plant gets exposed to viruses other than those that affect the crop and therefore the challenge that was done to reassure the producer to make sure that synergy wasn't going to have any harmful effect in their commercial hands cannot necessarily be assumed unless you have some knowledge of what's happening in the wild relative if that's important, however it's not a big deal.

MR. ROBERTS: Comments from other panel members. Dr. Falk.

DR. FALK: I just have one brief comment and it is actually the first comment I made yesterday, and it is regarding gene flow and it is again, I think it

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is legitimate to question whether viruses have any role in native plants in terms of their ability to colonize and spread where the viruses are actually pathogens of native plants.

MR. ROBERTS: Okay. Other panel members have concerns other than perhaps synergy or comments about synergy? Dr. Kramer.

DR. KRAMER: Thank you.

MR. ROBERTS: Let's revisit question number 3 and would it be possible to project the list as it exist at the moment, and Dr. Stewart let me ask you to sort of lead discussion on this.

DR. STEWART: During the time this morning when we put that up basically put it on the computer I have been revising it so, this one is not too bad and then I'll also read into the record some floriculture bedding plants and ornamental plants that are up here, so I just want to explain a little bit about how the list got there.

What I ended up doing was to take the USDA national agricultural statistical services list state-by-state, territory and protectorate by

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territory and protectorate to see what were the major crops that were listed as far as what was grown in the recent years and that's where that list came from and then I eliminated -- well that's not where that list came from, a bigger list, I eliminated ones that I

knew had wild relatives.

I ended up doing literature search and using various reviews, some primary literature in Google of all things to come up with this list, and then I'll read off another list. So what's up there so far is almond, asparagus, avocado, banana, barley, bean and this includes string beans, French beans, the common bean, (inaudible), black-eyed pea which is cow pea, chocolate, celery, chick pea, citrus, coffee, cucumber, eggplant, guava, mango, okra, olive, papaya, parsley, pea, which is (inaudible), peach, peanut, pineapple, pistachio, spinach, star fruit, sweet potato, sugar cane, taro, tobacco, watermelon, and cantaloupe.

The ones that are not up there that should be up there I think is plumb and the ones that I have added some notes to, asparagus has some naturalized --

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there's some examples of naturalized asparagus under bridges and that type of thing, very few -- I visited some arboretum while I was here, University of Texas arboretum, University of California, Berkeley arboretum which are pretty complete, so there is some asparagus growing under bridges, that type of thing in the 1930s, so it does escape cultivation. So that one has a notation.

Celery also has a few examples of naturalization I think. Sugar cane you wouldn't want to grow that in the Caribbean because of shatter cane, you don't get shatter cane everywhere so that's why I flagged that one. Sweet potato I removed because there is apparently some controversy as to -- number one sweet potato is not very productive as far as see, pollen, that type of thing, but it can cross apparently spontaneously with a couple of other epimeas (ph) species, and so the reason why I took it off, it's up there now but it's off kind of off my final final list, is because some of those species are agronomic weeds, so the sweet potato could be controversial, but (inaudible) good data there.

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The ones I removed yesterday you see that list, some of those like daffodil, tulip, chrysanthemum, gladiolus, ornothogulum (ph), geranium we added back onto another list, lima bean, apple, pepper and onions, there are some data to indicate that they can at least form hybrids, viable hybrids, you know long term robust hybrids, who knows, cantaloupe I also removed from the list, was domesticated in the new (inaudible) and there are wild relatives that might come up to New Mexico.

Other than that tobacco also has a few congeners but it's really highly selfing, so there are some reports of hybridization with nocianium (ph) tabacum, but it is hard for me to imagine it coming from tobacco country.

Any questions about that list and then we're

going to another list, and I'm just going to read that one into the record.

DR. HAMMOND: The addition of apricot and nectarine should probably go along there as other (inaudible) fruits.

DR. STEWART: Okay.

MR. ROBERTS: Dr. Tepfer.

DR. TEPFER: So are you planning to annotate this with some sort of star for all of the ones for all of the ones where there are some sort of feralled populations like asparagus or -- I mean --

DR. STEWART: Yeah. Next time EPA calls I'll make sure I can get a clarification. So yeah my best -- what I'm planning on doing is to do a pretty decent literature search in doing everything that I've done so far but more of it in the next two or three weeks. I don't want to add plants to the list, simply remove them during revision as it seems there is some evidence of hybridization.

And I will make notes of those that are -- the ones that are extensively naturalized where viable populations exist, I think those will be removed from the list. If it is a spontaneous you know every few years infrequent type of volunteers or naturalized plants they'll probably stay on the list, but with that denoted on there if that makes such sense. And of course this is going to get sent back around so, again I just want the plants to get on the list that could be on the list and remove them if there's sufficient evidence that hybridization could be an issue.

MR. ROBERTS: We have to do this carefully. I think what we need to do to be consistent with facts is to produce our list as our best shot at it at this meeting. We can caveat that list that pending further examination this list may be modified and then perhaps present an appendix to the minutes that if there's some subsequent analysis how that might change the list.

DR. STEWART: I don't see the list really changing all that much although I would like to put scientific binomials on there to make sure there's no ambiguity as to what we're talking about.

MR. LEWIS: Let me just add some comments what Mr. Roberts just mentioned. The list we're going to present now is a list that the panel agrees upon, now any changes you want to make after would be as an appendix that would not reflect the panel position but reflect your position, what you want to try to do is to have this list present here if you want to have a caveat saying we're presenting this, but there is some question about any changes to be make, you want to have an appendix that be added onto this but only reflecting your position, it will not reflect the

panel's position, so when the public looks at the meeting minutes they will be see this list in terms of the panel's position, so we want to make sure we reflect that again, all discussions occurring here want to be reflective in the minutes also.

DR. STEWART: That sounds good to me because I realize that I'm not perfect here.

DR. COOPER: I recognize my imperfections at least as much, so I don't know about prunus and I would therefore just simply raise the question is prunus domestica one of those things which cross hybridizes, is there evidence of that does, that question the inclusion of one or two of these plants.

DR. STEWART: I took a look at the stone fruit prunus specifically in literature search and apparently all of the citations that I could find they were be hybridized within that group by plant breeders, not in nature. Now when you go to uragia

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(ph) that's a different situation because there are a lot of wild relatives there.

Now, so could you imagine a situation where you have almonds growing in an old homestead someplace it's been abandoned so now you have some naturalized trees, sure.

DR. COOPER: Or even root stocks growing out from underneath.

DR. STEWART: Sure. That's the problem with perennials is they're not babysat. In the Appalachian mountains if you want to find some old, I guess I ws mentioning this to someone yesterday, if you want to find old homesteads just look for daffodils growing in the woods because they don't go away very fast, so in the Great Smokies where it was made a national park in the 1930s they ran out all the homesteaders, well you still have daffodils growing in the Smokies. So it is not totally black and white.

MR. ROBERTS: Are there any comments or suggestions for edits on this list? Dr. Bujarski.

DR. BUJARSKI: Just a short questions, do we concentrate on trees as well here, other trees than

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the crop.

DR. STEWART: So some of the trees that I'm aware of which are native like Poplars, Amilanchor (ph) which is service berry that's been made transgenic, Walnuts, those never made the list because I know they're a lot of wild relatives and that's the problem with a lot of forest trees, your Pines, Poplars, these are all I think all represent all the woody plants here and all the perennials here are all exotics, so the geographic center of distribution is not in North America I don't think for any of these.

DR. SHERWOOD: The other day we discussed the idea particularly in regards to ornamentals about putting down some type of guiding principal, are we not going to do that?

DR. STEWART: Well the perennial list is a tough one, what John Hammond did and what I will read into the record is probably the best approach and so what he did is he cataloged the ornamental plants that have been transformed, and so now we're crossing things off the list there that have wild relatives and have a potential to form hybrids.

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DR. SHERWOOD: But what about the things that not have been transformed do we exclude them?

DR. STEWART: Well I don't know because I went to the NASS yesterday, they list all of these really smaller crops even though they might have really significant economic value, they list them as floriculture, bedding plants, and nursery crops, and so they're not broken out as far as species that are grown each year, so I don't really know what to do at this point.

And I would like to address this systematically and I don't really see anyway to do that here at this meeting, maybe at the 2006 SAP.

MR. ROBERTS: Perhaps then we should describe along with this list sort of the extent to which we limited or delimited the consideration of the kinds of plant that we're explicitly considering and the ones that we're maybe were not considering or unable to consider.

And then you had some additional, are they ornamentals?

DR. STEWART: Yeah, it's the second list and

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so this is coming from Dr. Hammond. So this even though the USDA NASS does not produce state-by-state data on flower and ornamental crops there are several ornamental species that have already been transformed which should be considered here. And I also put as part of the minutes here I also said that our recommendation is that a systematic study be done at some point.

So on that list is antherium (ph), carnation, chrysanthemum, the geranium that's commonly grown as a bedding plant which the genus is paragonium, gerbera, gladiolus, hyacinth, lily, I think we probably won't include lily since there are a lot of native lilies, lisanthius, licianthis which is ustoma and John says this is also a native.

DR. HAMMOND: That is a native.

DR. STEWART: So there are a lot or orchids that are being transformed, insidium, dindrobium, colanthee which I recognize as an indogineus genus and cynbideum and philonapsis (ph). So I don't know about some of these other (inaudible) as far as wild relatives in the U.S. and a lot of times orchids grow

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much better in the tropics than they do in the continental U.S. so that will be something else that will be fun to look at. Once again a good example for

a floristic study in case the EPA wants to fund that go to the Virgin Islands. Ornothogulum which is another native, osteo --

DR. HAMMOND: It is not a native but there are populations that have naturalized again from home gardens.

DR. STEWARD: Osteospermum, petunia, poinsettia which is a native North American plant so that's probably not going on the list, rose, rose has wild relatives, so we need to look into that, turennia and tulip.

So what you're saying now is speak now or forever hold your pease at least until the next SAP.

MR. ROBERTS: Sort of. Basically what we're looking for is committee or panel endorsement of these to the best of their knowledge.

Discussion by panel members?

DR. TEPFER: So you want us to add names and things like that then perhaps?

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MR. ROBERTS: Add or argue for deletion.

DR. TEPFER: Kiwi you could probably add I would suggest, artichoke.

DR. STEWART: I'm pretty sure that artichoke does have wild relatives, I don't think kiwi does.

DR. TEPFER: Artichoke does?

DR. STEWART: I think it does, I'm pretty sure it does, so we don't want to -- I actually looked into artichoke. Kiwi I'm pretty sure does not have any wild relatives.

DR. TEPFER: I think watermelon is originally from Africa.

DR. STEWART: Right. And I didn't see any evidence for -- so that one -- it actually is off on my revised list, revised revised.

Any other candidates for the list?

DR. MELCHER: I would just like to be clear on whether the ones that Dr. Hammond made comments about are now on the list or off the list.

DR. STEWART: They're off the list if they're naturalized or have wild relatives in the U.S. So the ones that we commented on en route I think will be

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off.

DR. PORTIA: Carenbola, star fruit.

DR. STEWART: Star fruit?

DR. PORTIA: Yeah, it's grown in South Florida, I don't think it's native.

DR. STEWART: No. It's on there, star fruit is on there. Passion fruit, I don't know.

Does anybody know anything about passion fruit?

MR. SPEAKER: It does have wild relatives doesn't it?

DR. STEWART: I think it does but I don't know how, I don't know if -- there are some indigenous pasafluras but I don't know about their relatedness.

Once again I don't think that was a big one on the NASS.

DR. PORTIA: How about ficus, figs?

DR. STEWART: Ficus definitely has --

DR. PORTIA: There's ornamental and then there's --

DR. STEWART: I didn't include ficus because there are so many of them that have escaped

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cultivation.

Any other plants, your favorite plant, add your plant now.

MR. ROBERTS: We put the minutes together and we put this list together, I mean it will come with the caveat that this is the best we can do under the circumstances of the meeting, we well, there may be, there may be some post meeting suggestion by individual panel members for edits on the list and I sense the panel would suggest a more detailed systematic study by the Agency to truly create a sound list, is that a fair statement?

DR. STEWART: Sure. And something else to consider which is totally the opposite of what we're considering today is plant pathologists might imagine some day where you engineer castainia dintata, American Chestnut with a transgene that would confer tolerance or resistance to the Chestnut (inaudible) and then reintroduce it as a dominant tree for eco restoration where you actually want gene flow to happen.

DR. HAMMOND: That's not far from the truth.

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DR. STEWART: Right. I mean it is something that we definitely need to get a grasp on that and Dogwood enthracnos is another one where Dogwood populations are suffering in the U.S. and you can really use a genetic engineering approach for ecological restoration where gene flow is the target not the thing to avoid.

MR. ROBERTS: Okay. Well that will wrap up question number 3, let me now ask Dr. Kramer and others at the Agency if during the course of the discussion of the last two days there are questions beyond the original 21 that you would, on this topic that you would like to pose to the panel.

DR. KRAMER: No, we have no further questions, thank you.

MR. ROBERTS: Let me then ask the panel if there are some technical or scientific points that panel members think should be made relative to this topic that perhaps weren't covered in the individual questions, points that have not been previously expressed.

DR. SHERWOOD: Can we ask a question?

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MR. ROBERTS: It depends on the question, is it a question related scientific technical issues

associated with this topic?
DR. SHERWOOD: No.
MR. ROBERTS: Then it would perhaps best be asked informally after the meeting.
DR. SHERWOOD: I will informally ask what is next in this process after this report is developed and goes to EPA, what is the next step in this process.
DR. KRAMER: We'll carefully consider your suggestions.
DR. SHERWOOD: And who is we?
DR. KRAMER: The Agency.
MR. ROBERTS: There you go. Asked and answered.
DR. MCCLINTOK: Those of us on the permanent panel have heard this very often. Just to add to that, our work group is comprised of several representatives from the various program offices and as Dennis Suhay (ph) is representing OPP as a whole or representing OPPTS, but there are other members of the

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work group that surely would take all of these comments into consideration.
DR. SHERWOOD: Is there inter-Agency cooperation on the development of a response to this report or is it totally within EPA?
DR. MCCLINTOK: It would be within EPA but surely we do work with USDA and other agencies, surely.
MR. ROBERTS: All right if there are no further questions or comments from panel members I would like to first thank the Agency for their presentation that helped set up and provide background for our discussions and as well as your willingness to actively engage the panel during our discussions and deliberations, I think that was very useful in terms of helping direct our responses, helping us to understand the information that you are seeking and I think really contributed very much to having some productive discussions.
I would like to thank the panel members for coming prepared to discuss and once here being very active in terms of discussion on these topics. I

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think the information you provided the Agency is going to be very helpful to them. Of course I would like to thank the SAP staff for putting this meeting together, they work behind the scenes but it's a considerable amount of effort to assemble a panel as outstanding as this and get us here and make it possible for us to have this meeting. I would also like the thank the public commentors for taking their time and in some cases traveling to the meeting to present their viewpoints for us and that's always very helpful in the panel's deliberations.
Paul are there any announcements that you need to make before we close this session?

MR. LEWIS: First of all I want to thank Dr. Roberts for serving as chair for the meeting of these past two days and for leading us along in terms of having panel respond to the questions in charge during the course of the meeting here. And I want to thank all the other members of the panel for agreeing to serve on a panel here and being actively engaged, being an active player in terms of the preparing your comments before the meeting and providing a very

interesting and challenging deliberation we had in the past two days.

For members of the public thank you also for being active players in terms of watching deliberations that occurred here and for those that provide either oral or written comments we appreciate their remarks provided, it really provides a more scientifically engaged process for looking at your remarks and seeing how we can best look at those issues as we grasp with these topics.

And for my colleagues at EPA, always a pleasure for working with you and thanking our colleagues, the SAP staff in terms of working with me and putting this meeting together.

Members of the public as I mentioned before we anticipate releasing our meeting minutes that serves as a summary for discussion that occurred in the past two days in approximately six weeks, it will be available in both the office (inaudible) docket and the E docket system and also on the SAP website. Thank you.

MR. ROBERTS: With no further business this

session of the SAP is now closed. Thank you.

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I, Monica Knight Weiss, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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MONICA KNIGHT WEISS