FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

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VOLUME I

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Reported by: Frances M. Freeman

C O N T E N T S

DR. PORTIER: Welcome to the FIFRA Scientific Advisory Panel Open Meeting on Corn Rootworm Plant-incorporated Protectant Non-target Insect and Insect Resistant Management Issues. I want to welcome you this morning.

I would like to begin this morning by introducing the members of the panel. I'll ask them to give a brief introduction of themselves and their background. And we'll move around the table for this starting with Richard.

DR. HELLMICH: I'm Rick Hellmich. I'm from the USDA Agricultural Research Service, in Corn Insects and Crop Genetics Research Unit in Ames, Iowa.

I'm an insect ecologist. Over the last few years, I have been working with insect resistance management for Bt corn. And also, most recently, with non-target effects of Monarch butterfly.

DR. FEDERICI: I'm Brian Federici from the University of California at Riverside. I'm an insect pathologist. And I basically work on the

molecular biology and genetic engineering of bacterial insecticides based on Bacillus thuringiensis and Bacillus verrucous (ph).

DR. JEPSON: I'm Paul Jepson from Oregon State University. I'm an ecotoxicologist.

I work in areas of regulatory science associated with non-target invertebrates, mainly with conventional pesticides, but also with GM materials.

DR. ANDOW: I'm Dave Andow. I'm professor of entomology at the University of Minnesota. I'm an ecologist.

I have studied the natural enemies of pests associated with corn. And also I have been studying the evolution of resistance in corn pests to transgenic corn varieties.

DR. BARBOSA: I'm Pedro Barbosa, Department of Entomology, University of Maryland.

I'm an insect ecologist working on insect/plant interactions, three trophic level interactions and the ecology of parasitic insects and predators.

DR. PORTIER: Dr. Alexander?

DR. ALEXANDER: Martin Alexander. I'm an emeritus professor at Cornell University. My fields are soil science, microbiology, ecotoxicology and recently specializing in biodegradation of (inaudible) compounds.

DR. ANGLE: Good morning. My name is Scott Angle. I'm a professor of soil microbiology at the University of Maryland and also the director of the Maryland Agricultural Experiment Station.

I work on the fate and risk of genetically modified organisms in soil.

DR. NEHER: I'm Deborah Neher, soil ecologist from the University of Toledo in Toledo, Ohio.

I work with soil invertebrate communities. Primarily, nematodes, also, collembola and mites. Interested in their use in environmental monitoring. Also relating these communities, their composition to ecosystem function. And I'm gearing up for a project

beginning next summer also looking at their response to this product.

DR. PORTIER: Thank you very much. I'm Chris Portier. I'm director of the Environmental Toxicology Program at the National Institute of Environmental Health Sciences in North Carolina. And I also manage the U.S. National Toxicology Program.

At this time, I would like to turn the mic over to Mr. Paul Lewis, who is going to give us some details on administrative proceedings.

MR. LEWIS: Thank you, Dr. Portier.

I would like to welcome panel members and the public to this important meeting of the FIFRA Scientific Advisory Panel addressing corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues.

I would like to first thank the panel members for agreeing to serve and for the time and effort preparing for this meeting, taking into account their busy schedule and the time commitments preparing for this meeting.

I want to begin my remarks by providing a brief background of the FIFRA Scientific Advisory Panel and the panel composition.

The FIFRA SAP is a federal advisory committee that provides independent scientific peer review and advice to the agency on pesticides and pesticide-related issues regarding the impact of proposed regulatory actions on human health and the environment.

The panel is composed of seven permanent panel members. And panel membership represents several scientific disciplines, including, but not limited to, toxicology, pathology, environmental biology and related sciences.

In addition, the panel is augmented through a science review board where these members serve as ad hoc temporary members of the scientific advisory panel and provide additional scientific expertise to assist in reviews conducted by the panel.

And if you look on the listing of the panel members, we have broken down the panel

composition by permanent panel members and some ad hoc members of the FIFRA scientific advisory panel.

My role as a designated official to the FIFRA SAP is to serve as a liaison between the agency and the panel. I'm also responsible for ensuring provisions of the Federal Advisory Committee Act are met.

And as a designated federal official for this meeting, a critical responsibility is to work with appropriate agency officials to ensure all ethics regulations are satisfied.

In that capacity, panel members are briefed with provisions of the federal conflict of interest laws. And each participant has filed a standard government ethics report commonly known as a financial disclosure report.

I, along with the deputy ethics officer for the Office of Prevention, Pesticide and Toxic Substances, and in consultation with the Office of General Counsel, have reviewed the report to ensure all ethics requirements are met.

In addition, we have provided a sample copy of this form, a new form that was developed for members, for SGEs serving on federal advisory committees at EPA. It is available in the Office of Pesticides Programs Docket.

We have several challenging science issues being presented today and the next two days focusing on insect resistance management. We have a full agenda for today, and meeting times are approximate. Thus, may not keep to the exact times as noted due to panel discussions and public comments.

I want to ensure adequate time for the agency's presentations, public comments that are presented and panel deliberations.

For presenters, public commenters and panel members, please identify yourself and speak into the microphone, since the meeting is being recorded. And for panel members, we will be distributing overheads of all presentations that are available today, be it powerpoint slides or other visual effects.

For members of the public requesting time to make a public comment, we request that you limit your remarks to five minutes unless prior arrangements have been made.

For members of the public that have not preregistered by contacting myself, please speak to a member of our SAP staff sitting to the right of me over here to request time to make a public comment.

For this meeting, we have established a public docket of all background materials. Questions posed to the panel by the agency and other documents related to this SAP meeting are available in the docket.

And overheads will be available on the docket and will be available in approximately two to three days.

In addition, the primary background materials are available on the EPA web site.

At the conclusion of this meeting, the SAP will prepare a report as a response to questions posed by the agency, background

materials, presentations and public comments. This report serves as meeting minutes that captures the panel's discussion today and the next two days.

We anticipate the report to be completed in approximately four to six weeks. It will be available both in the pesticide programs docket and posted on our SAP web site. Thank you.

Dr. Portier.

DR. PORTIER: Thank you very much, Paul.

I would like to introduce Ms. Sherry Sterling, the acting director of the Office of Science Coordination and Policy.

MS. STERLING: Good morning. I just wanted to offer my welcome and my thanks also for the panel's participation in this very important meeting.

What I have come to see as I have worked with the SAP is that it is not only what you have here at these few days of very intensive discussion, but it is also all the preparation beforehand and then all the work afterwards in

getting the report out.

So while we're just seeing the tip of the iceberg, let me thank you for the things that have already happened and what is to come. So thank you.

As you know, we have a very important topic to take up, corn rootworm plant-incorporated protectants. We have basically two almost separate meetings going on.

Today we're going to be talking about the non-target insects. And then the following two days we'll be talking about the insect resistance management issues.

All important issues. And I think they are important and so interesting that there are many facets to these issues.

What I would say is that we're calling you together to help us work through the science portion of the issues. Today, the non-target pests. And then the insect resistance management will be in the other two days.

But I know it is tough to focus on the

science when these are such involving issues. But I trust that we'll go forward and have an interesting scientific discussion on these topics. And I want to thank you for that.

DR. PORTIER: Thank you very much.

Ms. Marcia Mulkey, Director of OPP.

MS. MULKEY: Good morning to all of you and greetings to everyone else who has gathered with us today.

On behalf of the Office of Pesticide Program, I am always honored and pleased to thank those of you who work with us in the context of these FIFRA Science Advisory Panels.

I believe that you make a huge contribution to good government, to the quality of our science, to our opportunities to be transparent, to be accountable within the scientific community and with the general public.

And all of that contribution that you add to what we do is valued by us and, I believe, valued by our public. And it is never more obvious than in this subject matter involving

genetic modification that the American people have a degree of trust in their government around these issues, which is not enjoyed in every part of the world on topics close to these.

And I believe that your work with us today is a very material part of our capacity to deliver to our people an open and credible government around these issues.

Today and tomorrow we bring forward some issues, as Sherry has already mentioned and as you, of course, already well know, some issues relating to another version of this technology, this plant-incorporated protectants involving Bt and this particular one aimed at controlling a pest in corn which opens up both some very exciting opportunities and some particular challenges.

And so we feel the weight of the responsibility upon us to work through this technology in our role in regulating this technology in a responsible, thoughtful and effective way, because we believe that the stakes

are significant in particular in this area.

In the pesticide program, we sit at the nexus between biotechnology regulation and pesticide regulation. And so we get a piece of both and not all of, as you well know, biotechnology regulatory responsibility of the United States government by any means, but our ability to see that universe of conventional pesticides and PIPs also allows us to bring to the public policy table some special perspective involving controlled technology in this area.

And so all of that has gone into our consultation with you on these particular topics set forth for today and tomorrow. And we hope we will bring to the table a meaningful framing for your advice. And we very much value the fact that you bring to the table, not only, as Sherry said, the work you have done immediately in anticipation of this session, but your life's work in many cases and, certainly, much of your recent professional work directly relevant to what we do.

I know that there will be somewhat

different panel members involved in tomorrow's issue and that there is some overlap. Because I will not be here tomorrow, I would like to take this opportunity to share our feeling that both panels are very important, that we are pleased that there is some overlap between them because all the topics are somewhat different, the extent to which we get advice that is contextual and in the larger context is always useful. And to thank those of you who won't be around tomorrow for today's vital service.

I really, really enjoy this part of our work. And while I will not sit through much of today's session, I want to assure you that I and people in jobs like mine up and down the organization pay very close attention to the content and the nature of these sessions. They really do make a difference.

So thank you.

DR. PORTIER: Thank you very much, Ms. Mulkey.

This is a significant issue with very

significant stakes. And I'm sure the panel recognizes that that is the case. We want to applaud the agency for having such an open scientific debate on some of the issues associated with a number of pesticides -- and not just these.

And we also look forward to an interesting scientific debate this afternoon.

It is important to note that this meeting has a broader scope than just the pesticides we're looking at here in the sense that some of the discussions we have will help to set OPP policy in the next few years in terms of how to evaluate some of these novel pesticides.

So I do think this is a significant meeting and it is going to be a very interesting debate.

Dr. Andersen, good morning.

DR. ANDERSEN: Good morning. Thank you.

I'm Janet Andersen. I'm the director of the biopesticides and pollution prevention division. And of course I want to add my thanks also to the panel and to the participants we will

have from the public today. Not only for the people who will speak here today, but also the people who have sent us in written comments also, or electronic as we get more and more of those.

It is my pleasure to get us launched right in today and to introduce the members of the biopesticides and pollution prevention division who are participating today.

Immediately to my left is Robyn Rose, who will be giving the principal presentations. Then Dr. Zig Vaitzus and also Dr. Chris Wozniak at the important computer monitor to make sure that all the technologies work for us to be able to proceed with this meeting.

So without further ado, I'm going to turn it over to Robyn Rose. Thank you.

MS. ROSE: Good morning. As Janet just mentioned, my name is Robyn Rose. And I'm an entomologist with the Office of Pesticide Programs, Biopesticides and Pollution Prevention Division.

This morning, I will be presenting our

preliminary risk assessment for soil, soil surface and foliar invertebrates for Bacillus thuringiensis Cry3Bb1 protein.

I will essentially be briefly summarizing these studies submitted to us by Monsanto and EPA's review of these studies.

I would like to acknowledge my colleagues that also did reviews for the ecological risk assessment, including Zig Vaitzus, Gail Tomimatsu, Chris Wozniak and myself.

Part of the EPA guidelines for microbial pesticides require testing on at least three natural enemy insect species and also honeybee testing. And we have adopted these for the Bt crops also.

Essentially, they are to choose from three of these beneficial insects. And typically, lady beetles, green lacewing and parasitic hymenoptera are tested.

So today, I will be summarizing the honey bee larval and adult tests, parasitic hymenoptera test, green lacewing, lady beetle,

collembola, Monarch butterfly tests.

All of these are laboratory tests. And then also some field evaluation studies that were submitted to us. And also earthworm studies, endangered species assessment. And as part of our environmental fate assessment, I'll be summarizing the soil degradation study.

So I'll be starting with the honey bee test where they tested larval and adult honey bees. And it is important to look at these insects as our beneficial pollinators.

This test was conducted based on a protocol titled, Evaluation of the Dietary Effects of Purified Bacillus Thuringiensis Cry3Bb2 protein in honey bees. And there is a larvae and an adult study. And this protocol was based on EPA's OPPTS guideline.

In the honey bee larvae test, the larvae were dosed with 1,790 parts per million Cry3Bb1 protein, which is considered 100 times the maximum concentration in pollen, which is an appropriate safety factor, since the method of ingestion for

honey bees, potential exposure to the Cry3Bb1 protein, would be through pollen.

In addition, a controlled substance was used for comparison. And a reference substance or a positive control was used, which involved potassium arsenate. This assured that bees were ingesting the treatments and that the study protocol was appropriate.

This was introduced to larvae in brood frames by pipetting in three microliters of the treatment directly into the brood cell. The frame was left to lay flat for about 30 minutes to allow the larvae to ingest the treatment.

A total of 80 bees were treated with each of the test controlling reference substance.

As I just mentioned, here is an example of a frame. These are actually already capped cells. And as I mentioned, it would be pipetted into a brood cell and allowed to wait for 30 minutes.

And then observations were made day eight and twelve to evaluate the level of capping.

And this is an example of capped cells here. Capping is essentially when brood cells are caps and larvae are pupating.

On day 12, these frames that were treated were moved into emergence cages. And twice a day the frames were evaluated to see the level of adult emergence.

All of the larvae survived to capping or pupation in the Cry3Bb1 treatment group. '97.5 percent survived in the control group.

All larvae in both groups that survived to capping did emerge as adults. So there was new statistically significant difference between the Cry3Bb1 treatment and control group in this instance. So we concluded that the no observable effect concentration is greater than 1,790 parts per million Cry3Bb1 protein, which is more than one times the level that the honey bee larvae would be exposed to in the field.

So we can conclude that in the field that development and survival of honey bee larvae will not be affected by Cry3Bb1.

In addition, a test on honey bee adults was conducted. This involved using 360 micrograms per milliliter of Cry3Bb1 protein.

And the activity of the protein was verified using the Colorado potato beetle in an insect bioassay. The Cry3Bb1 protein is a coleopteran active protein. It is particularly and specifically active towards chrysomelids. So the Colorado potato beetle is considered a sensitive species and appropriate to use to verify activity of the test substance.

Again, a control and reference substance were used also in this test.

And they were administered to the honey bees which were kept in cages by putting the treatment into a 12 milliliter vial.

Each of the cages had 40 adults. Each treatment was replicated four times. So a total of 160 bees received treatment control and reference substance. And there were daily observations of mortality and abnormal behavior.

The test was terminated on day 11 when

there was 40 percent mortality in the control group. EPA's OPPTS guidelines recommend conducting these tests until there is 20 percent mortality in the control group or for 30 days.

This test was conducted until 40 percent mortality, because 20 percent mortality occurred on day 3 or 4 and they wanted to carry the test out longer.

And the results of this study showed no difference in mortality between the Cry3Bb1 treated group and the control group. Therefore, we concluded that the no observable effect concentration of the Cry3Bb1 protein for adult honey bees is greater than 365 micrograms per milliliter, which in the study was reported as 20 times the concentration in pollen, but in Monsanto's written public comments, which are in the docket now, they actually acknowledge that this is actually only 4.3 times the concentration in fresh weight pollen.

So overall from the adult and larval honey bee test, we can conclude that Mon 863

expressed in a Cry3Bb1 protein will not cause adverse effects to honey bees under field conditions.

Next, I'll summarize the parasitic hymenoptera or the parasitic wasp test, which was conducted based on a protocol title Cry3Bb2 protein, a dietary toxicity study with the parasitic hymenoptera, Nasonia vitripennis, which is in the family pteromalidae. And this protocol was based again on our OPPTS guidelines.

There were two treatment levels in this group, a 400 and 8,000 parts per million Cry3Bb1 protein, which is equivalent to 1X and 20 times the maximum protein concentration in plant issue, which does represent a worst case scenario since the protein is expressed at its highest levels in the plant tissue.

Again, the protein concentration was verified by a Colorado potato beetle bioassay. And there was a control group using water and a reference group using potassium arsenate.

The parasitic hymenoptera were kept in

one-half pint paper containers during the test. And treatments were administered by mixing them with honey water. They were allowed continual access to these treatments throughout the test.

Observations were made of mortality, pupation and other clinical signs of abnormal behavior or to toxicity.

And this test was terminated on day 16 when greater than 20 percent mortality was reached in the negative control group.

At test termination, there was 24 percent mortality in the 1X treatment group, 58 percent mortality in the 20X treatment group and 23 percent mortality in the control group.

And although there was not a statistically significant difference between the 8,000 parts per million and the control group, there was an acknowledgment of this greater rate of mortality. So the no observable effect concentration was determined to actually be the 400 parts per billion or the 1X treatment group, and the LC 50 was determined to be 8,000 parts per

million of Cry3Bb1 protein or the 20X group.

Based on these conclusions and the fact that minimal exposure is expected to parasitic hymenoptera in the field, basically, they would be exposed to Cry3Bb1 either through parasitizing an insect that has ingested the protein or possibly by feeding on pollen due to this minimal exposure.

And the no observable effect concentration, we do not expect MON 863 to adversely affect parasitic hymenoptera under field conditions.

The next study that I will summarize is the green lacewing study. In this slide there is a picture on the top, which is the egg, on the bottom, a larvae, and to the right an adult green lacewing.

This test was conducted according to a protocol titled Cry 3Bb2 protein, a dietary toxicity study with green lacewing larvae, chrysoperla carnea, which was based on our OPPTS guidelines.

In this case with the green lacewing

test, the diet was administered to green lacewing by mixing the moth egg from the Sitotroga species with the Cry3Bb1 protein. So they are actually eggs mixed up with the protein in a water meal diet. This was not a diet specifically formulated for the green lacewing.

It was administered at -- (inaudible) parasitic hymenoptera, 400 and 8,000 parts per million, which represents 1X and 20 times the maximum exposure in plant tissue.

The activity was verified by a Colorado potato beetle bioassay. There was also a control and reference group which included potassium arsenate.

In this test, there were 30 test chambers which had one green lacewing larvae per chamber. There was a total of 30 insects per treatment group, allowed continual access to the treatment.

Observations were made on mortality, pupation and other clinical signs of abnormal behavior or toxicity. And this test was

terminated after 10 days when greater than 20 percent mortality was reached in the control group.

There was no pupation in the control or treatment groups in this test.

Looking at mortality rates of the larvae, in the 1 X group, there was 27 percent mortality. There was 23 percent mortality in the 20 X group. And 27 percent mortality in the control group. So there was no statistical difference in mortality between the treatment and control groups.

Therefore, we concluded that the no observable effect concentration for green lacewing larvae exposed to the Cry3Bb1 protein in diet is greater than 8,000 parts per million.

However, this test was conducted with MON 853 rather than MON 863, which are very similar products. And because they produce a nearly identical Cry3Bb1 protein variant, we concluded that it was acceptable to conduct this test with Mon 859 rather than Mon 863.

Based on this test, we concluded that Mon 863 will not adversely affect green lacewings in the field.

Next, I will summarize the lady beetle tests.

One study was submitted to us prior to granting an experimental use permit for MON 863, which involved the Hippodamia convergens lady beetle larvae fed pure cry protein in a lab. But since we were dealing with a coleopteran product here, we decided we wanted to take a closer look at the potential effects on lady beetles as a representative, beneficial beetle species.

So in our review of the first lady beetle study submitted for the experimental use permit, we requested additional studies be conducted using actual pollen from Mon 863, since this would be the primary route of exposure of lady beetles.

So three additional tests were conducted using pollen 1 on coleomegilla maculata adults. One on coleomegilla maculata larvae and another on

hippodamia convergens adults.

So first I'll summarize the initial tests submitted prior to the experimental use permit which used hippodamia convergens larvae, fed purified Cry3Bb1 protein. Again, at the same levels as the green lacewing and parasitic hymenoptera, the 1 X and 20 times the maximum protein concentration in plant tissue.

They were also fed a control group and a potassium arsenate reference group.

Observations of mortality and other abnormal behavior and signs of toxicity were observed daily. This test was terminated on 10th day after test initiation when greater than 20 percent mortality was reached in the control group.

In this test, there was 33 percent mortality in the 400 micrograms Cry3Bb1 protein group, 35 percent mortality in the 8,000 micrograms Cry3Bb1 protein group, and 24 percent mortality in the control group.

There was no statistical difference

between the levels of mortality in the control or treatment groups. Therefore, we concluded that the no observable effect concentration is greater than 8,000 micrograms Cry3Bb1 protein per milliliter, which was 20 times the expression level in plant issue.

And we concluded that MON 863 will not adversely affect parasitic hymenoptera under field conditions. However, we did want to take a closer look at feeding these beetles the pollen.

So the first of the three pollen feeding tests involved feeding coleomegilla maculata larvae pollen which was mixed with a dried tephritid fruit fly egg diet at ratios of 50 percent pollen to 50 percent diet. And this was based on the concept that the maximum level that lady beetles will ingest in the field would -- half of their diet would potentially be pollen.

The expression levels have been shown to be 93 to 101 micrograms per gram fresh weight MON 863 in corn pollen. And they did count the number of pollen grains in their treatments.

There was a control group that used a non Bt isoline from event Mon 864 pollen and a potassium arsenate reference group.

Again, there was one larvae per test chamber to avoid any cannibalism between the larvae. They were allowed continual access to the diet. A total of 30 larvae received each of the treatment groups and control and reference groups. And they were observed daily for developmental stage and mortality. And as the adult beetles emerged, they were weighed.

There was no difference in any fitness cause as far as the weight and developmental stage or mortality between the treatment and control groups. Therefore, we concluded that the no observable effect concentration for Cry3Bb1 protein expressed in pollen to coleomegilla maculata larvae is greater than the expression levels found in pollen. And we do not expect the C. Mac larvae to be adversely affected under field conditions by MON 863 corn.

Looking at the coleomegilla maculata

adult tests, they were treated with corn pollen that was assayed and determined to be expressing 37.4 micrograms Cry3Bb1 protein per gram pollen.

They were also treated with pollen from event Mon 846 which does not express Bt. They had another assay control which used bee pollen. Bee pollen is the actual pollen captured by bees and brought back to the hive.

And all of these pollen tests were -- treatments were mixed with an equal amount of the dried tephritid fruit fly egg diet. They were also fed a potassium arsenate reference group.

A total of 30 adults were fed each of the treatments. And they were allowed continual access to each of these diets, and observed daily for levels of mortality. This test continued for 30 days as suggested by our OPPTS guidelines.

At conclusion, there was 83.3 percent survival of lady beetles on the Cry3Bb1 pollen, which was actually slightly higher, although not statistically different from the 80 percent survival on the non Bt pollen.

Therefore, we saw no differences between the treatment and control, and concluded that MON 863 corn will not cause adverse effects to coleomegilla maculata adults in the field.

And the final test of these four is the Hippodamia convergens adult test. Both hippodamia convergens and coleomegilla maculata are common lady beetles found in corn fields. So these were appropriate test species.

Hippodamia convergens were fed the corn pollen plus honey in a 50 to 50 ratio. And the expression levels were found to be for the pollen used in this test, 55 to 73 micrograms Cry3Bb1 protein per gram pollen.

They were also fed a control group of the non Bt isoline corn pollen and a reference group.

There were 25 beetles per test chamber. The test chamber involved a one-pint container. Each treatment was replicated three times. So there was a total of 75 beetles that received each of the treatment groups. They were allowed

continual access to the diets. And this test was terminated after 14 days.

There were daily observations made on clinical toxicity, abnormal behavior and mortality.

At the termination of this test, there was 84 percent survival of the hippodamia convergens adults on the Cry3Bb1 pollen, 81.3 percent survival on the non Bt pollen. So again, there was a slightly numerically higher survival on the Bt pollen, although there was no statistical difference.

We concluded no adverse effects from MON 863 corn at levels that would potentially be encountered in the field.

So looking at the four tests as a whole, we do not anticipate any adverse nontarget effects to lady beetles in general in the field.

We look at collembola as a representative decomposer found in the soil community.

So the collembola test submitted to us

involved three treatment groups using .5, 5 and 50 percent Bt corn leaf tissue plus yeast.

The corn leaf tissue used was from event Mont 859, which as I mentioned is significantly similar enough to MON 863 that we found it acceptable to use this.

In addition, this actually represents a worst case scenario because the Cry3Bb1 protein is expressed at much higher levels in event Mon 859 leaf tissue than Mon 863.

These treatment levels represented 8.73, 87.3 and 873 micrograms corn leaf tissue per gram diet.

There was also a control group which used a non Bt isoline and also the .5, 5 and 50 percent non Bt corn leaf tissue. And there was obviously no expression of Bt in this corn leaf tissue.

And in this case, the reference group utilized thiodicarb.

10 day old folsomia candida collembola were used. There was 10 collembola per jars.

Four jars per treatment. So there was a total of 40 collembola that received each of the treatments in this test. They were allowed continual access to diet by giving them two milligrams of diet every other day. So the diet was never depleted. And this test was conducted for 28 days.

At the end of the test, the number of adults and offspring were counted.

There was no difference between the survival rate between the treated and control groups. Nor was there any difference, statistical difference in the number of offspring between the treated and control groups. Therefore, for collembola, we were able to conclude that the no observable effect concentration is greater than 872.5 micrograms of Cry3Bb1 protein per gram diet.

Therefore, we expect no adverse effects to collembola as a beneficial decomposer in the field under field level conditions. This 872 micrograms per gram is a much higher level than would ever be found in the field.

A primary route of exposure of

collembola would be through the corn roots where the expression of MON 863 is three to 66 micrograms.

The next study I'll be summarizing is the Monarch butterfly study. The agency did not actually request this study since we are dealing with a coleopteran active protein. We look more closely at beetles such as the lady beetle rather than looking at a lepidopteran like the Monarch.

However, Monsanto voluntarily conducted this study. Since they conducted it, they did submit it to the agency and we did review it.

This study involved using levels of pollen grains applied to leaves at 2, 50, 100, 200, 400 and 800 and 3200 pollen grains per centimeter square.

10 first instar larvae were exposed to each pollen level. This was replicated four times. So a total of 40 monarch larvae were exposed to each of these different pollen levels. Neonate first instar larvae were exposed for four days. Then they were removed from the leaves

which contain pollen and exposed to clean leaves through the rest of their develop -- for another six days.

They were observed after 48 hours, 96 hours and 10 days for survival and development. And the amount of leaf consumed was observed after 48 hours and 96 hours.

This test showed no adverse effects of Mon 863 corn pollen on the survival larval weight gain and consumption of Monarchs. Since these tests were conducted at much higher levels than would be encountered in the field, we do not expect any adverse effects to Monarch butterflies by Mon 863 corn.

As part of the registration of MON 863, the agency requested that studies be conducted in the field to look at community abundance levels of non-target insects as well as some of the target insects in the field.

So I'm going to first briefly summarize the study that EPA requested of Monsanto, and they submitted to us as part of the registration. And

then I will briefly describe some other studies that they submitted to us, a preliminary report to us that was supplemental information to what we actually asked them to submit.

So as part of the field study that the agency requested, they looked at the abundance of nontarget organisms in the soil, soil surface and foliage level of the corn fields.

This was a two-year study conducted in 2000 and 2001.

Thusfar, only a partial summary of the 2000 data has been submitted to the agency, which I will discuss briefly today. And a final report will be submitted to us when all the data has been analyzed. And we'll review it after it has been submitted. So this is a preliminary report at this time.

In this test, they looked at both Bt, they compared Bt and non Bt fields. Each of the Bt and non Bt fields either received no insecticide, a seed treatment prior to planting, a granular insecticide incorporated in furrows at

planting, a foliar insecticide used after planting to control adults.

The seed treatments and granular treatments are used to control corn rootworm larvae.

This study involved a split plot design where the main plots were the Bt and non Bt hybrids. The subplots within the main plots were the four insecticide treatments I just described, and each of the main plots was replicated four times.

The subplots which received either no insecticide or the insecticide treatments were 60 feet by 60 feet, included 24 rows. And there was 30 inches between rows. They used pan, pitfall, sticky trap and a dropcloth method of sampling, which I will briefly describe now.

The pan sampling was used to evaluate soil dwelling invertebrate. And this involved collecting eight inch rootballs of soil from each of the subplots. They were sampled during the V6, V10 and R1 growth stage of corn and then taken

back and sent through a modified Burlese funnel system to extract the insects or invertebrate from the soil. And they were extracted into ethylene glycol.

Again, we only have partial results from 2000. So these are preliminary results. But based on the preliminary results, there was no difference in the number of soil dwelling organisms collected from pan samples between the Bt and non Bt hybrids. Although, there was some effect seen from insecticide treatments, even with the same insecticide treatment among the two different hybrids, there was no statistical effect.

The predominant beneficial organisms found in the pan samples included spiders, immature and adult carabids, centipedes, rove beetles, diplurans and earthworms.

The next sample method included pitfall traps to look at surface dwelling invertebrate. This involved putting cups buried into the ground in the field and putting about 100 milliliters of

ethylene glycol into the cups.

This picture here is not necessarily the size of the cup that was used by Monsanto, but just a picture that I had to give a visual representation of what a pitfall trap looks like.

There was four traps per plot. Traps were left in the field for three days and then removed. Pitfall trapping was conducted four times during the growing season between the V 6 and R 4 growth stage of the field corn.

And this test also showed no difference between the number of organisms, beneficial and pest insects found in the Bt and non Bt hybrids. The most abundant species found in these pitfall traps included the most abundant natural enemies, were the spiders, immature and adult carabids and crickets.

In addition, there was a high number of tiger beetles, centipedes, millipedes, ants, rove beetles and carrion beetles. And the most abundant pest species found in the pitfall traps included sap beetles, scarab beetles, corn flea

beetles and a few click beetles.

Also, to look at the number of foliage dwelling invertebrate in the field, yellow sticky traps were used. Traps were placed in the field at canopy level. Again, this is just representative of a sticky trap in the field, not necessarily the exact way that Monsanto placed them in the field.

There were three traps per plot that were left in the field for seven days. And the sticky traps were put in the field four times between the V 6 and R 4 growth stage.

And again, no statistically significant difference was found in the number of invertebrate both beneficial and pest invertebrate found in the Bt and non Bt plots.

The most abundant species found on yellow sticky traps included the northern and western corn rootworm, as well as corn flea beetles, sap beetles and the corn leaf aphid, which is pictured there, but a little small and blurry, unfortunately.

The most abundant foliage dwelling insects as far as the beneficial insects were the Asian lady beetle, seven spotted lady beetle, convergent lady beetle, which is hippodamia convergens and the lady beetle cycloneda munda. In addition, spiders, parasitic hymenoptera, syrphids, green lacewings, brown lacewings, carabids, ants and damsel bugs were also found on these traps.

Finally, as part of this study, the dropcloth method was used to look predominantly at lady beetles.

And on these dropcloth method, the number of beneficial insects was not different between the Bt and non Bt hybrids. The key natural enemies that were looked at in the field that occur in corn fields in general and were found with this method include coleomegilla maculata pictured here, also orius insidiosus, the minute pirate bug, which is pictured on the right, and the parasitic hymenoptera macrocentrus grandii.

So in general, from the preliminary results at least of this field study, we have not found any adverse effects of MON 863 on beneficial non target invertebrate.

There is additional ongoing research that was -- a preliminary report was submitted to the agency which is supplemental to the abundance study which we actually requested. What has been submitted thusfar involves eight studies, seven field trials and one laboratory study.

These studies in general looked at the abundance of invertebrate in the field. There were specific tests that looked at collembola and/or carabids. There were tests that looked at the soil community. Specifically, at coccinellids or lady beetles. And specifically at nematodes. So I will briefly give you a couple of, basically, a one slide on each of these studies.

This was data from one year submitted to us. We do not have at this time a complete submission of materials and methods. Not all data has been analyzed.

It has only been one year of multi year studies that have been submitted thus far. So at this point in time, I'm just going to present it so you have an idea of some of the research that is still ongoing, but we cannot draw any conclusions from these preliminary reports.

We also acknowledge that in addition to what was submitted to us in this report, there are additional studies that are being conducted in the field right now to look at effects of Mon 863 corn.

The first study was titled, Effect of Mon 863 on non-target insects in corn: Results of visual inspections of transgenic corn for corn rootworm control.

This was conducted during the summer of 2001. It was a field trial to look at abundance of arthropods in the field, much like the study I just summarized.

They looked at Bt and non Bt hybrids, both without insecticides and also with seed treatment, granular treatments and foliar

treatments. And the abundance was evaluated using visual counts on the whole plant. Also, using pitfall trapping and Tullgren funnels, which is another form of the Burlese funnel that I described which looks at your soil dwelling organisms.

The next test looked at the effect of transgenic corn rootworm material on beneficial arthropods.

This one looked at specifically at collembola collected from pitfall traps, as well as visual observations made in the field. Again, Bt and non Bt hybrids were looked at. Of course, the Bt hybrids are Mon 863 corn.

Again, there was no insecticides, and the different insecticides on both hybrids looked at effects on collembola.

An additional study which looked at collembola was titled, Effects of rootworm resistant Bt corn and insecticides on springtails and community biodiversity.

In this case, the pitfall trapping was

utilized in Bt and non Bt hybrids both with and without insecticide treatments.

The next study looked at carabids or ground beetles in the field. This was titled, "Preliminary report 2001, carabid activity in large plot plantings of rootworm resistant hybrid corn.

Bt and non Bt hybrids were looked at with and without insecticide treatments using pitfall sampling. However, again, we have only preliminary, a preliminary report of this. Within this preliminary report, the authors did acknowledge a problem with this study because there was missing values due to animal damage and other unknown factors that damaged the pitfall traps themselves.

There was also a density gradient across plots and a large wetland that potentially limited the movement of beetles into the Mon 863 plots. So this study will be continued this summer and for additional years.

The next study looked at the

decomposers, the effect of MON 863 on decomposers and the rate of decomposition of the tissue in the field. The title is, Influence of Bt endotoxin expression in corn on plant residue decomposition and soil invertebrate community structure, a preliminary report.

There was three aspects to this study. The first one utilized litter bags filled with dried corn residue buried in the field 5 to 10 centimeters, which was appropriate for a tilled system to see what would happen if you tilled the tissue into the fields.

Another aspect of this study involved taking the plants out of the inside of the field and drenching them with water to bring earthworms to the surface and earthworms were collected.

And a third aspect used wheat straw put into litter bags to look at the effects of different environments, these different environments being the Bt and non Bt hybrids with the different insecticides treatment, no insecticide, the seed granular or foliar

treatments.

Another -- the only laboratory study that was part of this report involved lady beetles, the C Mac. lady beetle. It was titled, Non-target effects of corn rootworm Bt corn, a preliminary report. Coleomegilla maculata larvae were used in this. They were fed both aphids intoxicated with the Mon 863 protein pollen mixtures containing 0, 25, 50, 75 or 100 percent Mon 863 pollen in diet.

And the duration of development of each instar as well as pupal weight was looked at.

And a second part of this study looked at pollen mixtures with artificial diet. This looked at duration of larval development, pupal stages, pupal weight, adult walking speed, flip time, survival and fecundity.

So essentially, they were looking at different -- potential fitness cost of MON 863 on the lady beetle larvae. And this test is also being conducted on carabids. Although, no information was submitted at this time.

So as I mentioned, this was a very preliminary report. I wanted to point out to the panel that different types of field research that are ongoing. Additional studies are being conducted which were not summarized in this report. When these studies are completed, we expect that a report will be submitted to us with final results.

And I failed to discuss this last report. I'm sorry. There was a couple more reports.

This one is the preliminary report of the response of coccinellids exposed to corn rootworm resistant hybrids in the corn. This involves sampling coccinellids under field conditions using sticky traps and whole plants and Bt and non Bt hybrids.

There was also a study that I failed to mention, I jumped the gun a little bit here, looking at nematodes in the field. This study was titled, Bt corn suppression of Meloidgyne incognita and other nematodes.

Looked at a plant-pathogenic nematode. The plant-pathogenic nematode study involved using three-week old corn seedlings grown in pots and infesting them with the plant-pathogenic nematode at rates of 5,000, 10,000 and 15,000 nematodes.

Observations were made on weeks 2, 5 and 10 after infestation.

The second part of this test looked at a bacteriovorous nematode and an entomopathogenic nematode or a predatory nematode.

And this involved using four-week old corn seedlings grown in pots. The seedlings were removed from the pots, and these nematodes were tested with both the soil leachate from the corn seedlings and also a root extract taken from these corn seedlings. And a number of live and dead nematodes were determined.

Back to my ultimate results from all these is that these are ongoing studies with limited information. So we cannot draw any conclusions at this time other than to just look at what is being done at this time.

Also, as part of the invertebrate test, we looked at earthworms. The earthworm test was a 14-day LC 50 test. The 14-day LC 50 -- the LC 50 was shown to be greater than 570 micrograms Cry3Bb1 protein per kilogram of dry soil, which is 10 times the maximum exposure that earthworms would have in the field.

So we were able to conclude the no observable effect concentration for earthworms is greater than 570 milligrams Cry3Bb1 protein per kilogram of soil. And we do not expect Mon 863 to adversely affect earthworms under field conditions.

As part of our assessment for the Bt proteins as well as any pesticide, we look at potential effects on endangered species. Here I have pictured the American burying beetle, which is an endangered beetle.

It becomes a little bit difficult with these endangered species tests because you cannot directly test an endangered species in the laboratory. Therefore, you have to look at

exposure to highly sensitive species and potential adverse effects to them as well as potential exposure of any nontarget endangered species in the field.

Cry3Bb1 is a coleopteran active product that is specifically toxic to chrysomelids. And there are currently no chrysomelids listed on the endangered species list.

Based on this, we don't expect any adverse effects to endangered chrysomelids because there aren't any.

But we took a closer look at the Colorado potato beetle, since it is a sensitive species, and it is illegal to directly test these species, as I said, as well as exposure, potential exposure.

Most of the endangered and threatened beetles occur in caves or aquatic habitats. So their exposure would be minimal to MON 863. And we in general don't expect any endangered beetles to be in or near cornfields.

The one beetle that we took a closer

look at as a slight possibility of exposure was the American burying beetle which might occur in old fields or cropland hedge rows. But the American burying beetle essentially oviposits into decaying animal carcasses that are buried.

Based on the fact that they would be inside the decaying animal carcus, which then again is buried in the field, we don't expect this beetle to be exposed to MON 863 if it were to occur in an old field.

And finally, I'm going discuss one aspect of potential environmental fate of MON 863, which is the soil degradation study submitted.

In this test event Mon 859, lyophilized field corn leaf tissue was used rather than the MON 863. And as I have mentioned, the agency has concluded that they are similar enough, variants of the Cry3Bb1 protein that it is appropriate to look at Mon 859. Plus we viewed this as a worst case scenario since Mon 859 is expressed at much higher levels in the corn leaf tissue than MON 863.

The youngest whorl leaf tissue of 2 to 4 week old corn plants were used, which again represents a worst case scenario since the highest expression levels of Cry3Bb1 is in young leaf tissue.

And it was found that this was expressed in Mon 859 at 1,745 micrograms Cry3Bb1 per gram dry weight lyophilized leaf tissue.

Since they assumed that the leaf tissue could be incorporated into the top six inches of soil, this is what was looked at in this study. They looked at levels of 3 percent or 10 percent of dry weight leaf tissue per gram of soil.

So essentially, 3 percent or 10 percent of the soil would constitute the lyophilized leaf tissue. And there was also a control group which used a non Bt isoline from event Mon 846.

This soil was collected from the field in Kentucky. It involved a sandy loam soil which had all the natural microbes that would occur under field conditions. This soil was not amended. It was taken back to the lab. And an

insect bioassay was conducted using the Colorado potato beetle as a sensitive chrysomelid species.

16 beetles were used per replicate. And treatment doses, as I mentioned, included 3 percent of the soil or 10 percent of the soil being the dried leaf tissue.

In addition to the insect bioassay conducted with the Colorado potato beetle, an ELISA was conducted. However, there are problems with the ELISA because the ELISA does not let you know whether the protein that is found is functional or nonfunctional, meaning toxic or not toxic, not active protein, Cry3Bb1 protein.

It only shows extractable protein. And as I mentioned, does not distinguish between whether it is functional or nonfunctional.

Results of this test were based on the 10 percent leaf tissue in the soil as opposed to the 3 percent because the levels were not high enough to detect at the 3 percent level.

The DT50 or time for 50 percent of the protein to degrade was determined by insect

bioassays to be 2.37 days. The DT 90 was determined to be 7.87 days, which was not significantly different from the results from the ELISA test where the DT50 was 2.76 days and the DT90 was 9.16 days. After 28 days, the protein was not detected at all by the ELISA test.

Therefore, we were able to conclude that in sandy loam soils, the Cry3Bb1 protein likely degrades very rapidly under field conditions.

However, studies have shown that the Bt proteins will bind to clay and humic acid type soils.

Therefore, we have requested that in our preliminary assessment we are looking for additional field tests looking at a variety of soil types which will include clay and humic acid soils over a longer period of time and under field conditions.

We prefer seeing these fields from -- the actual soil from field conditions because it is possible we also want to include roots as well as leaf tissue because it is possible that the

root tissue is degrading slower than the leaf tissue.

So that essentially -- I was going to read the questions. Shall I wait?

DR. PORTIER: We'll read the questions later.

MS. ROSE: Prior to any points of clarification, I just would like to thank all of my colleagues for all of their help with these assessments and Chris for manning the computer for me today. I would like to thank Allen Dively (ph) for providing me with a lot of these pictures.

And I particularly would like to thank the chair and the panel today for the opportunity to present our EPA's assessment to you.

DR. PORTIER: Thank you very much, Ms. Rose. That was a lot of material to cover in such a short period of time.

Are there any questions of clarification from the panel?

Dr. Federici.

DR. FEDERICI: On the lacewing egg

feeding trials, it wasn't clear from the written material or from what you presented here that they actually ate the toxin. It said -- you used the word, in the egg, is the actual preposition that is used. So I'm wondering how do you know that they actually ate the toxin?

MS. ROSE: I agree with you that it's unclear. If you note in our questions, that's a question we're asking of the panel today, do we believe based on this way of administering the diet. We're unsure if they are really ingesting the protein or not because I don't believe it's a diet specific for the green lacewing.

Are they ingesting, is this appropriate or is there a better way is a question that we would like answered.

DR. FEDERICI: Now I have kind of a policy question, which maybe I shouldn't ask now to be answered. But let's suppose that you actually found an effect. Let's suppose that actually you have the right kind of feeding trial and it kills 50 percent of the lacewing larvae.

Then what?

MS. ROSE: That's a Janet question, a Dr. Andersen question.

DR. ANDERSEN: Then you have to do a risk assessment to put it into context. Our law requires us to look at the risks and the benefit of a pesticide. That would clearly be a risk area. And we would have to weigh that risk against the benefits of the product.

DR. FEDERICI: I have several other questions. But they are to later parts of it.

DR. HELLMICH: I have a couple questions. First of all, on the field invertebrate consensus studies, I understand that there were several treatments, four or five treatments. But you just gave the results of the Bt versus non Bt.

Were there impacts from the insecticide treatments in these cases?

MS. ROSE: Yes. There was impacts from the insecticides themselves. But if you looked at Bt and non Bt applied with the same insecticide, there was no difference between the two hybrids

plots.

So if you looked at Bt and non Bt with no insecticide, there was no difference between the Bt and non Bt as far as abundance. If you looked at the Bt and Bt both applied with a granular soil applied insecticide, again, no difference between the hybrids.

If you looked at the insecticides, there was an effect. I didn't present that because we're not looking at effects of insecticides today.

DR. HELLMICH: Okay. But I think the panel would be interested in knowing whether or not there is benefits. And in certainly comparing to --

MS. ROSE: I have a copy of the study with me. I do not have committed to memory the exact effects or results from the insecticides, but we can take a look at that.

I can give you that to look at.

DR. HELLMICH: You were finding effects from the insecticides compared to --

MS. ROSE: Correct. There was definitely a reduction in most of the species from the actual insecticides. But if you looked -- just looking at the effect of Bt versus not Bt, in each of the insecticide regimes, there was no difference between the hybrids.

DR. HELLMICH: I think that is an important point.

Now, going back to the honeybee study. I'm somewhat familiar with some of these tests that you can do with honeybees.

Why is the 4.3 X, why was that the limitation on that? You were feeding them in these little vials on top of these hoarding cages. Is that right?

MS. ROSE: You are saying why did they test it at 4.3 X or how did we determine that it was 4.3 X?

DR. HELLMICH: Why couldn't they go a little bit higher on that. Were the honeybees actually repelled by the -- what was the limitation there?

MS. ROSE: In the study, they showed where they actually took corn pollen and looked at the expression in dry corn pollen and found it was 19 micrograms per gram pollen. And I believe they were basing it on the 20 X of that.

But if you look at from their product characterization studies the expression in fresh weight pollen, it is actually a 4 X, which either way is a higher rate than expressed levels --

DR. HELLMICH: So when they conducted the test, they thought they were doing 20 X.

MS. ROSE: Yes.

DR. HELLMICH: But afterwards, they found out they had to revise that.

MS. ROSE: Right.

DR. PORTIER: Dr. Alexander.

DR. ALEXANDER: It's well known that the bioavailability of proteins in the soils is affected by the type of clay. Enormous differences with type of clay.

This would affect the availability for toxicity, availability for biodegradation. And

I'm surprised that neither Monsanto nor EPA has ever asked the question of the kind of clay. Not the percentage clay.

MS. ROSE: That is correct. We have the percentage of clay that was in the soil tested. And we know that it was field collected soil from Kentucky. But the exact kind of clay was not reported to us. But that is a good point that perhaps during the discussion it could be brought up again for the report.

DR. PORTIER: Dr. Angle.

DR. ANGLE: What is the concentration of the expressed protein in the stem tissue?

MS. ROSE: I have everything except stem tissue. I would have to go look that up for you, which I can do during one of the breaks.

DR. ANGLE: Any idea what proportion of the crop residue return back to the soil is comprised of stem tissue?

MS. ROSE: Again, other than knowing that you would mow (ph) down the entire plant -- there is also at least for the lepidopteran

actives I know that they have shown that the level of protein degrades as the corn plant cineses. So potentially by the time it is plowed into the soil is at a lower expression level than in the fresh stem tissue.

But the exact amount of tissue that is in the soil, I couldn't answer that.

DR. ANGLE: Do you think we could get that sometime today?

MS. ROSE: I certainly can try.

DR. PORTIER: Other questions? Dr. Andow.

DR. ANDOW: I would like to thank you for such a concise summary of a lot of information. I thought that was quite good. You mentioned that in comparing the toxins associated with Mon 853, 859 and 863, that you came to a determination that they were not really that different.

I was wondering if you could summarize the evidence that you used to come to that determination?

MS. ROSE: Can I ask John to do that?

I'm an entomologist. We have people that do those things. John Kough, if he could help out.

DR. PORTIER: Please introduce yourself.

MR. KOUGH: John Kough. I've done part of the review for the product characterization of the events that you were asking about, Mon 863 and Mon 859, I believe.

DR. ANDOW: Isn't there also a Mon 853?

MR. KOUGH: Yes, 853 and 859 are basically transformants using the same plasmid. The proteins in both these events are engineered from the wild type.

They contain either four or five amino acid differences, which were apparently introduced to increase or enhance their activity to the diabrotica pest species.

The difference between the 859 and the 863 is that the 863 has the protein with the five amino acid changes and the 859 and 853 have the plasmid with the alteration that only has four

amino acid alterations.

The tests looking at bioactivity between those two protein types at the level of sensitivity that can be detected with bioassays did not indicate that there was a significance difference in the bioactivity against the target pests.

And also I believe that many of the tests were done with the Colorado potato beetle because it's such a sensitive species.

In addition to that bioassay information, there is also indications on the biochemical characteristics that are used for the human health assessment, which include amino acid homology comparisons and in vitro digestibility.

And neither of those two assays showed a significant difference.

In summary, that information was used to basically say that there was not an indicated difference between the toxins in these two events.

DR. ANDOW: Just a quick follow up on that, then. On the amino acid changes, is this

based on extracted protein from the plants? Is it based on an analysis of the DNA in the plasmid? Or is it based on analysis of the DNA as it occurs in the plant?

MR. KOUGH: It is a DNA analysis. It is not confirmed, to the best of our knowledge, from actual sequencing of the expressed protein.

There was extensive analysis using a maltitoff, which is a mass spec type analysis, which indicated that a large portion of the protein is the -- the fragments that are generated from that are in the size range that would be expected. It doesn't confirm the amino acid sequence.

DR. ANDOW: So it is the DNA and the plasmid that --

MR. KOUGH: Yes. And it is sequencing -- I believe there is also analysis of the plant DNA that would confirm that too.

But right off the top of my head, I can't remember exactly which of the two it is. I know for sure that it's the plasmid DNA. But

there may have also been analysis of the plant DNA.

I could look that up for you.

DR. ANDOW: That would be very good if you could. Thank you.

DR. PORTIER: John, can I follow up with a real quick question? Are the maltitoff results in the public domain?

MR. KOUGH: Yes, they are part of the data package.

DR. PORTIER: Any other questions?

Dr. Barbosa.

DR. BARBOSA: I had a question relative to the nontargets. I'm curious to what degree the choice of nontargets to be tested are required by EPA relative to it being a choice on the part of Monsanto.

And a follow up related to that, the degree of choice involved in how the tests are to be conducted. Specifically, the exposure to the protein, whether it is to be in a diet or a fluid like water or pollen or et cetera, et cetera.

MS. ROSE: The first part of your question regarding the species picked, I assume you are talking about the predators and parasitoids?

DR. BARBOSA: Yes.

MS. ROSE: In our pesticide assessment guideline, subdivision M, we ask for three species from the list that I had shown earlier. Typically, that's lady beetle, parasitic hymenoptera. And it's typically nasonia vetripennis, which I think just has to do with being able to rear it in the lab. And the green lacewing --

DR. BARBOSA: But is it specified to type of insect or is it specified to species? In other words, parasitic insect would be a group.

MS. ROSE: Parasitic hymenoptera would be a group, lady beetles --

DR. BARBOSA: But you don't specify a particular species?

MS. ROSE: No, we don't. And it is very likely that -- it's typically up to the company to

come request. We have made those something of an unofficial standard.

If the company wanted to test a different natural enemy than the green lacewing, for instance, the minute pirate, they could do that, but we usually recommend having consultation with EPA ahead of time to make sure that that's going to be okay.

A lot of times we almost prefer them test a species that would potentially be exposed.

DR. BARBOSA: The other part was to what degree is there flexibility in how, in this case, the protein is given or provided to the nontargets?

MS. ROSE: There is some level of flexibility unless we specifically ask. For instance, with the lady beetles, we specifically ask they conduct some studies with pollen, since we knew that's how the primary route of exposure would probably be.

Typically, with the Bt crops, they use purified protein. And that, I think, has a lot to

do with just being able to do it in the laboratory and to be able to conduct the test without everything dying anyway just from the testing type effects.

And also, looking at purified protein is also often a worst case scenario. Because you can get much higher -- we want to have a risk or a safety level of 10 to 100 times field exposure, which is difficult to do if you are taking what they are exposed to in the field, that is at field exposure levels. By using purified protein, you can now bump up to a safety factor.

Again, if something other than the purified protein could certainly be used, and a lot of times we recommend to the companies you come and talk to us first before you conduct a test that would not be acceptable.

So there is some flexibility, certainly.

DR. PORTIER: Dr. Federici?

DR. FEDERICI: There is a question over there.

DR. PORTIER: Dr. Neher.

DR. NEHER: I had a few questions.

One, first of all, on a follow up on the decomposition study. You mentioned the lyophilized plant tissue. I was wondering was that plant tissue ground or were those fragments? What was the form?

MS. ROSE: It was ground.

DR. NEHER: Ground, okay. And as far as the environmental conditions, I was thinking, what, most of that litter in the field would typically land on the ground near harvest, post harvest.

Were the environmental conditions similar to those -- to post harvest?

DR. ROSE: Yes, and that is reported in the summary. I didn't go into that level of detail today due to time constraints. And I honestly don't have a lot of that committed to memory. But again, during the break I have the study with me and we can look at a lot of that.

DR. NEHER: Okay. There were a couple things that I thought might be perhaps

typographical errors in the report. Do you want those? Like there was something on the collembola. It mentioned coal as the substrate. That should be charcoal, I presume, something like that.

MS. ROSE: I'm not sure.

DR. NEHER: I can mention those later. I can tell you the actual pages --

MS. ROSE: Again, I have all of these studies with me so we can look to see if it was my error.

DR. NEHER: I have the page numbers and the report. I would be happy to go through those.

MS. ROSE: Excellent. Thank you.

DR. NEHER: On the nematode study, is it correct that there is no protein concentration reported for the root extract or the soil extract protein concentration?

MS. ROSE: The protein concentration from the product characterization studies was found to be 93 to 101 micrograms of Cry3Bb1 protein.

I think there was also a published study that showed -- I think that was for the roots. Yes. There was also a published study from a root expression assay that showed 58 parts per million expression in the roots.

DR. NEHER: And that was the expression in the extract? Or that was in the living root tissue. The nematodes were exposed to an extract of roots, was my understanding.

MS. ROSE: Yes, they actually took the living roots and produced from the fresh roots an extract.

DR. NEHER: And that was the concentration in the extract. Okay. That wasn't clear to me.

MS. ROSE: Well, it wasn't clear. That's why I was really trying to emphasize that these were so preliminary that we weren't given full methods.

And I don't know if they did an assay to see exactly in the roots they used. They didn't report that. So I don't know at this time

exactly in the roots that were used.

And I believe in the final report, that we'll get that sort of information.

DR. NEHER: That would be helpful.

One of the other items I wanted just clarification was reported in the earthworm study. In terms of the equation reported for computing percent moisture, the denominator in that equation was reported on page 3 of that document as net wet weight.

Typically, in soil physics they use net dry weight. I wasn't sure if that was a typographical error. But that would influence the computations of concentrations that are expressed per gram dry weight of soil.

MS. ROSE: I didn't actually review that study. If my colleagues could help. We can look that up for you.

Off the top of our head --

DR. NEHER: I think that's useful to double-check.

DR. ROSE: Okay. For the earthworm.

DR. PORTIER: Dr. Federici was next then Dr. Jepson.

DR. FEDERICI: I noticed in several different parts of the reports in the information we were given that the term chrysomelid specific, that Cry3Bb1 is chrysomelid specific.

And in general, Cry proteins are not family specific. So I wondered if you could either document that somehow.

One reason I have concern about it, either EPA or Monsanto may be backing themselves into a corner in that it wouldn't surprise me if some other families of beetles and species were eventually found to be sensitive to Cry3Bb1.

MS. ROSE: According to the Monsanto submissions, they have referred to it as chrysomelid specific. But we at EPA have recognized that it's a coleopteran active and have looked at it more.

That's why with the endangered species we did look beyond just chrysomelids. And that's why as far as our beneficial insect, we look

closely at lady beetles recognizing that we didn't want to just concentrate on chrysomelids. So I agree with you.

DR. PORTIER: Dr. Jepson.

DR. HELLMICH: This is just a follow up.

DR. PORTIER: Let Dr. Hellmich follow up for a minute.

DR. HELLMICH: But currently there are no other beetle families besides chrysomelids that have been found that have been affected by this protein. Is that correct?

MS. ROSE: I believe so.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: I just had a couple questions to ask about the acceptability of some of the testing.

So in the chrysomelid test and the parasitic wasp test, the tests were brought to a close once control mortality exceeded 20 percent. And that was deemed to be an acceptable criterion by the reviewer.

In the aphis molifera (ph) adult test,

there was an argument made, which you also accepted, that the test should continue beyond 20 percent mortality in the controls to enable a more comprehensive treatment and control comparison.

Now, in the chrysoperla test, the endpoint was pupation, and yet the test was brought to a close before pupation had occurred. So it didn't really allow us to evaluate any impacts potentially on the duration of the life cycle.

In any case, I would have expected pupation to be occurring at 10 days because at that temperature, chrysoperla carnea should be expected to stop pupating at eight days and I would have expected you to make some notes about that in the review.

So can you comment on the acceptability or non acceptability of data when control mortality exceeds 20 percent given the variation and the standards you have applied across the evaluations?

MS. ROSE: This 20 percent mortality in

the control or until 30 days is a guideline. There is no etched in stone that this is the way a test must be conducted. There is a lot of flexibility.

And we also consider potential risk to the insect. For instance, a green lacewing we consider the potential exposure in the fact that it's not a neuropteran active product as we're doing our reviews.

We did make note of that as we went through the review process and found that we didn't think that at field exposure levels that there would be a risk.

But you make good points.

DR. JEPSON: I'll be commenting later on what I feel an appropriate conclusion to draw from a laboratory test might be. And that's something you have asked for guidance on. I'll be talking about that. I think that's something that needs to be considered further.

I would note also for the nasonia test you cite pupation as one of the endpoints. And

this is a test on adults.

Did that test continue through the life cycle?

MS. ROSE: No. I'm trying to remember noting --

DR. JEPSON: There needs to be amendment to your evaluation --

MS. ROSE: I would have to go back and take a closer look at that.

DR. JEPSON: Yes. There seems to be a standard language you use between some of these tests and the evaluation.

And unfortunately, the organisms don't cooperate by having -- because -- you treat them at different life stages in their life cycle.

The only other thing I wanted to ask was I don't know of any data that explores whether or not the toxin -- how the toxin would persist in, for example, the chrysoperla diet, which was changed weekly.

As far as I can see in that study, there was an evaluation at the outsets before the mixing

took place to determine the toxicity of the protein. But then it was left in the chamber for a whole week mixed with water meal diets and eggs.

Are you confident that there was continual exposure to the toxin in those studies?

MS. ROSE: In most of the studies, and I can't speak for the green lacewing exactly, but in most of these studies, they did periodically take subsamples to double-check the activity of the protein. And then they checked again at the end of the test.

DR. JEPSON: The early study where there is reference to that -- sorry to interrupt, I think is the aphis molerifera (ph) study, which seems to be applied with standard of having bioactivity recorded throughout using the test organism as well as the ELISA studies of concentration.

That standard didn't seem to be applied in the other test or you didn't refer to it in your evaluation.

MS. ROSE: There also has been studies

conducted that have shown that at 80 degrees below, negative 80 C, that the Bt protein will remain active for about a year.

DR. JEPSON: But these tests were run at 21 through 28.5 -- I have forgotten the exact temperatures here. I'll note it in the report. They were running at high humidity and at relatively high temperature.

MS. ROSE: It is very possible that some level of the protein degraded. But again, that would also be happening under field conditions where they would be exposed.

If it's going to degrade -- in the lab it also would be degrading, particularly pollen which would be an exposure rate. Once it is shed from the plant, the expression goes down and is gone after a few days.

DR. JEPSON: I understand what you are saying. But pollen is shed for a period --

MS. ROSE: Yes.

DR. JEPSON: -- That exceeds the duration of this test.

MS. ROSE: Yes.

DR. JEPSON: And in any case, the laboratory test is not meant to be simulating what's going on the field. Because it manifestly does not simulate that.

What you meant to be doing is challenging the organism with a dose that in theory exceeds what it might be exposed to in the field.

So what I'm saying is I'm asking about the level of confidence you have that that high exposure level did actually persist throughout those tests.

MS. ROSE: Well, I, as the reviewer, clearly feel confident because I accepted the study. I brought all the studies with me. I will double-check that one also to see if I just didn't include it in my summary and perhaps they included that information.

If not, as we have our green lacewing discussion this afternoon, I'm hoping that a lot of these things come back up.

DR. JEPSON: I won't continue anything more now.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: I have some questions.

The CD you gave us, and there are these TIF files, and just for the panel members, the file that is 45653003 in the study the title is, research and the effects of corn rootworm protected transgenic corn events on nontarget organisms preliminary results, there is nine studies in there. And there is 70 pages here. So I think it is pretty important information.

You sort of indicated that the results for 2000 you have looked at those. But it seems like the results from 2001 are also in here. Is that true?

MS. ROSE: Yes. Unfortunately, I tried to present that clearly. But there still a little bit of confusion. The first test that I discussed was the abundance test. And that reported the 2000 results to us. And that was the study we actually requested Monsanto do.

Then, there was that second submission which had, you are saying, nine studies, but actually, there was eight and then there was just a sentence that said, there was a statement of John Lucy (ph) that said, we have no information to give you, but we know the study is being conducted.

So it was actually eight, seven field and one lab. And that was where I had the one slide. Essentially, I gave you the title and two or three bullets on each test.

From that study, it was one year. We did not have a comprehensive materials and methods given to us. Very little of the data was analyzed. We didn't feel comfortable making any conclusions from the little bit of information.

Because we ask a lot of questions of your opinion on the importance of field studies and what types of field studies, I wanted to make sure to let everybody know in the panel what is being done.

And as I think a lot of us know, there

is more being done than what has been submitted to us.

So you are talking about two different submissions. You have that abundance study, which had the 2000 results. And then you had those nine studies -- or eight.

DR. HELLMICH: In these eight studies, some of them have 2001 results. Is that correct?

MS. ROSE: Yes. Some of them they have 2001 minimal results. Nothing I felt comfortable -- when we at EPA took a closer look, it was partial data. It might have been like one rep looked at. It was so preliminary that we didn't feel comfortable drawing any conclusions.

DR. HELLMICH: But there may be more information available now.

MS. ROSE: Absolutely. That's why I said we're anticipating -- and these are continuing -- that will be submitted to us.

DR. HELLMICH: If it is available, it would be nice if we had -- could at least look at it.

MS. ROSE: We don't have that information. It is possible that Monsanto does. I don't know if we could have that in any short time frame.

But we anticipate when actual data has been collected, analyzed and written up, it will be submitted. I'm a little hesitant to look at things prematurely.

DR. PORTIER: Any other questions, Dr. Hellmich?

DR. HELLMICH: No. That's fine.

DR. PORTIER: Dr. Andow?

MS. ROSE: I'm sorry. Can I -- another back-up. You made a good point.

All of those additional studies were not data we required for registration. This is additional data, some being sponsored by Monsanto, some by USDA, some by other forms of funding. So it is additional supplemental information that is not required, at least from an EPA perspective, to register the product.

DR. ANDOW: A few questions.

First, when you were talking about the coleomegilla pollen consumption studies where they were mixing the diet with lyophilized tephritid eggs, you said that the 50 percent mixture was based on what you were expecting to be a maximum consumption in the field.

The registrant, I believe, said that that was an average consumption rate. And I just wanted to clarify is your position based on analysis that this is a maximum or is it really based on the registrant's assertion that it is an average?

MS. ROSE: It was based on the registrant's assertion, and I must have misstated it. I thought they had said up -- I that the submission said up to 50 percent of their diet was pollen.

Again, I have the study with me. So I can double-check that. So I misstated, then, if it was an average.

DR. ANDOW: I may be wrong too. But I was looking at that over the past couple days.

And that was the impression I had. I will address this later, then.

The second question is about folsomia. As you know, a lot of the collembola eat yeast primarily. So I wonder what evidence was there that convinced you that the folsomia were actually eating the leaf tissue that was being offered to them.

MS. ROSE: These were a lot of studies that I did reread recently. Being that they had a reference, and I believe they also had the reference in the collembola study, and when you have a high level of mortality in the reference group, that verifies that your methods are working and that they are ingesting the products.

And that's about the best we can do to assure that they are ingesting the treatments.

DR. ANDOW: And in the case of the leaf tissue with the arsenic, it could be that they are getting the arsenic on their cuticle and cleaning it off and eating it, because the arsenic is not inside the plant tissue, whereas the cry protein

would be inside. So I just was wondering about that.

MS. ROSE: That's a good point. And any recommendations on other ways of assuring that collembola ingestion for future studies is welcomed.

DR. ANDOW: Two more questions.

MS. ROSE: Excuse me. Zig wanted to make a comment.

DR. VAITZUS: Zig Vaitzus. I would like to make one additional point in regard to collembola ingestion.

Our main goal is to make an assessment of what happens in the field, not necessarily to totally examine a laboratory study. And if they do not ingest the leaves in the laboratory, I think it is a fairly safe assumption to say that they will not do so in the field and, therefore, there should not be an environmental effect. So it becomes academic to whether the test itself involve the ingestion or not.

I just wanted to make that point, that

our goal is to extrapolate from the laboratory into what happens in the field. And if they don't eat in the lab, they won't eat in the field, presumably.

DR. ANDOW: Well, as you know, folsomia (ph) is used in laboratory studies because it is relatively easy to rear on the yeast in the laboratory, whereas some of the other species are not so easy to rear in the laboratory probably because they don't just eat yeasts in the laboratory.

I would consider that to be very dangerous reasoning to rely on.

DR. VAITZUS: One last point. Therefore, we do have considerable information coming in on field studies on the effects of Bt corn on the field abundance of collembola species. Not just their total abundance, but individual species.

So we rely primarily on that for our risk assessment.

DR. ANDOW: Thank you.

I have two remaining questions.

One related to the endangered species analysis. One part of that analysis could be to what extent is corn grown to known endangered species habitat.

I have to admit I didn't analyze this segment that closely. But I'm wondering is that a part of your analysis?

MS. ROSE: Absolutely. I was trying to make that point during the talk, that we look at potential exposure. And being that most of the endangered beetles occur in caves and aquatic habitats, we don't have a big concern of exposure of Mon 863 in a cave.

There may be a little bit of pollen that will get into the water, but at such minimal levels that we didn't expect a risk to any endangered aquatic beetles.

The one that we found that had the chance of occurring in old fields was the burying beetle. And again, because it buries, we again didn't expect there to be exposure.

So we did look specifically at beetles and specifically at exposure.

DR. ANDOW: So for the burying beetle, you felt that its habitat was --

MS. ROSE: Would preclude it from exposure. Yes.

DR. ANDOW: So that it wasn't necessary to do the proximity analysis, really?

MS. ROSE: (Nodding).

DR. ANDOW: And then the final point is a question about how you relate to the control plants that get used in field studies, since most of the time the control plants are at best near isogenic matches so that there is actually quite a bit of genetic difference between the controls and their Bt counterparts even when they are as closely matched as seems reasonable for agronomically useful varieties.

In some cases, they are not even matched at all except that they appear to be agronomically similar.

Given that there are a lot of other

differences, genetic differences between the plants, I would just like to have you elaborate a little bit about the way you view this. Because if there are differences that you detect, they could be attributed to the other differences in the plants and not to the Bt difference.

If there are no differences, it could be because the other genetic differences are masking somehow the effect of the Bt. So it leaves sort of a problem in terms of inference.

I'm just wondering what is your, as a reviewer, what is your current way of thinking about this?

MS. ROSE: The Mon 846, the event Mon 846 which was used as the control was reported as a nearly identical or similar isoline of the MON 863.

Basically, what is the alternative, I guess, is what comes up in my mind of if we want to compare Bt to non Bt in the field to see if Bt is having an effect, the only way you can see if it's having an effect is to compare it to

something that doesn't contain Bt.

In my mind, we use the best hybrids or isolines that we have available, which I believe was the Mon 846.

But also, this is part of the reason for today's panel, is to address those sorts of issues.

DR. ANDOW: Thank you.

DR. PORTIER: I think there will be other opportunities. But Dr. Alexander.

DR. ALEXANDER: A fast question. I think possibly a fast answer.

Does the agency expect the submitting companies to provide information about confirmatory or negative information that exists in the literature and/or does EPA go through that information and put that as part of their assessment?

MS. ROSE: Both. Certainly, there is part of FIFRA that requires any adverse effects to be reported to the agency.

In addition, companies typically will

submit positive results from the literature. And then we do an extensive literature research in addition, particularly for the Bt crops. For the Bt crops being so new, that the information is limited enough that it is pretty easy to keep up.

DR. PORTIER: I had one question. It's a little bit multiphasic.

In looking at this overall set of studies that are done here, I'm curious about the sample sizes used in the laboratory studies in this setting versus the sample sizes used in the laboratory settings for an administered pesticide, a non biologically based pesticide. Are they roughly equivalent?

DR. ANDERSEN: If you are looking at a conventional chemical pesticide, most of these tests wouldn't even be done. We ask for far more data for these products than we do for conventional chemical pesticides.

The basic framework for how we approach looking at ecological effects for these products, we have relied on the specialized pesticide data

requirements for microbial pesticides.

So it is -- the guideline numbers that you saw provided were guideline numbers actually for microbial pesticides that we admittedly look at as a model and then adapt a little bit sometimes for these studies.

But you do not do a test for a parasitic hymenoptera, et cetera, for a conventional chemical pesticide. So there is no comparison.

However, as those tests for microbial pesticides were developed as required under FIFRA, they were brought forward to the SAP panel, reviewed just on a panel like this looking at the data requirements and guidelines that we were proposing and taking comments from the panel before they were finally put in place.

DR. PORTIER: But for microbial pesticides, I want to get into the toxicology issue here, you would generally do either dose response or much higher exposure levels with these sample sizes in order to test for the non effectiveness.

MS. ROSE: I'm not sure that the sample size number is actually because these are guidelines etched in stone. It comes down to, have enough insects been tested for statistical analysis.

And it is looked at on a case-by-case basis. I don't think there is a standard number that we can say -- off the top of my head having reviewed a bunch of these studies, I would say the numbers are pretty close.

DR. PORTIER: I'm trying to match dose versus number. In the classic toxicological paradigm, you are going to increase dose to make up for small numbers. And you use the increased dose to increase your power to be able to detect an effect.

In this situation, many of the tests that you are doing, you have not increased dose. You are actually using field level doses.

I'm wondering if you increased the sample size to take into account that you are using field level dose to look for a toxic

endpoint.

MS. ROSE: Not specifically. As I said, we look more at in general do we feel as though the test -- the sample size is large enough to be able to make some statistical conclusion from it on an individual basis.

We don't look at -- and what they require is it is tested at a safety factor at 10 X to 100 X.

With the microbials -- with the non-target insect studies, don't necessarily do this dose response.

The companies know where their toxicity is, and they will go with these maximum levels. And it's typically no observable effects.

DR. VAITZUS: I would like to add to that the use of a large dose is not so much for statistical purposes or whatever.

The intent of using a large dose in the biological pesticides realm was to limit the cost of doing an LD 50 at no effect level, an LC 90, whatever.

The intent was to do a limit test with a large dose. And if effects were found then, to narrow it down to the effect, a no effect level and the effects at field use rates.

And we rarely, if ever, ask for a study at field use rates. We always try to have a larger level so that we don't have to spend time in fractionating and doing a number of studies to determine the LC 50 or something like this.

DR. PORTIER: I would hope that's not the case. But in my comments later this afternoon, we'll get into this issue more.

Unless there is any pressing questions from the panel, we'll take a -- Dr. Federici.

DR. FEDERICI: I just want to make one comment.

If you have a highly specific protein, there is no way to determine an LC 50 or an LD 50 against a non sensitive insect. So that's why you use the high dose.

Maybe there is something that I don't understand about what you were saying.

DR. PORTIER: What I was saying was I was asking the questions -- in many of the studies that are done here, they are actually not doing dose response. They are doing the field tested dose or in some cases fractionating protein in product to get some sort of dose response, although the analysis is not done as a dose response analysis. It's done as T tests (ph).

But in the classic paradigm, you try to increase dose. And if you see nothing at a high dose, you feel pretty safe.

And that safety, that concept of safety is based upon the sample size, the level of the dose, et cetera. All of that factors into it.

To do a test at the same equivalent number of animals at a lower doses lowers your chances of detecting something if it's really there, and typically you would increase your sample size to make certain you haven't made a mistake.

That has not been done here. And that's what I was questioning.

Dr. Neher.

DR. NEHER: Just a point of clarification on the collembola study. There was a table on the percent survival and cumulative number of offspring.

Based on the conclusion that there was no effect, I questioned one of the datapoints on cumulative number of offspring.

For.5 percent Cry3Bb1, it had 100 percent survival. But the number of offspring is tenfold less than any of the other reports. I'm wondering could that be a typo? Or is it saying 20 instead of 200? That just raised a red flag for me.

MS. ROSE: Is this information from the submission or from our review?

DR. NEHER: This is your review on May 20 of non-target insect studies.

MS. ROSE: Without having that in front of me, unfortunately, I can't answer whether it is a typo or --

DR. NEHER: I just wanted -- for the

record, we need to double-check that because that's important.

MS. ROSE: Thank you.

DR. PORTIER: Without any additional questions, let's go ahead and take a 15-minute break. My clock says it is 10:32. We'll start again at 10:47.

(Thereupon, a brief recess was taken.)

DR. PORTIER: Our first commenter is from Exponent, Clifford Habig. If you could come up right here in the corner here where it says, public commenter, I would appreciate that.

Introduce yourself, give your affiliation, and then begin your comments.

DR. HABIG: Good morning. I'm Cliff Habig with Exponent, formerly Novigen Sciences. I appreciate the opportunity to present comments to the panel on issues concerning non-target insect testing and risk assessment for plant-incorporated protectants.

EPA has posed several questions to the SAP regarding non-target insect testing and risk

assessment procedures, specifically, for the corn rootworm product Mon 863.

However, many of these -- these questions are all generic in nature. And the focus of my comments will be on the generic nature of those questions. And because of the generic nature of the questions, they are applicable to all the other plant-incorporated protectant products. Not just the Mon 863 product.

And I will also draw comparisons to procedures that are used for more conventional types of products, both chemical pesticide products and, particularly, conventional microbial products in a very short time frame for these comments.

The first slide lists a few bullet points about testing and data requirements. And as we have heard already from some of the questions this morning and from some previous SAPs, there is a number of questions concerning the appropriate laboratory testing schemes for plant-incorporated protectants including the

appropriate dosing.

For instance, the microbial, conventional microbial testing guidelines use a maximum hazard dose approach where they test a product that is at concentrations well above the expected environmental concentrations.

You can do that if you use purified protein in the PIP testing. But it is very limited how high you can go if you use leaf tissue or pollen or something like that, just because it is limited by the expression and the particular plant tissue.

One thing, one option that the panel may consider is a core study set for all PIPs, and then supplementing that set with studies that are more specific for the particular type of PIP.

That will allow the test to be directed towards characteristics of that particular PIP. And include some considerations for potential exposure of different types of non-target organisms.

Traditionally, the EPA has used a tiered

approach to testing requirements for registration of pesticide products. And the criteria for moving on to higher tier testing is based -- first of all, one criteria is an exposure consideration, and then another type of criteria are risk and toxicity concerns from the lower tier testing.

And normally, movement from lower tiers to higher tier testing involves several levels of laboratory testing, both in the conventional chemicals and in the microbial, conventional microbial products before you get to field testing.

Traditionally, field testing represents the highest tier of testing for pesticide products. And in the past, for more conventional products, field testing has been conducted to address risk concerns that are based on laboratory toxicity data and estimated exposure concentrations that are estimated through modeling or calculations using generic databases.

Field testing for PIPs, however, follows a different rationale. These tests are not

conducted for risk based concerns from the results of laboratory data as were the more traditional field testing programs, but instead, they are designed to support the laboratory based risk assessment and to help address some areas of uncertainty or areas where right now there are not practical laboratory tests for some of the organisms.

One point that I would hope the panel would consider in its deliberations of field testing -- there are several questions asked about field testing. One thing I would hope the panel would consider in its deliberation of field testing, particularly the large census type studies, are the lessons learned from the Mesocosm type studies that were conducted in the late 1980s and early 1990s, which were large expensive studies. They have subsequently been dropped from the regulatory program.

Instead, I would hope the panel would consider alternative approaches, such as smaller, more focused field studies, semifield studies,

expanded laboratory tests and options like that to address specific questions and issues that arise for PIP products.

The risk assessment for non-target organisms, the EPA OPP has used a risk quotient approach. This is entirely conducted using laboratory toxicity data and the estimated exposures from modeling or generic databases.

The use of field data in the risk assessment process generally plays a supplemental and confirmatory role for the risk quotient calculations that are done based on laboratory data.

I would hope that -- the one thing I think is important in risk assessment across different types of products is to maintain consistency in a basic approach as you go across different types of products from PIPs to conventional microbials and conventional chemicals.

And also, another important consideration in an overall product evaluation is

a consideration of the overall safety by considering risks from all sorts of different potential sources of risk instead of just focusing on one potential type of risk in your product evaluation.

In conclusion, one option that I think might be worth exploring for the panel is to have a core data set for PIP products, plus a supplemental set, a set that allows some flexibility, allows you to consider potential exposure of various types of non-target organisms and the particular characteristics of the particular product under consideration.

And I would note that the Mon 863 product did follow this result with its concentration on additional coleopteran tests.

I also think it is important to maintain consistency in the basic risk assessment procedures across different types of pesticide products.

And it is also important to consider and balance all the various types of potential risk

when evaluating product safety.

Thank you for the time.

DR. PORTIER: Thank you, Dr. Habig. Are there any questions from the panel? No?

Thank you very much.

Our next public comment is by Dr. Jane Rissler on behalf of the Union of Concerned Scientists.

DR. RISSLER: Good morning. Thank you for the opportunity to comment this morning. I'm Jane Rissler with the Union of Concerned Scientists, a nonprofit partnership of scientists and citizens working for sustainable solutions to environmental problems.

In particular, in the food and environment program, of which I'm a part, our goal is to create a food system that encourages innovative and environmentally sustainable ways to produce high quality, safe and affordable food, while ensuring that citizens have a voice in how their food is produced.

We appreciate that the panel members

have taken time away from their other work to participate on this panel. I reiterate some of the comments earlier this morning about how valuable this work is. And we're grateful to EPA for expending their resources to hold three days of meetings on this subject.

There has been considerable discussion in the last two or three years about the quality of oversight at the three federal agencies that oversee products of agricultural biotechnology.

EPA clearly stands out in its efforts to gain expert advice in public settings from the scientific community.

In fact, as the Department of Agriculture is undertaking steps to remedy deficiencies in its oversight, we will be encouraging them to look at EPA and its use of the SAP as a model for increasing the scientific rigor of their reviews.

I have already communicated with the committee concerning the comments that UCS and Environmental Defense submitted to EPA in late May

on the proposed registration of MON 863.

Analyses by Drs. Angelika Hilbeck and Charles Benbrook contributed significantly to these comments. I brought some extra paper copies if they are needed and will give them to Paul Lewis sometime today.

UCS and Environmental Defense called on EPA not to register MON 863 because Monsanto has failed to demonstrate the absence of unreasonable risks as required under FIFRA. Monsanto also failed to provide a strong credible insect resistance management plan.

We concluded that the benefits of MON 863 may be modest due to its marginal efficacy and the declining use of high risk chemical insecticides for corn rootworm. MON 863 benefits may also be short-lived because of inadequate resistance management.

Turning to today's specific subject of non-target impacts, I will highlight only a couple of points since you have our detailed comments.

Both Monsanto's submission and EPA's

preliminary assessment conclude that the studies submitted by Monsanto indicate that MON 863 will not pose unreasonable adverse impacts on nontargets.

UCS and Environmental Defense disagree. We found that Monsanto's submission failed to demonstrate the absence of unreasonable risks to non-target organisms.

Let me be clear. We're not saying that MON 863 posts unreasonable ecological risks. In fact, we don't know the answer to that question.

There are insufficient good quality data on which to base a conclusion. Monsanto has yet to rigorously address environmental risks. We urge you, as you have already begun in the discussion this morning, to take a close look at the experiments, the data, the conclusion that both Monsanto and EPA have used to conclude that MON 863 is safe for nontargets.

As our comments detail, we believe that the existing set of experiments, because that set is incomplete and insufficiently rigorous, that it

cannot support such a conclusion. I offer the following examples:

Toxin expression data are inadequate for an assessment of risks, yet are critical. Credible data are needed on Cry 3Bb expression levels in all Mon 863 tissue types under a range of environmental conditions.

As a matter of fact, the question about stem levels of Cry 3Bb this morning caused me to think that there are no results in the publicly available material on the levels of Cry 3Bb in stem.

The equivalence of microbial and various plant-derived Cry 3Bb proteins to MON 863 has not been rigorously established, yet they are assumed equivalence is critical to the company's and EPA's analysis.

The field evaluation of coleopterans and other studies are incomplete and suffer from low statistical power and other shortcomings which limit the conclusions one can validly draw.

The assessment of impacts on soil

nontargets lacks multi trophic studies and is based on questionable exposure calculations. Neither Monsanto nor EPA addresses the implication of stacking other Bt genes with MON 863.

In conclusion, we urge you to ask and answer the question, what information is needed to assess the non-target impacts of MON 863? And then we urge you to ask whether Monsanto has generated that information. That is, are additional data needed, are the existing experiments appropriately designed and executed.

Thank you.

DR. PORTIER: Thank you very much, Dr. Rissler.

Are there any questions from the panel?

Dr. Angle.

DR. ANGLE: You had mentioned nontarget trophic level interactions. Are you looking at nutrient cycles? What specifically are you suggesting?

DR. RISSLER: Organisms eating organisms eating organisms.

DR. PORTIER: No other questions?

Our next public commenter is Mr. Robert Nadry (ph) on behalf of the National Wild Turkey Federation.

MR. NADRY: Thank you for allowing me to speak today. My name is Bobby Nadry. I'm with the National Wild Turkey Federation out of Edgefield, South Carolina.

Let me begin my comments to briefly describe the National Wild Turkey Federation to members of the Scientific Advisory Panel, if they are not familiar with our organization.

The NWTF was founded in 1973 when there were an estimated 1.3 million wild turkeys and about one and a half million turkey hunters nationwide.

Thanks to the work of wildlife agencies and many NWTF volunteers and partners, today there are an estimated 5. 8 million wild turkeys and approximately 2.6 million turkey hunters.

Since 1985, more than 164 million NWTF and cooperator dollars have been spent on over

21,000 habitat research and education projects benefiting wild turkeys and other wildlife throughout North America.

These totals include 251 scientific research projects totaling more than 3 million dollars.

The NWTF has a 450,000 member grass roots non profit organization with local chapters in all 50 states and three Canadian provinces. It supports scientific wildlife management on public, private and corporate lands, as well as wild turkey hunting as a traditional North American sport.

As far as biotechnology, the scientific staff of the NWTF has observed the emergence and adoption of agricultural biotechnologies and carefully examined the process utilized to evaluate the environmental safety of transgenic crops.

Today, transgenic corn is making it easier and more economical for wildlife biologists and NWTF members to provide supplemental food that

increases the winter survival of wild turkeys at northern latitudes.

In cotton growing areas, biotech cotton has benefitted wildlife by significantly reducing the use of insecticides that may disrupt the brood habitat.

Biotechnologies have also affected wildlife positively by enabling conservation tillage that reduces soil erosion, preserving water quality and improving habitats for many aquatic and terrestrial species.

Speaking specifically on the corn rootworm control technology, data collected to date suggests that the corn rootworm controlled biotechnology that you are evaluating, Monsanto event 863, will further reduce insecticide usage on many rural landscapes.

Obvious is the fact that approval and adoption of this technology will create wildlife benefits in the form of reduced potential for exposure of turkeys and other wildlife species to restricted use insecticides.

Accordingly, it is the recommendation of the National Wild Turkey Federation that the Environmental Protection Agency move forward with the approval of this biotechnology and future technologies that, A, generate wildlife and other environmental benefits, and, B, scientific evaluations have shown to be safe to wildlife and humans.

Thank you.

DR. PORTIER: Thank you very much, Mr. Nadry.

Are there any questions from the panel?

Thank you.

Dr. John Foster, University of Nebraska, Lincoln.

DR. FOSTER: Good morning. My name is John Foster. I'm a professor at the University of Nebraska, Lincoln. However, my views today represent my own views and only my own views.

I'm a professor of entomology, and I also hold a courtesy appointment as professor of plant breeding and genetics at the University of

Nebraska.

I have been responsible for gene deployment in resistive crops for about 25 years. First with USDA ARS and secondly now with the University of Nebraska.

I have been involved in the studies of transgenic corn expressing various Bt proteins for nearly 10 years. And I have been interested in the potential non-target effects for five years.

My research on nontargets has been funded in part by Monsanto, federal Hatch funds and the University of Nebraska, Lincoln.

I have seen the benefits that biotechnology can bring. And yet at the same time, I recognize the need to thoroughly assess the agricultural and ecological impacts of any new technology.

Having been involved with Bt corn and issues focused on the Monarch butterfly also has made several things clear to me. First, it is easy to focus on potential hazards of new technologies and lose sight of its benefits and

the associated risk of existing technologies.

Secondly, with a new technology, appropriate field studies are really the only way to understand the performance of a product.

Over the past three years, we have been conducting field research on the impact of MON 863 on non-target arthropods at multiple sites in Nebraska.

We have compared 863 with standard and soil insecticides. We have used increasing plot sizes as seed became available. And we have used a variety of sampling techniques. We focused on a number of groups of arthropods that we believe are ecologically important as brought out today and have economic importance.

Also, these groups are not impacted by the coleopteran active proteins that are expressed in the roots and above ground tissues of corn plant.

To date, when comparing the arthropods found in the plots of 863 and those in plots containing untreated isolines, we have seen no

significant adverse effects of 863 on any non-target group. Not a single group.

We have seen some differences in the communities among sites. But no significant impacts of the variety compared to its isoline.

Our analyses have included the ground dwelling beetles, the carabids, the collembola, the spiders. And we have used various sampling methods.

I have communicated with my colleagues at other institutions, other academics in the process of performing similar kinds of studies. And they, too, shared with me their results that found no differences.

With locations ranging from New York to South Dakota to Kansas and a variety of techniques and points of focus, large plot size in some of these studies represent comprehensive investigations. Obviously, the volume of data gathered to date will take us some time to evaluate and analyze.

Together with all of these results

generated by myself, my students and colleagues, these studies give me confidence that Mon 863 is a safe product with respect to nontargets. And hence, the EPA's ecological assessment is an accurate one.

Finally, I know what the currently used insecticides can do to the agri ecosystems in Nebraska. In Nebraska, these technologies currently used to control rootworm have obvious adverse effects on many non-target species. We use a lot of aerial application as well as ground applications.

I believe that the introduction of Mon 863 has the potential to bring clear and measurable ecological benefits to corn production systems in terms of reduced insecticide usage and, hence, worker exposure.

Thank you very much.

I will be available for the next couple days if someone wishes to ask me questions, so please grab me.

DR. PORTIER: Thank you.

DR. HELLMICH: I have a couple questions, Dr. Foster. You said you were increasing plot sizes. How big are the plots that you are looking at now?

DR. FOSTER: We're up to a half acre.

DR. HELLMICH: I have a report in front of me that was given to me by the EPA on some of the work that you have done on beneficials. And I take it that the information you are giving me now includes even more information that is not included in this report. Is that true?

DR. FOSTER: That's correct.

DR. HELLMICH: So based on further analysis, you see no detrimental effects, as you just said. In any of those plot experiments that you did, did you ever compare side by side Bt with insecticide treatments?

DR. FOSTER: Yes.

DR. HELLMICH: What did you find?

DR. FOSTER: We did find the insecticide treatments had an adverse impact on the beneficials -- on the non-target -- excuse me.

However, that's over the control of no insecticide.

But when you made the comparison of Bt and non Bt isolines, there was no differences. And those were all in the same plots.

Other questions?

DR. PORTIER: Yes. I had two questions. One pertains to the same comment that Dr. Hellmich was making.

Are any of the studies that you are recently describing available in the literature? Available in written technical form for the panel to look at? Anything that we can actually evaluate?

DR. FOSTER: No. As you know the process, those have been presented in public forum at the North Central Branch and will be presented at the Entomologist Society of America. And some of them were presented at the Brazilian Congress of Entomology in June of this past year.

Usually, the process is -- first goal is posters and presentations at national meetings.

And as the data is thoroughly analyzed, it will go into public forum as a reference journal.

DR. PORTIER: Just for my clarification to make sure I understand this. In my list, it says you are speaking on behalf of the University of Nebraska.

DR. FOSTER: That is not true.

DR. PORTIER: So you are here as a private citizen?

DR. FOSTER: That's true.

DR. PORTIER: Thank you very much.

Dr. Federici.

DR. FEDERICI: Can you give us some examples of the non-target classes that you looked at in carrying out these studies, the diversity?

DR. FOSTER: Sure. Actually, the most diverse set was a set of pitfall traps which gathered up the ground beetles, the carabids, the collembola, earthworms.

The set of data that is most analyzed to date is the carabids. We got everything in there, including mice.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: I'm interested in the carabids.

So you are saying -- approximately, what are the dimensions of the plots you are working with?

DR. FOSTER: Various sets of experiments.

But 60 by 60 are some of the smallest.

DR. JEPSON: The largest?

DR. FOSTER: Half acre.

DR. JEPSON: My concern is that carabid ground beetles can span the whole scale of an experimental field within a matter of days or weeks following treatment.

So without barriers, I find it difficult to see how any measurements of impacts can be assigned to a specific treatment other than the immediate acute effects of the pesticide that are undoubtedly measurable.

So I have concerns that this may underestimate the impact of the pesticide

treatment because of reinvasion from untreated parts of the field, but also that it may underestimate some of the potential benefits of the new technology because there is a general suppression of insects in the field from using acute pesticides in other blocks.

Do you have any kind of experience of that or is this something that you are concerned about?

DR. FOSTER: Yes, I do have concern. And you are right on all points if you assume that corn is grown in smaller areas with large riparian areas nearby. However, in some production systems, for example, in Nebraska where corn is grown with thousands upon thousands of acres of contiguous corn, then you find a different set of environment.

Indeed, we found that the carabids do move rapidly. You are right on target there.

And in small plot size, particularly near riparian areas, the only differences we saw that was meaningful, which would be substantiated

with the literature, is the abundance over the season versus plot treatment. Whereas in the larger plot size in the real world, then there were no differences.

DR. PORTIER: Any other questions from the panel?

Thank you very much, Dr. Foster.

DR. FOSTER: Thank you.

DR. PORTIER: Dr. Mike McKee from Monsanto.

DR. MCKEE: Mr. Chairman, members of the panel, I'm Mike McKee. And I'm responsible for ecological risk assessment at Monsanto Company.

I would like to thank you for the opportunity to discuss the scientific aspect of the Mon 863 risk assessment for the corn root testing program as it relates to the non-target organisms.

In general, the Cry 3 class of Bt proteins are selected towards certain coleoptera beetles. Monsanto has incorporated the Cry 3 gene into corn that encodes for the Cry 3Bba protein,

allowing control of the beetle pest commonly known as the corn rootworm.

A similar Cry 3 protein has been previously evaluated by EPA and registered for use in several products.

I would like to summarize Monsanto's risk assessment process for Mon 863 as well as the resulting data and conclusions. Most importantly, our approach has assured a robust and comprehensive risk assessment program tailored to the unique characteristics of the Mon 863 product and has yielded solid data supporting infinitive conclusions.

Furthermore, our Mon 863 ecological risk assessment was based on several key principles that together reinforced the conclusion that Mon 863 does not pose any unreasonable risk to non-target organisms.

First, the process was science based and utilized state of the art guidance on ecological risk assessment.

Second, the process directly

incorporated recommendations from recent EPA scientific advisory panel recommendations focused on ecological risk assessment.

And finally, we consulted with EPA through the risk assessment process to ensure that our approach was state of the art and consistent with the EPA standards and expectations.

Our risk assessment began with a laboratory based risk analysis as has been the standard practice for a number of other similar plant-incorporated protectants, including the Bt technologies for corn bore control that were reassessed at the EPA last year.

No effects were observed in the laboratory protein studies for Mon 863 at concentrations from 4 to 86 times the maximum worst case exposure concentration in the field.

For looking for even larger margins of safety, the LC 50 values were many times higher than these NOECs. These indicate minimal risk as per the OPPTS 1996 guidelines.

The studies to assess the safety to

green lacewing larvae have drawn a great deal of attention due to the unique feeding biology of the lacewing larvae. Therefore, Monsanto added a positive control to verify that the study can detect effects on lacewing larvae when exposed to a known toxic material mixed into the diet.

In the study, the lacewing larvae in the positive control group were significantly affected, whereas lacewing fed diets containing 8,000 parts per million of the Cry3Bb1 protein were not affected at all.

This concentration is a minimum of 86 times the worst case exposure level in the field. So we believe that these data are practical, they are science based, and they support a conclusion of minimal risk to the green lacewing.

Since Mon 863 is a known beetle active protein, we also recognize the need to obtain data on non-target beetles that is discussed in earlier SAPs. These data ensure that the Cry3Bb1 protein would cause no unexpected effects in species closely related to the corn rootworm beetle.

We initially tested the ladybird beetles at extremely high concentrations of 8,000 parts per million in the diet, and identified no adverse effects.

Subsequently, we conducted three additional studies with adult and larvae ladybird beetles with Mon 863 pollen and found no adverse effects after extended periods of exposure.

In addition to the ladybird beetles, Monsanto has conducted laboratory studies with representatives of three other beetle families and found no effects.

This reinforces the observation that the activity towards beetles is limited to the family chrysomelidae, which contains the corn rootworm.

Two other important families of non-target beetles, carabidae and staphylinidae were not assessed in the laboratory studies because methods were not readily available.

Instead, information on these taxa were collected in confined field studies of Monsanto and various academic institutions. MON 863, these

studies, did not cause adverse effects on these beetle families under the actual use conditions.

The soil incorporated insecticides, however, did show a trend towards reduced populations for several non-target organisms.

Taken collectively, these data indicate that event Mon 863 will pose no unreasonable risk to non-target organisms.

The risk assessment process also evaluated the potential for Cry 3 protein to persist in soil, as that subject has also been highlighted in SAP meetings.

Monsanto has submitted a soil degradation study for Cry3Bb1 protein using corn tissue added to field collected soil that indicated the protein dissipates rapidly. The design of the study employed exaggerated doses to simulate worst case soil deposition from a variety of mechanisms, including possible secretion, shredding of root hairs, degradation of biomass or pollen deposition.

The calculated DT 50 or timed to 50

percent degradation was 2.4 to 2.8 days. And the calculated DT 90, the timed to 90 percent degradation was 7.9 to 9.2 days.

There was no detection of the Cry3Bb1 protein by either ELISA or bioassay when samples were incubated over 21 days.

Monsanto recognizes that soil persistence data employing additional soil types and field use data would broaden the available information.

However, the existing data are sufficient to establish an acceptable margin of safety for the non-target organisms, using these conservative levels of estimating how high the exposure would be.

The rapid degradation of the protein serves to increase the margin of safety even more, further minimizing the risk.

Based on these observations, Monsanto believes that additional soils data are unlikely to change the fundamental conclusion that Mon 863 poses no unreasonable risk to non-target

organisms.

Several of the questions before the panel examined the need for field data and more specifically examined the need for census data versus focus studies on indicator species.

Traditionally, field data as a part of a regulatory testing scheme has been considered a higher tiered test, triggered only when risk is identified at a lower tier, usually in the laboratory.

Monsanto believes that it's important to recognize the collection of the field data for Mon 863 was not triggered by a risk conclusion from the laboratory assessment. Rather, the field studies were undertaken as an extra measure of proactive assessment that field studies were initiated to reinforce the findings of safety in the laboratory studies and to reduce the uncertainty around laboratory testing methodologies in this relatively new area of scientific investigation.

Therefore, Monsanto believes that the

field investigations should be very focused in answering specific questions or addressing particular areas of uncertainty.

For field investigations focused on particular indicator organisms, one to two years data should be sufficient to indicate any potential adverse effects.

Census studies for insect communities typically spread the resources too thin and do not allow rigorous analysis of specific hypotheses useful in regulatory decisionmaking.

Moreover, Monsanto feels that the need for field studies will likely decline as the laboratory assessment program is further refined and is strengthened.

Finally, to put the ecological safety of this product in context, one important consideration is the long history of safe agricultural use for the Bt products. In addition, any analysis of the potential risk and benefits of a new technology must be considered in the context of existing pest management practices

and systems.

The objective of any insect control program is to control the pest, but with minimal impact on the non-target organisms.

The implanted delivery system characteristic of this product limits potential exposure to non-target organisms. The Cry3Bb1 protein as expressed in Mon 863 is virtually nontoxic to non-target organisms, while existing organophosphate and pyrethroid insecticides currently approved and commonly used to control corn pests are toxic to an array of non-target organisms that occur commonly in agricultural fields.

The ecological safety of this new technology compared to existing widely employed pest management and practices is clear.

In summary, Monsanto has undertaken a rigorous and comprehensive risk assessment program to evaluate the ecological safety of Mon 863. The collective evidence of these laboratory and field studies for Mon 863 conducted by Monsanto and

other scientists consistently showed no unreasonable risk to non-target species.

In fact, researchers conducting the field studies have observed that Mon 863 fields can actually have a greater number and larger diversity of non-target organisms present than adjacent fields that were treated with conventional insecticides.

I want to thank you for the opportunity to comment.

DR. PORTIER: Thank you, Dr. McKee. Are there any questions?

Dr. Jepson.

DR. JEPSON: The EPA asserts in the literature that we have that test methods for things like carabid ground beetles and staphylinids are not widely available. And they also say that they are very expensive and therefore recommended moving to the field for kind of inventory purposes.

I just wanted to ask briefly, in terms of expense, how do you weigh the relative cost of

laboratory based toxicological studies versus field based investigations? Do you share the EPA's view that the lab tests are expensive and that perhaps one should therefore collect field data?

DR. MCKEE: I don't share that exactly, the same opinion, simply. But I do think that there is a developmental cost to get the studies up to where the standards are that we need to have to be able to have a reliable laboratory study.

So there is a great deal of information that needs to come around, round-robin testing and so on. But once the tests were put in place, I think that that would be a less expensive and meaningful way to move forward.

DR. JEPSON: Are you aware of the regulatory tests for precilus (ph) otherwise known as pterostichus cupreus that have been established?

DR. MCKEE: Yes. I'm aware of the test system.

DR. JEPSON: Now, you stated that tests

for -- this is a carabid ground beetle. You stated that tests for carabidae were not available. And that was why you elected not to examine impact, potential impacts on those taxa yet.

This is a testing procedure that has been validated by ring testing in a number of labs. And is widely practiced as a tool.

DR. MCKEE: My understanding on that protocol was that it was geared towards an application of an insecticide as opposed to a dietary uptake. And that was where I think that the -- the problem was that the protocol would need to somehow be modified to accommodate the plant-incorporated protectant.

DR. JEPSON: I will note this afternoon that it is regularly modified to take into account different routes of exposure and types of pesticide and dietary uptake as part of approved testing procedures under GLP. There's certainly scope for employing such tests.

I also wanted to add that tests for

staphylinids are also widely published that incorporate dietary pathways. There is a book called, The Handbook of Soil Invertebrate Toxicity Tests that incorporates a Phyllanthus cognatus (ph), a predatory staphylinid test.

This is widely known, widely cited. It is known within the regulatory community as well as the academic community.

Again, I somewhat take issue with the general statement that tests for these organisms are not widely available and not ready yet.

DR. MCKEE: I think that that's a welcomed discussion. Because I think that for the plant-incorporated protectants there has just not been a consensus type document put together for testing methodologies that would be relevant -- because there is a number of methods that were developed for chemical insecticides that are not relevant for plant incorporated protectants.

So a close examination of those that are and those that aren't I don't think has been done.

DR. PORTIER: Thank you. Dr. Alexander

then Dr. Neher.

DR. ALEXANDER: Two questions. The first data from every company that was producing chemical pesticides, or probably every company, indicates that the degradation rate of absorbed compounds is markedly affected by soil properties from zero to 100 percent relative rates. I'm sure Monsanto has similar data in its own files.

And I find it very difficult to accept your conclusion that you are not going to get much difference when you look at different soils. It is totally inconsistent with the available information published and unpublished on biodegradability of absorbed compounds.

DR. MCKEE: The reason that I made the statement that I did is not to downplay the importance of the different soil types, because I share your view.

The intent of my statement was to say that the safety margins that we have are sufficient that when we learn new information it is not likely to change the conclusion that we

currently have because of those large safety margins.

So I'm not disagreeing that the different soil types can have an effect.

And then I would add -- one more observation is that we do utilize the bioassay in assaying the material. And it is my understanding that from what we can see in the bioassay they are very efficient at removing the material that is in the soil even if it is bound.

DR. ALEXANDER: Well, that, I would question. And that leads to my second question.

And that is when you do a chemical assay, typically you look for recoveries. And in the results reported by Monsanto, there are no indications of the percentage of the compound recovered in the ELISA assay.

The original paper by Palm, which is the method you use for extraction, indicated that, what, 30 to 60 percent of the compound was recovered or 40 to 70 percent was not recovered.

So how do you know how much is actually

taken up by your insects and how available the residual fraction might be?

DR. MCKEE: I would have to go back and look at the specifics of the correcting through recovery. I agree with your assessment that in the ELISA that there was a certain amount that was recovered. There was a percentage. It wasn't all. Everything wasn't recovered.

So I would have to get additional information to get back with you. I recognize the importance of that.

DR. PORTIER: Dr. Neher.

DR. NEHER: I was wondering if you could elaborate just briefly on your comment about that the field tests may decline as lab tests are refined.

I guess my question comes from the point of view that my perspective is that the lab tests are testing a lot of direct toxicity, the field tests are getting at some different issues, some indirect effects, food chain, you know, getting into the food chain, sort of these trophic groups.

Some of the indirect and/or ecological effects.

I would like for you to elaborate a little bit on that statement for clarification.

DR. MCKEE: The basis for the statement comes mainly from a regulatory background in that the majority of the assessments recognize that if you are going into an insect community and you are controlling some aspect of that community, some pest aspect, that there will be some indirect effects, whether it be an insecticide, chemical insecticide or a plant insecticide or whatever.

And traditionally, we have not -- for the chemical insecticides, we have not pursued to a great deal the indirect effects, except when it comes to beneficials and some other insects.

So the basis of my statement was it was grounded in the regulatory framework that we currently use.

Now, that regulatory framework could change. But that's the regulatory framework that I'm operating from.

DR. PORTIER: Dr. Angle.

DR. ANGLE: I would like to follow up on Dr. Alexander's comment. And this is something we'll probably discuss later on this afternoon, but I would like to get your thoughts on it now.

Many of your procedures that you have used have essentially been designed to look at the worst case scenario. And then if you see no effect at that level, you can extrapolate down to lower levels and assume that there would be no adverse effect.

Yet when you selected a soil, you picked a soil that would probably show -- or I assume you guessed would probably show a rate of degradation that would be most favorable to Monsanto.

Why didn't you pick a soil such as with a higher clay content that would show a much slower degradation rate again using this kind of worst case scenario philosophy and then extrapolate back from that?

DR. MCKEE: First off, I can assure you that we didn't select the soil to get a desired result because -- it was simply the contract

facility that does those types of work was where we went to get the test done.

So we just simply used the soil that they use in all of their environmental fate studies for chemicals. We went to a facility that does chemical environmental fate studies and simply used the soil that they would routinely use. So it was their recommendation.

As I said, I think we clearly recognize that this is an important issue. And we are collecting more information on this. But my point is that, as I said, is that there is no indication that there would be a risk situation even if it was -- even if it didn't degrade at all and so -- based on the amount that would be going onto the soil.

So at this stage, this information will be supplemental. But it is very important, and clearly, we're going to get at.

DR. ANGLE: And so would you have a soil with a high clay content with high absorptive capacity as well as a soil with a higher organic

merit content than what's used?

DR. MCKEE: Yes. It will be very high in the clay content.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: I wanted to first commend Monsanto for doing on their own in terms of it not being requested by EPA studies on Monarch and on that system.

But my question relates to some comments that you made about making sure that your test on nontargets were focused on coleoptera because of the relevance of that type of test, and wondering what the rationale was in looking at the Monarch system at looking at the Monarch larva rather than the beetle species that do occur on milkweed as a nontarget, both common beetle species and, in fact, some crysomellids that occur on milkweed.

DR. MCKEE: The reason we did the Monarch study was admittedly we just knew that there was a lot of interest in the Monarch. And we wanted to make sure that we weren't subject to that question because this product does express

in pollen. And we didn't have -- we had some other target lepidoptera, but we didn't have any non-target lepidoptera. So that was the rationale for testing the Monarch.

As far as beetles, we have really focused on the ladybird beetle as being the surrogate for foliar feeding non-target beetles that might consume pollen.

So that was why we expanded that risk assessment and used that to address that particular concern.

The other aspect of non-target beetles was principally at a recommendation from the SAP as well as -- targeting the soil environment and to try and understand what is happening in the soil.

So that was the driver for why we expanded the ladybird beetle, why we tested the Monarch and then why we focused on the soil.

DR. PORTIER: Dr. Hellmich and then Dr. Federici.

DR. HELLMICH: Just out of curiosity,

did you test to see if rootworm beetle adults would be affected by the protein?

DR. MCKEE: Rootworm beetle adults?

DR. HELLMICH: Yes.

DR. MCKEE: I would have to refer to somebody else. I do not do that. I can check with some of my colleagues if you would like me to.

DR. HELLMICH: Okay. There is a study here that is entitled, Research on the Effects of Corn Rootworm Protectant, Transgenic Corn Events on Non-target Organisms Preliminary Results.

It is actually authored by Graham Head. So he may want to help you with some of the questions I have here.

But I think these are --

DR. MCKEE: I can try.

DR. HELLMICH: We'll see. First, I would like to -- so these were supplemental information that wasn't really required by the agency. But I think they are pretty informative.

Dr. Foster summarized some of the

research that he had. And there is a study, Rice and Bitzer (ph) at Iowa State, where at least in a preliminary analysis they found that the overall diversity of collembola species was actually higher in the Mon 863 versus an insecticide treatment.

Do you have anything further to report on those studies?

DR. PORTIER: Make sure you identify yourself for us.

DR. HEAD: My name is Graham Head. I'm also with Monsanto and I coordinate a lot of these field studies.

The work out of Iowa State has consistently shown that rare species of collembola are less common in insecticide treated plots. So you do get greater diversity in Mon 863 plots than in insecticide treated comparisons.

DR. HELLMICH: The studies that were done in Illinois, Zaborski (ph), do you have any updates on those studies, the ones with the litter bags and the earthworms and litter with wheat

stems?

DR. ANDOW: Can I just get clarification on that first result? Are you talking about 2001 data now, or are you talking about the 2002 data that appears in that study, the collembola study that you are referring to, that Rice --

DR. HEAD: I'm talking about my most recent update that I have heard from them. It wouldn't include --

DR. ANDOW: 2001.

DR. HEAD: -- the full three years of information.

DR. ANDOW: And 2002?

DR. HEAD: Yes.

DR. HELLMICH: So we only have 2000 data?

DR. HEAD: I believe you have the preliminary reports that separately describe 2000 and then 2001.

The Zaborski (ph) work is still -- I don't have much in the way of updates, particularly on the decomposition aspect of that.

That work is still being analyzed.

And in terms of mite communities, he had not seen any significant, consistent significant effects there.

DR. HELLMICH: And then, again, from University of Illinois, the Rob Weiderman (ph) studies with looking at fitness cost with coleomegilla, do you have any updates on that?

DR. HEAD: That work on the fitness cost mimics the work that we did internally also. And they actually have a paper that is in press on that work.

And they found no consistent or they found no significant effects at all on any of the fitness parameters that they looked at.

DR. HELLMICH: Let's move over to Virginia. Some of the work that was done with Dr. Youngman and some of his colleagues.

Curiously, they found that there was a plant pathogenic nematode that seemed to be reduced. I think growers would actually like something like that.

Do you have any updates on that research?

DR. HEAD: That work we're in the process of repeating. And most importantly, we're in the process of actually doing with a whole bunch of different varietal background. Because we're still not certain as to whether that's just a varietal effect versus an effect of the protein.

It is worth noting that field work that is being done at least out of Kansas State suggests there are no consistent impacts in the field on nematode populations.

DR. HELLMICH: That's Jerry Wilde's (ph) research?

DR. HEAD: That Jerry Wilde and his graduate student.

DR. HELLMICH: And I was going to ask you about him and also Dr. Fuller's (ph) at South Dakota. Do you have an update on his research?

DR. HEAD: Yes. Dr. Fuller has worked with very large scale plots. Those are actually four-acre plots on coccinelids in the field and

has not found any significant effects on any of the coccinelid species he has looked at comparing transgenic and nontransgenic.

DR. HELLMICH: Now, these data seem to be pretty important. Will they be available to the EPA before they can make their decision?

What is the status of these data?

DR. HEAD: The third year of information is basically complete. Reports are being written. We would expect that all of this work will be submitted in the form of different papers to peer review journals.

At the same time, as EPA pointed out, it is not regarded as necessary for the risk assessment.

DR. HELLMICH: Thank you very much.

DR. PORTIER: Thank you. Dr. Federici.

DR. FEDERICI: I have a few questions here, and I'm not sure we'll be able to ask you for answers. This is for Dr. McKee.

Going back to the chrysoperla egg feeding study, one of the controls was the

potassium arsenate. And you use that as a kind of positive control, kill.

Do you have any idea or any information on the diffusion properties of potassium arsenate into the egg versus the Cry3Bb1 protein?

DR. MCKEE: No. We don't have any of the data to show whether it is taken up by the egg or not.

The selection of the arsenate was simply based on the fact that it is classified as a contact-- I'm sorry, as a stomach poisoning historically in the literature.

And that you can tell that it's not very effective at controlling insects by dermal exposure. So that was the rationale for selecting, and we don't know that it was taken up inside the eggs.

DR. FEDERICI: Because it's a much smaller molecule than the Cry 3B.

DR. MCKEE: That's true.

DR. FEDERICI: Again, I don't want to dwell too much on this business of calling Cry

3Bb1 a chrysomelid specific. I think you really should back off of that. But being that you mentioned you tested a bunch of different beetle families, can you tell me how many families of beetles there are?

DR. MCKEE: I'm glad that Graham is up here too. I know the coleoptera is the biggest order.

DR. FEDERICI: Well, there are about 250 families. So we don't have to dwell on this.

DR. MCKEE: Right.

DR. FEDERICI: You have tested an extremely small -- I would be very surprised if some other beetle families, some other members of beetle families weren't sensitive to the toxin.

And I just don't understand why you want to push it as something that's chrysomelid specific. Just call it coleopteran specific and then go by whatever data you have.

DR. MCKEE: And I agree that you have to be careful about what you say. I think the reason that we kind of look at that is it is just an

amazing level of specificity that you see within the coleops.

It is pretty phenomenal. And I think that's why we're kind of preoccupied with it. But then to turn it around and say that it's only chrysomelids that are susceptible I think is dangerous.

DR. FEDERICI: One last question here to follow up on the question that was asked on the other side before. The statement about -- you made a very strong statement about the field data from one or two years being acceptable, that we should accept this.

Traditionally, though, in entomological studies, particularly with new products, people usually have three or more years of data.

So what is the basis for saying that -- I don't have a related question to this, but what is the basis for sort of almost telling us that we should accept one or two years of data?

DR. MCKEE: I guess that I'm not clear from a regulatory standpoint what data you are

talking about that is three to four years.

I don't think in the U.S. that we have been doing these types of studies for insect -- in field biodiversity studies for insects.

So I guess I'm not aware of that information that you are talking about.

DR. FEDERICI: No. What I'm talking about, you are looking at it from a regulatory standpoint. However, I mean, we're in a situation here where we have a very new type of technology. And there is a lot of public concern. And there is concern even among entomologists about non-target effects, because it's very difficult to predict what tritrophic effects, particularly over several years, are or might be.

So I would imagine that it would be reasonable, and I know a lot of these studies are ongoing, some by Monsanto, but many others now funded by the United States Department of Agriculture, for instance, because of the tremendous interest in this area.

So whereas you thought or suggested that

maybe there should be more emphasis on laboratory studies as predictors, I would take almost the reverse attitude.

I would say that with a new technology like this, we're wise to have several years of data from the field looking at different, not exactly detailed, census reports, but significant amounts of data over a period of several years to build confidence in this new technology.

And I think that would be to everybody's benefit. I agree that these are very significant proteins and they should result in pesticide usage. But I, as a person who works in these proteins, I want to see more data. There may be groups out there that I'm not aware of that there could be an effect on.

And because insect populations often vary, typically vary from one year to another, I would think in my opinion it would be better to have several years of data.

DR. MCKEE: I have to qualify what I had in there. It was definitely regulatory focused.

You are talking about some other acceptance issues that are really outside of what I was thinking about when I put the comments together.

And the reason that I envisioned that you could even do it in one year is if you have a very focused field study where you control a lot of the things, then -- a lot of parameters, then you can potentially get the information in one year.

So that's -- but I'm not really addressing the broader acceptance issue that you are talking about.

DR. PORTIER: Dr. Federici, I think that's something we'll bring into our discussion this afternoon in greater detail.

Dr. Jepson and then Dr. Andow.

DR. JEPSON: In talking to Robyn Rose this morning, I put her on the spot, I'm afraid, for which I apologize. But that's what this meeting is all about. I might do the same for you since you provided the data package.

I wanted to ask you first about the lab data and then about the field data. It is basically about acceptability or unacceptability and the standards which we follow in industry and in the regulatory world accepting the constraints on design of these experiments.

Now, this morning, I mentioned several problems I had with the way, for example, tests were curtailed for both nasonia and chrysoperla once the control mortality exceeded 20 percent.

In the chrysoperla test, that prevented the contract lab actually going to the point to where they measured the endpoint which they had cited at the beginning, which is pupation.

So I questioned whether or not that study was actually acceptable to you, to the agency, to me as a scientist. I just wonder why a study that fails to reach the endpoint because of high control mortality should even be on the table for us to consider.

And secondly, then, in your own internal data collection, including one of the bee studies,

you explicitly move beyond the 20 percent mortality threshold in the controls because it was argued that gave a more comprehensive comparison between the treatment and control.

So I looked for consistency at least. And I don't find it when looking at that. And also, at what stage does a test become -- we have heard when -- most of these tests were acceptable to the agency. But you submitted these. So I assume they were acceptable to you. But under what circumstances is a test unacceptable if this type of data is all there is?

DR. MCKEE: So you are putting me on the spot now?

DR. JEPSON: I am.

DR. MCKEE: Well, for the lacewing study, I can tell you that it was just a focus, too narrow a focus on the criteria for terminating the study, so that there was a -- we were too myopic at looking at what the termination criteria was.

The termination criteria wasn't

incubation, although that was an endpoint for the study. The termination was written in the protocol that it was 20 percent.

And so simply when 20 percent was hit, they terminated -- it was an external laboratory and they terminated the study.

DR. JEPSON: No. I agree with you about --

DR. MCKEE: So they wouldn't even have contacted me. I'm sorry. But they wouldn't even have contacted me because it was meeting the criteria, that part of it.

DR. JEPSON: Would you ever go back and say, well, that was carried out under GLP as part of the archive; now it is something we're going to submit to the agency, but would actually like you to repeat the tests so that we get to the endpoints that we requested?

Did you ever do that?

DR. MCKEE: Have we ever done that?

If it was serious -- if there was reason -- there is a whole bunch of things lumped in

here. But we would do that if there was reasonable -- if we thought that there was a reason that we were going to be missing a true risk. We certainly would do that.

In this case, we didn't make that decision.

DR. JEPSON: Thank you.

I also asked about the potential for decay of the Bt protein in the diet in the chrysoperla test. That was actually changed weekly. It was there at 21 degrees, 70 percent humidity for a whole week.

How much protein would have been left in parent form at the end of that period, do you think?

DR. MCKEE: Again, that's a difficult question. And we did discuss that during the course of the study.

The reason that we could not measure the protein, we didn't have a method that we could measure the protein and the mixture of eggs without having a separate validation and

everything.

And so what I had wanted to do was to change it more frequently. But the laboratory didn't want to do that because the handling stress on the lacewings would be too great. So we essentially just got boxed in to where we had to do it the way we did and live with the consequences.

But having said that, unless there were a lot of microbials activity in there, the protein -- we had it sitting out before where it is ambient temperature. And it will stay around if there is nothing to break it down.

DR. JEPSON: I have one brief further question about field testing. It's really about this scale question. I want to try and get to the heart of this for a second.

Some of these experimental measurements have gone over two years. And some of the plot sizes you have cited, some of them are very few rows wide, actually. The distance from the edge of the plot to the center of the plot in some

cases is literally a few feet.

Can you provide scientific justification for extending measurements into a second year if cosorial (ph) animals can traverse a whole field in a matter of weeks?

What possible continuity of effect, damaging or otherwise, could be measured in a second year if animals are so dispersive on the scale of your experiment?

Can you provide us with some help to see the value in these small scale studies?

DR. MCKEE: Well, again -- first, I want to say that I don't know for sure what a small scale is. So we're starting from not even knowing, you know, where does it become small scale and where does it become large scale.

But most of the studies that you are talking about with the smaller ones I think are the ones that Graham has been involved with. Because the Monsanto study is a 60 by 60 foot plot. So I assume that that's moderate to large scale.

DR. HEAD: Briefly, in response to your question, Paul, obviously at very early stages there is just a seed and a scale limitation on what sort of design can be put out there. So in essence, you try and put out as much as you can.

You are not necessarily going forward to a second year trying to strictly repeat. If at all possible, you are trying to improve things. That's one point.

The second is that there is an understanding that obviously the more mobile insects, invertebrates generally, you can not get a really good measure. You get something that is a very sort of conservative test of what is going on with them.

So you have to interpret the results on a taxon by taxon basis based upon what you know of their behavior and life history.

And then the other point is that this is why we go to a whole array of different cooperators, different tests. Both because they have different expertise, but also because at

least in a few cases we could go to very large scales and thereby actually compare the sort of results you get at the much greater scales versus the smaller scales.

DR. JEPSON: Thanks. That's something we'll return to this afternoon.

DR. PORTIER: I have a number of hands up. Rather than trying to remember the order, I'm going to start on this end and work my way back around. Dr. Neher first.

DR. NEHER: I had some questions about the procedure protocol involved in testing some of the beneficial nematodes by Lewis, et al. So I think this would be addressed towards Graham.

In particularly, I had some questions about the test with C. elegans, the bacterial feeding nematode, as well as the entomopathogenic (ph), the carpocapsi (ph).

On the C. elegans, I guess -- both of these were involved with a soil leachate.

My first question, I don't see any concentration of protein reported for that. And

it seemed like a pretty short period of exposure of soil to the water. What I read is like two minutes or something. I was wondering is there an estimate of protein concentration?

DR. HEAD: No, there was not.

DR. NEHER: Would that be something that you could measure and report? I think that would be helpful to relate to what these organisms may be exposed to in the field.

DR. HEAD: Yes. In terms of going forward, as I said, there is some uncertainty in the first place as to whether it's a variety effect versus a protein effect.

So we want to look at a set of different lines and compare there, but also repeat that basic test, doing some of the things you are talking about.

DR. NEHER: I think that would really enhance that and make that more convincing.

Another question regarding that that I think would also be more convincing to me, the survival test on that was 24 hours in duration.

One benefit of working with C. elegans is it has a short life generation time of three days.

I was thinking that I guess I would be more convinced about the survival data if at least a full generation had been followed so that you could also report information on fitness as well as survival.

And it seems like it would be doable on that particular species. Maybe there is further data we weren't presented with.

DR. HEAD: There isn't at the moment, but that is definitely on the list of things that that cooperator was interested in doing.

DR. NEHER: I note that there was some speculation on the entomopathogenic (ph) that the test has included a nonfeeding stage. What is the plans for follow up on that experiment?

DR. HEAD: Well, in that case, the nonfeeding stage is still the relevant stage. It is the stage that would be out there.

If anything, we would look at probably a number of different pest species just to better

understand for organisms that definitely are feeding upon plant tissue what is the potential impact.

DR. NEHER: I'm not an expert on all the different proteins, but it has come to my attention that there may be some Cry genes that may affect nematodes, for example, apparently Cry 14 Aa1 has been reported to affect nematodes as well as rootworm.

And given that fact, in some of these preliminary data on responding to some of the beneficial nematodes, I would favor additional experiments to follow that up. Because it does raise the question of if there is another one that could affect nematodes, maybe -- what about Cry3Bb1.

So I just encourage further investigation on the nematode studies.

DR. PORTIER: Dr. Neher, that will be part of your comments this afternoon. Correct?

DR. NEHER: Yes.

DR. PORTIER: Moving along, Dr. Angle,

did you have any questions for clarification?

DR. ANGLE: I just have one quick question in the general context of how this corn will be used.

In the midwest, fields are often very large, hundreds, thousands of acres. But here in the east coast, in Maryland, for example, our average corn field is 20 acres and you are never more than a quarter acre from a riparian zone with a number of other different species.

Is the intent for Monsanto to market this primarily to the large farms of the midwest, to the smaller farms in some other areas of the country?

DR. PORTIER: To take you off the hot spot a little bit, I'm not sure if that question's relevant to our scientific debate. Plus, I'm not sure that these are the right guys to talk about the marketing strategy of Monsanto.

Could you clarify why that's important for clarification here?

DR. ANGLE: I'm not an entomologist, but

I'm trying to understand the proximity to some of the non-target species. They would be quite different based -- whether or not you are in the middle of a thousand acre cornfield versus 30 yards from a major tributarian as the Chesapeake Bay.

DR. PORTIER: I'm going to turn that question over to EPA and ask them about their guidance for non-target species and how you look at guidance for non-target species of, say, Maryland versus Kansas in terms of what should be looked at.

DR. VAITZUS: As was indicated -- this is Zig Vaitzus. It was indicated that this is more of a marketing issue.

As far as we're concerned from the ecological effects area, we look at what possibly can be exposed. Not so much in what geographical location.

And as indicated in the endangered species discussion, we look at the proximity to the corn field of endangered species.

And currently, our assessment is based on the fact that this PIP is confined within the plant. It does not spread. It does not travel. It does not drift. The only drifting occurs from pollen for short distances for a short period, so that that issue of geographically where it should or should not be grown is not particularly relevant. It may be relevant, but it's not particularly prominent in our assessment at this point.

DR. PORTIER: Dr. Angle, did that help? Did you have other follow-up questions on that?

Dr. Alexander, did you have any questions?

DR. ALEXANDER: Not a question. Just a comment because the issue is raised by several members of the panel.

With the exception of one protein, every protein that I can think of that is not sorbed or a complex with aromatics in the tanning process is readily biodegraded.

So that if the protein is sitting in a

warm, moist environment, it ain't going to be there for very long. The exception happens to be keratin, K-E-R-A-T-I-N.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: I have a question, but I would like to get a point of clarification because the write-up that we had wasn't quite clear.

Would it be accurate to assume that in the chrysoperla experiment what was presented to the lacewings was eggs suspended in water to which the protein had been added?

DR. MCKEE: That's correct.

DR. BARBOSA: So I guess my question is or what I would like to ask you is the rationale for this approach in light of alternatives methods which involve incorporation of compounds into a dietary solution.

DR. MCKEE: When we initiated this testing program, which was quite a while ago now, at that time there was a literature that came out that said that there was a possibility of being able to formulate an artificial diet that the

lacewings could use and live on. And that that would have an advantage and that you would be able to get the protein inside.

We did attempt to do that, but our survival wasn't high enough on the artificial diet to switch. And so this test that we conducted had been used for microbials for a number of years.

And that was -- it was a test that was readily available and that had been used for Bt proteins, but microbials instead of plant incorporated. So we attempted to come up with another artificial diet and we couldn't.

So we incorporated the positive control and was able to see that it was a similar response.

I will add that the ladybird beetle, we had a positive control. And the response was somewhat similar in terms of sensitivity between -- to the arsenate. They are different orders. But I would just throw that out, that it wasn't -- that we didn't -- that we were extreme levels.

DR. BARBOSA: I was just wondering.

What year was this or what diets? Because there are diets around that have been tested as far back most recently as '89.

DR. MCKEE: For the lacewing larvae?

DR. BARBOSA: Yes.

DR. MCKEE: Well, what I was talking about in particular was the encapsulated version that --

DR. BARBOSA: Wax eggs, for example, is one of the alternatives that have been used in '65. I'm just wondering why.

DR. MCKEE: To me -- I was involved with that. I saw what was in the literature. We did do a review literature to see what other diets were available, but I'm not -- whether we just didn't picked that up or not, I'm not sure.

But at the time we were always bouncing it against what we knew that EPA had reviewed and accepted in the past. And this study was one, this one that we did.

So we looked for reasonable alternatives within that time frame. So this was probably

around '98, '97.

So it's possible that other alternatives are there, but it still has not been elevated to a standardized test protocol yet.

DR. BARBOSA: I don't mean to put you on the spot, although he has already started that trend, what are the criteria for acceptable? There are diets that produce adults that lay 1,000 eggs in a couple months. That seems like pretty healthy individuals.

DR. MCKEE: Yes. It seems like what you are describing -- I have to tell you that I was not aware that there was a replacement ready to go into a standardized study that would be acceptable to the agency.

I was just not aware of that. This one was the only standard study that I was aware of.

DR. VAITZUS: This is Zig Vaitzus. I would like to add the Agency's point of view to this.

We also like to look at the natural diet of the insect. Because as I mentioned earlier,

the ecological effect is of most importance to us, not so much the academic toxicity in pure form.

It is very commonly accepted that the insect eggs are the most common diet of lacewing larvae. For that reason, we like to use that particular system even if the Bt toxin doesn't get in there because out in nature they would eat those eggs whether the toxin is on the outside or not -- inside or not.

DR. PORTIER: Dr. Andow.

DR. ANDOW: I guess it's lucky I'm following up here. It seems that if the approach is to try to get -- use the laboratory to estimate sort of maximum hazard, that you are -- by going to an artificial diet, you can actually increase the concentrations of the toxin to the levels that you would like to have them be. But that's just comment.

My question was hopefully just a simple one. I was wondering if you have any information on the LC50s of the arsenate for the lacewings or any of the others that you were working with?

DR. MCKEE: We know what it -- I guess I'm not exactly sure. For the lacewing, we have around an LC 50 in our study using it as a positive control. So in this test system, the LC 50 is around 400 parts per million.

That's just in the neighborhood. It is reported as 1,000, but you have to correct for the amount of arsenate that is the solution.

So if you put in around 400 part per million into moth eggs and stir it up, you will kill about half of the lacewing.

DR. ANDOW: So you are sort of basing that on the study that the Wildlife International --

DR. MCKEE: Right. We do not have a lacewing study where we know exactly how much that they consumed to compare that to.

That's why I mentioned -- in the coleoptera, the other study, we know that they were consuming the material because it is a direct -- they directly take it in. It is not a special feeding apparatus.

And we saw similar LC 50 range for that study.

DR. PORTIER: Dr. Jepson. Any additional questions?

DR. JEPSON: No, I'm fine.

DR. PORTIER: Dr. Federici.

DR. FEDERICI: I just want clarification on what you said about the chrysoperla (ph) and feeding on the egg. You said they are going to eat the eggs anyhow. Do they consume the whole egg or do they just pick it up and suck the juices out?

DR. VAITZUS: You are addressing me. Is that correct?

DR. FEDERICI: Yes.

DR. VAITZUS: Our information is that they suck the juice out of it.

DR. FEDERICI: Right.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: I want to visit this honeybee test again.

I'll have a question for you. And then,

Robyn, if you have any extra information, I would appreciate that also.

At the time that this experiment was conducted, it was thought that the amount of protein they had was 20 X. Then that was modified so that it was 4.3 X.

And it seems like it would have been very easy to redo the experiment and go up to 10 X or whatever it is.

Just looking at all of these experiments, the only one that sort of falls below the 10 X seems to be this one. Is that true?

DR. MCKEE: That's true.

DR. HELLMICH: Now, when they conducted that experiment, did they use newly emerged bees, field bees? What did they use?

DR. MCKEE: It is newly emerged. Within 24 hours, they will take the frames out and put them inside of a cage and collect newly emerged bees.

DR. HELLMICH: Why was there a discrepancy with the 20 X at the time this was

written, and what has happened since then?

DR. MCKEE: When we first -- when we initially did the study, the lead line at that point was Mon 859. And because these are done as maximum hazard dose studies, you have to link the testing to the expression level.

So subsequent to that, Mon 863 became the lead line and we have a whole another submission package that goes in on MON 863.

It had different expression levels. So it had lower expression levels in leaf material, but it had slightly higher in the pollen. So we have to readjust those values for that. That's how the adjustment occurred.

And the reason that we have not submitted a subsequent study was because it was 4.3 X to the no observed effect concentration.

But really, the regulations, as I read it, the ratio of the LC 50 needs to exceed at least 10. So with the NOEC as 4.3, then the LC 50 is going to be at least 2 X times higher than that. It's just based on my experience. That's

on my personal experience.

And so that was why we did not submit another study to the agency.

DR. HELLMICH: Any idea why the controls were a problem with this particular study?

DR. MCKEE: No. The reason that that occurred, that extended beyond the 20 percent, was because it was done late in the season. And we didn't have an opportunity at that point to restart it because it was late.

I had used Abbot's (ph) formula a lot in these types of studies to correct for mortality. So I authorized it to continue on. And we felt like it was fairly compelling.

DR. HELLMICH: So my question to the EPA, are they aware of any other honeybee data that would support that there is no effect from this that you are willing -- that you know of?

MS. ROSE: Not with the Cry3Bb1 protein. But we have seen similar situations with mortality and the controls. That's not unique with these laboratory tests with the honeybees.

But no, I don't know of any other studies with Cry 3Bb1 in honeybees.

DR. HELLMICH: That's all. Thank you.

DR. PORTIER: I'm going to ask one question.

Do you have written down guidelines or standards for the statistical analysis of the data that you present to EPA?

DR. MCKEE: No. There is no -- speaking from a registrant's perspective, it is mostly by convention. We'll seek whatever guidance that we get from the agency and the type of test that they might prefer at that particular time. And we'll try to stay abreast of that. But we don't have anything written down.

But one of the questions that -- what we usually do is we test at a level of protein high above the maximum exposure concentration. And then if we have any effects there, then normally we would want -- if we had over 50 percent response, then we would want to titrate the doses down. Then it would go into a dose response as

you were talking about earlier today.

That's just the rule of thumb that we use.

DR. PORTIER: We have had Dr. McKee on the hot seat for over an hour. Are there any other questions from the SAP for Dr. McKee? Clarification questions?

Any questions from EPA? We have kept you out of this conversation to some degree. Do you have questions for Dr. McKee for clarity?

Thank you very much.

DR. MCKEE: Thank you.

DR. PORTIER: Are there any other new public commenters? That is my list of public commenters.

Is there anyone else here who has not presented before who would like to make a public comment?

Barring that, I believe we are finishing nearly this morning's session. Before I close it, I was going to give you an opportunity, Dr. Andersen, please.

DR. ANDERSEN: Thank you.

Some of the discussion today has been about what the protocols are for the studies we have looked at.

During the break, we have gathered them, microbial test guidelines, also a scientific advisory panel report that some members of this panel actually participated in, both EPA's presentation and the panel's actual report from December 8th and 9th in 1999 that partly considered -- at least one of the topics there was looking at the ecological effects -- the non-target organism data that we would ask for these types of products.

And I would like to leave that with the panel if it is useful for them.

Also, during the break a few of you expressed trouble in being able to open some of your files. One of my staff has been expert at being able to try and figure that out. If you have your computer here right now and would wait a minute, I would like to have Mike Mendleson (ph)

look at it, see if he could solve that problem.

Otherwise, there are some studies that some of you would like to see and we would like to try and be able to provide those to you. We will try and do that over the lunch break.

Thank you.

DR. PORTIER: Thank you very much. I, in fact, look forward to seeing what sage comments this panel had back in 1999.

Mr. Lewis.

MR. LEWIS: Just to clarify or to add on to what Dr. Andersen mentioned, I know many -- a few panel members have a problem with opening the CDs that we gave to you before. If we can meet for those folks who have problems in our break room, bring your laptop with you and our EPA colleagues will work with you about getting those files operating properly. Thank you.

DR. PORTIER: Before we close, I want to reiterate a point that Dr. Rissler made during her public comments and commend the agency for providing this public forum for this discussion.

The considerable amount of time we spent this morning on public comments as well as the agency comments I think is a benefit in this area. It is a high profile, a very public interest area. And this is a great opportunity for the public to be involved in it.

Again, I want to reiterate what Dr. Rissler said. This is a very good thing the agency is doing.

We're right on time. I expect to open this afternoon's session at exactly 1:30. And I look forward to seeing you back here. Thank you very much.

(Thereupon, a luncheon recess was taken.)

DR. PORTIER: Good afternoon. I want to welcome you back to the FIFRA Scientific Advisory Panel meeting. This afternoon, we'll be answering questions put forth by the agency on corn rootworm plant-incorporated protectant non-target insect and insect resistant management issues.

Are there any comments from the agency

prior to us beginning with the questions?

DR. ANDERSEN: No, thank you. Not at this time.

DR. PORTIER: In that case, Ms. Rose, if you could begin with the first question, please.

MS. ROSE: This is actually the first half of the first question.

Please comment on the relative strengths and weaknesses of such field data versus laboratory feeding studies performed on a limited number of indicator organisms for purposes of hazard assessment.

DR. PORTIER: Thank you very much.

Dr. Jepson.

DR. JEPSON: Thank you very much.

I should have said so beforehand, but thank you for inviting me. I also admire the process and accept that we're concentrating on criticisms. But one thing that deserves again to be recognized is the fact we're having this meeting in the first place deserves praise and that we're worrying so much about this data also

is particular noteworthy.

I'm going to give a long answer to this first part. And then if we could move quickly on to the second part, Robyn, the two parts of the response are linked, really.

So the EPA invited me, my first ever trip to the United States, to Baltimore in 1992 and sponsored a workshop on ecological issues arising out of the expected approval of Bt transgenic technology.

And at that meeting in 1992, I was asked to address the selection of test organisms, the design of test methods and questions arising in terms of laboratory and field data and the interaction between those.

And I valued that invitation back then and 10 years later I value the opportunity now. So in addition to the FIFRA October 2000 SAP report, I'm also going to refer to a paper that myself, Brian Croft (ph) and Graham Pratt wrote from that meeting at the request of the agency to summarize the procedure for selecting species to

discriminate ecological risks posed by this technology.

And one of the things we argued was that the selection of test organisms needs to be representative of the system we're working in. There needs to be potential to rear them and culture these organisms.

The sensitivity and potential sensitivity of the organisms given the specificity of the toxin need special concern. And also the potential for ecological recovery of the organism.

We're not just looking for sensitive organisms physiologically. We're looking for sensitive organisms ecologically and addressing our concerns at those.

And in that paper, a number of laboratory based tests and testing methods were reviewed, that at that time and still to this day provide, I think, an excellent opportunity for the agency and industry with appropriate public comment to develop a consensus on the most appropriate testing methods. And we'll be

referring to those in our reports.

My first comment, really, is that the assertion in the preamble to this question, if you look at the packet, that extensive and difficult soil coleopteran tests might be a difficult thing to pursue relative to collecting direct field data I somewhat take issue with.

I have referred already to this book, The Handbook of Soil Invertebrate Toxicity Tests. And I also want to refer to The Handbook of Ecotoxicology. And I'll be referring specifically to test methods cited in both of those volumes that relate to specific protocols for, for example, carabidae and staphylinidae, which if pursued under modified form would allow an evaluation of potential impacts of these toxins at elevated levels for laboratory purposes.

And in both cases -- in all cases, I'm recognizing the requirements of regulatory toxicology for repeatable tests that can be carried out in a number of labs that have been evaluated by ring testing procedures.

I'm also going to refer to two publications of the Society for Environmental Toxicology and Chemistry, 1994 and 1999, which summarize the procedures for evaluating any product such as the ones we're talking about with respect to developing laboratory protocols and field protocols and how one balances the relative data value of those two.

I'm not going to go into detail about that now.

I'm also going to talk about publication of Barrett (ph) in 1992 and a large number of people representing the pesticide industry and the beneficial arthropod regulatory testing group, which, again, indulged in a large degree of method development over a number of years, which has led to regulatory standards for pesticides and non-target invertebrates. I think increased notice ought to be paid to these tests.

There is a scope for building greater -- an improved test battery in a relatively simple process, I would argue. And I know we're looking

at the tests as have been specified by the current regulations.

However, we have all been struck by some of the limitations of that. And I'm basically drawing the agency's attention, industry's attention, it doesn't need to be drawn to this because they are already carrying out these tests on a large number of pesticides in many cases and against natural enemy taxa for regulatory approval in other parts of the world. There is no surprises there.

So what are the strengths of laboratory derived data in brief? The strength of laboratory derived data, if it is collected properly, is that you can determine the potential for a lack of harm.

So if you subject an organism to a high dose and no effect lethal or sublethal arises, and clearly, there are many different measurements that can be made, then you can assert with some degree of confidence based on 50 years of experience in this area that harm is unlikely to

arise in the field.

And I believe it's widely accepted as a consensus scientifically and in the regulated community that these tests cannot be used to determine levels of protection or harm for terrestrial invertebrates, in particular, in the field.

Normally, these tests will be used as a trigger for some further inquiry or testing or some further risk assessment that incorporates other data. Not solely the data involved in the lab test.

Organisms are not exposed through their life cycles in the lab, whereas they are in the field. Levels of exposure in the field are always somewhat uncertain and variable.

No ecological processes ensue in the lab. Reproduction over several generations rarely takes place. We're not seeing that kind of balance between birth and death and immigration that leads to a given population density in the field.

Sublethal effects and fitness effects are not commonly measured, although they can be. Organisms in the field are subject to stresses such as starvation and parasitism, which they are not subjected to in the cushy conditions of a laboratory.

So I'm asserting that a test can somehow give you guidance for a lack of effect in the field. If an effect is actually detected, I think you are on much more shaky ground than if an effect is not detected.

So in terms of using a trigger for further testing, I'm moving on to a point here, not just rambling on, I hope, the agency presented me with a challenge of weighing up lab versus field, when I do not believe that's an appropriate comparison. Those are two ends of a spectrum to me.

There is a type of test method that again is being defined as the extended laboratory test. Okay, Dr. X, you have shown that this trisulfide might have a reduced feeding rate

subjected to this protein. Well, let's get more realistic conditions of exposure, not super exposure, and expose animals in a cage in a laboratory to eggs that it would be consuming on a transgenic crop, and see whether or not there is any potential for exposure at all.

So an extended laboratory test will often deal with issues that arise in a simple laboratory experiment.

And then we have the whole world of so-called semifield tests, which all have been well developed and established where you put a cage or a barrier around some corn plants, for example, in this case.

You can find organisms. You have control in treated areas. And you look at the specific fate of individual marks or populations of organisms that you have introduced.

So laboratory versus field, well, that's a difficult one for me to address because they are such different environments, as it were. But viewing a laboratory test is something that can

lead towards a suite of still further laboratory or simple field scale tests. So I think can deal with the public concern, agency concern and the industry's capacity to respond to EPA requests far more efficiently in my view.

And again, I'm going to refer you to the handbook, Free of Ecotoxicology and the C. tac documents because I believe these have a level of credibility that would gain recognition from all the different parties in these debates.

So I'm basically arguing perhaps there is a case for a second stage of response where extended laboratory tests could be carried out under certain circumstances.

Now, I mentioned this morning some challenges I found with respect to the individual laboratory tests to do with when you curtail tests and when you don't. And it was explained by Mike McKee from Monsanto that good laboratory practices standard operating procedures exactly often specify when a test should cease.

So that that lack of flexibility of

course is in the system. And we understand the reasons why it's there. However, this standard has been variably applied in the public docket records that we have seen. And I still find that quite difficult to deal with.

I still am concerned about uncertainties associated with levels of exposure and the amount of material in the diet. And all of the questions I raised this morning, which are already part of the record, leave me less certain than I would like to be at this laboratory stage.

So now I'm going to move on to field data. What are the strengths of field data?

I'm going to get on to what we mean by the field. But broadly, they measure ecological impacts, as Deb said this morning. We look at population and community impacts, indirect and direct effects. And they all get bundled together in a net outcome in terms of field exposure.

You can determine a level of hazard in a real world situation through your various laboratory and other tests. You have triggered a

need for further inquiry. You don't need to undertake field tests from a regulatory perspective if a complete lack of effect has been found, unless you are so uncertain about a new technology that you feel the field work needs to be done anyway.

And somewhere in this current debate we're still in that phase of discovery about these commodities rather than this kind of balance regulatory process that we'll have in a number of years time which sometimes would not require a field evaluation, where it is required at the moment.

So again, there is widely published research internationally on the design of regulatory test procedures to determine both the level and extent of effects from an ecological perturbation such as a pesticide or plant-incorporated protectant.

This deals also with the use of toxic standards which I will address and will address as a panel in the report. Some of the tests cited by

Monsanto I believe failed to find effects with some of foliar applied products where I would have really expected to see those.

If we apply a toxic standard to the arguments for the laboratory testing, why hasn't that been applied to the field data in the same way? Why, when some of those tests deemed to be invalid on the basis of a lack of effect from a known toxin? That's something that deserves to be looked at.

Also, the literature I'm referring to talks about statistical power, replication and the challenges posed by replication, the need for replication, but also the challenges of having plots that are large enough. And again, I'll refer to the literature in the report. And that may be of value in the further debate that happens -- I'm just winding to an end now.

So there are some essentials in my book, at least, for field studies. You need to preselect the site. Surveying a number of sites to determine which sites harbor the natural

enemies which you are looking for, perhaps the season before or earlier in a given season is a prerequisite as far as I'm concerned, a quality standard.

Then sampling methods of known efficiency need to be used. Pitfall trapping efficiencies are immensely difficult to work out, of course. But having some surface searching or suction sampling or some back-out method to at least evaluate sampling efficiency guards the agency in terms of the likelihood detecting an effect in the first place, should one occur.

And the scale layout and design needs to match in some way the scale of commercial application of the product. And that doesn't mean having an experiment the size of Nebraska. It does mean understanding what the limits of the experiments are. And I don't believe we have addressed that properly in the documentation that I have reviewed.

Some knowledge of the taxa under observation is required. And some knowledge of

the durational persistence of activity of the material is very necessary.

But ultimately, what I have been talking about this morning, scale is of absolute importance, of absolute importance in determining the scientific validity of these experiments.

Again, I'm going to refer you to literature. I have photocopies of this, which I'll leave with Paul, but also will be referred to in the report, literature on patterns of dispersed larva invertebrates, including carabids, staphylinids and spiders between plots, literature concerning matter population dynamics of carabid beetles on a farm scale, in sprayed farms. And validation of that with large scale, long term field studies. And also literature on the abundance of collembola, for example, which are amongst the prey of these carabid beetles in small scale versus large scale studies.

It's no good arguing that we can look at nondispersive species in small plots. Because if they are eaten by carabids that are there because

there was a control plot nearby, you are going to get dynamics that are a function of your design layout and scale, not dynamics that are a result of the treatments.

And I believe in regulatory toxicology we have enough of a sense of these issues now to be able to design criteria for field studies and guide our interpretation as to what or what is not valid.

So data from experiments where significant movement occurs between the experimental treatments is not in my view scientifically valid after a certain date. So beyond a few weeks after a product has been applied as a conventional pesticide, you simply stop making measurements, because they are no longer valid.

Redistribution of organisms takes place. This can artificially depress populations in the treated field if you are using a toxic pesticide. So the potential benefits, for example, in biodiversity in terms of a less harmful

plant-incorporated protectant could be underestimated, which I believe it should be of a concern to ours.

And also, certainly, it is the case that the impact of the conventional pesticide is underestimated.

And I would argue as I put to Monsanto this morning that observations of more than one season with in-field plot experiments may act as guidance for the design of larger scale studies or monitoring, but should not be used in any way to shape of you of the ecological impacts of any material because organisms redistribute themselves between the plots and you are not measuring a true treatment effect.

This is statistically invalid and ecologically nonsensical.

So laboratory versus field strengths. It depends on what you mean by lab and what you mean by field, I would argue. And of greatest value to me would be the development of a rigorous lab test battery, some of which I believe we have

seen.

I think some of the tests which we were able to review are of exceptional caliber and quality, particularly those carried out in-house by Monsanto on bees and -- yes, mainly, the bee studies and some of the coccinelid studies.

But there are other procedures out there and regulatory protocols to follow up with minimal modification.

I believe extended laboratory tests, which are simple to request, simple to carry out, they can be replicated and checked elsewhere, need to be addressed in more detail, and barriers and cages need to be thought about because they offer options which the field does not offer.

Those are the ends of my comments to Part A. And I wonder if we might move to Part B and then have the follow-ups to those, because the two are connected, or do you want to just deal with Part A first?

DR. PORTIER: We'll deal with Part A first.

DR. JEPSON: So that's all I have to say for the Part A. And if there are some supplementary or associate --

DR. PORTIER: Dr. Barbosa. Any additional comments?

DR. BARBOSA: I guess I would add that along with the comments that we just heard that implicit in contrasting lab and field is almost an assumption that they are asking the same questions.

And it is not clear certainly in terms of the documentation and in terms of what we have heard this morning that that is always of a case. And indeed, in some cases it might be very different.

So it may be, again, as suggested, that we need to look at these as separate issues rather than one.

The only other comment that I would make is that one of the advantages the laboratory test is indeed the ability to control variables.

And sometimes this simple advantage has

to be paid attention to. I found that in a number of experiments that were presented more attention could have been given to the simple issue of designing the laboratory experiments.

In particular, the use of appropriate controls so that the result and the conclusions from the particular research would be useful and of value.

And then finally, I think it is very critical certainly for a panel such as this, and I would also imagine for EPA, to ensure that the work that is conducted, the research that is conducted is described in appropriate and enough detail so that they can be valuated and the resulted can be evaluated.

That's not only in terms of the design of the experiment, but statistical analysis and statistical design, which, again, is critical to determining the value of the results that are obtained.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: Paul, you are writing up

this section. Right?

DR. JEPSON: I'm taking notes.

DR. HELLMICH: I think we have to be careful here because undoubtedly I think that the laboratory tests and the field tests are going to have certain roles in this.

When we're looking at the information that has been given to us where we're looking at the tests that have been outlined -- I think that Janet passed some information around with some tests that are required of the companies, and there was a science advisory panel in 1999 that said that in addition to these tests, these battery of tests that include certain insects -- I'll go ahead and read this.

That non-target insects should be selected based on their having an ecological association with the crop plant or target pest, their termination in which non-target organisms of the test should be done on a case-by-case basis for each plant construct taking into consideration the biology of the transgenic plant, the

ecological interactions with the crop plant and other organisms, and the organs or the means and probability of distribution of exposure through plant pollen, plant residues, root or organ exudates. And that the non-target insects should likely be susceptible (ph) to toxin because they are phylogenetically related to the target pest.

And I think that, from what I have seen, Monsanto did a good job of selecting other insects that were ecologically associated with the corn insects, with their selection of other beetles.

In some cases, these tests could be done in the lab because they were lab cultures of these beetles, and that was appropriate. In other cases, it is not quite so clear, so I think then you do have to go to the field test.

I think that we should distinguish between what is necessary for an evaluation and what we consider to be critical and what would be nice if we had unlimited resources.

I think sometimes a group of scientists can get around a table and say, yes, it would be

nice if we did this if we had 100 or 1,000 ecologists and an unlimited budget. But we have to focus on what we consider to be the critical data that we need. And I think in this case those data are available.

I think that when we look at this a little bit more closely, I think Chris had some concerns about some of those statistical procedures. I know in former science advisory panels we did focus on that. And we take for granted that scientists involved in this are following statistical procedures and the EPA has evaluated so that it has sufficient power.

I think we need to be careful that -- one of the speakers this morning talked about mesocosm type of analysis that they did and then abandoned 10, 20 years ago because it just got too complex. Again, it is getting back to the place where we need to be efficient and be intelligent in the selection of the type of test that we're doing. Obviously, it is going to be a combination of laboratory test and field tests.

I guess we're sort of in a position where any of these things can be improved. And there was a workshop where some of us participated in early in the summer where I think that clearly there will be ways that these things can be improved in the future and hopefully become more efficiently so that all parties are satisfied with it.

But again, I think we have to say, as the rules are right now, how do we rate or consider these tests, how valid are they. So those are my comments for right now.

DR. PORTIER: Do any other members of the panel have comments on this question?

Dr. Andow.

DR. ANDOW: Thank you.

I saw this question as being what is a good way of identifying hazards, to what extent is the field methods useful for identifying hazards versus the laboratory methods for identifying hazards.

So I don't think the question that --

the way I'm going to address it, it doesn't get into the risk issue at all. It is to what extent can we identify hazards to the different methods.

I would like to say on that -- I would like to agree with what Paul was saying about the field issue. I think that there are several points that make it so that in a field experiment, it may be difficult to identify a hazard even if it's there.

And so, for example, generally, field experiments have a large amount of environmental variance. And they have a relatively small number of replications. So that if you wanted to -- for example, if I'm looking at effects of different things, say, on European corn bore densities, to look at a 20 to 50 percent effect on a European corn bore density, I have calculated it might take as many as 100 replications in order to establish that level of an effect in the field, where as I could establish that with many fewer replications and a lot less work in the laboratory.

So that there is a certain amount to be

gained in the laboratory compared to the field.

The second reason that Paul addressed is the density in the field. Too often I have conducted field experiments where the insect of interest is just not abundant enough. You find like one every 100 plants. And so you can never find a treatment effect.

Third, as he mentioned, the plot size. And arthropod movement is an issue.

Fourth, he mentioned it, but I would like to expand on it. Sampling effort. This is particularly important, I think, for the soil arthropods, is that the number of pitfall traps or the number of targeting traps that you take is really important in this regard because there is so much variation from trap to trap.

So if you don't take enough traps, then what you have is you have an estimate of the plot mean that is not very precise.

You have introduced a lot more variance that is essentially within plot variance. It is not even between plot variance, the kind that you

would like to reduce. It is within plot variance. And it is going to end up showing up as between plot variance.

That then reduces your statistical power tremendously. For example, we did a study where we were looking at collembola with regards to different types of treatments.

And what we found is -- so we put I think nine pitfall traps in there. Then we used the information from the nine to determine how many we really needed to get a reasonable estimate of the density of the collembola in the plots.

We calculated out from that that we probably needed 12 in the plot in order to get a reasonable estimate so that the estimates were precise enough that that wouldn't appear in the error variance, so that if there were actually treatment mean, treatment differences, we could detect them.

So I think that that's really important to look into as well. But to do that, of course, requires a lot more work in the field. So it

starts to tip it in the direction of the lab.

Now, the other side is if you do a big field experiment and look at a lot of different species, the odds are that you are going to find some significant differences. So you are going to get some false positives as well.

So you are going to have to do follow-up work in any field result, even if you find a positive result, in other words, a difference between the treatments, to ensure that that actually is going on.

So there are a lot of, I think, pitfalls on that side.

The laboratory experiments though, I think, have to be well designed and controlled. I identified probably six just really basic ones that I find that many experiments don't actually meet.

And that whatever test species you use, you have to use -- that the main foods of the test species actually occur in the test locality, that the food offered to the species actually contains

toxin and actually is consumed, that the life stages are exposed appropriately and that you have proper scientific controls, and we'll get into that more, you have sufficient replication and sufficient numbers of insect screens so that you can make inferences from the data and that you use a system that actually exposes the organism in relevant ways, either the whole plant or plant parts or an extremely high dose, I think, is the main thing there.

And then in terms of how to select species for testing, I think that there are several criteria that one can use.

One can sort of use criteria that we have that are -- they are basically anthropogenic in origin. So things like why might a registrant want to test monarchs? It's because monarchs are of considerable cultural significance to Americans.

There is a category of species of -- bald eagles are one of them too. If anything was going to affect bald eagles, people would have a

lot of problems. In Australia if you are affecting koala bears and kangaroos, people would have a lot of problems with that.

There are species of cultural significance that I think we can identify would be a concern to a lot of people.

Anyway, there are a number of categories like that that one can then say, okay, have we actually covered these categories in our approach.

On the other side, one can look at ecological criteria. For example, we could talk about -- on the one hand, we talk about natural enemies, which are sort of more anthropogenic.

On the other hand, we can talk about secondary consumers. So natural enemies include things that eat weeds, whereas secondary consumers are only -- so that there are different things that are evoked when one looks at it ecologically versus anthropogenically.

I think that there is a relatively limited group of ecological functions and anthropogenic needs that one can list off and use

that as somewhat of a framework for thinking about species selection issues.

DR. PORTIER: Thank you.

Any other comments from the panel?

I will put in my comments that, basically, I don't see a disagreement amongst the panel on the issues. I haven't heard anything that is an obvious disagreement. I will reiterate my comment about sample size and, in fact, refer you back to the 1999 SAP where you, in fact, asked us that question specifically.

And our answer was to establish the effect level you are looking for, look at the coefficient of variation and use that to guide you in terms of sample size. And I think that recommendation would still hold.

In looking at the studies that have been put forth to us and the types of analyses done, as a statistician, I do see some deficiencies in the way in which these analyses were done. Most specifically, in the survival studies, there is classic tools and survival analysis that provide

much greater statistical power than the T tests that are predominantly being used at the ends of these studies.

And I think that could definitely benefit these types of assays.

I think we have a fairly clear and consistent answer to you here. Did you have any follow up at all on this question? Is this clear enough?

DR. ANDERSEN: That's good. Thank you.

DR. FEDERICI: I just have a question.

With respect to the field studies, correct me if I'm wrong, I envisioned this, that if the preliminary data are considered sufficient enough to go ahead with limited registration, that the amount of this corn that would be planted would be thousands of acres at least. Is that correct?

So the reason I say this is that the field -- the opportunity to conduct really large, what would be considered by traditional methods of analysis in the field, the plot sizes, the

opportunity is to have very large plot sizes and ample opportunity for replication and statistical power.

Having said that, then, are there particular organisms with respect to this corn that you would pick for study, or would you just say target 20 different invertebrates?

I'm directing this to Paul. How would you go about that?

DR. JEPSON: The words full inventory, to me, strikes terror in my heart. If I was asked to conduct a full inventory of a field study, I would go straight to the Smithsonian and the Museum of Natural History and I would get taxonomic experts from 150 different groups.

So the idea of making things more specific helps everybody. It helps the agency in terms of what the heck is going on. And it helps industry decide how long this piece of string is.

As it is a request for a full inventory at the moment, and I'm sure it was more sophisticated than that, but if it wasn't, it is

rather open ended.

Number two, it is extremely costly to conduct large scale field studies of the type that would be implied by the comments I'm making. I'm specifically addressing the limits to interpretation of small scale studies.

Number two, it would be very difficult to detect, even in a large scale study, say, 30 percent reduction in fitness of carabid beetles. Extremely difficult.

However, it is possible if you know what effects can arise to conduct observations in real time in agriculture to see whether or not these types of impacts are happening. So I'm not particularly envisioning very large scale, multi-treatment, multi-field studies because it simply is very difficult to put together the taxonomists and the other groups necessary to do this.

Where this has been done, I should mention, this is -- normally, people look at functional groupings of organisms rather than

particular species. So we're really asking for a huge amount here if we're expecting a full inventory of specific attacks (ph) on individual fields.

Because of the variance, as David said, in numbers over time. It doesn't mean the effects aren't important. What it means is it is very difficult to detect them in single studies.

These effects would emerge from observations over whole systems over time. That's the thing that makes them so difficult and challenging to work with. So a long answer to a short question.

With the level of knowledge, if the corn system -- for the gentleman from Nebraska that spoke this morning, for example, there is in general a quite sophisticated knowledge of the kind of invertebrate biodiversity in these systems.

I think it would be possible kind of by EPA eco region by eco region, for example, to say which 10 beetle species could be included in the

list.

But that's quite a challenge and I have not previously thought about that.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: In listening to Paul's remarks, it seems to me that the field survey type of analyses are quite daunting in the sense that it is unclear to me, at least, what an appropriate indicator species would be. Because I would suspect that if one were to go into any habitat, any community, whether it is a managed habitat like an agri ecosystem, typically, what you are likely to find are a handful, one or two numerically dominant, species and an incredibly large array of organisms that are essentially rare.

What are the implications of that? Well, the implications of that are that we don't necessarily know that the numerically dominant species is the species that structures that community or that is key to the interactions that maintain that community.

And if we were to go to the rarer species, even on a functional basis, it is not clear that it could be done in a statistically rigorous, sufficiently rigorous way to make determinations.

So I'm a bit -- and again, we may be getting into Part B here, but I'm a bit at a loss in terms of the concept of an indicator species.

DR. PORTIER: With that --

DR. JEPSON: I don't think it is on the agenda, really. I don't think anybody is envisioning the possibility of indicator species. More like groups that may be more or less sensitive.

DR. JEPSON: Before we go on to B, let me make sure I have some basic feeling for what we have said. I think we have noted that more increased notice should be given to existing expert comments on laboratory protocols and different types of laboratory evaluations.

That in direct answer to the question, the lab test provide stronger support for lack of

an effect and potentially more cost efficient approach for demonstrating a lack of an effect, but may have limited utility for doing an overall risk assessment under real field conditions on trying to make guesses or predictions about what will happen in the real field conditions.

And that it's not one or the other. That the question should have talked about the complementary nature of these two types of tests.

In looking at the actual specific case in front of us, modifications to GLP, modifications to the study protocols could have been better documented. We would have had an easier time of looking at it, if that were the case. That all of these test procedures seem to still be maturing into a more regulatory paradigm and that this is where it is right now and that's good enough.

We got six basic issues related to good laboratory practice in this area.

Noted the importance of species of cultural significance. Something that I don't

think we had ever discussed before other than the one SAP meeting we had on the Monarch butterfly, specifically.

And that large field studies maybe need more careful assessment for their utility before we begin to go down that path.

Did I sort of capture everything?

Dr. Andow.

DR. ANDOW: I guess the thrust of my comments was that the laboratory studies may actually be more effective at detecting potential hazard than the field study.

Just to complement what Paul was saying in terms of being able to assert the lack of an identifiable hazard, it may also be more effective at identifying those potential hazards as well.

DR. PORTIER: In fact, I think that's what Paul was saying in the sense that since they are more sensitive or likely to be more sensitive, lack of an effect makes you more comfortable that there probably isn't one.

But seeing an effect doesn't necessarily

mean it is going to happen in real life in the field. I believe that's what Paul had said. I think we got that interpretation.

Dr. Alexander first.

DR. ALEXANDER: If I could ask a devil's advocate type of question, one that I have asked myself as a microbiologist. And we have looked at the same kinds of problems for many years. We have books and books on the effects on microorganisms, on indicator species, on processes.

My irreverent or devil's advocate question is this: Which species, indicated species, groups of species, categories, whatever one wants to have are really important for the soil ecosystem, as a functioning unit or as something we want to preserve?

And the answer as far as the microbial activity is concerned is I don't have a clue in the world with all the publications we have had including some or our own work.

DR. PORTIER: I think that's part of

what we will discuss in the next question. So if you bring your rhetorical in the next half of this question, if the panel could try to address that as part of Part B, that would be useful.

Dr. Barbosa.

DR. BARBOSA: I just wanted to add something to what David just said. I realize this is an evolving process. But I just wanted to speak to the issue of consideration of -- because of the power of a laboratory approach, of other response variables beyond mortality.

And although obviously mortality is important, but I think there are opportunities without additional costs for determining other -- for using other responsive variables that could be very informative.

And this is not in the category of it would be nice, but I think it provides many ways in which fitness of an organism is reduced without seeing it expressed in mortality.

DR. PORTIER: If I might add to your statement, it was one thing I was going to bring

up. But it dropped. But I will bring it back up now.

That is that one thing clearly that would have been very nice and useful in the context of this evaluation that I heard in the questions this morning was a decent measure of exposure in the animal, a biomarker of some sort so that we know they ate the crop.

I think that would be an extremely useful tool in the context of strength of evidence here.

Okay. With that, I think we will move to Question B.

MS. ROSE: Can I ask for one point of clarification? It has been mentioned a couple times of conducting large scale field studies. I'm curious what you would think would constitute a large scale field study.

DR. JEPSON: It very much depends on the organism, unfortunately.

But I certainly know what a small scale is. Virtually, all the studies we have looked at

in this review I would call small scale in that.

If you are using pitfall traps and sampling the carsorial forna (ph), those animals are going to be moving between multiple plots. That's one definition of small scale.

But for lady bug, a whole field is relatively small. So that makes it very, very difficult, and one reason why I'm emphasizing the need to use cages and (inaudible) bioassays wherever possible to get around some of these problems.

Large scale, you know, we can't -- there is no -- it is one-half turn above. No, we're not saying that.

But if you are carrying out a study where you want a second year of monitoring data, the scale of the experiment has to be tuned to that kind of time scale.

So you are talking about 10s of hectares for it to -- which we're not going to do.

However, if you wish to have data that spans two years, you have to tune the scale of the

study to match up to that requirement.

DR. ANDOW: I would like to disagree a little bit. The ideal, I think, is where you are heading in that what you have is you have a population that is basically interacting primarily with -- internal to the plot than sort of flowing among plots.

But I think that with some information about the flows among plots, one can interpret some of these other field experiments as well.

DR. JEPSON: I would go along with that.

DR. ANDOW: And so that one has to be sensitive to how scale affects the interpretation perhaps more than just having a really big experiment.

DR. JEPSON: Really big, just for the sake of it, is pointless. You have to have a really, really good reason for doing it.

DR. PORTIER: That was Dr. Jepson.

Dr. Federici?

DR. FEDERICI: When I mentioned large scale field trials, I didn't mean field trials per

se. You are going to have large plantings of the crop anyhow. And you design your studies to go in and then do the sampling in there. So it is a matter of the type of sampling you do.

I certainly didn't mean that you just go out and do large experimental plots of 10 acres replicated 40 times or something like that. None at all.

DR. PORTIER: I think that's clear in our response. We're not asking for that type of study.

Ms. Rose.

MS. ROSE: The second part of Question 1 is, the panel is requested to comment on the logistics, validity, cost and expected scientific gain, if any, of conducting a census of the invertebrate community versus concentrating the studies on specific indicator organisms.

In addition, please comment on suggested indicator groups such as carabids and staphylinids in the case of Cry3Bb1 that would be most likely to provide the agency with meaningful data for

assessing the potential hazards to non-target invertebrates from corn rootworm plant-incorporated protectants.

DR. PORTIER: Thank you. I think we have gotten a little bit into this question already.

Dr. Jepson.

DR. JEPSON: First of all, I will suggest to the panel now that we add some remarks about functional group analysis, because this can get you into the realm of being able to carry out large scale field studies without a huge amount of taxonomic expertise, but from which you can still get a great deal of value.

I would also like to note that many of us in the room are aware of work that is currently going on which isn't part of the package which relates to other commodities of the sort that, say, Galan Dively is doing at University of Maryland, where he is using principal response curve analysis and really quite sophisticated statistics to interpret these effects.

So we must not leave the room with the sense that nobody is pursuing this work in a sophisticated and interesting way. There is some excellent work going on.

I would also like to mention that there is a link between soil health, how ever defined, and biodiversity of invertebrates.

And the leading exponent of research in this country is John Moore at Northern Colorado State University who works with Peter Deroiter (ph) in the Netherlands.

The one thing they have demonstrated is that the more disrupted the agri ecosystem through plowing and spraying, for example, the more uniform the phenologies of organisms tend to be. You tend to get gaps in the distribution abundance of organisms that then allow nutrients to leak from the field. Loss of nitrate is extirpated by greater levels of perturbation.

So it was a level at which an assumed knowledge in which we operate, which I'm sure many people are aware of, but I think we need to put a

preamble into our report that this is why we're concerned about broad diversity issues in agriculture and how relief from toxic pesticides is potentially going to improve a whole variety of measures of soil health in longer term. So that's why we're so interested in this.

So logistics, validity, costs, scientific gain of censuses of communities versus specific studies. Rather than go back over the things I had just mentioned, all of which I think apply, this is a coleopteran active material and there are a number, approximately 250, families of coleoptera.

The last thing we're going to do is make measurements on 175 of these and you'll have some level of confidence.

I think -- but carabiditae and staphylinidae are both significant players in agri ecosystems in a variety of trophic striata. And they are important predators of crop pests. And there is concern in the agricultural community about preserving these organisms. So they would

seem to be relevant organisms.

And there are published test batteries, as I have mentioned, the laboratory, extended laboratory, semifield and field level for representative carabids and staphylinids.

And we'll refer in the report to the different groups of carabids that you need to bear in mind. There are some of the o venturin (ph) field boundaries and penetrate the field each year. There are some that breed in the fall. Some that breed in the spring. Some have surface active larvae, some subterranean larvae.

I think all of those say you need to have some understanding of the ecology of the organism before you construct a test and have some estimate of the potential exposure.

Why a carabid is interesting in other terms possibly as indicator taxa. Well, one reason they are interesting is that they are very sensitive to fairly mild perturbations.

They don't have very high reproductive rates. Many taxa are wingless. So they are among

the first taxa to become locally extirpated in sprayed systems. They disappear from sprayed systems.

So as an indicator of effects of intensive agriculture and pesticide use, particularly things like organophosphates and pyrethroids and other materials, they are rather sensitive indicators by virtue of their life histories. That makes them very, very interesting.

But also sensitive to small perturbations, a 20 percent reduction in fitness of a carabid ground beetle I can assure you would likely be very significant. Because of the low dispersal rate, low population replacement rates, these are more important questions to ask of carabids than perhaps of coccinelids.

But the impacts are scale dependent. So I have my continuing concerns about scale that obviously we'll address in the report and need to be brought in mind in interpreting the data.

So the relative value of census versus

specific studies, I think I would personally make a case for barrier based on cage studies as an initial stage, possibly even with marked organisms, because you get a much better measure of what is happening where you can confine these insects versus where you are just monitoring numbers in pitfall traps over a whole season.

Pitfall traps are activity dependent traps. If you increase prey availability in a field because you don't apply a pesticide, you will decrease carabid movement because they have lots to eat. So you will catch fewer in a pitfall trap.

We have known this for three quarters of a century, but we don't seem to take it into account necessarily in interpreting the data from our field experiments.

So some measure of mobility is actually quite important because they are activity dependent traps.

So we'll summarize the available literature for test protocols for other beetle

families, including chrysomelid beetles that occur in wheaten fields which are important in Europe, certainly important food for birds.

That's really all I have to say. I think I'm an advocate for an intermediate scale of testing and evaluation that isn't at either of these polar extremes. I think you will discover more and the public confidence will be higher as a result of doing this, potentially.

That's all I have to say.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: I guess the only thing I would add is more or less akin to Paul's suggestion about functional groups. But to think perhaps in terms of ecosystem or habitat functions might be another approach to be considered.

And that is not to look at any given particular species in that we don't necessarily know the dynamics of that habitat and whether that represents -- the role of that species is ecologically duplicated by another species. But to look at functions, that is, decomposition,

levels of predation, levels of parasitism as a measure of significant changes as opposed to the numbers of an individual which may or may not, depending on the circumstance, have an influence on the dynamics of that habitat.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: I have had the opportunity over the last couple years to observe several research groups that are trying to tackle this. And I would agree that Galan Dively seems to be at the forefront of this in that he has shown some innovations and some good leadership.

From talking to Galan, I think it is becoming very clear that when you jump into this and from what I have seen from a lot of the researchers that you can very quickly just become overwhelmed with the numbers of taxa and the complexity of the investigation.

It does need to be simplified. I think that as people keep mentioning going back to the functional groups and maybe finding one or two representative taxa within these functional

groups may be a good way to -- a good compromise for approach from these studies.

On the other hand, I hear David and Paul saying that the amount of information that you get versus the money you put toward it may not be as efficient as it would be with laboratory tests.

At the same time, I think there is a cultural need to take this a step further in that these field studies with a little bit more involved -- I would like to think that over a few years, maybe even fairly quickly, it would become pretty obvious it is not going to being necessary to repeat this over and over again.

Certainly, by then maybe we'll come up with the most efficient design for answering these questions.

But I think that we're here right now because of the nature of this product. And I think that we're -- certainly, we have not investigated these questions before for other products and it is new territory.

I would like to think that all the

people that are putting literally years of research into this, that -- I think there is some opportunities for people to share information, to get together and to come up with what they consider to be the most efficient protocol so that people across the country don't keep reinventing the wheel and that maybe we can help some people to -- well, maybe save some careers because some think that we're really investing a lot of time on this.

I think there is a lot of room for improvement.

DR. PORTIER: Any other comments on this question from the panel?

Dr. Neher.

DR. NEHER: I just wanted to follow up on what Rick was saying in terms of the approach that Dively is using because I think -- and back to this principal response curve.

I guess one approach -- useful result of that approach can be, I'm not advocating that everybody go out and do these censuses, but in

that situation, he is in a position to apply that technique which will then help identify particular candidate indicator taxa. Because then we can identify those that may be particularly sensitive or tolerant.

So I think one of the benefits from those kinds of studies is that we'll be able to narrow those groups down or identify particular ones, and then those can be used and studied in the semifield or laboratory studies in further detail.

But I just wanted to make that connection. That's a mechanism for identifying candidates.

DR. PORTIER: Dr. Andow.

DR. ANDOW: I'm going to address the question related to carabids and staphylinids, specifically, and suggest that carabids that could be screened would be one of the bembidion species. There are three that we commonly trap. Bembidion quadrimaculatum (ph) tends to be the most abundant one of all those.

They are little guys. They are numerically abundant in corn fields in the upper midwest. And they are primarily predacious. And they probably have a reasonably high reproductive rate. And people have worked on them in the past. So this is one potential candidate.

Taroxcus malanarious (ph) is one of the larger species that we see of the carabids. It is also primarily predacious. And of the larger species, it's probably the most abundant of the primarily predacious large carabids and it probably has a relatively low reproductive rate. So that would sort of bracket those things.

There is a whole group of medium sized species, however, that -- some of them are primarily predaceous. Some of them are primarily seed eaters. But there is a group that will also -- that is a little bit more omnivorous and then you might actually find them eating decomposing corn tissue, which of all those species I can't think of the names off the top of my head.

UNKNOWN SPEAKER: Amara.

DR. ANDOW: I have amara listed. I wasn't sure if amara was one of them. But those would be useful as well, I think.

Then if you go to staphylinids, one possibility would be stenus flavicornis, which is a relatively larger staphylinid, make it a little bit easier to work with. But it is not tremendously abundant, but it is common enough that you can pick it up at good frequencies.

It is sort of dodging Pedro's point about needing to look at some of the more rare species. But on the other hand, the common species are the ones that are going to be possible to test and to find and to do work on.

So those would be some of the ones that I would just throw out there for consideration. Specifically, carabids and staphylinids.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: It is a good suggestion, but it is important to bear in mind -- Rick made the point about what would be nice and what do we want from a regulatory perspective.

Some carabids and some staphynilids have been run through the mill, as it were, in terms of determining whether or not it is possible to culture them.

And some of these animals are very, very difficult to culture because they have their cannibalistic larvae and because they have very low reproductive rates and the eggs tend to have very low fertilities.

So there is one pterostichus species, cupreus, which I do believe occurs here, which has been acquired as the kind of regulatory test organism in Europe because it can be relatively easily cultured compared to others.

I think it's important to bear that in mind as well as coming up with lists of organisms that are abundant in the given system.

So again, striking balance and not indulging in excessive expenditure, making use of what is already known is also a part of it.

DR. ANDOW: I guess part of my comment was to eliminate the hapalines (ph) for

consideration because they are primarily seed eating. They do a lot of seed eating. They feed a lot on weed seeds.

DR. PORTIER: Dr. Alexander.

DR. ALEXANDER: Without belaboring the point, I maintain that my devil's advocate question has not been answered.

DR. JEPSON: Can you remind us of the question?

DR. ALEXANDER: The question is that given the concern with individual species, with groups of species, with functional groups, with indicator organisms, what is the convincing evidence that any of these are important for the things that we are looking to soils for? To grow crops? Obviously, plant parasitic organisms are important. But for growing crops, for maintaining quality.

Or is it as in the definition, and I will apply this to soil health and health. What is a healthy individual? A healthy individual is a person who doesn't say he is unhealthy.

That's about all we can say there. Now, given all the concern we have with effects on soil, I would like to know which soil organisms, which soil processes, microbial, invertebrate or otherwise, are in fact important for the things that we want soils for.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: I'll just give a brief answer. Obviously, this is a subject of intense debate and activity. But I would refer you to the work of John Moore. It's published in science and it's of excellent quality.

Basically, if I can summarize that healthy functioning soil, microbiology soil invertebrates, soil bio diversity, as it were, high levels of bio diversity are consistent with healthy functioning soils.

For example, loss of nutrients from soils that surely that is important. And they have demonstrated cultivation practices and other processes that lead to greater losses of nitrate from systems than those that don't.

However --

DR. ALEXANDER: I would maintain that's semantic obfuscation.

DR. JEPSON: It may be just the way I'm saying it at this stage in the afternoon. But if you can demonstrate a loss of nutrients if you deplete --

DR. ALEXANDER: That's important.

DR. JEPSON: That's all I was trying to say.

So if we avoid the semantics and get down to the nitty-gritty, I think there's data to support this.

In terms of a surface active forna (ph) we do not as a routine in this country monitor invertebrates on a large scale anywhere. We haven't done it historically. There is no current plans to do it despite the biological observatory programs of NSF. Long term ecological research sites, we don't look at invertebrates in agri ecosystems. That's to the loss of all of us.

It leaves industry wondering what they

can measure and what it means. It leaves EPA in a position where they can't interpret the data sets in the context of what actually occurs there.

So all I can say, where this data has been collected in temperate systems that are equivalent to those where corn is grown, there is a direct link between diversity of the animals we're talking about and the equilibrium population densities of pests.

So we want more of them. So we reduce the frequency of pest attack. And there is a very large literature that supports that.

DR. PORTIER: Dr. Andow.

DR. ANDOW: So in terms of why certain carabids, perhaps -- I refer to work conducted by David Weiss (ph) of Kentucky where he has been slowly but surely accumulating the evidence that linking decomposition food chains primarily through -- upwards of collembola through the ground, predaceous ground fauna and suggesting that if you -- well, he has shown in a number of experiments if you add decomposing organic matter,

you can increase the collembola which then increase the carabid fauna.

And the area that he's heading in is linking the decomposer food chains to the above-ground plant food chains, plant based food chains, because the carabids link in to feed on some of the insects that feed on the plants themselves.

So they form -- could form an important nexus between the decomposition food chains and the above-ground food chains.

And a little bit of work that we started to conduct in the corn system suggests that it could be bembidion or some of these other predaceous carabids that are key in the corn system itself.

So I wasn't just pulling them out of the air. But we do have a little bit of evidence that they may be functionally important as well. It is not convincing enough to say that that's the main reason to do it, but that's the direction I think that a lot of this work that is being conducted on

these decomposition food chains at the invertebrate level, at the arthropod level are linking in.

DR. PORTIER: I hate for us to get into a debate we can't end. So if I can sort of capture what we have just said in the course of the last few minutes, I would argue that it goes something like this: The choice of what we test and the choice of how we test it is driven by practical limitations many times rather than sound scientific decision about what should be tested based on knowledge of how an ecosystem works.

And that the science advisory panel would suggest that science continue in the direction of trying to find out more sound reasons for choosing models for testing than just practical reasons of ability to measure them and ability to culture them in a lab.

Have I captured the general idea, that we would like to strive toward something which is more tied to the importance of it in terms of goals set for quality of soils, quality of

existence on the planet in some sense.

Are there any other comments on this question?

I think what I have is just a few points that -- some indications of which indicator species might be best used, and again, this comment about how to choose the indicator species, some discussion about the intermediate approaches rather than field and laboratory, again, looking at things like barrier and cage studies, functional groups, trying to locate one or two representative taxa or habitat groupings or ecological groupings. And these don't have to be disjoint of each other.

Have I captured the basic points there? And there was also the point that Dr. Hellmich made about a cultural need for an emerging technology, a cultural need to feel comfort with it.

I think this falls down to the basic issue in science that it is almost impossible for us to prove a negative. It is easier to prove a

positive. In this case, we have to gain some degree of comfort that when we see a lot of negative studies, that, in essence, provides sufficient weight of evidence that we believe nothing is happening.

And that's part of, I think, the scientific culture of taking this a step further.

So the need for field studies will probably continue for a longer period of time, the need for broader array of studies simply as scientists gain comfort that we are actually approaching this problem properly in protecting the public and the ecology from these types of new emerging products.

Have I captured everything?

DR. JEPSON: Very good.

DR. PORTIER: I think we're still -- we are set to break at 3 o'clock. We could go on to Question 2 and break after Question 2. That puts us a little bit behind.

What would the panel like? Simple vote. Do we take a break now?

All those in favor, hands up. Those opposed, no hand. How many want to take a break now? Two. We'll keep going. Let's go to question Number 2.

Democracy in action.

MS. ROSE: Question 2, please comment on the adequacy of the two year field abundance study for making a determination of the potential risks from commercial use of event mon 863.

DR. PORTIER: Dr. Federici.

DR. FEDERICI: I want to preface my specific answer to this question, which is rather brief.

And just point out related to the last question is that there are -- it is unfortunate that Steve Naranjo couldn't be here because he is doing long term field studies in looking at a smaller group of insects.

And this is turning out, I think, to be very interesting. That's in a cotton system. And Bill Moore are also working with a cotton system with several other investigators throughout the

southeast, are in, I think, the second year or maybe the third year of their study.

So the reason I mention that is we're going to learn a lot from those studies which are much further along that will impact how I think we look at these new beetle products that are coming on line.

I was troubled, to be honest, with data that or let's say the lack of data that I saw in what I was supplied with, that I had availability, that were available to me.

Then also, Robyn, in your discussion this morning, you indicated that these are very preliminary results. Here is what I have to say. This study is very preliminary. Although based on the high specificity of Cry3Bb1, significant non-target effects would not be expected. Especially in comparison to those that occur with synthetic chemical insecticides. These studies should be carried out for at least three years. Especially as this is a new pest control technology. Much more extensive and ongoing

studies with Cry 1Ab corn have shown no significant effect between -- excuse me, have shown no significant differences between the effects of this corn and non Bt corn on non-target organisms.

And the same thing is true in the case of the cotton studies from everything I have seen. This provides a useful foundation for assessing Cry 3B1 corn. Nevertheless, the limited nature of the Mon 863 studies that have been provided can only be used for what must be considered a very preliminary assessment.

How you decide to use that, I don't know. But I don't think what you have now is adequate.

DR. PORTIER: Dr. Andow.

DR. ANDOW: The short answer would be to agree. I guess I reviewed the material and I didn't find any data that were reported for two years at any one site. Moreover, much of the reporting of the data is incomplete.

So even if a two-year field abundance

study were adequate for making the risk determination, the present data aren't sufficient to make such a determination for Mon 863.

So then, I sort of turned to the details of what are in those two reports, this 45538206, which is the field experiment from Monmouth, Illinois, for 2000, and 45653003, which is the reporting on the eight or nine -- I guess it's eight experiments, some of which are field experiments and some are laboratory experiments.

Basically, if I apply the criteria that the data are presented that the density of the insect -- or the arthropod that is being examined is sufficiently high, that the sampling effort is sufficiently precise, and just those three criteria, it is sort of eliminates all but, in my view, just three comparisons out of that whole data set because most of the species are relatively rare that are being --

And this is based on my experience working in the corn system and what kind of densities that I have seen and when I am able to

detect differences among treatments.

So it is a little bit -- it's subjective in that sense. This is my subjective opinion. I won't make any bones about that.

Furthermore, the power of these tests are relatively low. They tend to be all F tests with one degree of freedom in the numerator and three degrees of freedom in the denominator. There is some reporting of pseudoreplication in at least one of the studies. So that's an issue.

What it means, though, is that -- one can look at the data once they accumulate and address this issue of statistical power by doing or having or seeing a meta analysis of the multiple experiments, which require then a detailed discussion of the error variances associated with each experiment and tell you how it is that you combine the results.

That would actually be quite instructive once you get the data to see what it would actually do for you.

On the theoretical question of whether a

two year field study would allow adequate determination of risk, I would say that -- I would be highly skeptical. And that's because risk will be a function of the -- first of all, that there is going to be high variability from year to year in such an experiment. But risk will be a function in part of the extent of local use of Mon 863, which cannot be experimentally assessed at this time or, in fact, any time prior to registration.

You have to have enough theorems (ph) to do that.

So that one doesn't want to bank all of the evidence on ecological risk based on even a multiple field experiment such as these.

Then I just want to elaborate that in fact these studies could be used to identify the hazards as I discussed before.

It is sort of the multiple similar results of a field study that could be quite valuable. But again, it is sort of building it up in a meta analysis of all those things that would

ultimately be convincing.

And then as I mentioned earlier to you about the issue of isogenic controls, when you start dealing with field experiments, then you do have to be concerned about that. I'm not exactly sure what the best way to handle that is.

But one way is to try to combine laboratory experiments on the toxicity or -- on the effect of the trans gene product itself to show that it does have the same kind of effect that you see in the field, so that essentially you try to get mechanistic associations with field results.

Or else to have multiple comparisons of different types -- different varieties with Bt and without Bt so that you are not relying on just a single varietal comparison.

And if it shows up in multiple varietal comparisons, then you are more likely to believe that it is related to the product itself and not to other variations in the variety.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: One reason I was asking a lot of questions to the Monsanto crew when they were up here was because the information that we have is primarily from 2000.

I understand that the information, the data for 2001 and 2002 will become available shortly, so that will be three seasons worth of field studies.

The other thing I want to point out is that the invertebrate abundance studies, at least as I understand it, aren't really required for this registration. This is information that is being provided because Monsanto feels that it would be good to know.

I don't exactly disagree that -- I think that once the 2001, 2002 data are made available, and they should be made available fairly soon, that there may be adequate information there to at least allow the EPA to see whether or not this product is safer than an insecticide.

I keep on coming back to that because in a lot of cases, you're just comparing Bt and its

isoline.

But I think it is necessary also to compare this product with the conventional forms of control. And time and time again when researchers do that, there is that huge impact from the insecticide compared to this event.

So I think that the data, those eight field studies that are being conducted right now, and I know a lot of the people that are involved in this, and I know that they are good researchers, and I would hate to slight the work that they are doing because it has been done, it is just a matter with a little bit of time it will be made available.

And I think when that is done, that the invertebrate abundance studies will clearly suggest that this product is better than -- is similar to the isoline controls and that it is better than the chemical treatments.

DR. PORTIER: Dr. Federici.

DR. FEDERICI: Rick, I have very little doubt that what you say is true. I believe that

this corn is probably very safe for most of the nontargets. And that if this new data -- the more recent data come in, as Dr. McKee from Monsanto said this morning, that the fundamental result will not change. I believe that.

But I don't believe the data is here in what we were shown and asked to evaluate. That's the point that I'm, the primary point that I'm making. At least I didn't have -- the data that were in my packet I would feel very uncomfortable with just giving you a go-ahead.

But we are just advisory to you. And you make the decision. So once you get more data, maybe everything will be fine.

As far as the isogenic comparisons, there is going to be so much variation in soil and other geographical regions, rain, all kinds of other things, that I think that's going to more or less eliminate individual varieties and the effects that you might have in those -- that is, non Bt versus Bt corn.

And from everything we have seen with

the cotton and the corn that have been out there, there is no comparison of the effect that chemical insecticides -- basically eliminate most of the non-target organisms.

Basically, I'm in agreement with you. It is just a matter of whether the data are here or not.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: Just to enter a mild note of controversy. I think if the question is: With the study designs that we have been shown over the time scale of persistence of conventional pesticides, is this product as acutely toxic as the conventional pesticides, then that's the question that these experiments are designed to answer.

If you were to say on the basis of these results that over two years we have demonstrated a lack of harm with these small plot sizes, I just simply do not believe that's a scientifically valid conclusion to draw. Even though I also believe the effects will be very small if

detectable at all.

I don't believe it is possible to draw that conclusion from the experiments that we have seen designed.

So I think the question relates more to the comparison with the conventional products over the time scale the conventional products are active than the duration of persistence of this material because of redistribution into the treated areas following the end of the year.

The animals just walk from one plot into the other. You simply can't draw those conclusions in my view.

DR. PORTIER: Dr. Andow.

DR. ANDOW: On the issue of comparing the Bt effect with, say, an insecticide effect, I would just put in this one word of caution is that many of the experiments are designed as whole plot, split plot experiments where the whole plots are the varieties and the split plots are the insecticide treatments.

Usually, there is more than two

insecticide treatments. And often three in this design.

So that if you actually look at the power in the analysis, the power in the analysis is bias towards detecting an insecticide effect, because those are going to be F 2 sticks tests, whereas the Bt effect is going to be an F 1 3 effect.

So you are going to have to see a lot bigger differences in the Bt to find a statistically significant effect there. And that's just the nature of the design.

DR. PORTIER: Any other comments on this question?

I don't think we really disagree that much on this point. I think currently with what we have, the agency is being told that we don't think it is adequate in terms of direct answer to the question for making a determination about potential risk.

And there were a number of reasons. The primary nature of the data, it would have been

nice if they had been a little bit longer, although there is some controversy on whether longer studies would have been useful with this particular design.

Multiple years in the same site. The concern about the low power for this particular type of design. But that may be fixed by using more complicated statistical tools like meta analysis.

The use of the word risks here was raised to some detail. And that risk itself has to focus on all of the data. Not only all of the data pertaining to laboratory studies and the field studies, but also the actual density of use of the eventual product. So that was a difficult issue to look at.

Again, we raised isogenic controls, which had a lot of discussion about this morning in terms of clarifying where they are coming from.

And I think there was also consensus in the feeling that the studies that are in the pipeline will help to alleviate a lot of these

concerns.

Did I miss anything? Captured most of what we said?

We're going to break in a minute. Are there any questions from the agency for clarity?

MS. ROSE: If I can make one or two points of clarification. This question was intended for that first study that I summarized from the talk this morning where EPA did request Monsanto conduct a field abundance. And actually, we asked for a field census study during preregistration meetings.

So that was not voluntarily submitted. We did ask for that one study. The other eight studies you are referring to were more done to expand upon the science, not for the regulatory perspective. And those were very preliminary results.

So we did actually request one of the studies. That was, I believe, what this question was intended for, was that one study.

Not just the adequacy of the results

because, yes, they are preliminary, but also the adequacy of the test itself. And I think a lot of that has to do with methodology as far as field size, number of traps, et cetera, which I'm not sure how much we have touched upon.

DR. PORTIER: Would you like more discussion of those design points?

MS. ROSE: It depends. Is everybody going to be upset if we delay the break?

DR. JEPSON: I think we can address questions of design in the report. We haven't looked at it kind of item by item breakdown yet. But we certainly will do that.

MS. ROSE: I just wanted to clarify that.

DR. JEPSON: We were asked basically to tackle all the major headings without necessarily going into a lot of detail. But that's something we will be looking at.

DR. PORTIER: For the record, if there is something you want to say specific about design, we need to hold it in the oral comment.

Now, there has been considerable discussion about pointing out literature that addresses alternatives in design, new approaches that are coming up on the designs of these studies.

If that's the implication of what you will put into the report or as an appendix to the report, I don't think we need to get into that discussion here to have it included.

But if there are specific points about the design you would like to raise, I think, Dr. Jepson, you should do it now.

DR. JEPSON: I think David has adequately summarized these. The question of what to do with organisms of low abundance, the question of numbers of traps and choice of sampling method and sampling frequency, which we will look at, and questions of scale, which is obviously an interest of mine, as you have heard. We'll be talking about those.

I actually have a feeling that we have probably touched on most of the things. But Robyn just mentioned can we make this specific to that

study, which is kind of new. So what we'll do is now focus those remarks to that particular study.

And I don't think we have discussed that as a group yet.

DR. PORTIER: Well, now is the time to do it because anything we discuss has to be discussed in the public forum.

So if there is specific recommendations about design that you want to make other than pointing out general design criteria, I think we need to do that now and discuss it here.

Dr. Andow.

DR. ANDOW: I guess if you look at that, the Illinois Monmouth study, we really have the year 2000, that's the one you are referring to. Right?

MS. ROSE: No. Actually, it is not any part of those studies that were in the one packet. It's a separate study, which I do have a copy with me, which was titled, Field Abundance Evaluation, that has its own MRID number.

DR. ANDOW: Isn't that 45538206?

MS. ROSE: That could be.

DR. ANDOW: That's the Illinois Monmouth 2000 study as opposed to the other ones. Is that correct? I just want to make sure it's the --

DR. JEPSON: While Robyn is looking, I would like to note that I only gained access to these reports this lunch time. So if you are expecting today comment that we will necessarily need to make in the report, we're going to have to disappoint you.

If you want this to be record of this meeting, I'll gladly come back tomorrow during the IRM meeting and summarize our feelings so it's a matter of public record.

But given the time scales involved and the seriousness of this question -- and we do intend to address the experimental design of that particular study. But we can't do it specifically now.

DR. ANDERSEN: I think it's 8206?

DR. ANDOW: Yes.

DR. ANDERSEN: I might point out that on

the set of slides you got, I think we're talking about Page 9 where it begins with the field studies and the field study abundance, so that if that helps you look at the materials.

DR. PORTIER: Why don't we at this point -- we're partway through Question 2. But there is clearly some discussion about the design issue that will have to occur.

Why don't we at this point take a break, come back and finish up this question and then go on to number 3 -- and see what we can do on the design issues for Question Number 2.

If that's okay with the panel. We'll break for 15 minutes. According to my clock, that will put us back in here at 3:30.

(Thereupon, a brief recess was taken.)

DR. PORTIER: Welcome back to the FIFRA Science Advisory Panel meeting.

Just before we took a break, we were working on Question Number 2. We had pretty much provided an answer to what we thought was the full issues for Question Number 2.

Ms. Rose had asked us to give very specific comment on the two year field abundance study.

During the break -- and the issue that came up was that a couple of members of the panel had difficulty actually reading that study because of the format it came on the CD and the proper software, et cetera, associated with that and haven't had time to really get into the details of that study to provide good comment on it.

What we have decided to do is that a small subpanel from the SAP will get together after we close the SAP meeting today. That subgroup will look at this study in greater detail and come back tomorrow morning. And at the very start of the SAP tomorrow morning, we'll provide a report, a public report of their findings in terms of this particular study and the design issues associated with this particular study.

That will not allow this panel, since we will have a new panel sitting tomorrow, the opportunity to comment on the subpanel's comments.

So I want to poll the SAP that's here whether that is sufficient for you. The comments that come back tomorrow will not be the comments for this entire SAP. It will be the comments of the subgroup.

No dissention? So that's what we will do.

Before we go to Question Number 3, are there any other points for Question Number 2?

DR. HELLMICH: I have a question -- a clarification for the EPA. When we look at these experiments, the way you have presented it, you are looking at the Bt versus the isoline.

But I think in some cases it is more relevant to look at the Bt versus traditional forms of control to assessment that they are safer than that.

As Dave pointed out, the power of the statistics in this case is such that it would be easier to do that.

So what I'm saying is that it would be a lot easier for us to evaluate whether or not this

hybrid, this event is better than, traditional forms, rather than an isoline.

So can you clarify what it is that you want exactly?

DR. ANDERSEN: I think we're actually looking for a bit of both. We're overall looking at for the assessment of this product by itself and for where there is relevant data that you want to comment on scientifically looking at some of the alternative products and other methods that are used now for control of this insect.

We'll take all of that scientific advice that you give us into consideration as we make a regulatory decision.

DR. PORTIER: Dr. Andersen, if I might ask a question on follow up, then.

We could certainly as a science advisory panel talk for the next few minutes about what is the more appropriate control scientifically. But I guess the issue of whether you use an unexposed control group versus a chemical pesticide control group is more of a question of policy for the

agency than it is a scientific issue for us to look at.

Are you in agreement on that or not? Or would you -- I mean, this is a difficult issue in the sense that if it is the policy of the agency as to whether or not all new pesticides must be compared against an untreated control, an unexposed control, or is it the policy of the agency that it should be better than what exists out there?

DR. ANDERSEN: It is even more complicated than that because of some aspects of the law, specifically.

The law actually, just so everyone understands, the law actually says that EPA cannot deny the registration of a new product simply because there is an existing product that also controls the same pest -- in my terminology, not in the legalese of the law, but that's essentially what it says.

However, in looking at what we are directed to do by the statute and the regulations

associated with it is to manage and balance the risk versus the benefits.

And in doing that, we do do something called a comparative risk assessment as we look at it. So we will take into consideration what are the risks from the other ways and the benefits from the other ways that you could control this pest or this combination of pests, set of pests as we look at it.

So that part of balancing the risks and the benefit is the part that I think we feel that is inherently governmental and that that is our responsibility.

And what we're looking to the panel to do is to give us scientific advice on our risk assessment. And certainly this risk assessment has had some discussion about the chemical pesticides that are also used to control this pest and some indication of the other aspect, the cultural controls that are used to control this pest.

I may not have answered your question, Chris, from the way you are looking at me.

DR. PORTIER: I guess I was looking for a simpler answer.

Do you want the panel to discuss the issue of whether or not a field study of the type we're looking at here should include a chemical pesticide and how to choose that chemical pesticide, et cetera, and how to control for it when looking at these types of pesticides.

DR. ANDERSEN: I think that actually would be of value to us. Yes.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: It seems to me that this dichotomy in relationship to the question requires, then, answering two separate questions. Because if one is comparing the relative merits of two control modalities, I can envision that one year is more than enough. If a question is, does this new control modality have significant impact in terms of abundance of organisms, be they non-target or whatever, the answer to that question might be very different.

So it would seem to me that it has to be

treated as two separate questions.

DR. PORTIER: Anyone on the panel want to try to tackle this?

Dr. Federici.

DR. FEDERICI: I still have to read the whole report, which I'll do sometime today, I guess. However, if the data show and the chemical insecticide treatment data are in there, then what Pedro said, one year may be enough. Because I think the results are going to be so dramatically different between the chemical insecticide treated plots in the Bt and non Bt plots that it makes it a fairly straightforward comparison.

DR. PORTIER: Let me try to be a little more specific on the question. Assuming that the chemical pesticide treatment has already been evaluated by the agency, so there is existing data on the chemical pesticide regarding some of the non-target species that might be affected, what is the value of the additional study?

Dr. Jepson.

DR. JEPSON: Firstly, in the mean,

non-target invertebrate data is not requested as part of the data package for registering conventional pesticides in the United States uniquely. Although, that's something that ought to change in my view. But that's another debate for another time.

Secondly, comparing with a conventional treatment is completely defendable and a good idea, even if you already have that data because of course each circumstance and each set of situations varies.

And it is part of a formal experimental design and you get a particular outcome to your question.

There is another reason to have conventional pesticides in there, though, that they can act as something of a toxic standard. But therein lies the controversy, because, of course, the way this material is delivered to the organisms is completely different to a conventional pesticide. So that may be a challenge.

If, however, in one of these studies you don't get an effect with one of these comparative treatments, it must tell you something about the ability of that experimental design to detect an effect if an acutely toxic pesticide actually doesn't give you a result in these studies.

I think we will come back with a short response on that. But I think the agency probably has its act together pretty much on this. And the idea of making comparisons with the conventional treatment is probably what it is all about in the mean, as I said.

But you can also exploit those conventional treatments to tell you whether or not the experiment has to the power, as it were, to detect effects if they exist.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: I would like to fall back on the monarch case as an example. Again, the work that Galan Dively did where he put in an insecticide treatment where we had Bt, non Bt pollen looking at the effects with the monarch

caterpillars, that from a cultural perspective, a lot of the people that came and looked at the impact of those studies, they said that was the part of it that really convinced them -- these were just general people on the street that, yes, this was -- that the effect wasn't as bad as what it has been made out before.

So I think, as Dave suggested before, in some cases we have to consider the cultural realities of this. And I think that in this case that I would just suggest that we do compare it with the insecticide treatments.

DR. PORTIER: Dr. Andow.

DR. ANDOW: I think Paul Jepson's suggestion that the insecticide could act as a toxic standard, in other words, you so choose a deliberately toxic insecticide rather than the most commonly used insecticide, so that if the experiment doesn't see differences associated with that toxic insecticide, then one would have the whole -- it's like the use of the arsenate in the other things. And that seems to be of valuable

use.

When one starts to talk about conventional, then you start getting into conventional where and for whom. Then EPA, I think, should tread very gently on those eggshells, because if you are sort of trying to say that it is the conventional method, then you are introducing sort of a subgroup of farmers that you are particularly interested in serving with these decisions as opposed to just any farmer who is out there.

DR. PORTIER: Dr. Andersen.

DR. ANDERSEN: Just one thing that I tried to make clear as I made the statement, is that we would be interested also in other methods that are used to control this insect such as cultural methods that I think you have to look at the whole situation.

So I do think your point is taken that you don't want to just look at the situation necessarily for ones that are using a particular chemical pesticide or the most toxic, but the

whole situation, looking at it --

DR. PORTIER: I think you will get a much more thoughtful response to that in the morning.

DR. ANDERSEN: Thank you.

DR. PORTIER: Any other comments from the panel?

Let's move on to Question Number 3.

DR. ROSE: Question 3. The agency solicits the panel's comments on an appropriate design for evaluating the toxicity of Cry3Bb1 proteins to lacewing larvae.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: This is an issue that is perhaps a little bit more focused than others that we have dealt with so far. It revolves around the protocol that was used to determine toxicity of a protein.

And to be very brief, after reviewing the materials that we received, I would suggest that the protocol that was used doesn't take into consideration some alternatives that are not only

available in the literature, but that have been available for some time that perhaps at least I would contend might have been somewhat more appropriate for these types of tests.

And basically, they involve the use of surrogate eggs, be they wax eggs or perhexiline (ph) eggs. A variety of other options that have been used in tests with crysoperla are fairly effectively. But more importantly, also provide, unlike the protocol that was used, the incorporation of test materials into a defined diet for the lacewing.

And there are -- I'll provide more details in the written report. But there have been for a number of years a variety of diets that are reported in the literature that will produce high quality adults that can then be incorporated in something along the lines of a wax egg. Basically, a droplet of treated or untreated diet encased in a fine wax covering that can be used and have been used with chrysoperla.

The only other things that I would add

would be that it also may have been appropriate. Although chrysoperla may not rely heavily on pollen, it has been reported to feed on pollen.

Some might have been an appropriate addendum to the protocol. And that is to test the impact of a transgenic pollen.

The last point that I would make, I guess, would relate to the choice of chrysoperla. Although many of my biological control brethren have an inordinate affection for chrysoperla, in this situation, perhaps another organism like orius insidiosus may have been a more appropriate choice based on reports of its relative importance in this particular agri ecosystem and the clear importance of pollen to this organism.

And so I relay that as a final comment to this, related to this issue.

DR. PORTIER: Dr. Andow.

DR. ANDOW: A large part of my comments would reiterate the first point that Pedro made. But in terms of the exposure system, I think it also needs to be raised here that from a chemical

perspective, I think it should be questioned whether or not the Mon 859 transgene product really is a good enough mimic of the Mon 863 transgene product.

From a purely chemical perspective, they are different chemicals, although they are similar. But what we're talking about here is do they actually have the same non-target hazards. It is just a question that I think should be raised.

The other points that I would like to bring up has to do with replication. In terms of my reading of the supplementary material, there is really only one replication of the experiment. There is one batch of chrysoperla eggs that were used. They sort of split it into three groups of 10 in terms of how they reported it. But they didn't really describe how that happened and so on.

And it would be useful to have at least a couple, three, true replications of the experiment so that you know that it is not really

related to the -- something related to the source material that you are using.

Then finally, the total larval sample being only 30 larvae is really quite small. Accepting that mortality at 10 days is a good measure of a potential effect and with their controlled mortality of eight larvae out of the 30, then a test treatment would have to have at least 17 dead, 57 percent mortality have a significantly -- statistically significantly higher mortality than the control.

This is double the mortality of the control mortality. So you are sort of raising a fairly high -- by having so few larvae, you are having to detect a very big effect in the experiment. So it sort of compromises the ability of the experiment to detect as a maximum hazard experiment.

That, I think, is a problem -- I guess I would -- typically, in these kind of experiments, we go to at least 100 per treatment. And sometimes a little more. Rarely up into the 200s.

But still, if you have 100, then one death is one percent mortality. So you are still -- so you are likely to detect 10 percent differences in mortality if you have 100 plus.

You get a better sense as to whether there is anything going on.

In addition, what happens when you do this is that -- when I looked at the data very carefully that was delivered, it looked like it was possible that the Bt toxin was causing mortality a little earlier than the control occurred.

But of course, it would be way over-interpreting the data to say that the data even -- that the data supports that. But what it does indicate is that if you had more larvae involved, then you could actually look for those kind of effects, which would be a little bit more sensitive than just pure gross mortality up to age 50, say. So I would make those points.

And then finally because of the problems with the exposure system, I'm not sure that it

makes any sense to try to estimate a NOEC and try to assert that it exceeds or doesn't exceed the MEEC (ph).

With a better exposure system, it would be easy -- you would have a sounder basis to make those kind of conclusions.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: I will try not to repeat myself too much. But the first comments relate to exposure.

Firstly, having Bt in the diet's broth, was the bioactivity and the quantity of the material evaluated at the beginning and at the end? If not at the end, I personally have doubts about whether or not the Bt persists in that diet in the current protocol.

But certainly we don't seem to be in a position to comment on that.

I would consider requesting the lab to modify the SAP. Not necessarily to suspend the experiment when there is 20 percent control mortality. Or if there is, to require an

experiment to be conducted where a control mortality is less so that there is a chance to reach the endpoint they had in mind at the start, which was pupation.

If you don't reach the endpoint you have defined for the experiment, that's the reason for calling that study unacceptable in my view.

It also struck me that at a slightly higher temperature you might get slightly more rapid development, and that would help in this case.

I phoned back home yesterday and got a post doc of mine to check on the development times of this organism. And certainly within eight days at kind of 22 degrees you would expect pupation to be taking place from emergence from eggs.

This trial was suspended at 10 days and no pupation had yet occurred. I'm not criticizing the lab for that. It's just that 20 degrees I think or 21 nearly degrees might be a little bit cool.

Secondly, I think an endpoint that looks

at something like development going through to pupation and possibly then emergence is best than one that looks at survival alone.

So it just -- that's the robustness of the tests, really. So if they can continue with those animals they have been getting to pupation, then there is no reason why you shouldn't also measure eclosion from the pupae.

In addition, I would agree with the comments Pedro Barbosa has made about the use of the egg procedure in the first place. I'm not so concerned about whether or not they were exposed.

I think if the Bt is in that diet and they are probing the diets and feeding with those pencil-like mouth parts, it seems likely that some exposure would occur. It may be there is a dye with very fine presence in the gut. I don't know.

Pedro also mentioned why this species. Of course, we're trying very, very hard to get Bt into this organism when is that necessarily the right organism. But that seems like that's kind of an unreasonable thing to say, probably, at this

stage.

Thank you.

DR. PORTIER: Are there any other comments on this question from the panel?

Dr. Federici.

DR. FEDERICI: I question this one statement here. This may not be a solution, that is, the use of aphids, to the problem because lacewing larvae are also said to feed on the aphid body fluids which do not contain the cry proteins. The cry proteins are confined to the digestive tract of the aphid.

Do you have any evidence to support that? Aphids are phloem (ph) feeders. And as far as I know -- I don't know that the cry protein actually enters the phloem.

MS. ROSE: I don't know. I have heard that there is a study that has shown that where the Cry protein is binding in aphids that the green lacewing would not be exposed, which is why we have not requested a green lacewing study.

I have heard explanation that spider

mites, I don't know if the panel has any comment on that, may be a better organism to use as a prey.

DR. FEDERICI: Well, spider mites feed differently from aphids. I think this statement might be wrong. I'm just curious. Can we ask somebody --

MS. ROSE: I had heard it having to do with the binding, as where it binds in the -- but I don't know completely about that study.

DR. FEDERICI: Maybe Dr. McKee or somebody from Monsanto can answer whether you know whether the cry proteins actually enter the phloem?

That would be pretty unexpected.

DR. VAITZUS: I think that the statement as you read it says exactly what you are saying occurs.

DR. FEDERICI: It says cry proteins are confined to the digestive tract of the aphid. I'm saying that they don't even get into the digestive tract of the aphid, because they don't get into

the phloem.

DR. VAITZUS: So the question is --

DR. FEDERICI: It's a point of clarification unless Monsanto has some data to indicate that they do get into the phloem, in which case could be very important and very interesting.

DR. MCKEE: This is Mike McKee again. My understanding is there is a publication -- I'll have Graham Head come forward.

DR. HEAD: This is Graham Head of Monsanto.

The two studies that I'm familiar with, one by Hilbecks Group and the other by ourselves, both indicated that there was not Bt present in the phloem for the aphids to ingest in the first place?

DR. FEDERICI: So this statement is wrong in here? I just wanted to clarify that. Some people may think there is actually --

DR. HEAD: The Cry3Bb specifically or --

DR. ANDOW: No (inaudible) --

DR. HEAD: Yes. That was from Cry 1 studies.

DR. PORTIER: Any other comment by the panel?

Dr. Andow.

DR. ANDOW: Actually, I did see Robyn nod about the species. And I guess I would also support Pedro's suggestion that orius might be more appropriate -- orius is much more abundant in most maize fields than the chrysoperla.

Its early instars usually are plant feeders so that they will be exposed to the Cry toxin from the plant directly. And they hang out in the pollen. And it is probably true that they are eating pollen as well.

So that they are much more abundant. They probably have a pretty good effect on a lot of different prey species, above-ground prey species, including the mites and thrips. So just a suggestion -- and also corn bores.

DR. PORTIER: Again, I didn't hear much controversy from the panel in terms of

disagreement.

Some comments about the diet and how it is used here and potentially use of possible other organisms instead of chrysoperla, ones that more readily eat the pollen.

Some concern about validation of the trans gene product of 859 versus 863 to make sure that they are, in fact, identical or at least identical for purposes of regulation. Concern about lack of replicates and some confusion of the design in terms of three groups of 10 versus 1 group of 30.

Considerable concern, and I would agree with this, in terms of the overall power to detect an effect. The validation of the active protein during the study, we have talked about that quite a bit, in the feed itself. Changing the standard operating procedures to allow for pupation to take the study to the endpoint that it was intended to be taken to.

And there are a couple of comments in here that might require better documentation. Did

I miss anything there?

Shall we move on, then, to Question Number 4?

MS. ROSE: Question Number 4 deals with degradation of the Cry3Bb1 protein in soil. And there are four parts to it.

The first part of the question is: The panel is requested to comment on the advisability of testing additional soil types and for having soil persistence studies for up to three years.

DR. PORTIER: Why don't we certainly go through A and B together.

MS. ROSE: B states what soil types would need to be tested and what duration is needed for soil persistence studies.

DR. PORTIER: Before you give your answer, Dr. Angle, do you think that's the proper grouping, to do A and B together and then C and D together?

DR. ANGLE: Yes. Thank you.

First, I would like to thank the EPA for allowing me to participate in this review. And

secondly, I would like to follow up on the comments of Jane Rissler this morning and compliment the EPA for a very good way of getting at some very difficult questions. I have been quite impressed by the level of discussion today.

I would also like to follow up in her comment that this is something that the USDA needs to be doing a lot more of. So if we have any USDA folks in here or people who have an influence on what they do, I think it would help them quite a bit if they could follow a similar process.

The answer to the first question is actually quite simple. Let me just say we, the three discussants, have not discussed this issue yet. So there could be some different opinions from mine.

The first question, just to read it again, the panel is requested to comment on the adviceability of testing additional soil types and for having soil persistence studies for up to three years.

I think the general answer is yes. It's

a good idea with some qualifications.

There is a need to study persistence in other soils. I think we have seen some acknowledgement of that fact already by the EPA and some tacit acknowledgement by the part of Monsanto that it would probably be a good idea.

While it was certainly not intentional to use a very sandy loam soil, that would show a very rapid degradation rate that from their perspective would be a best case scenario. I think it would be much more adviceable to use a soil in a situation, environmental protocol, that would be a worst case scenario using a soil with a high exchange capacity and incubating that soil under temperatures just for example of low temperature and slightly on the dry side.

With that said, I doubt that the persistence even under a worst case scenario will be much longer than figures cited by Monsanto and the EPA report.

As noted earlier by Dr. Alexander, this protein is not really particularly different from

other proteins which are incorporated into soil on a continuous basis. This soil is well-adapted for degradation of these materials.

So even in a very different soil, while the degradation rate might be slightly longer, it is probably not appreciably or significantly longer, at least in my opinion.

However, despite having said that, I think it is important that this work be done in additional soil simply because this is a question that the public will always answer. This is a very basic question.

Persistence of a chemical, whether it is genetically modified protein or a chemical, the very first thing they always ask is how long does this thing last in the environment.

Well, we have some good data already suggesting it may degrade quickly. I don't think you can say for certainty that it would survive, it would persist longer in other soil. So I believe it should be tested in at least two other soils, which I will discuss in a minute.

Let me address this long term issue of testing and persistence, in this case suggesting that it should be monitored for up to three years.

When you are looking at a protein that has a persistence in days to a very few number of weeks, testing for up to three years is probably not appropriate.

But in general, what we typically look at is persistence testing for a period where you can no longer test that or detect that material generally for one or two extraction and testing periods beyond your date of the last detection, which usually isn't more than a couple weeks, at most a month.

A couple other comments are somewhat related to this whole issue of long term persistence. This kind of comes out of some work that I think the EPA has brought into either rightly or wrongly so for a number of years now. And that's when a chemical, whether it's a protein or a pesticide, become sorbed to soil components when it is released later on, whether that's

months or years or decades, it will retain the same level of toxicity that it had when it was sorbed on to the soil.

From my past work with EPA, this has been a common area of discussion. We have been through this discussion many times with some of you in here. But let me just give you my take in the this whole type of thing.

As these proteins are released over the long term, and again, this can be months to years later, it can be released at a rate that is so low that in effect they will have no measurable toxicity in the soil.

So For that reason, I don't think rerelease back into the soil solution is an important consideration.

Secondly, when they are released, months to years later at a very low rate, they will be degraded very quickly. There is no reason that degradation rate two years from now will be different from the observed degradation rate that Monsanto has reported.

So what is released will be degraded very quickly. Probably before it can have biotoxic effect.

Then finally, as was mentioned by Monsanto, this is all really a moot discussion anyway because of concentrations that are most likely being added to soil are below those that can detect -- below that where a toxic effect can be detected.

On some extent, this is, I believe, really an academic discussion. I know in industry and in the regulatory groups, academics can be quite frustrating because we often want answers to questions, but we sometimes don't know why we're asking those questions, which is great for publishing papers and advancing your academic career, but it doesn't always help with the regulatory process. Yet, we still continue to ask these types of questions because that's the system that we work in.

So just to wrap up a couple comments on Question Number 1. I think we probably do need to

look at a couple other soils. I would recommend that we look at two different soils. I'll discuss them in just a minute.

I don't think you need to look at these for three years, but rather for only a very short period of time after the proteins can no longer be detected in soil regardless of the method that you are using for detection.

For the different types of soils, I guess there is an acknowledgment, this may already be happening, that you are looking at a soil with a higher clay content. That should be a clay with a high exchange capacity. There are different types of clay. And these clays have different exchange capacities.

You certainly want to be looking at one that has a high exchange capacity. And also looking at a soil with a high organic merit content. Various organic materials in soil can bind these materials and then potentially release them at a later date.

I guess at this point I'll turn it over

to one of the other discussants.

DR. PORTIER: Dr. Alexander.

DR. ALEXANDER: I'm in substantial agreement with Dr. Angle, with a few exceptions.

Let me go back to a logic from my own thinking. The ELISA data are very interesting in that it allowed me to do a kinetic analysis of the disappearance, which Monsanto apparently has not done, at least hasn't reported that we have seen.

Proteins are typically degraded by growth link biodegradation, which means the biomass increases continually. The biomass increases continually, then the rapid -- the degradation looks like that.

It becomes more and more rapid with time because you are getting a larger and larger biomass.

When I plot these data, the ELISA data that way, there is in fact an initially rapid increase in degradation, and then it slows down. And that's not what you expect for a large biomass which appeared. Something seems to be happening to

the protein.

If one plots it as if it were a first order kinetics, in fact, it is a reasonably good plot. But biodegradation of growth supporting compounds should not be first order kinetics.

So it suggests that something else is limiting the rate of degradation. Something makes it less available to microbial activity and that less availability is affected by the first order kinetics.

And that's likely going to be a sorption of some sort.

And proteins are sorbed to a varying extent. And this is why one needs to have different soils. And to expand what Dr. Angle said, it is not only simply a cadon (ph) exchange capacity, but there are two major types of clays. Expanding lattice, which means the clay goes like this and has little spaces in between, and a non-expanding lattice.

If a protein gets into that expanding lattice, it is not available for degradation very

quickly. If it comes out there, then it becomes available more readily.

So I think the answer is soils have different cadon (ph) exchange capacities, different clay minerologies. And the percentage of clay is important, but very often far more important is the type of clay which never appears in the EPA documentation.

And also as Dr. Angle said, the organic matter. EPA in one of the publications cited talks of humic acid type organic matter. There is no such thing.

Humic acid is an extracted fraction which doesn't have the physical properties of soil. It doesn't have the nano porosity of soils. It just is an extracted fraction which serves for many good scientific purposes, but is not the real soil itself.

So the answer is, several soils, different clay types, different organic matter types or different organic matter percentages and different cadon (ph) exchange capacities.

Then in terms of the length of time involved in degradation, I think it is very difficult to arbitrarily choose three years.

I think there are several factors, not only absorption, which make me think that the degradation is more slow than this one sample that Monsanto has tested. Firstly, they used the wrong tissues. Secondly, they ground the tissues.

Both would give you much more rapid biodegradation than if the compound were in roots and in intact grooves.

There is also no concern with the fact that corn roots grow deep into the soil. And at lower depths in the soil, we have lesser microbial activity. We have often have poor moisture relationship. We have lower nutrient, inorganic nutrient availability for microbial decomposition. So the process may be slower. Now, I think ultimately that it will be degraded.

The question also arises as to whether the material which is not readily biodegradable is bioavailable for effects on invertebrates. And

that I think is something that has to be addressed.

This raises the question of what is now called sequestration.

Many organic compounds become physically sequestered in the soil. And they are not readily extractable as in the very mild extractants used for the ELISA test.

In fact, the National Research Council have a report coming out very shortly on this particular problem on the bioavailability of organic compounds which become entrapped in the soil lattice.

Proteins have a structure about 15 nanometers across. Soil surface area is mainly in pores in that size range. And if a protein is entrapped in one of these pores and absorb, and that appears to be what happens with many compounds that have been tested, then, in fact, it is not going to be readily biodegradable.

This poses the question also whether they are going to be toxic. And that is a

question that I don't think can be resolved.

So I think relative to the persistence in the soil data, one needs to have more soils, one needs to have a persistence or a testing time adequate to indicate the availability of the compound and its degradation.

As Dr. Angle points out that if a compound is released from an unavailable form, the concentration may be so low that it be biodegraded and not particular issue.

I agree with him. I agree with him completely.

On the other hand, there is a question that only data can resolve as to whether this is, in fact, a reality.

So specifically, in answer to the questions, additional soils should be tested. The testing period should be long enough to determine whether the compound is still going to be bioavailable in some form.

And the soil types there are going to be really appropriate for major crop growing or the

corn growing areas in the country.

I think there are a whole series of questions that can be resolved reasonably quickly. There is one other question which I think belongs under C, and that is, what happens to the large part of the protein which is not being extracted.

And that is -- Monsanto, I believe, has done no recovery studies. The published papers with one exception have done no recovery studies. And the one paper which did it said we're not recovering too much of the compound out of the soil.

So we need to have some recovery studies and to know that we are, in fact, recovering the available fraction or most of the compound available or unavailable.

Thank you.

DR. PORTIER: Dr. Neher, before you comment, let me ask a question, since I was a little confused by one of the things Dr. Alexander said. I want to make sure I heard it properly.

I also looked at the degradation data

for whether or not it would match first order kinetics.

You stated that -- and I agreed with it, that it does appear to match first order kinetics. Yet, you are still concerned about a resorption.

To some degree, that grates against my scientific intuition in the sense that either the data supports beyond first order kinetics or it doesn't.

And since the data does not appear to support greater than first order kinetics, why force a design to address something which may never appear?

DR. ALEXANDER: It is not resorption.

The fact that it looks like first order kinetics -- does not follow growth kinetics there, the first time.

It suggests that there is a major effect of soil type, that the availability of the compound is governed by something intrinsically other than the ability of microorganisms integrated to compound. That's the only point for

citing the kinetics.

DR. PORTIER: And that's the second question I had for you that I didn't understand.

Why would that -- why is that the case if it follows first order kinetics?

DR. ALEXANDER: Because it shouldn't follow first order kinetics. No protein decomposition that I have ever seen when it is freely available is first order.

DR. PORTIER: And yet, everything I have seen in terms of -- I do mammalian systems inside the body. But in a linear range, when you are not at V max (ph) on some proteolysis constant, it is first order.

DR. ALEXANDER: The difference is that mammals don't increase logarithmically. And that if you have a protein available in the unit time, for example, assuming bacterial growth, you have 1 cell, 2, 4, 8, 16, 32.

DR. PORTIER: I got it. Thank you.

I hope everybody got it. Thanks.

Dr. Neher.

DR. NEHER: Generally, I'm in agreement with what both Scott and Martin have said. I will just try to restrict my comments to some aspects that they did not cover.

One is just a quick review in terms -- I'm going to take more the perspective in terms of kind of the biologically active component here in its interaction with soil in terms of the proteins being expressed in the root tips. And I also note that really near that root tip is -- right behind that would be where the acting growing regions of the roots are.

This is also an area where a lot of the cells would be sluffed there at the cap and leaving them behind in this elongation zone and the root hair zone. This is also where a lot of the activity in the riser's fear is going to be far as interaction with microbes and invertebrates that are feeding on those microbes.

So when I start to think about protein activity, I think more of the riser's fear instead of the bulk soil or the concentration in the root

itself, but what is going on in the riser's fear that they are exposed to and where their activity is.

Another point in the report about the degradation study, just a couple suggestions on the reporting format. When it looked at -- there was a table, I'm looking at mortality of the Colorado potato beetle larvae, and the percent mortality with different times of soil incubation as percent mortality for each -- for me, it would be also helpful to add an additional column that had a cumulative mortality.

It would just be easier for me to assimilate that information in my mind just to add one more column.

And on the percentage -- looking at the curve fitting parameters, it has percent error with positive and negative values.

I'm not sure that you can really report error as negative values. To me it seems like absolute values would suffice in terms of that. I don't know if anybody else wanted to comment on

that.

Do you report errors in negative? I'm not familiar with that.

DR. PORTIER: I don't remember seeing that part.

DR. NEHER: It is on the review of the soil degradation study, Table 5, Page 9, last column.

It shows up on -- the similar thing shows up on Table 7.

DR. PORTIER: Which data document is that?

DR. NEHER: The review of aerobic soil degradation study submitted by Monsanto. It is dated July 10, memorandum.

DR. PORTIER: Does anyone else in the panel have a comment on this?

DR. NEHER: Do you see where I mean? Flip to about Page 9. That's Table 5. And then on Page 10, Table 7.

MS. ROSE: I have the actual study with me.

DR. PORTIER: I want to take a little time to look at it before I comment.

DR. NEHER: That was just my response. But I would like to have a second on that in case I misinterpreted that.

My thought, if you are expressing percent error would be expressed as an absolute value, or sometimes if I think negative, I start wondering is it really a zero or are we really talking -- what does a negative mean. Anyway, just clarification on that.

Just to second what Martin was saying about the degradation of the plant materials, it seemed like the decomposition was under ideal conditions. I think it would be good to look at.

And under worst case scenario, larger plant fragments and under cooler temperatures. A situation where we would expect to have the slowest, a slower decomposition just kind of to cover the basis on worst case scenario.

There is the issue about absorption on to soil particles. One thing I think about is,

okay, what happens if that is consumed an transferred into the organ as -- what is degradation like after ingestion.

That's a question that kind of raises in my mind in terms of what is that degradation like. Is it transferred in the food chain or does it just continue to have a similar degradation as if it were not ingested.

The only other thing that really hasn't been mentioned, and this may be irrelevant, if the degradation is very quickly and that is that we really don't have much information about movement or translocation of protein in soils in terms of vertical or horizontal movements.

Other than that, I think that's all the comments I have that are unique or different from what Martin or Scott have said.

DR. ALEXANDER: Just a comment on the protein movement. They don't move.

DR. PORTIER: Any other comments from the panel on this question?

I think in Table 7, if I'm reading it

right, that percent error is not standard error. That's percent -- that's specific area against the predicted value versus an observed value. And yes, you sometimes would place it as a negative if your error is in the direction of underpredicting versus overpredicting.

DR. NEHER: Okay.

DR. PORTIER: Dr. Andow.

DR. ANDOW: Question for EPA.

I didn't really look at this material. But if Cry 3Bb does degrade with first order kinetics, it was pretty clear from previous panels that the Cry 1Ac or Cry 1A toxins did not degrade with first order kinetics.

Have you thought about why there might be a difference here? Is there a difference, or is it really basically what Dr. Alexander is suggesting, that maybe they both don't have first order kinetics but it just looks that way for this one?

What is your position on this?

MS. ROSE: That's part of the reason

that we're bringing these questions to the panel, actually.

DR. PORTIER: Any other comment from the panel on this particular half of this question?

I'm not sure I got all the points here. But I think the answer to the first question was, yes, with some conditions.

Certainly, at least -- the argument was at least two different soil types, looking at variations in amount of clay, type of clay, organic fraction, cationic exchange capacity, a number of other issues raised about looking at multiple soil types.

Also, possibly some variation in the environmental variables. So you have a lot to play with here.

Three years -- we had some difference of opinion. I don't know if Dr. Alexander was pushing fort three years or not. But clearly, Dr. Angle was saying that three years was definitely too long for something with a half life that appears to be on the order of three to ten

days.

Dr. Alexander was pushing for something longer, but I'm not sure if he specified three years or not. You might want to correct me on this.

Considerable discussion about first order kinetics and why that occurs and what that might mean.

I don't think we went into a lot of description about how we might resolve that question for you as to why this may be the case or not in this specific example. But clearly, it is a flag that was raised.

And then some issues on reporting, I think, is basically what we covered.

Dr. Alexander, did you have anything to say about the length other than longer than 20 days?

DR. ALEXANDER: It is very difficult to say. I'm working on samples now where the compound has been there for over 40 years. And we would have expected based upon half life kinetics that

it would have disappeared after two years.

To give a straightforward but vague answer, I would say until the data suggests that there is an insignificant level, however, the protein is still present.

And that could be after three weeks. It could be at three years.

Anything more -- I don't see a 40-year study as we're doing now. But most of my graduate students don't want to hang around that long, with one exception.

DR. PORTIER: Dr. Neher.

DR. NEHER: As a follow up on that, I guess, I think of at least one growing cycle and a chance after post harvest to look at the decay of that plant litter seems important to me.

It's kind of along the same line as long as it is not present. I don't think there is any magic time.

DR. PORTIER: Okay. If we could go on to the second half of this question, Part C and D.

DR. ROSE: At least the third quarter of

this question is, are these studies truly expressing the time to 50 percent or 90 percent degradation of Bt protein in the soil or whether they are only determining the level of detection of Cry3Bb1 protein in the soil?

Discuss the acceptability of these studies for a preliminary risk assessment to evaluate the fate of Cry3Bb1 in soil.

DR. PORTIER: Is this separate enough from part D to go separately? Yes?

Dr. Angle.

DR. ANGLE: I personally found this to be a hard question to answer. I almost saw it as a philosophical question, not a scientific question.

To me, it's analogous to the old philosophical question: If a tree falls in the middle of a forest and no one hears it -- you know the rest of that.

And I want to go back to my comment earlier about academics and regulators. Really, these are questions that academics always want

answered. But I'm really not sure that the EPA will be better off for necessarily answering this question.

If protein degrades or absorbs in soil, yet it doesn't show any biological effect either now or in the future, does it really matter to anyone.

I suppose that it depends on your perspective on this particular question.

I know for a fact there are some people that say even though you can't measure it, if it's still there, it is important. There are other people there that say, no, if it has no effect, then it is not important. Again, it depends on your perspective.

That's why I found this to be such a difficult question to wrestle with.

With that said, let me note that I clearly think that we need monitoring of these proteins and soil. And that it should be one of the very first types of risk assessment conducted in these studies.

In this case, we have pretty good hindsight. We know what the protein will do. We have a fairly good idea with some caveats of how quickly it will either sorb or degrade in soil.

But while this is true for most of the proteins that we study now or that we can imagine studying in the future, there will be some exceptions, as was noted previously.

We have to be on the lookout for those exceptions. I don't think this is one of them. I think this probably exhibits fairly normal order degradation rates in soil. But there will be some exceptions in the future.

And while this isn't the one, we have to be on the lookout for them.

So I would say is that the answer is that we really don't know, but to some extent we always have to argue that it may not matter since the bioassay in my opinion is really the baseline determinative of how important persistence will be.

I don't think anybody is recommending

that we do away with the bioassay that was conducted or that it's not a good, appropriate bioassay for this type of study.

DR. PORTIER: Dr. Neher, we'll switch to you this time.

DR. NEHER: I also found this a bit challenging to answer, but I took a slightly different tact to this.

I guess one thing I think about with these degradation studies that are trying to get at 50 percent or 90 percent is it is like the protein is put there and then you are following that one dosage through, where, in reality, this would be expressed continually or repeated times through the growing season.

So I start to think, so what does a 50 percent or a 90 percent really mean in that context because it continued to have repeated dosages throughout the field season.

Back to related in terms of linkages in with the microbes and invertebrates feeding on microbes, how does this degradation -- a question

raised in my mind in terms of the duration of this impact, we don't really know once that toxin is transferred within the soil and litter food chain.

And perhaps Martin can help me with this one in terms of -- I'm curious -- maybe we just don't know in terms of an issue about whether sorbed materials remain biologically active or not.

Those are my three points.

DR. PORTIER: Dr. Alexander.

DR. ALEXANDER: The answer to your question is some sorbed materials are biologically available and some sorbed materials are not biologically available. There are too many mechanisms of sorption.

My comment to this question suggests a degree of duplicity on the part of the pesticide office.

For a chemical pesticide, you say, I want all the chemical present in the soil. I want 100 percent recovery. But I don't care about the biological activity whether that relates to it.

I want to get a good method for a chemical analysis. Biological aspect is something else. And a lot of the chemical pesticides that are detectable by vigorous chemical analysis are not biologically available.

You are asking get the other way around in this case. You don't have a method which gives you quantitative recoveries. One doesn't even know the extent of recovery.

So how can you determine whether a 50 percent or a 90 percent disappearance is appropriate. I think you have to decide on what basis you want to go.

I'm answering in a similar fashion as Scott did. If you are interested in the biological availability, then you do a biological test. That does not reflect the total concentration present.

If you want to know the total concentration of the chemical present, then you have to have a quantitative recovery from the soil and then use that as the basis.

My inclination is that since the issue is one of the biological availability and not the chemical availability, that the assay should be on a biological basis, and the extraction method should be one that parallels the bioavailability and not the chemical procedures.

The same would apply to the chemical pesticides.

DR. PORTIER: Any other comments?

Dr. Federici.

DR. FEDERICI: I have a question for the EPA in terms of what is your concern here?

Most insects don't feed on soil directly. There are things like earthworms and some other things that do. I'm just curious what is the point of asking this question?

MS. ROSE: This particular question, not getting into the whole idea of asking for this type of study, was a little bit, I think, more simplistic in my mind of based on an insect bioassay, is it appropriate to call these a DT 50 or DT 90.

Are we truly looking at 50 percent or 90 percent degradation based on the insect, the Colorado potato beetle bioassay, or using the same test, is there another term that would be more appropriate to describe what we're really looking at.

DR. PORTIER: But if I could follow up on --

MS. ROSE: I'm also appreciating the additional comments, which are useful.

DR. PORTIER: If I could follow up on Dr. Federici's comments, this is what I was going to ask as well.

Again, this is not my field. So maybe my simplicity here makes some of the questions a little clearer.

I can see two things you might want to know. The peak bioavailability in the soil in terms of what it might do in some effect within the soil either to invertebrates in there or whatever, but peak bioavailability would be something important to know.

But then bioaccumulation over time, does it bioaccumulate from season to season. Are we going to run into a problem 10 years from now with so much of this protein in the soil that we're not readily prepared for it.

Are those the types of questions you are trying to get at?

MS. ROSE: Actually, the question regarding whether a three year study is needed gets to your bioaccumulation comment. That's why we were asking is a three year study needed. And that would be just to see if you've got, say, continuous Cry 3Bb corn for three seasons, would there be an accumulation.

That goes backwards in our questions a little bit.

DR. ALEXANDER: I have one comment about the D T 50 or DT 90. These are completely appropriate for a compound that disappears with first order kinetics.

As if, as was pointed out, one of the other proteins is not, then it is totally

inappropriate. And there are more than 20 separate kinetic patterns for biodegradation.

A half life for DT 50 would give you completely the wrong answer if it were growth link kinetics or second order kinetics or a mixed order kinetics.

So I would be very careful in using such values arbitrarily.

DR. PORTIER: Any other comments from the panel?

I don't even think I'm going to attempt to summarize this one. I'm going to let the experts try to do it for you in the write-up, because I only caught a few things concerning redefining the question and then doing the right study.

Any additional comments?

Okay. If we could go to part D.

MS. ROSE: The final part to Question 4. What, if any, difference would it make in the values of these ELISA-based studies if clay particles to which the Cry3Bb1 protein might bind

are present in the soil being tested.

What measure should be taken to ensure that the test is not measuring inactive protein fragments.

DR. PORTIER: Dr. Angle.

DR. ANGLE: I have a fairly short comment on this.

First, that this will occur. This has affected the current set of data that was presented to us. So it is not a hypothetical concern.

The effect will be greater in soil with greater binding capacity regardless of what that binding capacity is due to.

But clearly, there was some binding, there must have been some binding in the soil that was used in the data that was presented to us. Again, this is not a hypothetical concern.

ELISA measures all fractions of the proteins, whether they are bound or free and whether sometimes -- whether they are whole or sometimes whether they are even partially

degraded.

That's why I did not know the true extent of the measure that takes place with this particular procedure.

It is clear that it accounts for both all active and many of the inactive fractions. What this will do in the end is to overestimate the amount of the protein that persists in soil.

Real life persistence is, therefore, likely to be overestimated by using this procedure or the ELISA procedure.

That gives me some confidence that as we talked about before we are using a worst case procedure here. The rate of persistence will either be as measured in the test or less, but it is probably very unlikely that it would be greater.

And because of that, I'm quite confident that we will have a level of protection built into the risk assessment evaluation using this procedure.

DR. PORTIER: Dr. Alexander.

DR. ALEXANDER: First a comment. Clays are important, but organic matter is also. So I think when asked, put the two together.

And in essence, every one of our major soils used for corn production will have clay present. There aren't too many soils used in agriculture which are basically sands. So we do have -- there's always some clay there.

Again, I think we -- we have three separate kinds of assays. One is a rigorous chemical assay there which one doesn't do for proteins because we don't have that kind of chemical assays.

One is an assay such as the ELISA procedure. One is a bioassay. And I think the only way that you can guaranty that a true chemical assay or an ELISA assay is a reflection of the active material is to measure active material, which is a biological test.

So I think that -- it always has to be calibrated against biology. And the agency has to decide to what degree are they going to rely on a

biological procedure which has low precision. That's bad from a regulatory viewpoint. And to what degree are they going to rely on a surrogate procedure, which has good precision, but maybe not overly relevant.

DR. PORTIER: Dr. Neher.

DR. NEHER: My comments will be brief. It was more on what measures can be taken to deal with it.

I guess the thought I had was in terms of the -- I would recommend doing -- calibrating the effect of binding and recovery efficiency for each of the soil types that are tested.

Particularly, focusing on those worst case scenarios whether it is the sandy loam that has been tested previously, the clay would be a worst case scenario and a humic. Just to know what the binding and recovery efficiency can be as a matter of defending that procedure. That would be my recommendation.

DR. PORTIER: Any other comments from the panel? I think that was, again, pretty

straightforward.

Basically, we're told this occurs, that the ELISA technique is going to be measuring a lot of different aspects of it.

There is a trade-off between what you are going to do in terms of the bioassay versus the ELISA technique. One could also potentially require the development of a bacterial assay with a transfected reporter gene that would detect it as well. That would be a different type of bioassay.

But it's a trade off. You have a mix here. That is something you are going to have to decide on.

And then the one recommendation -- I believe we had that recommendation in part C as well. And that is that a preliminary study of recovery efficiency with known amounts of protein put into known types of soil I think is one thing that might provide you some better insight into what is active and what is not active.

DR. ALEXANDER: With the proviso that

the protein is allowed to stay in soil for some time to allow for any reactions, abiotic reactions to occur in a sterile soil.

DR. PORTIER: Any other comments on this question? Is that clear?

If we could go to Question 5.

MS. ROSE: Question 5. Please comment on the agency's non-target invertebrate and soil fate assessment.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: I assessed that the ecological risk assessment, that Monsanto followed the EPA guidelines, that they did incorporate recommendations from the Science Advisory Panel, particularly the 1999 Science Advisory Panel. And I quoted some information from that previously.

In that sense, it did focus on lady beetles. And they did three or four studies on lady beetles.

Additionally, in that vein, they focused on carabid and staphynilid field studies. I guess there is some debate whether or not a lab study

would have been more appropriate.

Also, they did lab studies on three other families of beetles, including tenebrionidae and curculionidae, which I think is commendable.

Looking at this data, there is no observable effect levels that I can see that are greater than 10 times -- none of the effects were greater than 10 times and no observable effect level, except for the adult honey bee. And we had some discussion about why that was, because of the changes in the events that they were using.

On the invertebrate consensus, I think it depends on what your measuring stick is. If you are comparing the studies that I have seen, even some of the preliminary studies that have some very obvious results, if you are comparing them with insecticides, organophosphates or pyrethroids, that most of the studies suggest there is no unreasonable effect to -- no unreasonable adverse effect to the non-targets, at least compared with the insecticide studies.

On the other hand, if you want to

compare them to the isolines, it is very difficult to prove a negative. But at this point, I don't see any red flags. There is no evidence to suggest that there would be an unreasonable effect.

We have done a lot of talking about scale issues and how long the studies should be run. I should note that some of these questions would be better answered if there was more material available so that larger scale studies could be done if that was appropriate.

On soil degradation, I will refer to the conversation they just had over there. I think it may be appropriate -- first of all, I would like to say from my assessment of this, that the protein does appear to degrade very quickly and that there may be questions about whether or not tests should be done in the future to include other types of soil. I think that's legitimate.

I think there may be -- Deb said something about maybe doing some tests with bigger pieces and maybe with colder conditions just so

that they have all their bases covered. I think that may be appropriate too. So those are my comments on this.

I guess I should just say at the end of this I have spent a lot of time working with European corn bore Bts. And a lot of us have been saying that we were looking forward to these Bts because the potential savings or reduced environmental effects may be substantial.

DR. PORTIER: Dr. Federici.

DR. FEDERICI: I'll just read a short paragraph that I have here.

While most of the data presented in this study shows little likelihood of adverse effects on non-target organisms, the high control mortality in the lacewing and hymenopteron studies is troubling.

The methodology used in this study seems crude and should be improved to lower control mortality. With respect to the soil fate studies, these should be longer in duration to determine whether there is any significant bioaccumulation

from one year to another.

Given that specificity of the Cry3Bb1 significant non-target effects would not be expected, nevertheless, it is important to undertake studies of longer duration to test this.

In the end, these studies will likely show that Cry3Bb1 corn will be a much more environmentally compatible pest control technology than synthetic chemical insecticides.

DR. PORTIER: Thanks.

Dr. Jepson.

DR. JEPSON: I wasn't going to go through each of the constituent tests, we're probably pleased to hear. But I tend to concur with the previous two.

I have been referring throughout the day to the need for more conversation and more consensus building over appropriate tests.

And these comments, however, must be based on the tests as submitted and the relationship that EPA has had with Monsanto in requesting this material and Monsanto's efforts to

actually produce it.

With regard to the lab testing, I think I found some flaws, I felt, with the chrysophyte (ph) study that I had some difficulty with accepting that was a reasonable test.

The other tests to a greater or lesser extent seems reasonable. There is no basis on the moment to conclude that there is any particularly adverse effects emerging from lab data.

With regard to the field data, surely we should have some statistical criteria to decide whether or not an effect differs -- a treatment differs or does not differ.

I think it is just too early to say from the field data we have presented what is happening. We all have suspicions of hopes or otherwise about what may be occurring in those various plots.

But even given the doubts we have about the design for the study, it just seems too early to say. And I find it difficult to argue for, reach a conclusion on such preliminary findings at

present, despite the direction they have shown. There must surely be a statistical basis for reaching the conclusions. Until you can reach that, I'm not sure that you can validly claim anything other than review the data that stands at the moment and just check how it is going.

As I have also mentioned, I think scale is a problem. So that must limit our ability to make broad reaching extrapolations to the real world.

That's all I really have to say.

DR. PORTIER: Dr. Neher.

DR. NEHER: I will take a slightly different approach to this. I wanted to focus more on some of the soil invertebrate tests.

I concur that based on the evidence that we have so far that in a comparison to conventional pest management practice, it appears that the Mon 863 has less impact on non-target inverts than some of the conventional ones.

I also want to applaud EPA, Monsanto as well, for looking at some of these non-target

invertebrates. I feel like the target of this protein is towards invertebrates and not necessarily the microbial side of the soil food web.

So I think we're targeting -- the aim is in the appropriate part of the food web focusing on it.

Microbes are vitally important in decomposition. However, I do -- some of the non-targets, I think, we are -- the nematodes, the mites, the spring tails, these are some of the groups that are being looked at, are in that riser's fear where the toxin are exuded, they are consuming and/or dispersing microbes, whether or not these microbes are actually ingesting this protein or not.

A question that just comes to my mind, and perhaps this is more academic, but we also have case histories of problems with introduction of genetics. And that is, my question is what if -- the expression of this protein, does it have any effect on expression of any other plant

defense? Do we know that? I don't know.

I guess -- is there any change in the susceptibility to any other pathogen or pest dealing with this? I just revisit in my own mind kind of the case history on male sterile cytoplasm, which ended up leading to susceptibility of corn to southern corn leaf flight.

Anyway, this is something that I keep in mind, do we have trade-offs? I don't know.

Just a few things I wanted to bring up in relationship to the data evaluation reports. I'll just start with -- some of these are a bit on the detailed side, but I want to make sure they were in public record.

First, starting on the one with the collembolan, it is the May 20 report called, Review of Ecological Non-target Insect Studies for this protein. On page 16 where it is describing the folsomia candida protocol, it mentions that the media is eight to one plaster to coal breeding substrate.

I think that was just a typo and it should actually be charcoal.

DR. ANDERSEN: Correct.

DR. NEHER: And then just reiterate the one check on that same document, Page 18, to determine if number of offspring was 20 as typed or perhaps 200 on the number of offspring for the .5 percent.

MS. ROSE: Actually, I'm not sure if that was a typo or not because I couldn't get my hands on the study this morning. But I did speak with somebody from Monsanto over the break who said, same thing, he wasn't 100 percent sure if it was a typo, but that he knows it was not statistically significantly different from the control.

So 20 may be correct. But there was no statistical difference.

DR. NEHER: If there is no difference, then, since it is a tenfold difference, I start to wonder about the power of the test in that situation. Because that's a pretty big

difference, tenfold difference in offspring.

I really see survival and the reproductive fitness as the two kind of big areas we want to target in the studies.

DR. PORTIER: Dr. Neher, how many of these do you have? Because the two you have just done could have been handled as an appendix to the report or a direct correspondence between you and the agency for clarification of the issue.

If some of these points impend upon your interpretation of the study in answering question Number 5, then please pursue them even further. But if they are just corrections for the agency to put into their documents, I think they can be conveyed as either an appendix or a direct correspondence from you.

DR. NEHER: Okay. I just misunderstood the directive from this morning. I thought you wanted us to cover these things.

Then I'll just skip down to the nematode assay in terms of just a few comments. One, I think it became clear this morning from the public

report that there was no protein concentration reported in the leachates.

Another comment I had in terms of root extracts versus soil extracts, I thought as far as non-target nematodes, it seemed to me that the root extracts may be more realistic than soil extracts when looking at the non-target effects.

And there is the question about whether C. elegans would be the appropriate nematode species to look at, that certainly the lab rat, the model nematode, but it's not very commonly found in soil or in the riser's fear. I don't know of anybody that has found it. I certainly haven't.

There are certainly assays for some other bacterial feeding nematodes that are more common in the book that has been cited previously, including the pectous species and others. So there are some standardized procedures for that.

My opinion would be that they would be more relevant in terms of looking at non-target impacts.

In those cases, I think -- I would recommend that the test be extended to at least one generation. I think that's feasible for nematodes in culture, especially, bacterial feeding nematodes. Those could be from a few days to two weeks max, those kinds of tests.

I'll conclude with that.

DR. PORTIER: Are there any other comments from the panel, Dr. Barbosa.

DR. BARBOSA: In line with the comments we just heard, perhaps a relatively minor point, but I just wanted to make a comment for the record, that, in my opinion, the choice of nasonia vetripennis stands in stark contrast with the attempts to utilize species that are relevant in this system given that this is a gregarious endopasitoid (ph) of fly pupae.

I think almost any other choice would have been more appropriate.

The only other thing I would like to comment is, this may not be a point that is hugely important, but again, there is design problems

with some of the experiments in which there is stark contrast between treatment and control. Particularly, in relationship to nontargets, the protein is delivered with honey in one case and controls are plain water, which may or may not increase the levels of mortality in controls and make comparisons perhaps look better than they would ordinarily.

DR. PORTIER: Any other answers to Number 5? I'm going to ask if you have any other comment for the agency in a minute. But strictly on Number 5.

Dr. Andow.

DR. ANDOW: I guess I would say -- I have focused on the field studies and I have focused on the green lacewing study and then I have also spent a lot of time on the coccinelid studies. And in particular, on the coleomegilla maculata studies because those are -- because I like coleomegilla maculata. I have worked with it a long time. I know it quite well. It is also the one that I thought was the one most likely to

be exposed to high levels to the corn plant.

And based on looking at these, I would disagree with some of the panel members and say that I see that the data are insufficient to indicate that there is no unreasonable effect. And there is not really a measuring stick issue.

When I look at the C. mac data, the main thing that I see is that there is an argument that it is difficult to rear them on a pure pollen diet, 100 percent pollen diet. Yes, some labs have difficulty rearing them on 100 percent pollen diet.

When we first started working with them, we had some difficulty getting high survival on 100 percent pollen diet. But basically, we learned that it was the water presentation that mattered most.

Once we could get that out, we typically get 90 percent survival on a pure pollen diet of immatures and we can get long survival of adults for a number of days.

So that, in fact, I think it is possible

to do the test to actually look to see what sort of maximum potential hazard there is.

Secondly, whether or not the average feeding of -- by coleomegilla in the field on pollen is 50 percent or the maximum stated maximum of 50 percent, what we know is that there is a time after -- partway through anthesis when essentially the coccinelids have eaten up all the aphids.

Basically, all that is left is either other coccinelids or pollen. And C. mac tends to feed on the pollen at that time, whereas the other species tend to feed on C. mac and themselves.

So I think there is a period of time when C. mac actually will have a very high percentage of its diet just pollen. And these are the larval stages. I think it actually is meaningful from the field perspective to look at a higher rate of pollen exposure.

And then in addition, we found that when you actually mix foods, in our case we have looked at mixing of pollen and aphids, mixing of pollen

and European corn bore eggs, what we find is that many of the characteristics of development and survival of -- development of the immatures tends to track the better food, the eggs or the aphids, rather than the pollen.

Pollen when it's fed alone always shows slower development time compared to the other two.

We find that when you mix them together, they tend to track the better food. So that it is not clear to me that by mixing these you are just sort of wiping out any other things that you could have seen when you mix the tephrited eggs with the pollen.

Now, on the other side in Appendix E of the supplementary material, the Illinois study does use pollen diets mixed with an artificial diet where it is just the pollen in different types of mixtures.

Actually, I think they may even have some just pure pollen diets. But they ran into the problems that we ran into early on, which is their control mortality is very high. It makes it

difficult.

We found that it was very difficult to detect a lot of different defects of foods for C mac. So I'm unconvinced that the C. mac studies really allow us to say that we have actually looked in the proper way for effects.

Then finally, the sample size here is also quite small in two of the studies where treatment ends are only 30 adults. Again, it limits what we can actually detect.

That's just a supplement to comments I have made on the green lacewing study.

Then on the field study, if you look carefully at the study that was requested by EPA, what you find is that on the pan trap samples, there are no effects of insecticides. On the pitfall traps, only spiders are affected by insecticides. And on the sticky traps, you get coleomegilla, macracentrus and orius that are affected by the insecticides. But if you look at the data, it is only because the foliar insecticides are killing them. There is no

differences in the soil insecticide treatments.

And then finally, if you look at the non-target pests, of which many were tested, there are no insecticide effects. So I think it is going a little bit too far to say that we know that the insecticide effects have a smaller effect -- that the soil insecticides have a smaller effect on the non-targets than any of the other treatments, except for perhaps spiders, the spiders.

So I think that it's inconclusive to say that we have no unreasonable effects.

DR. PORTIER: Any other comments by the panel?

Dr. Jepson.

DR. JEPSON: This is the talk about the experiment that we'll have a further discussion about. And I think I'm right in saying that it was the soil insecticide tefluthrin and the foliar insecticide, permethrin.

Yes. Because with pamethrin (ph) on the soil, you would only really expect to affect

spiders. They are hypersensitive to pyrethroids. But many of the other animals will not be affected because of binding and lack of bioavailability.

So perhaps those results do line up a bit more -- closely to what you would actually expect to happen.

With tefluthrin, I'm really not sure there is any evidence at all of invertebrates' impacts of properly applied -- it's a granular product applied at drilling, I think, is it or -- was it Force?

MS. ROSE: Yes. Force was the foliar applied and the granular and Goucher (ph) was a seed treatment.

DR. JEPSON: So you wouldn't expect very much happening with that data.

DR. ANDOW: My point was not what I expected, but that the data don't indicate that the insecticides have a larger effect on non-targets than either the DT or the control.

DR. JEPSON: I'm sorry.

DR. PORTIER: I had one other comment.

Again, that's following up with what Dr. Andow said about sample size in these studies.

In the previous Scientific Advisory Panel report, I want to use the exact wording here, I guess I'm not going to use the exact wording here. I'll just read it out from the previous panel report for the record again because I think it is something that -- there are some subtleties in here that the agency didn't take into account in this particular situation that I would like to have reconsidered by the agency.

This was in the questions concerning sample sizes, Based on this position, the consensus of the panel was that the agency should provide applicants with detailed recommendations regarding experimental design and data analysis.

The agency should consider how the data will be used and established in acceptable level of statistical power. Based on these decisions, appropriate tests and sample sizes can be determined.

Case in point to determine a maximum

hazard dose, the agency and applicant should agree on a statistical test and level of statistical power. Then the applicant can use their experimental coefficient of variation to determine sample size and replicate number.

It is difficult to determine whether the agency's current recommendation of 10 per replicate for LD 50, LC 50 tests and 30 -- this was bird and fish and 100 insect per applicate for hazard testing are adequate without knowing the coefficient of variations and the desired levels of power.

Again, I think had we had a discussion here, a presentation here of the agency saying upfront, these studies are intended to detect at minimum a 20 percent change in mortality, these studies must have at least an 80 percent statistical power for detecting that 20 percent change in mortality, then it becomes clear to us that that has or has not been achieved in the studies that we're looking at given the adequacy of the design.

And so I would have liked to have seen something of that as guidance from the agency or a response from the registrant.

DR. PORTIER: Dr. Neher.

DR. NEHER: Just a quick point. I'm not sure I made it clear or not.

In terms of -- I think the data -- I just find the data presented inconclusive about the effects of this protein on both pathogenic and beneficial nematodes.

There seem to be some inkling there are some reductions, both in the pathogenic one and the bacterial feeding example.

And it is hard to determine whether that is issues related with the experimental design or whether there is truly an effect. And if so, what the nature is. So I encourage follow up on that because it is the one component I can say that is hard to make some sweeping statement about no effect.

DR. PORTIER: Last comment on Question 5?

Have we answered your Question 5 well enough?

DR. ANDERSEN: I actually do think we would like some clarifications.

One of them relates back to the issues of chemical insecticides. I think when the discussion was asked of us of about how we looked at it, we do look overall at all the alternatives that might be there.

I just might say as you are going to look at that study, you have to recognize that it was only one or two insecticides and not everything.

So I think we have to be careful how we -- if we do all look at it, and is the agency's responsibility overall to balance the risk and the benefits, we'll do that.

But I'm hearing some disagreement amongst the panel members. I think we would like to see some kind of clarification from you, if possible, about what you might make as a recommendation on whether or not some of these

studies such as the lacewing study or the hymenoptera study need to be redone.

DR. PORTIER: For this specific case?

DR. ANDERSEN: For this specific case.

DR. PORTIER: And this is all still part under 5, or you're adding another question? Still part under 5.

DR. ANDERSEN: I think it's under 5, yes.

DR. PORTIER: So those two specific studies. Does anyone have an opinion as to whether they should be redone or not?

Dr. Jepson.

DR. JEPSON: I had written down in my notes that I would give the company the option of doing an extended laboratory test, if that's within your current guidelines, you know, something that's a more realistic exposure. And that applies specifically to the hymenopteran.

For the chrysoperla test, I just don't like the test. I don't think the company should have passed it on to you. I think it should be

repeated. But that's my personal opinion.

DR. PORTIER: I think under the law the company has to pass on all of the tests, whether they are good, bad or ugly. All the information that they have used to develop registration gets passed on to the agency.

Dr. Andow, did you have a comment?

DR. ANDOW: No.

DR. PORTIER: Dr. Barbosa.

DR. BARBOSA: I would definitely concur in terms of the chrysoperla experiment in terms of its needing to be repeated. It simply has too many significant flaws, both in terms of experimental design and the appropriateness of protocol.

DR. PORTIER: Any disagreements on that assessment?

Any other comments?

Is that sufficient for those two?

DR. ANDERSEN: Yes. Thank you.

DR. PORTIER: Okay. I believe that ends Number 5.

Now I would like to ask the panel if they have any other comments that don't necessarily fall under these five questions that they would like to make for the agency.

Dr. Federici.

DR. FEDERICI: This is just for clarification for Dr. Andersen. What are the consequences of having to redo the chrysoperla studies? I mean, it doesn't seem that it would take that long to do those studies.

DR. ANDERSEN: Those are the decisions that the agency will have to make based upon what the recommendations are from the panel. We will have to decide how we consider that in our risk management decision on the product.

DR. FEDERICI: The reason -- I'm thinking in terms of some of the data that has been reported at meetings recently on field effects where chrysopid populations are being monitored in the field. And I can think of at least two different studies where there doesn't seem to be an effect at the field level.

I'm just asking the question to see how you --

DR. ANDERSEN: Are you referring to studies on Cry3Bb1?

DR. FEDERICI: No.

DR. PORTIER: Any other comments from the panel for the agency?

I had one question for the agency. Janet, I don't know if you are the person to answer the question or not. Will the agency be bringing before the Science Advisory Panel the question of the health effects, potential for health effects in the evaluation of the potential for health effects for Cry3Bb1 any time in the near future?

DR. ANDERSEN: It is not our intention to do that. We have taken comment on this protein a couple of times because of the nature of proposing tolerance exemptions, et cetera.

It currently has a temporary tolerance exemption and has been evaluated for this protein as well as others that have been -- for this event

as well as the 859 and others. So there is an existing one.

We have not had any significant comments on health effects of it.

DR. PORTIER: That was for the record just so I would know what was coming down the line.

The other question is not a question, actually. If there are no more comments from the panel, I'm going to close very soon.

Dr. Hellmich.

DR. HELLMICH: When the EPA considers the comments -- you know, it is very difficult to prove a negative. And I haven't seen any red flags here in whether or not your tests were conducted appropriately. I'm not sure that warrants -- well, warrants a negative decision on anything.

Tests can be conducted later. And as I mentioned before, some of these experiments would benefit if there was more product available, if larger scale experiments were necessary.

So you have to consider that some experiments that probably may be done in the future would be jeopardized if we were worried -- if certain things didn't happen because some of these other experiments were holding it up.

DR. PORTIER: Dr. Hellmich, you are expressing sort of the same degree of concern that Dr. Federici was expressing in the sense that just because this test is on the books, just because this test is part of the regulatory request, the fact that this particular example of this particular test or this particular compound is insufficient or has design deficiencies doesn't necessarily -- doesn't necessarily mean it has to be redone given the other breadth of data that is in front of you.

Is that what you are trying to express here?

DR. HELLMICH: I think the question is the timeline and when it should be redone.

DR. PORTIER: What would be your recommendation for that?

DR. HELLMICH: I don't know if we can comment on some of these things. We're just supposed to comment on the science and not on the --

DR. PORTIER: I'm trying to get to the science question here. Because the science question is one beyond the regulatory question in the sense that just because it is required in this particular study, might have failed in design flaws, do they have to get it again before they register the product or not? That's the risk management decision.

Our comment on that was that it was insufficient; we would like to see a new test. But I think Dr. Federici's comment was more feeling some concern about, well, there is a lot of other data there. And do we actually have to have this test this time.

I want to make sure the panel's comments there are captured. If there is some concern here, I don't want to let it go.

DR. FEDERICI: Let me just expand a

little.

I'm trying to look at this from a scientific standpoint in a holistic sense looking at data that I know is in press or is coming out of a variety of different studies.

And they show that if you look at chrysoperla populations in the southeast, in Arizona, on cotton and on corn in Maryland, there are no effects, this is Cry 1Ac and Cry 1Ab, those two, seen in the field.

In addition to that, we have other data that have been published on laboratory experiments that have been quite heavily criticized. And having worked with these proteins for more than 20 years in a variety of different types of non-target studies that come along with our work, I -- the tests that have been done are flawed.

But if it's only for a preliminary assessment, given all the other data that are available, I would not want to see a registration held up on the basis of this particular chrysoperla study -- as much as I didn't like it.

DR. ANDERSEN: Maybe the scientific way to ask the question would be to say, does the panel believe that this protein -- from their scientific expertise, does the panel believe that this protein is likely to cause adverse effects to lacewings in the field?

DR. PORTIER: Or potentially --

DR. ANDERSEN: Potential.

DR. PORTIER: I guess I would have to turn it the other way around. Is the data sufficient, the broad spectrum of data, not just that one study, sufficient to imply that it is not likely to affect lacewing in the field?

DR. ANDERSEN: That's good.

DR. FEDERICI: Again, I would say in terms of a preliminary -- if you use this term preliminarily, which is used in a lot of these, I think -- I would say that based on my experience, based on the total knowledge of what is in the literature, the answer to that question would be, no, that I would -- my assessment would be that there would be no adverse effects on chrysopids in

the field with MON 863.

DR. PORTIER: So we have a bit of a conflict.

Dr. Barbosa, you were much more in favor and, Dr. Jepson, of having these studies. Is that still, again, still the case when this broader question is put forward?

We don't have to reach consensus here. I just want to make sure we have captured everybody's opinion.

DR. BARBOSA: The only way I can respond to what has been said is that what has been said makes one critical assumption, and that is that the field tests were designed to answer the same question as the lab test. And I don't believe that that's the case.

And although there is more information that provides some insights, they are not equivalent questions so that the answers can't be made equivalent.

DR. PORTIER: Dr. Jepson.

DR. JEPSON: I think we're beginning to

set the hurdle too low. I think we're being asked to speculate based on experience and our views of the technology when actually what we are meant to be doing is viewing the scientific quality and validity of the studies as presented.

And some of those fall well short. They don't provide us with a statistical basis for discriminating treatments in the field data. There are design flaws in the field data that we need to have addressed for the longer term. And some of the lab studies were incomplete and with inconsistent standards applied to them.

So bluntly, I think we don't have sufficient data upon which to make a judgment at the moment. Whether or not the decision would be any different if we had better, more rigorously applied tests that are more consistent, that's not what we're being asked to comment on, and I don't want to comment, and I don't think we should.

So that's simply put in my view.

DR. PORTIER: Dr. Hellmich.

DR. HELLMICH: My opinion is that there

is a three year registration. I don't think that lacewing populations are going to be at risk at all over the next three years if this product would be registered.

That's based on comments from my experience with this and what --

DR. PORTIER: But in terms of this particular study, the one we're talking about, its value in reaching that decision, does it need to be repeated?

DR. HELLMICH: I'm not convinced that it is fatally flawed. It may be it could be improved.

DR. PORTIER: Any other comments from the panel? You have clearly gotten a mixed response on this. I think that's clear.

DR. FEDERICI: I just want to respond to Dr. Barbosa's comments.

It is true that the lab study has a different purpose than the field assessment. I think that if -- there is a good possibility that if you really want to find out if chrysoperla is

sensitive to this toxin, if you get enough of it in there, it may be.

If the original studies on, for instance, Cry 1Ab are valid, that shows that -- at least the data said to me here is an insect that is sensitive to the toxin. So, therefore, it could be possible that they would be sensitive to -- it is unlikely, but it's possible, it could be sensitive to Cry3Bb1.

However, the field is a different situation altogether. And there you are looking at, in my opinion, the real, a more real world than you would find in the laboratory.

So the laboratory studies are very good for telling you where to look. But despite what kind of results, let's say you showed a fairly high mortality in the laboratory, that would not mean to me that you are going to see that kind of effect in the field.

I think that's what we're really ultimately after.

Now, I don't like the particular set of

data, I have said that already, that were provided here. I don't like it. I think it would be nicer to have better studies done. It is a little surprising to me that at the time we have been at the evaluation of the effects of these various transgenic plants on non-target organisms that the companies haven't come along with better systems, more statistically reliable techniques.

The high control mortalities in all these studies bother me. I have said that several times. I don't like the data the way it looks now, but I don't think the data reflect what will go on in the field situation. That's the bottom line for me.

DR. PORTIER: Any other issues, comments from the panel?

Dr. Andow.

DR. ANDOW: In the past, there has been suggestions that EPA consider some of the soil processes, soil -- ecosystem processes as potential endpoints for essentially non-target areas because it is virtually impossible to do

non-target species work on the species in the soil.

And I guess I would like to reiterate that that's a good idea to be considering. Things like nitrogen transformation rates and things like that might be useful for understanding does this have any effect on soils.

DR. PORTIER: Okay. With that, I will note to remind everyone that tomorrow morning we will have a report from a subpanel at the beginning of the SAP meeting in the morning on specific issues of the design of the studies that we were mentioning previously.

I, in my experience on the Science Advisory Panel, have been through a lot of different things. But I want to point out that this is really the first time that the agency and the registrant have put forth so much data for us to look at.

I think the atrazine (ph) was the only other example. And I'm still not sure we got everything to look at for atrazine. But this time

we saw a lot of information from both sources. And I think that opens up the process, and it is very positive towards moving these issues forward. And I want to thank you both for doing that.

And I want to thank the panel for a very stimulating discussion.

Mr. Lewis, do you have any closing comments?

DR. LEWIS: Just a few brief remarks. I would like to thank Dr. Portier for, again, serving as chair for our meeting today and for his upcoming service as chair for the next two days on the insect risk management discussion.

For those members of the audience, we'll be beginning tomorrow at 8:30 focusing on the IRM discussion with that beginning our meeting with a subgroup question on Question 2 that Dr. Portier has discussed.

I would like to thank the panel members today for their great service, their contributions for the discussion today.

For those of you departing, again,

thanks for your service. And for those of you remaining for the IRM discussion, I'm looking forward to working with you the next two days.

If I can have the panel in the next five minutes meet briefly in our workroom just to go over some administrative issues as we work in terms of writing our report, meet in about five minutes in the workroom.

Thank you. Have a pleasant evening.

DR. PORTIER: Before we leave, Dr. Andersen, did you have any additional comments? Ms. Rose?

DR. ANDERSEN: I think we have kept the panel long enough. Thank you very much for all your good comments today. We really appreciate your work efforts and what's to come. Thank you.

DR. PORTIER: Thank you very much. This meeting is now closed.

(Thereupon, the meeting was adjourned at 5:40 p.m.)

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I, Frances M. Freeman, 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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