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Veterinary Medicine Advisory Committee Meeting

AquAdvantage Salmon

September 19 - 20, 2010

Monday, September 20, 2010

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Veterinary Medicine Advisory Committee Meeting
AquAdvantage Salmon
September 20, 2010

VMAC members present:

David F. Senior, ACVIMN-SA, ECVIM, VMAC Chair (Acting)
Craig Altier, D.V.M., Ph.D.
Michael D. Apley, D.V.M., Ph.D.
Dicky D. Griffin, D.V.M., M.S.
John B. Kaneene, D.V.M., Ph.D.
Jodi Ann Lapidus, PhD.
Alan G. Matthew, Ph.D.
James D. McKean, D.V.M., J.D.
Robert H. Poppenga, D.V.M., Ph.D.
Paul C. Stromberg, D.V.M., Ph.D.

Temporary Voting Members present:

Gregory Jaffe
Gary Thorgaard, Ph.D.
Alison Van Eenennaam, Ph.D.
Kevin D. Wells, Ph.D.

Veterinary Medicine Advisory Committee Meeting
AquAdvantage Salmon
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Page

Announcements
by Aleta Sindelar, RN, Executive Secretary, VMAC 7

Opening Remarks from CVM Center Director
by Bernadette Dunham, DVM, Ph.D. 9

Welcome
By Joshua M. Sharfstein, M.D., Principal Deputy Commissioner, FDA 9

Overview of the New Animal Drug Evaluation Process
by Steven Vaughn, DVM, Director, Office of New Animal Drug Evaluation, CVM 15

Opening Remarks from CFSAN
by Michael Landa, JD, Acting Director, Center for Food Safety and Applied Nutrition, FDA 27

State of World Fisheries
by Yonathan Zohar, Ph.D., Director and Professor, Center of Marine Biotechnology, University of Maryland 28

Committee Questions and Answers 52

Atlantic Salmon and Risk Issues Associated with Fish
by Eric Hallerman, Ph.D., Professor of Fisheries and Wildlife, Department Head, Virginia Tech 60

Committee Questions and Answers 87

AquaBounty Technologies
by Ron Stotish, Ph.D., CEO 97

Committee Questions and Answers 112

Regulation of GE Animals at FDA
by Larisa Rudenko, Ph.D., DABT 121

I N D E X (cont.)

	Page
Molecular Characterization of AquAdvantage Salmon	
by Jeff Jones, DVM, Ph.D.	135
Committee Questions and Answers	145
Phenotypic Characteristics of AquAdvantage Salmon	
by Donald Prater, DVM	158
Committee Questions and Answers	178
Announcements	
by Aleta Sindelar, RN, Executive Secretary VMAC	197
Food/Feed Safety Assessment of AquAdvantage Salmon	
by Kevin Greenlees, Ph.D., DABT	198
by Kathleen Jones, Ph.D.	206
Committee Questions and Answers	213
Environmental Safety Assessment	
by Eric Silberhorn, MPH. Ph.D., DABT	233
Committee Questions and Answers	252
Claim Validation	
by Evgenij Evdokimov, Ph.D.	260
Committee Questions and Answers	263
Durability Plan and Post-Market Requirements	
by Jay Cormier JD, Ph.D.	264
by Barry Hooberman, Ph.D., DABT	269
Committee Questions and Answers	273
Public Comments to the VMAC	275
by David Edwards Biotechnology Industry Organization	277
by Darrell Rogers Alliance for Natural Health-USA	280
by Alejandro Rojas Aquaculture Resource Management	284

I N D E X (cont.)

Page

Public Comments to the VMAC(continued)

by Michael Hansen
Consumers Union 288

by Wenonah Hauter
Food and Water Watch 292

by Jaydee Hanson
Center for Food Safety 296

by Nina Mak
American Anti-Vivisection Society 299

by Eric Hoffman
Friends of the Earth 306

by Anna Zivian
Ocean Conservancy 310

by William Muir
American Society of Animal Sciences 314

by Ann Kapuscinski
Dartmouth 318

by Jane Rissler
Union of Concerned Scientists 323

by Leo Broderick
Resident Prince Edward Island 326

by Lisa Weddig
National Fisheries Institute 330

by Dave Conley
The Aquaculture Communication Group 331

Charge to VMAC:
Questions of clarification from VMAC to any presenters
by Larisa Rudenko, Ph.D., DABT 333

Discussion among VMAC 334
by David F. Senior, VMAC Chair (Acting)

Question 1: *Do the data and information demonstrate that the rDNA construct is safe to AquAdvantage Salmon?* 335

I N D E X (cont.)

	Page
Question 2: *Do the data and information demonstrate there is a reasonable certainty of no harm from consumption of foods derived from AquAdvantage Salmon?*	357
Question 3: *Do the data indicate that AquAdvantage Salmon grow faster than their conventional counterparts?*	378
Question 4: *Are any potential environmental impacts from AquAdvantage Salmon production adequately mitigated by AquaBounty Technologies' proposed conditions of use?*	379

Keynote: "---" indicates inaudible in the transcript.
"*" indicates phonetically spelled in the transcript.

M O R N I N G S E S S I O N

(8:03 a.m.)

Announcements

by Aleta Sindelar, RN

MS. SINDELAR: Hi. I am Aleta Sindelar. I am the Exec Secretary for the Veterinary Medicine Advisory Committee. I have a few announcements to make for the start of this meeting.

First, I would like to have you turn off all of your cell phones. Second, those that have come into the hotel may have had to get parking tickets. Parking is free, so please have those validated at the front desk.

We have new press here from yesterday, and so I would like to introduce the FDA press officers here who are here to assist our Veterinary Medicine Advisory Committee members as well as any staff in any particular interviews that are requested by the press and our invited speakers. I would like to make the comment also: No press interviews will be made prior to the close of deliberations. Please stand when I call your name: Siobhan DeLancey, FDA Press Officer, and Mike Herndon, FDA Press.

I also have a statement regarding the conflict of interest that is read before every meeting, so let me begin:

"The following announcement addresses the issue of interest with regards to this meeting and is made part of the

public record to preclude even the appearance of a conflict of interest at this meeting on September 19th and 20th, 2010.

Federal conflict of interest laws preclude the participation of committee members and consultants in advisory committee meetings if they have a conflict of interest, unless a 'Waiver of Exclusion' is granted by the Agency.

The Associate Commissioner for Special Medical Programs, FDA, has appointed Mr. Gregory Jaffe, Drs. Gary Thorgaard, Alison Van Eenennaam and Kevin Wells as Temporary Voting Members for this meeting.

Based on the submitted agenda for this meeting and a review of all financial interests reported by the Committee participants, it has been determined that all interests in the firms regulated by the Center for Veterinary Medicine, which have been reported by the participants, pose no potential for conflict of interest at this meeting.

In the event that the discussions involve specific products or firms not on the agenda for which FDA's participants have a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the public record.

With respect to all other meeting participants, we ask in the interest of fairness that that they address any current or previous financial involvement with any firm whose products they wish to comment upon."

Thank you, and I would like to pass the baton to our Director, Dr. Bernadette Dunham.

Opening Remarks from CVM Center Director

by Bernadette Dunham, DVM, Ph.D.

DR. DUNHAM: Thank you very much, Aleta. And thank you again very much for coming. We really appreciate the public participation in this very important meeting. We appreciate the press who has also come to attend this meeting. And more importantly, we appreciate everybody here from the Veterinary Advisory Committee members. Thank you so much.

Before I go any further, it is honor that I am pleased to announce that our Principal Deputy Commissioner, Dr. Joshua Sharfstein, is here and would like to make a few opening comments. Dr. Sharfstein?

Welcome

by Joshua M. Sharfstein, MD

DR. SHARFSTEIN: Thank you, Dr. Dunham, and thank you all. I bring greetings from Dr. Margaret Hamburg who -- the FDA Commissioner, who could not be here but wanted me to pass on her appreciation to all the work -- for all the work that is going to happen today.

Let me specifically say to the Advisory Committee: Thank you for your time in preparing for this meeting, the time at the meeting, your independent and candid thoughts in assessing the data and making recommendations to the Agency.

No decisions have been made by FDA and today and your input very much matters, so --

I also want to thank the many FDA staff who worked very hard to bring about this meeting and to work and review this application. I think the really excellent documents that we have released providing background on the product at issue, talking about the legal and scientific background as well as summarizing all of the data upon which we are basing this application review. It is really extraordinary, and really, from my perspective, having seen many different types of discussions happen at FDA, really about as open and complete really as anything that I have seen at the Agency.

And also to the public, I want to thank you for coming, for engaging on the issues, for presenting -- those representing -- for submitting written comments. Everything that you provide will, I know, be reviewed and receive serious consideration. And not just today. As you know, there are also opportunities for public engagement tomorrow on the topic of labeling and also, depending on how the process goes from there, at other points in time. We very much appreciate all of the input that we are getting. This is a very unique and important area for the agency to be working in and we value all of your contributions.

And to everybody, I just want to wish a very productive day. Thank you.

DR. DUNHAM: Thank you very much, Dr. Sharfstein.

I also would like to just acknowledge a few people that we have here that are quintessential to helping us. As you have already met, Aleta Sindelar is truly the master of ceremonies in holding everything together and I do thank you. Lisa Burns is handling our transcription. Ryan Cumin* is doing audio and microphones and Joanne Kla has been assisting with our computer, along with many folks, as you know, outside that are helping us pull this all together. Thank you, very, very much.

So, once again, good morning. On behalf of all of my colleagues at the Center for Veterinary Medicine and the Food and Drug Administration, I want to extend a very warm welcome to everyone attending today's Veterinary Medical Advisory Committee meeting.

This is a very special day for all of us as we discuss the review of the first genetically engineered food animal. This technology holds great promise for the world's food supply, but we recognize that as the first of its kind, we are sailing uncharted waters. However, it is the science that will lead us as we chart these new waters.

The in-depth scientific review of this fish, the AquAdvantage salmon, is not something we have taken lightly. We have an amazing group of scientists at CVM and they have applied their vast expertise to the careful and thorough

review of the data. Our most senior and experienced reviewers analyzed the data and information provided. They reached conclusions unanimously at all risk-based stages of reviews, and I would personally like to thank each and every one of them for all the hard work they have put into this review. And you met many of them yesterday as we went through the educational outreach portion of this meeting.

We are now ready to present these conclusions to the VMAC and the public for additional comments and recommendations prior to reaching a final decision. I hope you will take and keep an open mind as you listen to the presentations that will be given today.

The Advisory Committee is responsible for assessing whether or not CVM has met its obligations under the regulatory framework that Congress has given us. We appreciate your interest in this meeting and we look forward to hearing your comments, and I look forward to a very productive and polite discussion.

And now, it is my pleasure to introduce you all to our Advisory Committee members. And as I say their names, I would like them each to stand so that you can see them.

Our Chair for the Committee is Dr. David Senior. He is Associate Dean, Advancement and Strategic Initiatives, at the School of Veterinary Medicine from Louisiana State University. Thank you.

Dr. Craig Altier, Associate Professor, Department of Population Medicine and Diagnostic Sciences, the College of Veterinary Medicine at Cornell University.

Dr. Mike Apley, Associate Professor, Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University.

Dr. D. Griffin, Professor, Beef Cattle Production Management Veterinarian at the University of Nebraska, Lincoln.

Our consumer representative is Gregory Jaffe, Director, Biotechnology Project, Center for Science in the Public Interest, Washington, DC.

Dr. John Kaneene, University Distinguished Professor of Epidemiology, Center for Comparative Epidemiology, Michigan State University.

Dr. Jodi Ann Lapidus, Assistant Professor, Division of Biostatistics, Department of Public Health and Preventative Medicine, Oregon Health and Science University.

Dr. Alan Mathew, Professor and Head, Department of Animal Science, University of Tennessee.

Dr. James McKean, University Professor and Extension Swine Veterinarian, Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University.

Dr. Robert Poppenga, Professor of Clinical Toxicology, California Animal Health and Food Safety Lab,

School of Veterinary Medicine, University of California at Davis.

Dr. Paul Stromberg, Professor of Veterinary Pathology, Department of Veterinary Biosciences, Ohio State University.

And our subject matter experts are Dr. Gary Thorgaard, School of Biological Sciences and Center for Reproductive Biology, Washington State University; Dr. Alison Van Eenennaam, Cooperative Extension Specialist, Animal Genomics and Biotechnology, Department of Animal Science, University of California at Davis, and Dr. Kevin Wells, Assistant Professor, University of Missouri, Animal Science Research Center.

So again I want to give a very warm thank you to you today for making this what I know will be a very interesting Veterinary Medical Advisory Committee meeting. So, thank you so very much.

And now we will proceed and move forward. We will probably have Mike Landa, who is the Acting Director for the Center for Food Safety and Applied Nutrition, joining us, and when he does, I will introduce him. He has a few remarks to make.

So until he arrives, we will move forward, and I believe Dr. Steve Vaughn is going to now give a presentation. He is Director for the Office of New Animal Drug Evaluation at

the Center for Veterinary Medicine.

Overview of the New Animal Drug Evaluation Process

by Steven Vaughn, DVM

DR. VAUGHN: Good morning, everybody. What I would like to talk to you a little bit about today is just give you a little bit of an overview and background relative to the new animal drug evaluation process. Hopefully, it will answer some of the questions that I am sure you have relative to how we apply the statute in our evaluation process.

(Slide)

So first off, let us start. Obviously, anything that we do has to be within the powers that are granted to us, responsibilities that are granted to us, by Congress and in the law that is in the Federal Food, Drug and Cosmetic Act, and I will be talking a little bit more about that.

We are subject to a number of other laws as well, as the National Environmental Policy Act, Clean Water Act, National Aquaculture Act and a number of others that would apply particularly here.

We then can further interpret the law in the form of regulations, and those are published in the ***Code of Federal Regulations*** and those regulations also have the force of law. And then we can further interpret those regulations and talk about our current policies, our current thinking, and publish those in non-binding documents that are known as “guidances.”

So those are really the three categories of documents that provide us our authority and explain our current thinking. The first two have the force of law; policies and guidance do not.

(Slide)

So, dealing with the law, let us start with the definition of a new animal drug, and I hope this will help clarify why we are regulating genetically engineered animals as new animal drugs.

First off, the definition in the statute: "Articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals and articles intended to affect the structure or function of the body of man or any animals."

Now, if you look at the definition, the definition is not based on what the entity is but rather on its intended use. And so it is a very broad definition, and Congress intended this because they envisioned that there would be many different types of technology that we would have to address over the years. And so this is sort of an umbrella definition to make sure that anything that has an impact on a therapy or the production of animals is captured by this statute. And so if you look at articles intended to affect the structure or any function of the body of man or any animals also falls within that definition when we are talking about genetically

engineered animals.

(Slide)

So what we need to do at FDA then is adapt our scientific and regulatory approaches to address the specific science that is pertinent to that technology to make sure that it demonstrates safety and effectiveness. Any approach that we take to evaluate this as a new animal drug must meet the statutory requirements for safety and effectiveness, meet the regulations, and sometimes we have to make some adaptations in the way we approach that because of the nature of the entity that we are evaluating.

(Slide)

So in this particular case, a new process was developed and was tailored to address the safety and effectiveness of this technology. The intent of this new process is to meet the safety and effectiveness standards as outlined in the statute, evaluate the drug to ensure that any specific hazards or risks associated with the technology are addressed, resulting in a safe and effective product in the marketplace.

(Slide)

Now, as we are mandated by the Food, Drug and Cosmetic Act, any new animal drug may not be sold in interstate commerce unless it meets one of the four process approvals that we have. And the first one is the one that we

are talking about today, and that is an approved New Animal Drug Application under Section 512 of the Act.

We also have provisions for generic animal drugs that would be approved, abbreviated New Animal Drug Applications. We can do a conditional approval, which is under Section 571 of the Act which came to us through the minor use/minor species legislation. And then the 4th legal way to market a product is through indexing. And this also came to us through the minor use/minor species legislation for very minor uses in animals -- it would be, for example, in zoo animals where it would be very difficult to conduct a complete battery of studies that would be necessary for evaluation.

So those are the four ways, and we are talking about the first one today, the New Animal Drug Application.

(Slide)

So what does an NADA mean?

First off, it means the product is safe and effective for its intended use.

Again, go back to the definition: What causes it to be a drug is its intended use. And so under those conditions, we are trying to evaluate the safety and effectiveness. And then, the methods, facilities and controls used for the manufacturing, processing and packaging of the drug are adequate to preserve its identity, strength, quality and purity. This is an important principle, and it has been

specifically addressed in the evaluation of this particular product.

So what are the components that we have to look at under a New Animal Drug Application?

First off, we have to look at human food safety. Is it safe that any of the tissues that might be derived from the animals that have received this new animal drug are safe? And so traditionally we would use a battery of toxicology studies, residue chemistry studies, and if it was an antimicrobial, we would address microbial food safety. And you are going to hear about how the human food safety was addressed today.

We also deal with target animal safety, which is the safety to the animal, in this case, the salmon.

We look at environmental safety, and you will hear about that today. And effectiveness, manufacturing chemistry, and the labeling.

And then we have a catchall, which is all other information. So any other information that might not have been submitted to address one of these specific components will be caught up at the end and be included in a larger, sort of a data dump, if you will, to make sure we haven’t missed anything.

(Slide)

So, let me run you through very quickly the definitions of effectiveness and safety.

First, effectiveness. It is based on substantial evidence consisting of one or more adequate and well-controlled investigations such as **a** study in target species, a study in laboratory animals, a field investigation, a bioequivalence study, an in vitro study. It can be any number of different types of scientific design studies that enable us to reach a conclusion.

The 1996 Animal Drug Availability Act gave us a lot of flexibility to be able to apply different types of science in the evaluation of effectiveness, and this is what is specifically listed in the statute. And we can use any combination of one or more of these types of studies in the evaluation of any new animal drug.

(Slide)

So, based on these studies that are conducted by experts qualified by scientific training and experience and then evaluated by those same type of qualified experts, to evaluate the effectiveness of the drug involved, these experts then can come to the conclusion, fairly and reasonably conclude that the drug will have the effect it intends, that it purports to have or is represented to have, under the conditions of use prescribed, recommended or suggested in the proposed labeling.

(Slide)

Definition of safety is, first off, we have a very

wide definition, broad definition, of safety; enables us to make sure that we are covering a lot of area so that we are making sure that, as I said before, it is safe for humans and the environment, and the animals that are receiving the drug. And there are adequate tests by all methods reasonably applicable that show the drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling.

(Slide)

So the Act also further directs us to determine whether the drug is safe. We have to consider a number relevant factors.

First, the probable consumption of such drug and any substance formed in or on food because of the use of the drug; the cumulative effect on man or animals of such drug, taking into account any chemically or pharmacologically related substance; safety factors which, in the opinion of experts, are appropriate for the use of animal experimentation data; and whether the conditions of use in the proposed labeling are reasonably certain to be followed in practice.

So, as I mentioned, we really look at four areas of safety -- human food safety, target animal safety, environmental safety and user safety. The fourth category is safety by the people who may be administering the product.

(Slide)

So, the New Animal Drug Application is a systematic approach to document evidence that the drug products are safe and effective and the approved drug product consists of the drug, the packaging and any labeling.

(Slide)

So we describe the document in evidence and we communicate to the public -- let me start there -- we communicate to the public in a number of different ways.

First off, we start with of a Freedom of Information Summary which provides documented evidence that we relied on to make our decision. We also publish an environmental assessment wherein we describe the impact, or lack of impact, on the environment and how we arrived at that decision, and then the drug labeling. And there is going to be, I believe, a hearing tomorrow to talk about labeling.

We also codify the approval in the ***Code of Federal Regulations***, and there is a ***Federal Register*** announcement announcing to the public that the approval has occurred. And all of these documents are accessible by the public.

(Slide)

So why did it take us so long to get to this point? I know that is another question that is out there.

Well, first off, we worked for many years with the sponsor trying to advise them regarding the types of information that we would need to be able to meet the

statutory requirements. And this was something we started working with the sponsor very early on and there was a lot of thought that went into how to approach this new technology and how to meet those statutory requirements.

Then we had to determine how we were going to regulate these genetically engineered animals. And once we had figured that out, then we worked for a number of years to make sure that we had achieved broad consensus across the rest of the Federal government that this technology should indeed be regulated as new animal drugs. And now that we have secured that decision, the review process has proceeded very expeditiously.

(Slide)

I want to stop for a second and just mention that this has been challenging for us as new technology, and to meet that challenge, we have assigned our most senior and experienced staff to work on this project. You are going to hear from them today. And I just want to make a comment that I believe that these folks are the best scientific experts that could be had to be brought to bear on this challenging technology. And these are very experienced folks that are recognized nationally and internationally for the work that they do in the new animal drug arena. And I can’t compliment them enough. And these scientists not only fully understand the safety and effectiveness standards at an expert level but

they understand how these standards can be met, taking into account the characteristics of this technology.

So with that, I would be happy to answer any questions that the Committee might have.

(No response)

DR. VAUGHN: Okay, thank you very much.

MR. : We have one question.

DR. VAUGHN: Okay.

DR. THORGAARD: I wonder if you describe in a little bit more detail about the process of reaching a consensus with the other agencies about how to proceed with the question.

DR. VAUGHN: Actually, I would defer that to Dr. Rudenko. She has spent an enormous amount of time, as we all have, working, talking with other agencies both domestically and internationally.

As you know, there is a lot of discussion that is going on not only here with USDA and EPA and other agencies but also in the international arena, in the Codex arena and so on as far as food safety is concerned. So, Larisa?

DR. RUDENKO: I am terribly challenged when it comes to --- (microphone off) so, thanks, Steve.

The shorter version of this, because it goes on for a very, very long time, and I have noticed that Dr. Randy Lutter is in the audience as well, is that when we -- at the end of the Clinton Administration, there was a series of

OSTP/CEQ documents that gave case studies that indicated how transgenic animals or genetically engineered animals might be regulated by the Agency. And we felt on behalf of the Agency that they would indeed be regulated under the new animal drug provisions of the Act because that gave us the maximum flexibility and the maximum utility for applying the power of the Act to regulate both animal health, the environment and human health.

We spent a number of -- hundreds of person hours developing various matrices to show how we would maximize coverage of all of the risks that might be encountered and worked that through in an interagency agreement, the Office of Science and Technology Policy at the White House, so that we had full interagency buy-in across the U.S. government. That was done at the highest levels of the government, including staff levels of interaction, to come up with some of these risk matrices.

At the end of the day, we published our Guidance for Industry 187 which clarified the legal authority under which we operated, indicated very clearly what the Agency had authority over and what we would cede to other agencies, which would be full enclosure and research purposes, for example, at NIH. But we are not interested in doing a full NADA for research mice, for example.

And then we went to full notice and comment from the

public. We went through that and we adopted Guidance 187 in final form.

We have continued to work with our friends and colleagues across the U.S. government as issues come up through the OSTP, Agricultural Biotechnology Working Group and the Animal Biotechnology Working Group.

We have worked very closely with OECD in establishing standards. We have worked closely with the Codex Alimentarius Commission. There are a number of folks who are sitting behind me now who have worked on various components of both writing and passing the Codex Alimentarius guidelines for the safety assessment of food from rDNA animals.

So we feel that in many ways the U.S. has taken a lead position in developing a regulatory policy for this and developing a risk-based regulatory policy for this and in gaining the support of our peers across the country and across the world as we move forward on it.

DR. VAUGHN: Thank you. Any other questions for me?

(No response)

DR. VAUGHN: Okay. Thank you very much.

DR. DUNHAM: Thank you very much, Dr. Vaughn. I appreciate that.

It is my pleasure now to introduce you to Mr. Michael Landa, who is the Acting Director for the Center for Food Safety and Applied Nutrition, CFSAN. Mike, please?

Opening Remarks from CFSAN

by Michael Landa, JD

MR. LANDA: Thank you, Bernadette. Good morning, everyone. I promise to keep this meeting running smoothly by making my remarks quite brief.

FDA Center for Food Safety and Applied Nutrition is pleased to be engaged in this process with CVM.

As many of you likely know, CFSAN will be holding a public hearing tomorrow in this room on the labeling of food derived from AquAdvantage salmon. This is a good example of how the Agency can leverage its expertise across centers to consider the scientific and regulatory aspects of products emerging from new technologies.

I want to emphasize an important point, and that is that today’s meeting conducted by CVM will focus on safety and effectiveness issues related to the AquAdvantage salmon and that tomorrow’s hearing conducted by CFSAN will focus specifically on the labeling of food from that salmon.

We are having a hearing on food labeling on the heels of today’s VMAC hearing so that if the application for AquAdvantage salmon is approved, we are in a position to make a decision about labeling, if necessary right away.

Data gathered as part of the NADA process could have a bearing on labeling should we reach that issue. That is because the issue, in a nutshell, is whether there are

material differences between food from AquAdvantage salmon and non-GE salmon, differences, for example, in composition or functionality.

Again, however, it is important to note that the issue of food labeling is only relevant if FDA approves the application related to the AquAdvantage salmon.

If you wish to attend tomorrow's public hearing and if not pre-registered, you may register onsite tomorrow morning. Again, the hearing will begin at 9:00 and it will begin in this room.

Again, CFSAN is pleased, very pleased, to be engaged with CVM as the Agency continues its evaluation of the AquAdvantage salmon.

Thank you. Have a good day, and more importantly, a good meeting. Thanks.

DR. DUNHAM: Thank you very much, Mike. I appreciate that very much.

It is my pleasure now to introduce Dr. Yonathan Zohar, who is the Director and Professor for the Center of Marine Technology at the University of Maryland. He is going to give us a presentation regarding the State of World Fisheries. Dr. Zohar?

State of World Fisheries

By Yonathan Zohar, Ph.D.

DR. ZOHAR: Okay, good morning. My pleasure to be

here today and I think that my role is to set the stage, introduce the discussions of the day today by talking about the state of the global fisheries, the promise of aquaculture, and the role of biotechnology. You can see micro-injection of -- into early fish embryos -- in helping aquaculture deliver its promise.

(Slide)

I think that we all know that the world is facing a major fishery crisis. It has been captured abundantly by the media, like the tragedy of the ocean in ***The Economist*** as well as the promise of aquaculture, again in ***The Economist,*** a few years later, same venue, the tragedy of the ocean and the promise of aquaculture.

(Slide)

And the bottom line of the crisis is the following.

We all know that the world population has been on an accelerated increase. We are almost at 7,000,000,000 people right now, and more people eat more fish. And as a result, we witness an increase in the consumption of seafood, fisheries product, that is not only the result of more mouths to feed but also an increase in per capita consumption of seafood. That is a global trait -- people go off red meat into poultry and off poultry into seafood. So here is an example: 10 to 17 kilograms per capita consumption of seafood over the past 4 decades, and that is global.

(Slide)

As a result of all of that, we have become a society of very efficient fishers. We have these huge like factory fishing ships. We have these huge containers that are actually guided by airplanes -- we will get back to it. Huge nets, as well as all kind of huge numbers of traditional fleets.

This is a picture I took in Qingdao in China a few years back, and it goes for miles, boats like that.

(Slide)

And also, don't forget fish is the last major hunt-and-gather animal crop. You do hunt for foxes or for deer for food but you don't hunt for chicken or beef or pork, but you do hunt and gather for fish. This is cod. Or for crustaceans, for seafood.

(Slide)

And as a result, the oceans are being harvested at maximal sustainable yields. The ocean cannot give any more. Despite the fact that we are becoming more sophisticated fishers, the ocean cannot give any more. This is a different --- so on, but this is the important trend, that commercial landings has leveled off at about 80 million tons.

And it is actually worse than that because this is the same kind of data but from a different publication by Daniel Pauly in ***Nature***. Same data. The leveling off at about

80 millions tons of the reported fisheries. But look: There is about 20 percent of what is, or is estimated, as illegal and unreported catches so that we are catching, really, much more than what we report.

(Slide)

Which led to this quite well-known study by Boris Worm and some other science fisheries scientists, that was published in ***Science*** in like November ’06, “Impact of Biodiversity Loss on Ocean Ecosystem Services.” And the same day, this paper was picked up by all the major media venues such as like ***USA Today*** and others. 90 percent of the oceans’ edible species may be gone by 2048 if we don’t do anything about it. And this is straight from the study of Boris Worm and the group that showed that really we are running out of fish.

These are number of taxa that are considered collapsed. And he went, they went, around marine systems around the world, like shown here, and they counted the number of taxa from 1950 based on historical data that are considered collapsed and it goes from zero to like 80 percent here, and this is like the annual number and this the cumulative number, and if you extrapolate this down, you will see that by 2048 you are going to be running out about 90 percent of our edible fish species in the ocean.

That is the bad news. The good news, that at the

end of the paper, the authors write that yet, available data suggest that at this point these trends are still reversible. But basically, we need to leave the wild stocks alone for those stocks, for these trends, to change and to reverse.

(Slide)

And this is another look at the state, rather sad, of the world fisheries. This from the FAO SOFIA report, "State of the Ocean Fisheries and Aquaculture," the latest one, '08, and this shows you the percent of stock that assess to be overexploited, depleted or recovering from depletion, 28 percent, and leveling off that are overexploited, depleted or recovering, 52 percent, and a little of a trend of an increase are the stocks that fisheries sold that are fully exploited and only 28 percent, and leveling off is the fisheries -- sorry. Yes -- that is right. 28 percent in leveling off are the fisheries that are considered overexposed and depleted or recovering. 20 percent, and decreasing, unfortunately, are those that are underexploited or moderately exploited. So this is not good. And -- but that is the, as I said, the sad state of the world fisheries.

(Slide)

Let me give you some examples now.

The bluefin tuna, a giant of the ocean -- those are fish that are like swimming along the Atlantic coast of the United States down to the Gulf of Mexico. The eastern -- the

western population and the eastern population is the one that is in the East Atlantic and in the Mediterranean. Those are like 5 to 1,000 pounds, animals that swim around the globe, being caught quite intensively, especially the Eastern stock and the Mediterranean stock, by those persain boats that are guided by airplanes, and unfortunately, fishing time occurs at the spawning season as the fish aggregate for the spawning and then minute schools that are spotted from airplanes and then they are being circled and captured, and in many cases they go into the --- today and seafood market or to cages to fatten them until they are ready to be harvested.

(Slide)

So, these are the statistics on the bluefin tuna stock that continue to be devastated by quite intensive harvest and the harvest has been going up, and because -- and this is from the International Commission for the Conservation of Atlantic Tunas, ICCAT, and because they published some quotas which is like -- this is like the red line, total allowable catches, the catches went supposedly down. But again, the most alarming fact is this one, that those are like the unreported estimate. So actually we continue to overfish tuna quite aggressively., and that is the bluefin tuna.

(Slide)

And this is like the fishing mortality, fishing pressure, and abundance of the giant bluefin tuna, or these

breeding stocks, this 5 to 1,000 pounds fish. And as you can see, where the East Atlantic where most of the fisheries occurs for the bluefin tuna, the Mediterranean stocks, there are much more fish there. The fishing pressure keeps going up and the abundances of the broodstock, of the spawning stock, goes quite steeply down and it is a much worse situation in terms of the broodstock here in -- along the coast, of the Atlantic coast of the United States into the Gulf of Mexico. The Western stocks fisheries fluctuate both because regulation is better and because we are running out of fish. We are really down to very few bluefin tuna broodstocks in our like west Atlantic and Gulf of Mexico stocks.

(Slide)

Plummeting fisheries catches in the northwest Atlantic -- this is overall fisheries -- very sad situation. This is like the global capture production for Atlantic cod. Going very fast down, as we know.

(Slide)

And here is the promise of aquaculture. Since the year 2000, there is a beginning of global production of Atlantic cod through aquaculture, which has been on the increase. So again, the crisis and the promise of aquaculture.

(Slide)

Capture production of American plaice -- there is no

aquaculture of this fish, going down. We are running out of this -- of those like major, you know, seafood fishery species.

Now we are here to discuss Atlantic salmon. This is the state of the global capture of -- product capture production, fisheries, landing of Atlantic salmon goes down. And if you think that what stays here is wild Atlantic salmon, it is not. Probably about 80 to 90 percent of it account from escapees from cages, from aquaculture. We will talk about it in a minute again.

(Slide)

And again, the role of aquaculture. Aquaculture -- North Atlantic salmon has been on the increase here and we are going to talk about it more during this entire day. Everywhere that you look, like more locally, around, you know, our backyard here, the Chesapeake Bay, the Chesapeake oyster has been decimated, a classical case of combination of overfishing and pollution of a bay, like the Chesapeake bay.

(Slide)

The same is for the blue crab. The blue crab is like, you know, the abundance of the blue crab is on the way down although we think that maybe something better is happening now.

(Slide)

So, based on all what I just said in this brief

review, we are witnessing an increasing what we referred to as a “global seafood gap,” which is this gap between the demand, the rising demand, as I said, per capita, the rising demand for healthy seafood and that at best, leveling off landings of fisheries products. And this gap has to be filled by aquaculture.

I mean, we need to be able to feed the growing world population with seafood, and the only way to do is through aquaculture and it is a huge challenge for aquaculture. And actually, based on everything that I said here, the situation is actually worse because we are not leveling off at fisheries. Actually, we are like overfishing and depleting our stock so the challenge of aquaculture is actually larger than that.

(Slide)

And responding to that situation, aquaculture production has been growing. As capture fisheries has been leveling off, aquaculture production has been growing. This is -- I mean, there is more recent data, but not much on the databases at all there. And aquaculture, global aquaculture, has been growing at a pace of about 10 percent per year, which makes it the fastest growing agricultural industry in the world. And right now, it is about 63,000,000 tons annual production whereas the global catches are at about 70,000,000. So almost half of the seafood that we eat currently comes from

aquaculture production.

(Slide)

Here is the U.S. perspective. Many commercial fisheries, Atlantic and Pacific, are overfished. I showed some of them. I don't know if you know, but the United States is the world's second largest importer of seafood. About 80 percent of the seafood consumed in this country comes from overseas. Seafood imports contribute about $7,000,000,000 to $8,000,000,000 annually to the United States trade deficit, which is the largest among all agricultural products contributed to the trade deficit.

Aquaculture, as a result, is the fastest growing agroindustry in the U.S. but you still rank only number 14 in the world value of its aquaculture and it is about $1,200,000,000 industry, a lot of room to grow.

(Slide)

I was also asked to talk a little bit about -- quickly about the practices of aquaculture. How do we practice aquaculture? Most marine aquaculture production, and that is what we are referring right now to, the marine aquaculture production is a practice in these kind of floating cages in coastal areas.

This is one of the beautiful fjords in Norway where we grow Atlantic salmon, like those square cages, or round, big, round cages. They are all in like coastal areas so far

and there is a lot of residents that are using this beautiful coastal area.

There are a lot of issues and controversies between the coast use and the aquaculture use in these like coastal floating cages as well as all kind of other adverse impact on the environment. We will talk about it in a minute again.

All this is like in the southern part of Spain, these huge cages where they stock the bluefin tuna. They overfish and put them here to fatten them until they are ready to go to the Japanese market at peak sushi time and quality.

(Slide)

There is a lot of talks now and NOAA has been trying to develop a policy for offshore aquaculture so you kind of mainly to address the issues of adverse effect on the environment. And it means taking the cages about three miles, off three nautical miles off the coast, so there is less impact on the coastal environment. And in many cases because of those water is stormy and so on, you are talking about submerged cages that they can go under the water in case of storms et cetera.

(Slide)

And there is still a lot of aquaculture that is done in this type of ponds, mostly coastal ponds. The water come usually from beautiful coastal areas. In many cases, in South/Central America, in mangrove areas, so the water come in

and go out, and it is mainly used for shrimp production but some finfish as well, and a lot of issues of polluting the environment and as well as the fish.

(Slide)

Okay, so we said aquaculture has a major challenge that it is facing to fill this increasing gap between the rising demand and the dwindling supplies of fisheries products. How can aquaculture meet this challenge? And this is where we get a little bit to the role of biotechnology.

Aquaculture currently is a 63,000,000-ton, $78,000,000,000 industry. It must increase production at least 2 to 3 times by 2030 to fill this gap that I was describing.

For that, aquaculture must become more efficient and cost effective. For that, it must overcome biological obstacles, you know, several biological obstacles, and for that it needs strong input from modern biology and biotechnology -- and I am saying modern biology and biotechnology because I am referring here like to going from the genes into the whole animals.

So we biologists, we people who do marine biotechnology and aquaculture biotechnology, need to help aquaculture meet this challenge.

(Slide)

And here is some of the bottleneck in commercial

aquaculture that biotechnology can help open, and if we had plenty of time, I would have gone through each one of them, but I won't, but they are in the area of reproduction, that most commercial important fish do not spawn at all in captivity or undergo unpredictable spawning so closing the life cycle; early level development where we have low survival.

We are going to talk about growth, obviously, that we want to accelerate. Nutrition, the whole issue of harvesting fish -- to feed fish so replacing fish meals and fish oils, there is a lot of work going on there and we will be getting there. We will stop harvesting fish to feed fish. I can talk about this at a different time or later, if you want.

Disease/health management is a big issue in aquaculture. Overall performance, improved performance, selective breeding, and I am going to talk also briefly about interaction with the environment and because -- so those two issues grows and interaction with the environment are in the context of today's hearing and session.

(Slide)

Jumping right into the genetically engineered fish, in this case for accelerating growth and as a result reduce the cost of the production, so what we do here, obviously, we take a foreign gene that we biosynthesized in the lab and we

micro-inject into a very early embryo. This is like at a two-cell division, even earlier. We hope that some of the injected genes will integrate into the genome from one copy to just a few copies and as a result, those genes will drive the phenotype that we are interested in -- in this case, accelerated growth. Fishes will just make it faster to the marketplace.

(Slide)

A couple of examples. This is from Bob Devlin's work in Coho salmon. This is not the AquAdvantage. Genetically engineered Coho salmon.

Bob Devlin in 1994 and on used a construct that is an all-fish construct like is the aquaculture one that has a sockeye salmon, metallothionein promoters, or promoter that is --- that keeps driving the expression, the engine, that keeps driving the expression of the gene, and this case it drives the sockeye salmon. Grown salmon -- and here you can see -- this is from Devlin's ***Nature*** 1994 paper -- how much faster the genetically engineered fish grow compared to the control ones, the farmed ones, and those are fishes at 1 year of age. And here you can see a little bit more quantitative, that fish that make it to about 2-1/2 kilos, 5, 6 pounds, in about 2 years, compared to the control, much faster.

(Slide)

This slide is taken from Choy Hew and Garth

Fletcher, very early study in 1997. It is like the earliest version of AquAdvantage. And again, you can see the phenomenal faster growths in the transgenic fish, or genetically engineered fish, compared to the control.

Now if you look at this, if I look at that -- you know, I have been involved with aquaculture for 35 years, and, you know, it is an industry that must grow, as I said, and it is an industry that is struggling, and if I look at that, and I think about the benefit of this offer to the industry or to the fish farmer, there is no doubt in my mind that this type of technologies need to make it to the industry, especially that you can use the same technology, transgenic technology engineering, for additional traits.

You can produce fish and there is work -- Tom Chen is a pioneer in this area, the University of Connecticut. You can use this technology to develop fish that are resistant to disease. Disease costs aquaculture billions of dollars annually. You can use this technology to make fish that have a better environmental tolerance.

Those of you who know the aquaculture industry in the state of Maine or in New Brunswick, Canada, they know that the winters in many cases are too cold; the fish succumb to -- it is too cold. They stop growing, they die, their immune systems become compromised. They are very susceptible to diseases and pathogens and so on. Well, we can do things

here. Increase reproduction, reproductive efficiency, increase a nutritional value.

By far, the most farmed and consumed fish in the world are the carps and the catfish, and those are fish that are from freshwater. They don't carry the Omega-3 type of benefits that come with the marine fish. And there is a lot of work going right now in trying to do some genetic engineering to have those freshwater fish carry the right nutritional value. Those are herbivorous fish. They use less fish meal, less fish oil. And if we do it right, they can produce the right health benefit.

And we can also develop, and there is work in this field, fish that are like bioreactor. They produce -- they over-express vaccines and then you eat the fish and you get vaccinated.

And I don't know if you know, but there is already one genetically engineered fish that made it to the market. It is a zebrafish that was genetically engineered to fluoresce, over-expressing the genes for like green, red, yellow fluorescence proteins, and -- but that is the coral industry, the ornamental industry.

(Slide)

So I think, my opinion -- it is my opinion based on my experience -- that this technology is going to make it to the aquaculture industry, but, but -- and there is a big "but"

here -- it has to have all the right safety measures. Everything is going to be discussed here, earlier everything that we heard like 20 minutes ago, so when we consider introducing transgenic fish for growths and other traits, we have to address public risk, public concerns, food risk, food safety, risk assessment, environmental risk and so on. I think that we have to use all fish constructs and I think that that is obviously what the AquAdvantage and others have been doing.

We have to calibrate the transgene to the physiological range, very important. We don't want to over-express a hormone in like pharmacological level. And I will get back to it in a second.

We want to make the transgenic fish sterile, and that is what is proposed here for AquAdvantage. But I don't think this is enough. I think that we want to contain genetically modified fish in land-base, resituating in biosecure, aquaculture systems. I will talk about it in a minute.

And then, obviously, as is the case here, we want to conduct detailed risk assessment studies both for like consumer, the environment, the health of the fish, the health of the consumers and so on.

(Slide)

Now, back to that calibration theme. That is very

important. I borrowed another slide from Bob Devlin, Coho salmon. Obviously -- and this slide looks complicated but it makes two important points.

Obviously, when you are introducing a foreign gene, in this case for growth hormone, you are manipulating the endocrine in the hormonal system that is responsible for growth. So, what Bob Devlin did here, and I know the same was done for AquAdvantage, they went and measured the levels of the right hormone, growth hormone, IGF1 and T3, in the blood of the fishes, not -- and this is not in the edible tissue; it is in the blood of the fish -- that were genetically transformed.

And what you see here, that although there is, as you expect, in the blood circulation of the fish an increase in the levels of the right hormone which are responsible for the phenotype that I just showed you, all these levels, although increased, remain very well within the physiological level. And this is what I am referring to as “calibrating” your transgene. Point number 1.

Point number 2. If you compare these increases to what you see in domesticated Coho salmon that for generations have been selected -- selectively bred for fast growth, it is not different -- it is the same.

So the increase that you observe in the hormonal level in genetically modified fish is similar to that you will

be observing in domesticated fish that did not undergo genetic engineering, just selective breeding. And we have been eating selectively bred domesticated salmonids and other fish, including Atlantic salmon, for many years.

So those are the two points that I wanted to make in this slide.

(Slide)

Now, switching gears now to my last topic, the one that addresses aquaculture in the environment in the context of this risk assessment, environmental risk assessment, again, same slide showing how most aquaculture practices are being conducted right now around the world. And the issues are that -- not to say “pollution” -- chemical interaction. There is a lot of kind of organic matter runoffs, if you will, from the cages into the environment that may harm the environment.

Biological interactions are the escapees. Given if -- when you have an Atlantic salmon growing in the Pacific Northwest and it escapes from the cages, by definition it is a non-native species. So there is a biological interaction and there is a disease transmission that aquaculture is being blamed for disease being transmitted from the farmed fish to the wild fish.

But I, as somebody who has been working in aquaculture for so many years, I am saying, you know, there

are two sides to the coin here. And the flip side of the coin are those practices that are really not good for the fish, either. I said in the winter the conditions are too cold in many cases. Disease goes both ways, by the way, and so there is a lot of sub-optimal condition. These fish are exposed to all kind of pollutants, toxins that are in the water, harmful algal blooms which is a big problem, and so on.

(Slide)

So for that reason -- and obviously, I don't think that these practices has much of a future for everything that I say but also because obviously some groups that are concerned about the environment, Greenpeace, will continue to try to do everything to avoid these practices from developing. And actually, in the United States, if you want to grow fish in cages, you cannot do it anymore; it is very, very difficult.

(Slide)

So for that reason, my group and others around the world have been developing alternative ways of sustainable marine aquaculture and it is all about land-based technology, fully contained, resituated land-based marine aquaculture, and it is -- you can start with city water, you make your own seawater, or if you have a pristine source of seawater in a well or something, you use it. And it is all about microbial communities and this is where the biotechnology that remediate

the water. They remove the waste produced by the fish and the water cycles again and again and again.

And obviously, those systems that can be -- that are land-based generate no pollution, they are disease free, they are very clean and produce a very clean product, as green as you can get a fish. They are very flexible because you can tailor the temperature, you can tailor the water chemistry, you can tailor the duration of the day and so on and so forth and in substance are generic.

In my mind, the key word to success in aquaculture is diversification, and you diversify in this fish. And in the context of today's hearing, they are biosecure. They are completely biosecure. They are fully contained. And in my mind, those are the only systems that should be allowed, or approved, to grow genetically engineered, and by the way, no native species as well which we do now by virtue of the full containment. They are applicable for rural and urban location.

We in Baltimore developed like the urban marine aquaculture operation in the city of Baltimore. They don't need to be by the ocean, they don't need to be by the coast; you can make your water. They can go and be placed by the airport or by the fish market, and as such, they have also reduced carbon footprint.

(Slide)

Just showing you some nice pictures about these technologies, so those are kinds in our operation, relatively small, but it is -- this is in a basement, so it is your aquaculture in a basement or in a warehouse, 12 foot in diameter but 3,200 gallons. Excellent quality of water.

People say that those systems that I think should be used for AquAdvantage are not economically feasible. Well, they are, for a few reasons. Look at the quality of the water. This is gilthead seabream, high value marine fish. High density, so they grow dense fish, and density is much higher than what it is in the cages.

(Slide)

Look again -- this is harvest size for this gilthead seabream ***Sparus aurata*** marine fish. Beautiful water. High quality fish.

(Slide)

Again, look at the quality of the water, and this water has been -- it is the same water, for nine months. These fish are nine months old at market.

(Slide)

And this is another reason why those systems are absolutely commercially feasible. Well, maybe the capital initially is more, but, I mean, this is what it takes for this fish, the gilted seabream, we have been working on to grow to market side in the floating cages. It is about 410 grams, a

little bit under a pound. In 19 months, in our system, because we tailor everything to the requirements of the fish to allow optimal performances, those fish make it to the market size in about 8 months. So people tell you, well, they are not commercially feasible. I think they are. There are all kind of tradeoffs that we are talking here.

(Slide)

And people tell you this system is very energy consuming, lots of pumps and this and that. But what we do, there is a lot of sludge, organic matter, that is produced by the system that we do not want to release to the environment, obviously. So again we went into marine biotechnology and we used methanogens, marine microbes, on the marine environment. Those are all beneficial microbes, by the way, that are existing in the marine environment that convert the sludge to methane, to natural gas, that we capture to offset the energy cost of the operation. And here you can see where I have just --- right of the fish tank and we can start a generator right off the fish tank. So this really reduces the cost as well make sure that the technology is completely environmentally sustainable.

(Slide)

Diversification I mentioned. So this system allows you to diversity for species, cobias and other fish that has been fished out that we are working on. Bronzini, the

European sea bass, is another fish that is high value, that we have been working. You can grow crabs, and we have been doing that, the blue crab of the Chesapeake Bay in this system. And now we have some funds to work on oysters in subsystems. So you can diversify, which is a huge big benefit.

(Slide)

And this is my last slide. Very recently there have been a lot of data coming from Steve Sommerfeldt at the Fresh Water Institute in West Virginia and his group showing that Atlantic salmon can absolutely be grown in a full contained system. He does it in freshwater. In seawater, Atlantic salmon may perform a little better, but still they perform real well, high densities, huge -- very good, huge densities, very good quality of water. This is from Steve's tank. They make to commercial size. And here they make it in about a little bit under 2 years to harvest size of about 2 kilo, 4.5 pounds. Very beautiful fish. Maybe a little bit under what it takes in the floating cages.

So my bottom line message: I absolutely see in it AquAdvantage Atlantic salmon and other genetically modified fish would be only grown in these type of systems.

(Slide)

I leave you there. This is our -- the Center of Marine Biotechnology that now is called actually the Department of Marine Technology at the Inner Harbor of

Baltimore.

You are all welcome to visit. There is a lot of neat work going in aquaculture biotechnology over there.

And thank you very much for your attention.

DR. DUNHAM: Thank you very much, Dr. Zohar.

I will turn over to the Veterinary Medical Advisory Committee and ask if you have any questions of Dr. Zohar.

Committee Questions and Answers

MR. JAFFE: So I had a question. You said that about I guess 60,000,000 tons of fish come from aquaculture every year. And I am curious: What -- how much of that comes from inland tanks versus ocean ---

DR. ZOHAR: Very little.

MR. JAFFE: Very little?

DR. ZOHAR: Well, most of it actually comes from ponds. I mean, by far the major farm species are cod, in China. China produces maybe 80 percent of the entire aquaculture production. So, by far, you know, it comes from ponds.

But all the marine fish, and the marine fish is not much, you know -- this may be, I would say, in the 15, 20 percent range of these 60-some million tons of marine fish, including salmon. And by far, they are coming from floating net pens, floating cages.

The recirculating land-based tanks is a recent

development. Several of us in the area want to make sure aquaculture is going to be environmentally responsible, and this led to the development of this technology that in my mind, in 20 years from now, are going to replace the net pens.

There are places in the world that has already been moving, removing the net pens, the cages from the water, because of Greenpeace, because of environmental concerns and so on. So the whole industry was removed from the water.

Our only alternative -- we have to grow seafood, so we have to do it somewhere. We will do it through these recirculating tanks that are going to be used to grow, in my mind, genetically engineered fish in a way that is completely protected from the environment but also protect the fish from adverse environmental effects as well.

MR. JAFFE: If I can, one other question. You also mentioned -- I know there is a big issue between the wild caught fish and using that as feed for the aquaculture. And is that also the case for the inland facilities that you are talking about? They would still use --

DR. ZOHAR: They do, but much -- well, inland -- well, there is -- I would more refer to it like as freshwater to -- as opposed to marine.

Most of the marine farm fish are carnivorous ones and they need a relatively large amount of proteins and oils and most of them right now are coming from fish that are being

harvested, so like forage fish and so on that are reduced to fish meal and fish oil.

But we are changing it. Now, carp is like -- more like a herbivorous fish or an omnivorous. The same is for catfish. So they need much less of fish meal and fish oil. They still use some, but much less. And shrimp is in between. Shrimp, there is a lot of aquaculture of shrimp, and we are trying to reduce the fish meal and fish oil consumption.

I mean, obviously we cannot harvest fish to feed fish. Right now, the statistic is that about one-third of these like 60,000,000 tons of harvested fish are reduced to fish meal and fish oil and most of this fish meal and fish oil are being used in aquaculture. This needs to change and we are, I think, on the verge of doing it.

DR. ALTIER: I have a question regarding the growth rates of these genetically engineered fish. You showed two graphs from the literature, one for Coho and for Atlantic --

DR. ZOHAR: Right.

DR. ALTIER: -- that was over a certain period of time. Are you aware of data that describes how these fish continue to grow after that time period?

DR. ZOHAR: Not really. I really know -- I think most of the data that I remember published and maybe you will hear more later during the day from AquAdvantage, from AquaBounty.

I think that most of the -- most of the studies done in the world brings it to like the market size, which is between two to three kilograms. I am not sure how it goes afterwards, no.

DR. ALTIER: I have another question, unrelated. What kind of stocking density can you achieve in these closed systems, specifically for Atlantic salmon, either kilograms per square meter -- cubic meter or fish per cubic meter?

DR. ZOHAR: So it is about -- the density that you can reach is about between 80 to 100 kilogram per cubic meter. It is huge. It is about like two-third to three-quarter a pound per gallon. Salmon -- so in general I would say for the marine fish, between 70 and 100 kilogram per cubic meter and in an excellent water quality.

For salmon right now, the --- that I showed you from still some of all the -- it is like partially reused systems. Our systems are like 100 percent recycle. It is the same water for like the whole cycle.

DR. ALTIER: Thank you.

DR. WELLS: One point for clarification. You stated, I think, that 80 percent of some wild caught fish are actually escapees.

DR. ZOHAR: Right.

DR. WELLS: What species was that?

DR. ZOHAR: Atlantic salmon.

DR. WELLS: Atlantic salmon.

DR. ZOHAR: Well, it is -- the estimates are that along the East Coast of the -- most of the --- along the Northeast coast of the United States, up to 90 percent of the fish captured, harvested here, are from -- are escapees from aquaculture. The same in Europe, the same like in Norway or in the UK, like major salmon farming countries. Between 50 to 90 percent of the returns, the fish that you harvest, come originally from cages.

DR. WELLS: And what proportion of those escapees would be originating from an all-female triploid?

DR. ZOHAR: I don't know. The industry has been using more and more these all-female triploid sterile fish but not all the industry has been using it. So I guess a fair proportion but not -- I would say less than 50 percent, probably. Not -- the industry, not all the industry is using those triploid fish.

DR. WELLS: And do you have an estimate of the proportion that would be coming from inland facilities as opposed to floating nets?

DR. ZOHAR: It is very known most of this comes from nets. I mean, there is very little -- the inland facility that the Aqua salmon, by the way, are for the small production, so small -- much -- there is much small production going on over, you know, on land. But those are like the

juvenile salmon before they are being stocked into the sea. And so those are the freshwater phase of the salmon. And there are probably no doubt escapees from there as well. But the vast majority of the escapees are from the net pens.

And again, those are domestic fish. Those are not wild fish. Those are like, you know, selectively bred fish for many years. And the same is, by the way, on the West Coast. There are escapees of Atlantic salmon from Atlantic salmon cages in the Pacific, in the Northwest Pacific.

Okay, no more questions? Yes, one more?

DR. POPPENGA: With regard to aquaculture worldwide, do you see any trends towards more production in countries with less stringent regulatory programs in place with regard to biosecurity or environmental issues?

DR. ZOHAR: There is no doubt that if you want to, in some countries, especially in Southeast Asia, in Central/South America, if you wanted to grow fish in net pens or in floating cages, you can do it relatively easily or in ponds that have like flow -- what we refer to as “flow through ponds.” You can do it much easier than you can do it in some of the European countries or in this country.

Actually, the first country in the world that removed a sea cage operation from the water was Israel in the Red Sea. A whole industry of about a few thousand tons of fish production was removed from the ocean because it is a

pristine quarry fish. So some countries are as strict as the United States but some obviously, yes, I mean China, Southeast Asia, Central/South America. There is a lot of aquaculture development there with much less regulation.

DR. EENENNAAM: Along those lines, could you give some estimate as to what other countries are doing with regard to genetically engineered fish and if there is anything approaching commercialization in other countries?

DR. ZOHAR: Well, I am not an expert in like what exactly other countries are doing. I know that in the EU there is a long -- a lot of like, you know, anti-generic genetic engineering sentiments for whatever what crop it is, whether it is a vegetable crop or an animal crop though there is research and some of my colleagues in the European Union countries are working on developing transgenic genetically engineered fish for different traits like some of the ones that I listed here.

And beyond that, I know that there is research going on in Japan and there is a lot of interest in Japan in genetically engineered fish. but this research, I don't know how it works with like vis-à-vis the industry and so on. And that is pretty much what I know. I don't know much more.

DR. ALTIER: So this is a question about the additive effects or not of genetically engineered fish in your closed systems. You showed the group from West Virginia grew

the salmon in closed systems that had very remarkable gains in weight.

DR. ZOHAR: Right.

DR. ALTIER: Would one expect that a genetically engineered salmon would be even more efficient or would in fact the -- just improved environment of that tank, overwhelm the genetic engineer effect and you would see no change or little change?

DR. ZOHAR: Well, I think they are going to perform so well because in terms of growth. So those were like non-genetically engineered, obviously, right? And they grow almost as well as in the net pens. And they do so -- they do a little bit less, I think, because it is freshwater, it is not seawater. They do as well, or they do, you know, as well as they do because, yes, in --- you can really regulate, tweak, tailor, as I said, the environmental conditions.

So all these tradeoffs you can -- the temperature can be tailored not to go down to those like subzeros or close to zero temperature that you have in Maine or in New Brunswick, Canada.

Food conversion ratio -- food conversion ratio, by the way, is also better in this recirculated system because you have much more control in this tank system compared to just throwing feed into like huge cages.

So for all these reasons, so I think that

genetically engineered fish will perform like much better because, you know, they have -- were transformed to grow faster in this phenotype and they can do very well in recirculated system and actually I think they should only be grown in such systems.

DR. DUNHAM: Well, thank you very, very much and I appreciate the discussion, questions and answers. Dr. Zohar, thank you for an excellent overview of the state of the fishery industry. Thank you.

We will switch computers and we will have the next talk in just a moment. Thank you.

(Pause)

DR. DUNHAM: We have got more presentation before we arrive at our break.

It is my pleasure to introduce you to Dr. Eric Hallerman, who is Professor of Fisheries and Wildlife, Department Head, at Virginia Tech. He is going to talk about Atlantic salmon and risk issues associated with fish. Dr. Hallerman?

Atlantic Salmon and Risk Issues Associated with Fish

by Eric Hallerman, Ph.D.

DR. HALLERMAN: Thank you, Dr. Dunham. Good morning, everybody.

My mandate this morning is twofold, first of all, to acquaint you with Atlantic salmon, including its natural

history, aquaculture and means of genetic improvement, and, second, to provide you with an overview of ecological risk assessment for transgenic fish in general.

(Slide)

Let us start out, if you will, by meeting the fish. Atlantic salmon was named ***Salmo salar*** by Linnaeus, the father of taxonomy, more or less as the fish that leaps. In terms of taxonomic classification, it is a chordate, a vertebrate, a ray-finned fish, a member of the order of family and genus that bear its name.

In terms of morphological characteristics, Atlantic salmon expresses a number of traits that are characteristic of all members of family salmonidae and a number of traits that are characteristic of that species alone. But somehow, to speak of taxonomic classifications and morphological descriptions misses the point.

(Slide)

The Atlantic salmon is an iconic species, if you will, the king of fish. The Pictness people who lived in Scotland a thousand years ago were so taken by it that they incised an image of it in stone. It is mentioned in the Magna Carta. Thousands of scientific papers have been written on it and hundreds of books, and yet, many aspects of its biology, genetics, and especially its ecological adaptations, remain rather poorly characterized.

(Slide)

One of the most salient features of Atlantic salmon is its complex life cycle. Here I will show the anadromous life history that involves migration to the ocean. There are also other life histories. Adfluvial life histories have to do with migration to a large body of fresh water -- for example, an American Great Lake. And non-anadromous life histories involve having the entire life cycle occur in freshwater. In any case, all of these life cycles bear certain features in common.

On the spawning ground, the female digs a nest, termed a “redd,” in gravel. She deposits her eggs in them and the male fertilizes them. The eggs hatch in the gravel and the young fish that hatch, still bearing a yolk sac from aliments remain in the gravel until that yolk sac is resorbed.

They then emerge into the water column where they spend usually one or two years in freshwater. They take on a certain characteristic coloration characterized by those dark spots along the latter line called “parr.”

At some point, as they ready to go to sea, they take on a more silvery coloration and they undergo a range of osmoregulatory changes in order to prepare themselves for life in saltwater. The poled smolts, or juveniles, migrate out to saltwater where they remain for one, two or more years. At that time, the migratory adults return to freshwater to spawn

again. There is some degree of sexual dimorphism -- the males are more colorful; the male also have a modified jaw called a “kype” which they use for fighting for territory and for access to mates.

(Slide)

I simplified just a bit just now. Atlantic salmon males exhibit alternative reproductive strategies. Precocious parr do not migrate. They mature in their first or second year. They are small. And what they do is they sneak fertilizations while the large males and the females spawn, sometimes to great success.

Grilse migrate to sea for one year. They mature medium size and they return to sneak fertilizations also. Parental males migrate to sea where they spend two, three or more years and mature large, and they return. They defend territory and they court females, much like I described in the previous slide. It is key to note that the expression of early maturation is related to growth rate and as such has both genetic and environmental determinants.

(Slide)

It has long been recognized that salmon exhibit a high degree of homing for spawning. Andrew Young, who conducted one of the first mark recapture studies in fisheries, noted as early as the mid-1800s that each river has its own peculiar race of fish and each race finds its own

river with most perfect precision.

Different selective pressures upon life history traits and their respective ecosystems give rise to two phenomena -- local adaptation and, over time, to genetic differentiation among populations.

(Slide)

Atlantic salmon has a broad range. Above, I showed the range of the anadromous from migratory stocks and below, I show the range of non-anadromous, or non-migratory, stocks. It is key to note that Atlantic salmon has declined precipitously through most of the range along these hatched areas in certain regions. It is also important to notice that even within the regions that are shown in the solid red or the solid yellow that a lot of losses have occurred at the population level in local areas within those ranges.

(Slide)

The general decline of Atlantic salmon has been recognized by governments and conservation agencies worldwide. Atlantic salmon is listed on the IUCN Red List in the category of least concern. And conservation actions pertaining to Atlantic salmon at the international level are coordinated by NASCO, the North Atlantic Salmon Conservation Organization.

In Canada, the Inner Bay of Fundy populations are listed as endangered. In the United States, Atlantic salmon are now extinct in most of the rivers in New England where

they once occurred. Atlantic salmon in rivers of Maine were listed as endangered under the U.S. Endangered Species Act in the year 2000. And a recovery plan for the species has subsequently been developed and adopted.

(Slide)

Most of the Atlantic salmon in existence then occur in aquaculture operations. And it is appropriate at this point that we consider aquaculture of the Atlantic salmon. Aquaculture overall yields 69 percent of global salmon production of all species, and almost all of the Atlantic salmon are consumed by humans.

(Slide)

In these next several slides then, I will present an overview of the production cycle.

The freshwater portion of the cycle takes about 12 to 18 months. It involves spawning of the broodstock, and here I show the stripping of eggs from a female. They are then fertilized by the milt of a male.

The incubation of the eggs and the alevins occurs in a variety of different systems. What I show here is the classical heath tray system. When the young fish resorb their yolk sacs, they are transplanted to usually indoor tanks where they go through the -- they become parr and they go through the transition to become pre-smolts. The pre-smoltification often happens indoors but sometimes also in outdoor tanks, as

I show here.

(Slide)

The saltwater portion of the cycle takes 18 to 24 months and usually occurs in marine net pens. Here, I show a photograph view as we would see it from the site, and here I show a line drawing showing a lot of the supporting infrastructure under the water line. This is the one system that is commercially proven.

(Slide)

Other grow-out systems have also been proposed and tried to varying degrees for production of Atlantic salmon. These various production systems pose different combinations of production characteristics. They include ocean ranching, land-based saltwater systems, sunken cave systems, as Dr. Zohar showed moments ago, and recirculating aquaculture systems.

(Slide)

After the fish reach harvest size, which varies a bit depending on the market that you are meeting, whether it is for whole fish, for filet or for steak markets, they are harvested often using a fish pump and they are processed.

(Slide)

From its origins as a distinct sector of aquaculture in about 1970, production of Atlantic salmon has grown rather dramatically to the point where today production is about

1,500,000 metric tons. Leading producers far and away are Chile and Norway, and following at some distance behind them are Scotland, Canada, the Faroe Islands which are up there, the United States, Russia, Tasmania and a variety of other countries.

(Slide)

It is key to note in the context of our talk today, and building on what Dr. Zohar said moments ago, Atlantic salmon production poses its own ecological impacts, notably including locally eutrophication, amplification of past site and disease problems, and genetic impacts of escapees on locally adapted wild populations.

(Slide)

Clearly, cultured salmon are not wild salmon, so to set the context for the talk about genetic engineering of salmon, let us consider the full range of practices utilized for producing Atlantic salmon for commercial aquaculture production.

The most noteworthy, of course, is classical selective breeding, and there are several noteworthy Atlantic salmon breeding programs. The leading one far and away is the AKVAFORSK program that was brought forward starting in 1970 as a collaboration between the Norwegian government and the private sector. Subsequently, it has been privatized as two different companies, AquaGen and SalmoBreed.

Originally, AKVAFORSK used mass selection for growth, but subsequently moved to a state-of-the-art index-based selection program involving within and between family selection for growth, age at maturity, disease resistance, and flesh quality. Their salmon now grow twice as fast as wild salmon with a significantly greater feed conversion efficiency.

Other vertically integrated producers, starting in about the ‘70s and the ‘80s, started their own selective breeding programs. Their salmon are somewhat less high performance than the AKVAFORSK salmon.

Starting about 10 years ago, the USDA, through the ARS, has a National Coldwater Aquaculture Center in Maine that is involved in selective breeding of Atlantic salmon. It is key to note before moving on, though, that virtually all producers use selectively bred Atlantic salmon.

(Slide)

As came up in the previous talk, biotechnology does interface with Atlantic salmon production, first of all, in the production of all female stocks.

The interest in this is that by producing all female stocks, you avoid the precocious maturation of males and the loss of production in flesh quality that comes with that. While female production is achieved by sex-reversing ancestors of the fish that will go into production, what you are doing

is you are producing XX-neomales and crossing those with normal females in order to get all XX, hence all female fish. These fish are in commercial use.

The second important application of biotechnology in Atlantic salmon production has to do with triploidy induction.

(Slide)

It is of interest because it induces reproductive sterility. It is achieved by blocking the last step of meiosis by blocking the extrusion of a second polar body by applying some sort of a shock, in the case of Atlantic salmon, usually by applying hydrostatic pressure. This is in commercial use.

There is one key caveat that I would mention here. The males, their gonads may mature and they may exhibit reproductive behaviors and actually achieve matings, but their young are not viable because they have unmatched chromosomes. Hence, the production of all-female triploid stocks is preferable.

(Slide)

And then the third application of biotechnology is the topic that is before us today, and that is gene transfer. There have been two notable gene transfer experiments involving Atlantic salmon, both of them arising from collaborations between Choy Hew and Garth Fletcher’s groups.

The first involves an anti-freeze polypeptide. Its

transfer was aimed at protecting salmon in super-cooled water from freezing so they come into contact with nucleating ice crystals. Insufficient freeze resistance was achieved in this line of research; to my knowledge, has not been brought forward.

But a second experiment is the one that led to the topic before us today, the transfer of a growth hormone gene into Atlantic salmon. Four- to six-time growth rate enhancement was achieved early in life, such that the production cycle has been cut roughly in half, also, at a 10 to 20 percent improvement in feed conversion ratio. But the prospect of shorter production time, reduced costs, improved efficiency and profitability, it is not surprising that certain aquaculturists are very interested in producing these fish.

That is the salmon that we are talking about today, and I would like to take just a moment to broaden the scope a bit and talk about other gene transfers that are of interest to aquaculture.

(Slide)

A large international effort aims at developing transgenic aquatic organisms. This includes some two dozen different finfishes, at least six crustaceans, and at least seven mollusks.

(Slide)

In particular, at least 18 species of growth hormone transgenic fish have been developed for potential use in aquaculture. But the scope of gene transfer work, as I indicated, is much broader than growth hormone alone.

(Slide)

The many transgenes initiatives include reporter genes simply to understand the action of the promoters that turn on and off these genes and the host and a variety of genes that have to do with traits that are of interest to aquaculturists, including the growth hormone and anti-freeze polypeptides that I have mentioned; free genes that have to do with conferring broad spectrum resistance to bacterial diseases; several genes that have to do with metabolism, perhaps having to do with better utilization of substraits that are not low utilized by non-transform fish, and two genes that have to do with achieving, by transgenic means, reproductive sterility of the fish.

Transgenic fish have also been proposed for use as bioreactors for producing human biopharmaceuticals and as reporters for environmental contamination. This work has gone forward in at least 11 countries.

(Slide)

But the heart of my talk and my charge for the rest of the time that I have before you this morning is to talk about the environmental safety of aquatic organisms.

I will begin by noting that aquaculture production goes forward in a range of culture systems from floating net pens to onshore ponds to recirculating aquaculture systems that offer different degrees of bioconfinement.

Escape from these production facilities is more or less likely, and there are two questions that I pose to frame the rest of my talk. First of all, pick interbreeding with wild populations pose genetic and evolutionary harms to receiving populations. And secondly, that heightened predation, competition or other processes pose ecological harms to receiving ecosystems.

(Slide)

Against this background then, how will I approach defensible risk assessment and risk management for fishes or how will I explain that to you? What I will do is I will answer this question by showing a framework of risk assessment principles and by citing supporting examples from the empirical literature.

(Slide)

This is a very basic description of a generic risk assessment framework. The first thing you do is you identify potential harms, negative outcomes on receiving populations or ecosystems. Then you identify the hazards that might lead to the harms in our narrow context; that would be the transgenic stock itself.

You would assess the probability of exposure, in our case, the likelihood of escape and persistence of transgenics in our receiving ecosystem.

Then we would assess the probability of harm, given exposure to the transgenic fish.

Risk, then, or the probability of harms being realized, equals the probability of exposure times the probability of harm, given that exposure.

(Slide)

Okay, let us apply this approach then to the ecological risk assessment for transgenic organisms.

At the outset, we must recognize that this has to be considered on a case by case basis, starting with the host species, the gene construct itself, and the integration of that -- in other words, the particular genetic line resulting from the breeding of a single transgenic founder that resulted from the integration of the transgene into a particular place in the genome and then the receiving ecosystem.

(Slide)

A well elaborated risk assessment, risk management, for transgenic fishes has been developed and for the full treatment, I refer you to this book that I show here.

I don’t have time to go through this whole framework in a talk of limited scope, so what we are going to do instead is focus on estimating the risk associated with genetic and

ecological processes. We will focus on the frequency and exposure assessment and especially on the consequent effect assessment.

(Slide)

In this context, then, should fertile transgenic fish escape from aquaculture operations, current interbreeding of transgenic fish in wild populations pose genetic and evolutionary harms to receiving populations. I would start by emphasizing that such interbreeding, or introgression, is a risk pathway but not a risk end point. It is not a harm in and of itself.

The possible harms that are an issue here are loss of local adaptation to a locally adapted wild population; reduced genetically effective population size which implies loss of genetic variation, hence loss of the raw stuff by which this population would respond to changing selective regimes in the future. Then the extreme case, the severest harm that could be manifested would be the extinction of the receiving population.

(Slide)

We must assess the likelihood of harm being realized, given exposure. Realization of harm requires occurrence of a chain of events and risk assessment is the estimation of the likelihood of that chain of events occurring.

First of all, we would assess the probability of escape of transgenic fish from an aquaculture facility, of immature fish surviving a sexual maturity in the wild, of encounter between sexually mature transgenic and wild fish, of successful mating occurring, of the offspring surviving and themselves reproducing, and of successive generations of introgressed fish surviving.

(Slide)

That last part of the risk pathway raises the key question: Would the transgene be purged from the population or would it persist?

The key unknown parameter in this context, then, is the fitness of the transgenics and their offspring. Darwinian fitness is defined then as the ability to survive to reproductive maturity and to produce viable offspring.

Well, then, what is the fitness of transgenic individuals?

(Slide)

Empirical observations of transgenics show a number of interesting observations. First of all, the overgrowth of cartilage in some lines of the most growth heightened transgenic fish, including the transgenic Coho salmon that Bob Devlin produced.

Other traits include higher oxygen consumption rate, higher critical oxygen concentration, lower critical swimming

speed, high willingness to risk exposure to a predator in order to feed, lower viability of young, decreased disease resistance, decreased resistance of stress -- and I show here some data from my own group -- smoltification poorly tied to natural cues, and other observations.

These observations collectively suggest that transgenic individuals are less fit than non-transgenic individuals.

(Slide)

The negative impacts of expression of transgenes on fitness have led some investigators to suggest that transgenics pose no significant genetic risks. However, empirical observations of growth hormone transgenics also show heightened growth rate, heightened feed conversion efficiency, larger ultimate size which may convey advantage in terms of securing mates, and increased osmoregulatory ability.

Other transgenic lines may show heightened disease resistance, increased ability to use various substraits, or other traits that may increase fitness.

(Slide)

The weakness of this approach is that trait-by-trait assessments do not assess the integrated phenotype of an individual which evolutionists such as Theodosius Dobzhansky called the “target of selection,” the individual. Especially if there are tradeoffs among fitness-related traits, how then

will we predict the fate of a transgene in receiving populations and hence the likelihood of harm being realized?

The solution is to consider the effect of a transgene expression on the net fitness of individuals.

(Slide)

The net fitness model -- it was developed by Muir, Howard and their colleague -- involves measuring six fitness-related traits concerning critical points in the life cycle. These include juvenile viability, age at sexual maturity, mating success, female fecundity, male fertility and adult viability.

What the model does -- it is a demographic model and attracts the transgene frequency in the population size. And ultimately what it does is it predicts whether the transgene will become lost or whether it will become more frequent.

My colleague Bill Muir is in attendance today and will talk about application of the net fitness approach to the salmon at issue before us.

(Slide)

Now, what the model does then is it, as I mentioned, tracks the transgene frequency. And the issue here is tradeoff between various net fitness components. So here we are trading off between the daily viability of juvenile transgenics and the mating advantage of transgenic males. Depending what that tradeoff is, we have regions and parameter

space where that transgene will become more frequent or where it would become less frequent.

The interesting thing is at certain specific combinations of these net fitness components, you have a zone where the transgene could spread, and because of the loss of juvenile viability, threaten the extinction of the receiving population. That is just as the Trojans thought they were getting a gift from the Greeks but really they were getting Greek soldiers that led to the break of the siege. These are called “Trojan gene effects” because that female fish thought she was getting a very fit male, but instead, her offspring, his offspring, have lower viability, has a Trojan gene effect.

I show here three other tradeoffs among net fitness, components that lead to Trojan gene effects.

I would note in passing that similar predictions were made by a model with a very different approach that was put forward by Phil Hedrick.

(Slide)

Well, what do empirical results say about the net fitness model?

Muir and colleagues are currently measuring all 6 components of net fitness in red fluorescent zebrafish. We are expecting elimination of the transgene with time because early viability issues -- these are the glow fish that were mentioned by Eli Zohar moments ago. There were 20 replicate

mesocosms that were started with hemizygous fish -- in other words, that one copy of the transgene. We expect 75 percent fluorescent fish in the first generation.

What we see with time is that the frequency of the transgene declined; it is now zero in three of the replicates in generations that I don't show here in these interim results.

(Slide)

So what does this tell us?

The model is working as expected. What we are noticing here because of the error bars about these means is that stochastic processes result in variability about the predicted outcome.

(Slide)

It is important before going on that we recognize the limitations of the net fitness modeling approach. For certain key species, few data exist yet to parameterize and run the model. We saw in the last slide that there is variance associated with the predicted outcomes.

Perhaps most critically, the data that we need for natural conditions, especially for conditions of limiting resource availability which is typical of the wild, under fluctuating conditions which is characteristic of the wild, these change selective regimes which are important for affecting the fate of the transgene in the receiving

population, the model does not account for introgression of the transgene into different genetic backgrounds, which is important, just shown by the work of Bob Devlin with transgene rainbow trout. The model does not account for selection of other loci in the genome that might increase the transgenics over time.

This is an area of active research. The upshot is that we have a lot to learn about the likelihood of genetic harm being realized due to the interbreeding of wild and transgenic aquacultured fish.

(Slide)

So to summarize the sequence of slides, could interbreeding with wild populations pose genetic and evolutionary harms to receiving populations?

My own assessment as an individual is that our ability to make quantitative predictions is still rather limited. Risk may generally be low, but it is likely to be a non-zero.

(Slide)

So let us move on then to the second question here. Another area of potential harms could heighten predation, competition, or other processes, pose ecological harms to our receiving ecosystems.

(Slide)

A generic protocol for assessing ecological effects

of transgenic fish before they would ever enter a receiving ecosystem is as follows:

We would determine the potential exposure of the ecosystem to transgenic fish and characterize the ecosystem in terms of the biotic and abiotic components as well as spatiotemporal variation in those components.

We would determine the ecosystem resources and services used by the transgenic fish or potentially contributed by it.

We would identify ecosystem components likely to interact with the transgenic fish.

We would define and prioritize potential harms.

We would design experiments to assess the phenotypic traits and the critical environmental variables.

We would identify factors contributing to uncertainty, and that point, we would predict ecological consequences from empirical studies.

(Slide)

Let us put this in a more concrete sort of a context.

Of the potential ecological impacts that are before us, the two key concerns in my estimation are competition with natural populations and predation of pond natural populations.

Competition with natural populations was first examined in straightforward laboratory studies. For example,

6 laboratory fitting trials of size/mass Coho salmon, 1 transgenic, 1 not transgenic, were conducted by Devlin et al. They would throw feed pellets into the tank and for the first 3 that were contested, transgenics consumed 2.5 times as many pellets as the non-transgenics. Overall, it consumes nearly 3 times as many pellets.

This led to the inference that expression of a GH transgene increased the ability to compete for food. Similar studies have been done with other species and with broadly similar results.

But, of interest, subsequent studies with more elaborate designs have shown more nuance in terms of the inferences that we can get from these sorts of studies.

(Slide)

One such experimental design recognized that food availability in nature is often limited. So Devlin et al. cohabited transgenic and non-transgenic Coho salmon competing for different levels of food. What they found is that the transgenic outfeed the non-transgenics except when food availability was high and all individuals could express their fullest growth potential.

Perhaps most interesting, when food availability was low, the dominant individuals, usually transgenic, directed aggressive and cannibalistic behavior to other fish and they dominated the acquisition of food. All groups that contained

transgenics either crashed -- the numbers became very low or went all the way to extinction -- while groups of non-transgenics exhibited reasonably high survival and the biomass in those particular tanks increased.

The key inferences we reach here is the effect of the transgenics differed with environmental conditions, and more generally, the characteristics of the receiving ecosystem effects are assessment of ecological risk.

(Slide)

Another key ecological risk pathway is posed by predation of transgenics upon natural populations.

Sundstrom et al. evaluated predation by transgenic and non-transgenic Coho salmon upon fry as prey in hatchery and naturalized stream environments. Under the hatchery conditions, the transgenics grew dramatically, larger than the non-transgenics, and exhibit stronger predation effects even after accounting for initial size differences. In contrast, under naturalized stream conditions, the transgenics grew only a little bit larger than non-transgenics and the magnitude of the difference in the predation effects was much reduced.

The subtle inferences we can take away from this, first, is that environment influences predation intensity, and then at least for this particular pathway for these particular fish, laboratory studies may overestimate the predation risk.

But the key inference I want you to take from this is that use of naturalized environments will be critical for obtaining reliable risk assessment data.

And just in passing, I would note that there are other factors affecting risk assessment for the ecological sorts of risks that we have not dealt with very effectively yet.

First of all, impacts of predation can cascade, as we call it, through feeding webs. We call these “top–down” effects of predation.

Secondly, and perhaps most importantly, the scale and frequency of introduction of transgenics into a receiving ecosystem will have large bearing on ecological risk.

And, lastly, aquaculture escapees can outnumber wild fish in some ecosystems. That is, you may have hundreds of thousands of fish in floating net pens. Even a small percentage of them escaping may outnumber the wild fish in nearby receiving ecosystems.

(Slide)

So, returning to the question I posed at the outset of the sequence, could heightened predation, competition and other processes pose ecological harms to receiving ecosystems?

My own assessment of the emerging picture is that, under a range of ecological conditions, there would indeed be considerable risk of ecological harm being realized.

(Slide)

A key point here is that risk assessment cannot be considered in isolation. Risk management impinges on risk assessment.

Recognizing that risk is a product of the probability of exposure times the probability of harm given exposure, we can minimize the risk by minimizing the probability of exposure. That is, ecological risk may be minimizing by culturing the transgenic fish, as Dr. Zohar indicated, under strict confinement, which would include onshore culture and recirculating systems, with the practice of reproductive confinement and effective operations management.

(Slide)

That last piece, operations management, is sometimes overlooked. It is critical.

Some of the key aspects include ensuring that culture activities promote confinement, that we are preventing unauthorized human access, that there is regular inspection and maintenance, and that there should no marketing of live fish, as live sales pose an escape pathway.

(Slide)

So, to place this whole sequence of slides into context, here is my assessment of the stakes of ecological risk assessment for transgenic fish.

Regarding risk assessment, development of quantitative risk assessment is presently incomplete, especially given -- especially regarding the likelihood of harm given exposure to the hazard.

We need more studies quantifying net fitness, especially under near-wild, or wild, conditions.

We need advances in understanding certain fundamental genetic or ecological issues, for instance, the likelihood of outbreeding depression should transgenic and wild fish interbreed, genotype by environment effects, and ecological interactions in the wild.

Regarding risk management, there is the need to demonstrate the effectiveness and economic viability of aquaculture production under confinement conditions.

(Slide)

In a talk of half-an-hour of scope, I can only begin to introduce these topics. So for those that are interested, I will reference some key contributions to the literature.

First of all, regarding Atlantic salmon, this book by the National Research Council and this one edited by Eric Verspoor. Regarding production of Atlantic salmon, this book by Stephen Willoughby, and there are other ones as well. Regarding risks posed by animal biotechnology in general, this one by the National Research Council. And regarding transgenic fish, particularly Atlantic salmon, this one by the

Pew Initiative on Food and Biotechnology and this one that was published by CABI Press. Thank you.

DR. DUNHAM: Thank you very much, Dr. Hallerman. That was an excellent presentation. We will now turn back to the Veterinary Medical Advisory Committee to see if you have questions of Dr. Hallerman.

Committee Questions and Answers

DR. EENENNAAM: Yes. Thanks for your presentation. I was wondering if you could contrast the risks from the escape of the Norwegian salmon that you mentioned that grows a trifle fast; it sounds like a similar phenotype to the fish under discussion today and as compared to genetically engineered fish that can grow twice as fast.

DR. HALLERMAN: Sure. I don't want to sidestep -- Bill Muir will be talking about it specifically this afternoon.

But to summarize what he said, what we -- what the data that we have so far from work that my group has done, though it hasn't covered the whole life cycle yet, is that risk is not very high.

Is it higher than what you would get from a selectively bred salmon? That would depend a lot.

I would not advocate for a moment production of transgenic salmon without confinement, be it physical and reproductive. Simply practiced, that risk assessment -- that

-- rather, practice that risk management, then my risk assessment would be that the risk would be less than conventional selective breeding and production in net pens. Did I answer you?

DR. EENENNAAM: I guess I am just trying to get a handle on unique risks that are associated with a fish that has the same phenotype as has been achieved by natural breeding.

DR. HALLERMAN: Ah, now I am on your wavelength.

The issue here is that the growth hormone transgene is not subject to the same regulation within the fish, the same homeostatic regulation as the native gene. And some of these sorts of traits like smoltification not under the same sorts of ecological controls that you find in the wild fish is a critical sort of a finding.

That is traded off also, though. There are other sorts of indications that the viability of these fish is much less than you would find with the wild-type fish or the selectively bred fish. So it is a trade-off situation. And it is hard for me to answer you in a direct way.

DR. STROMBERG: Is there evidence that fish that are raised in onshore containment, if they escape, do they in fact subsequently migrate up streams and spawn or are they reproductively isolated from the natural population?

DR. HALLERMAN: If they are produced onshore, with

good confinement, they shouldn't be escaping.

But to get to the heart of your question, fish that escape from net pens tend to hang around in the area where they are released. They may or may not join in spawning migrations to join in with other fishes. They will participate in spawning and their young will be less viable. That has been well shown empirically especially in work in central Norway.

DR. WELLS: I am curious about one of the assumptions built into this, and I suppose Dr. Muir will speak to it later, but if you introduced a gene to a population, whether it is a transgene or some allele that is increasing frequency due to domestication and you provided some selective disadvantage, it seems as though that would be selective pressure to change the behavior.

For example, if a female typically would support -- or would choose a larger mate and her offspring don't survive due to this, that perhaps you're selecting for females that choose smaller mates. In most of the models, is the assumption that the female behavior for mate selection is a constant and that that would not be changing over time?

DR. HALLERMAN: The model does not account for selection at other loci across the genome. I mentioned that in that one slide.

But to answer your question more directly, we have a

pretty good empirical line of observations looking at what happens when selectively bred fish interbreed with wild populations. And what you find is that initial loss of fitness -- and remember that these animals may be escaping all the time -- does lead to the decline of many of the receiving populations.

The best examples of that are again in central Norway but there are also other examples in Ireland -- some of Tom Cross's work -- and there is other work coming out of Oregon State University, but that also happened to Pacific salmonids.

My point is that selection doesn't play out very quickly and that that loss of fitness that you get in those first few generations could be critical to a small population that might be facing extinction anyway because of demographic processes.

DR. MATHEW: Do I understand correctly that commercial production basically is focused on a larger female population and sterile males?

DR. HALLERMAN: The practice in aquaculture varies. Many people raise mixed sex fertile individuals.

The state of the art would be all female triploids, yet those fish cost a little bit more at the seed stock stage, so the utilization of those is not uniform across the industry. Did I answer you?

DR. MATHEW: My question really is: Does that production methodology then lead to reduction in receiving -- the receiving population because you have a higher population of females and sterile males in the breeding population now?

DR. HALLERMAN: If they were to do that in commercial culture, would that have less impact upon receiving populations -- is that the question?

DR. MATHEW: (Nodding of head)

DR. HALLERMAN: Yes, it would. The triploids tend not to join the spawning migrations. If they are females, they would not, because their gonads do not mature, hence the source of hormones that would drive reproductive behaviors wouldn’t be expressed and those fish would not join spawning migrations and the ecological effects would be less.

DR. McKEAN: I would like to follow up on that question.

Under standard industry norms today, what percentage -- how well would you reach sterility, reproductive isolation? How many would not make --

DR. HALLERMAN: I have seen data that show that triploidy induction on the scale of thousands of fish that are produced is on the order of 99 percent. It ranges between, say, about 98 percent to 100 percent, but sort of a rolling average as that.

How effective that is on industrial scale of

millions of eggs, I have never seen data to that extent.

DR. McKEAN: Thank you.

DR. ALTIER: So just to continue that question about triploidy -- so are -- a couple of questions. Are triploid females completely sterile or do they have some eggs that could be fertilized?

DR. HALLERMAN: They are effectively sterile. Their gonads don't develop. You have a little ribbon of tissue that histologically you can find, but it doesn't look like an ovary.

DR. ALTIER: So another question. Of the 1 percent or so that fail to become triploid in this procedure, are they in any way, the females, distinguishable at any stage in their life from -- physically from triploid versus diploid?

DR. HALLERMAN: What you could do rather straightforwardly is take samples of their blood and you look at the nuclei of the erythrocytes of the red blood cells and they are half again as large as normal because they have an extra complement of chromosomes. So there is an easy way to check for that. Outwardly, they would look the same.

DR. POPPENGA: I am sort of curious to get your opinion regarding the state of the regulation of these transgenic fish right now. Do you feel that it is adequate as constituted now in terms of an environmental impact?

DR. HALLERMAN: Well, you are asking this while we

are right in the heart of the oversight process. I think I could answer that a lot better after we know how VMAC decides to go forward. Is that an evasion or is that a straightforward answer?

(Laughter)

DR. POPPENGA: Well, that was pretty evasive, but --

DR. KANEENE: I was curious about your net fitness model. You provided some results. Did you have a chance to validate the model?

DR. HALLERMAN: Did I have a chance -- I am sorry?

DR. KANEENE: To validate the model? What data did you use to validate the model?

DR. HALLERMAN: Sure. Well, the two people whose work that I cite are with us today. Bill Muir will be speaking this afternoon and Anne Kapuscinski is also in the room, so I would ask them to answer that question when the moment arises.

DR. KANEENE: Okay.

DR. EENENNAAM: I just wanted to follow up on your comment about differentiating triploids and diploids, although certainly blood is easy. Can you comment on the feasibility of doing that on a commercial scale --

DR. HALLERMAN: Okay.

DR. EENENNAAM: -- and cost?

DR. HALLERMAN: Excellent question. If you are

spot-checking, say, some percentage of the stock, it is cost effective. If you are checking every individual, that adds some price to the cost of every egg of every fry or whatever life stage that you are buying, and that could be critical to determining whether it is economic --- but sorts of confinements that we are talking about.

DR. McKEAN: The confinement discussion that we had earlier was a very focused confinement. We talked about pens or inland ponds. How do you rank that ability to control or confine the fish?

DR. HALLERMAN: If we raised these animals onshore with reproductive confinement with effective operations management procedures, in Panama where even if they escape, they would not be in a suitable ecosystem. That is pretty effective confinement. Compare that to conventional aquaculture where they are in a net pen and where the one thing that is between them and the open ocean is the net -- that is a dramatic difference.

DR. McKEAN: I was wondering about the significance of the eagle in one of your slides.

DR. HALLERMAN: Predation risk -- actually, the eagle isn’t the one that is the most significant in my own eyes. It is more often seals. Seals are very persistent about trying to get in there at that rich food source. And there are applications where they put a second net on the

outside to keep them out.

You can also put nets over the top of the net pens to keep avian predators out of there. Those are reasonably effective.

What you are left with, then, are storms, human error, those sorts of things, and there is sort of a round average over a wide range of practices across the industry and there is a lot of variance about the mean.

A typical sort of loss on the order of a percent is really typical. That can still be a lot of fish. If you have 1,000,000 fish in your facility, that is 10,000 fish that can escape.

DR. McKEAN: And you talk about 1 percent in the fished -- in the penned --

DR. HALLERMAN: Yes.

DR. McKEAN: -- fish as opposed to -- I was primarily interested in inland.

DR. HALLERMAN: By inland, you should have 100 percent confinement. The only issue then is unauthorized entry of humans, and that can be taken care of.

DR. McKEAN: Thank you.

(Laughter)

MR. : Eric, on that note --

(Laughter)

DR. THORGAARD: Eric, I had one more question here.

Could you comment on whether net pen escapes in Maine, has had any impact on conservation issues for the wild Maine Atlantic salmon?

DR. HALLERMAN: You asked a question that has wrinkled many a brow in this town before. I would answer that by pointing out that Tim King and his group have done studies of genetic differentiation of the five Down East river systems and he claims he can still see the signal of aboriginal genetic differentiation among those populations. In other words, despite all of the presumed introgression that has occurred with aquaculture stocks, the wild stocks have persisted.

Has there been an impact? That is hard to say. Have the aboriginal stocks hung on? Miraculously, yes.

DR. DUNHAM: If I may, I would like to thank Dr. Vaughn, Dr. Zohar and Dr. Hallerman for a very excellent presentation this morning.

(Applause)

DR. DUNHAM: And now, if I may, I would like to request that we return by 10:30 so that we can move forward and have again more time for questions and answers. So take a break, stretch, have some coffee and some juice and we will see you back at 10:30. Thank you very much.

(Whereupon a break is taken.)

DR. DUNHAM: Okay. We now have a few more

presentations before lunch. It is my pleasure to introduce Dr. Ron Stotish, who is going to discuss some of the aspects of AquaBounty Technologies for us. Ron?

AquaBounty Technologies

by Ron Stotish, Ph.D.

DR. STOTISH: Thank you, Dr. Dunham. And I would like to thank the meeting organizers, the chairman, and the members of the VMAC committee for allowing me to address this meeting.

(Slide)

AquaBounty Technologies is a technology company. We are science and technologies and genetics. Our strategic intent is to provide molecular-based solutions to the global aquaculture industry. Said more simply, we hope to provide tools for a safe and responsible support of what you heard this morning, the “blue revolution,” the expansion of global aquaculture.

(Slide)

The company was incorporated in 1991 initially to look at some antifreeze protein technology, again in ---. The name changed in 2000 and again in 2004 as we eventually came to be known as AquaBounty Technologies and we went public through an IPO on the London Stock Exchange in 2006. The AquAdvantage technology was acquired from the University of Toronto and Memorial University of Newfoundland in 1996 under

a license from those universities.

(Slide)

You have seen these statistics. And one of the things I was gratified from the earlier speakers, and I want to thank the earlier speakers -- first of all, they had prettier slides than I have, but also their statistics and some of the graphics that they used were far superior to some that I will show you. But I will be able to go through this quickly.

According to the State of the World Fisheries in Aquaculture, a report in 2006, wild capture fisheries have peaked at roughly 90,000,000 tons per year. The aquaculture composition, or contribution, to the world seafood supply has been increasing at roughly 6 to 8 percent per year and reached a total of about 51,000,000 tons by 2006, the per capita consumption, 16.7 kilos for every man, woman and child on the planet from that 110,000,000 tons of seafood that has been produced by both methods.

(Slide)

These statistics led Chairman Omura to say in the meeting in 2009, the FAO meeting on the state of the world fisheries, that they were about to reach a milestone. Nearly 50 percent of the seafood consumed worldwide was to be provided by aquaculture. You saw some of the history this morning.

(Slide)

In 1960, there were 3,000,000,000 people on the planet. That had doubled by 2000 and will double again perhaps by 2050. FAO estimates and other international body estimates have emphasized the fact that both land-based agriculture and aquaculture resources are stressed, as are wild caught fisheries, and without improvements in productivity and efficiency, it is hard to imagine how we will meet the protein needs of the developing population over the next 20 to 30 years. That is particularly true when you think of developing markets where you now have emerging middle classes with a higher requirement for a better high protein diet, and in many instances, the healthy kind of diet that we Americans are used to.

(Slide)

But we are here to talk about salmon, and I borrowed this slide from a marine harvest online presentation and I have a few of their slides here.

(Slide)

You have heard earlier that in 2000 NOAA and the U.S. Fish and Wildlife Service declared the Atlantic salmon an endangered species under the Endangered Species Act. That order was broadened subsequently and in 2009 expanded. It is important to state that there are no commercial wild Atlantic salmon fisheries in the United States.

(Slide)

So where do all the Atlantic salmon that we eat come from?

Well, this is a major cultured finfish. In 1982, there were 13,000 tons farmed, just a bit more than came from the wild caught fisheries. By 2007, that had grown to almost 1,500,000 tons of farmed salmon. Atlantic salmon is about 90 percent of all farmed salmon and it is about 50 percent of the salmon sold in the United States.

U.S. imports -- and this is another important statistic -- 97 percent of the Atlantic salmon consumed here. In 2007, that number was over 450,000 tons of imported salmon.

The nutritional benefits are well known. It is a high protein food, high in Omega-3 fatty acids, a heart healthy food for the American diet.

And I think you have also heard, and I hope persuaded, that aquaculture has a significant role here in meeting the developing population and the growing population’s need for a safe and sustainable food.

There are, however, concerns associated with the present day culture of salmon, and as I said, it is a major cultured species.

(Slide)

Don’t bother with all the small numbers here. The really important stuff is down at the bottom. And this is

simply the USDA/ERS data showing the Atlantic salmon imports into the United States from 2003 to 2009.

I mentioned in 2007, a peak year, over 450,000 tons of Atlantic salmon were imported. Where did it come from? It came from Chile, it came from Canada, it came from the United Kingdom and Norway. Those are the first four exporters to the United States.

About that time, there was a major crisis in the Chilean salmon industry, and as you can see, the import numbers decrease over the next few years. That crisis was an ISAV outbreak in the Chilean industry which has decimated the industry. A similar outbreak occurred in the Norwegian and English and Scottish industries about 10 years ago.

So this is an example of the effect of natural disease in an aquaculture industry and the effect on the economics. That also led to historic high prices for Atlantic salmon in the United States earlier this year.

(Slide)

What are the concerns associated with the culture of the species Atlantic salmon?

The industry, as you have heard, is almost entirely focused on net and/or sea cage aquaculture. There are concerns -- seals and sharks tear the nets, try to get at the fish, fish get out. There is an opportunity for disease transfer, and as Dr. Zohar mentioned this morning, the door

swings both ways. These fish can either be disease reservoirs or they can be susceptible to disease from wild populations. One of the common features that you see in the press, for instance, is the sea lice. They are not really lice at all; they are small invertebrates. But they are particularly damaging to young fish when they attach themselves to the young fish. And they can be transmitted from the wild to the cultured fish.

The impact on the coastal waterways, the environmental quality, the potential for water pollution you have heard a lot about and I won't talk a lot about. But suffice it to say that there are significant issues associated with the production of this quantity of Atlantic salmon.

Last, but not least, the ISAV outbreak in Chile decimated that industry, knocked it down perhaps 70 percent or more, and it still hasn't recovered.

One of the more interesting ones is the -- there is also a simultaneous battle between the wild caught fisheries, particularly on the West Coast, and the aquaculture industry that also resides there. And the mere appearance of the Fisheries Minister from British Columbia in an aquaculture meeting was viewed as highly controversial and was derided publicly in the newspapers.

(Slide)

Land-based aquaculture you heard a little bit about

this morning. It is becoming an alternative that is considered by a number of responsible people in the aquaculture segment. It is a very small industry at present, but after the incident in Chile and the effect on the Chilean industry, this is now being widely considered as a viable alternative. The facilities can be similar in principle to trout production, for instance, that we already have here in the United States. It could be similar to the salmon hatcheries in the facilities that exist around the world already.

The key here is that this offers, as you have heard, a prospect for better biosecurity. It dramatically reduces the opportunity for escape and any impact on biological diversity or interaction with wild populations. And although there are significant issues of cost associated with the use of these facilities, there are also benefits, and significantly a reduction of the transportation costs associated with moving large quantities of salmon, for instance, from the south of Chile or from the north of Europe to consumption centers here in the United States.

AquAdvantage salmon has growth characteristics, as I will show you in a moment, and I won’t show you a lot of data because I have been asked not to, but I will show you that some of gross characteristics of AquAdvantage salmon address and mitigate many of the concerns associated with the

environmental impact and as well as some of the ecological and economic impact of salmon aquaculture.

(Slide)

I won't go through this in a long way. This is again simply showing that the juvenile portion of the salmon life cycle takes up a fair amount of time.

(Slide)

Another slide that I borrowed from Marine Harvest online presentation -- and it is very nice, if you are interested in salmon farming, I highly recommend it; it is basically everything that you wanted to know about the industry. But they show, as an example -- you heard about genetically improved lines from selection programs, genetic selection programs -- even the rapidly growing fish that have been selected in those spend a year or more as juveniles. The more common ones can spend a year to -- and the numbers that were used earlier, so I won't use different numbers -- were a year to 18 months to 2 years as juveniles.

That basically occupies a fair amount of the time for the daily growth of the salmon. The salmon is evolved this way. It is the way its growth is regulated. It is regulated by photoperiod, availability of food, and of course water temperature.

(Slide)

The AquAdvantage salmon construct was produced, as

you have heard, by Choy Yew and Garth Fletcher, and their approach was to address the issue of growth in that early portion of the salmon life cycle.

The gene construct you will hear a lot more about later today, but the gene construct was prepared using the promoter from the ocean pout and a freeze gene, a cDNA from the structural gene for the salmon growth hormone, and that construct was injected into those fertilized eggs.

The progeny were examined and they looked for fish that had incorporated the construct into their genome that were capable of expressing that construct and expressing the growth hormone, had a rapid growth phenotype and were capable of passing that construct onto their progeny -- in other words, that had been incorporated into the gene line.

Since that time -- and this initial event occurred in 1989 for those of you who think this is a new product that has been rushed to market -- the initial fish was constructed in 1989. Subsequent to that, AquaBounty has bred this using conventional genetics over 10 generations, many of which have been the subject of the so-called “durability evaluation” looking for the lack of change, stated in a positive way, the fact that the gene does not change over time and has not changed over these many generations; that the gene is always in the same location; that the gene is expressed and that the phenotype accompanies the presence of the gene. So, this is

an example of a food that has been studied over a long period of time.

(Slide)

Commercially, the fish will be produced again using the techniques of molecular biology by confirming the genotype of the broodstock -- and there was a question yesterday about, “How do you know that the fish that you are breeding has the appropriate genotype?” That can be confirmed with specific molecular assays.

Those fish are selected, and then the milt from those neomales are used to fertilize wild type eggs. The net effect of this is to produce a hemizygous progeny which has a single copy of the AquAdvantage gene construct.

Those eggs are then rendered triploid, and if you have looked at the data that is available on the FDA website, our triploid validation was validated to greater than 98 percent. And if you look at the data in the tables, you see a lot of 99.8 percent in those validation runs.

Before the eggs are released, they are assayed again, using the fluorescence activated cell sorter which basically identifies the DNA content per egg and the triploid, the extent of triploidy, and basically confirmation of the release specification prior to being packed into boxes which would be labeled and shipped via a secure distribution to eventual grow-out facility.

The grow-out facilities will be approved prior to receiving the AquAdvantage eggs and those facilities will be inspected by the FDA and we will have filed an environmental assessment. So this is analogous to distribution of a veterinary drug where you have a release specification for your product, the product is shipped in a labeled -- containing the label and instructions for use of the veterinary drug, and shipped to a confirmed facility that has received FDA inspection.

(Slide)

These are the growth characteristics of the early stage AquAdvantage salmon. And again you have seen data, but if we simply look at 200 grams, the AquAdvantage salmon reaches 200 grams in roughly 150 days. This is data from our hatchery.

The unmodified genetically identical non-transgenic sibling -- in other words, these are sisters -- they differ in a single gene locus, the AquAdvantage locus. It takes 350 days to reach that same 200 grams.

Again, that may not be interesting to the general public, but if you are in the business of growing salmon and you are familiar with their life cycle, that is an important distinction.

(Slide)

Looking at the grow-out period, the time beyond that

initial growth rates, you can see that the growth rates eventually begin to normalize as the fish get larger. And the real growth benefit in AquAdvantage salmon is in that really first year of life for that growth to that first 1,000 grams of kilo weight.

And again, picking a point, 2 kilos reached in something less than 500 days and nearly 700 days for the genetically identical except for the AquAdvantage gene construct.

(Slide)

These are the fish. The fish -- these are sisters. The fish in the foreground is approximately 200 grams; the fish in the background is nearly a kilo. These fish look like unmodified Atlantic salmon. People who see them think they are very attractive fish. We happen to think they are lovely fish, but we have done a lot of work with these fish over the years.

(Slide)

The fish is, of course, being regulated under Guidance 187 which you heard about and I won’t take a lot of time because Larisa is going to talk about that.

(Slide)

I will tell you we have a very precise product definition for this product. It is a triploid hemizygous all-female Atlantic salmon. And it has a single copy, a large

gene construct at a very specific location in its genome. We have evaluated this construct and the stability of that genome over now 10 generations.

The claim? Again, again not very interesting, and you won't see it advertised even on late night TV because significantly more of these Atlantic salmon grow to at least 100 grams between -- within 2,700 degree days than their comparators. A degree day is simply the temperature in degree Celsius times the number of days -- 10 days at 10 degrees would, for instance, be 100 degree days. Again, not exciting to the average person, but if you are in the business of growing salmon, that is an important distinction.

Limitations for use? And again, this is critical. These Atlantic salmon are produced as eyed eggs for grow-out in only FDA approved, physically contained, freshwater culture facilities. These are offered for sale in labeled containers through secure distribution analogous to the delivery of a veterinary drug. They are sold only to producers who have FDA approved facilities.

(Slide)

The salmon was originally developed as a superior production animal. We realized sometime after the initial studies that this rapid growth phenotype conferred opportunities for other cultivation systems, like land-based cultivation, that made it superior in other respects to the

unmodified Atlantic salmon.

It can address some of the concerns that we have talked about, and you have already heard a lot about them so I am not going to talk about escapes, I am not going to talk about water quality, I am not going to talk about environmental impact on coastal waterways and so forth. You have heard all of that. But what I would propose, or posit to you, is that AquAdvantage salmon, because of its growth rate, represents an economically viable, environmentally sustainable solution to some of those issues.

The growth rates, because it grows more rapidly and because it is more efficient, can reach harvest weight in about half the time. That very fact allows it to be grown economically in a contained production system. You can grow unmodified salmon in a contained production system, as you heard this morning, but this fish dramatically alters the economics and makes those systems even more appealing and more attractive.

Along with that contained production, you have reduced disease exposure in both directions, as Dr. Zohar said; no interaction with wild populations or ecosystem; no -- in principle -- no affected biodiversity.

And an aspect that we haven't talked much about: You have the opportunity for regional production of a fish that is today grown in very remote locations either in the

south of Chile or in the North Atlantic. Because this fish can be grown in contained facilities, it can be grown closer to population centers. We can bring an industry back to the United States that we lost nearly 20 years ago for environmental and economic reasons. Implicit in that is we could have a sustainable source of high quality seafood protein closer to populations and, in essence, fresh fish closer to the cities where the fish are consumed.

This clearly offers the opportunity of an environmental footprint because in addition to reducing the transportation cost -- and an example would be flying salmon from the south of Chile costs roughly $1.50 to $2.00 a kilo in transportation cost. But that is not the only cost. There is a huge cost associated with the environmental footprint, of flying those 747s from the south of Chile to the New York market, for instance.

(Slide)

By growing these fish locally, you have the benefit of that fresh and sustainable seafood product, but you have it without that environmental footprint and with a reduced transportation cost.

So therein lies the possibility, and this is one of the benefits of this technology -- again, I won't go over all of the slide. We at AquaBounty do not believe that this solves all of the world's problems in food security and all of

the world's problems in biodiversity. But we do believe that this is an example of the application of a technology that can address many of those problems and can do so sustainably.

With that, I will yield back to the Chair.

DR. DUNHAM: Thank you, Dr. Stotish. To the VMAC now, do you have some questions?

Committee Questions and Answers

DR. EENENNAAM: I will just follow up on your comment -- oh, I am sorry; Alison Van Eenennaam -- your comment that fish could be grown closer to the population centers. At the moment, the proposed package we are looking at is to grow them in Panama.

Could you discuss a little bit about what your thinking is with regard to a company as to where they might be grown in the future and how additional facilities that might come on line would go through the permitting process to show that they have security for the fish?

DR. STOTISH: I am sure Dr. Dunham and Dr. Rudenko have some thoughts on this, too, but let me address your question; it is a very good question.

In one of the features of applying for a New Animal Drug Application, and in this particular instance, as you heard yesterday, was -- they are basically interested in the entire life stage of the animal, including its effect on the environment and its -- the ability to produce this fish

safely. We established the location that is listed in the environmental assessment in Panama as an initial production facility to demonstrate that, number one, you could grow this salmon outside its normal geographical range; number two, this fish could be grown safely in containment; and also to demonstrate that was a -- not only an economic development opportunity for a lot of countries, including the United States, but that this fish can now be grown closer to those population centers, as I mentioned.

Our view, and I started this talk by saying that we are a technology company, we are a technology and genetics company. We do not wish to be the next Marine Harvest. We do not want to be the world's largest salmon producer. We want to supply technology and solutions to that industry.

But we had to establish that the safe -- and demonstrate the safety of this product in cultivation. We believe that we will be able to have facilities inspected and complete EAs and file applications to the FDA beyond the initial approval, should an approval be granted, that would allow the use of other facilities. And we believe that those sites, not unlike drug manufacturing sites, will be inspected and certified and acceptable and periodically inspected by the Agency.

Now having said that, I have told you the company is here. Perhaps the Center would like to comment.

DR. DUNHAM: That is true. If there is going to be additional requests, they would come through as supplementals. They will be reviewed. This particular focus is the claim that you are going to hear and location and that is the only thing that we will be reviewing. The companies, as with any drug company, can come back and supplement. We do have to go through review. We do have to do inspections for any future facilities that they request.

MR. JAFFE: As sort of a follow-up to that, so, the application that we are looking at -- oh, sorry, Greg Jaffe -- the application that we are looking at today is for the inland facility in Panama. My understanding is that is four tanks or outdoor ponds or tanks or something. Can you give me an idea of what the capacity is? I mean, how much salmon will be imported to the U.S. from those? How much is going to be grown on a yearly basis harvesting?

DR. STOTISH: Well, the capacity from that location would be of the order of a few hundred tons, for instance. The kinds of facilities that we are thinking will be constructed in the United States and other locations are perhaps of the order of 2,000 tons, consistent with the sort of systems that Dr. Zohar talked about earlier this morning.

And I would also add that their physical containment, as Dr. Hallerman pointed out -- he made a very important point and I will take this opportunity to emphasize

it -- physical containment. We are biologically contained and we have redundant biological and physical containment. Physical containment can be reached with a variety of facility designs and the FDA and the CVM in this instance will inspect those facilities to assure that they have not only suitable design but suitable operation.

And the last key that Dr. Hallerman mentioned is the management component and restriction of those sites from unauthorized personnel, so --

But the facility design aspects, the capacity of those facilities and so forth, will really be in the hands of other people and then will be reviewed by the regulators.

DR. RUDENKO: Ron, I would like to add something in response to your question, Greg.

We are focusing on one application with one set of conditions. It is nice for AquaBounty to talk about their future business plans. You are not evaluating those future business plans. We are not evaluating those future business plans. We are evaluating one set of conditions with specific conditions of use for this one approval. Should other proposals be brought forward, they would require a complete supplemental application and complete review and inspection.

So I would urge you to concentrate on this particular application and if you want to talk with Ron about his business plans, I am sure he would be happy to share them

with you.

DR. STOTISH: Dr. Rudenko always has the last word. But I agree with her.

(Laughter)

DR. WELLS: Kevin Wells. I am curious about in this specific location, in this specific application, how -- what your plans are for either maintenance or introduction of genetics? Is the plan essentially to have a closed, genetically closed, population and all further improvements will come out of this population, or will there be -- are there plans for this facility to introduce new genetics from other efforts within your company or elsewhere?

DR. STOTISH: That is a very good question, and I should have been more clear.

Our hatchery is located in Prince Edward Island. The eggs are produced and shipped from that location. Our genetics program resides there. The grow-out locations are simply for grow-out.

So we are beginning programs in genetic evaluation, and many other programs. For instance, we think that the data that we have on feed efficiency understates the efficiency of the salmon. So there are a variety of aspects that we are continuing to study as a part of our genetic improvement programs.

The key is that the way that we are producing these

fish, we can introduce the AquAdvantage gene, as you see, from the neomales into a variety of other lines, and those fish can be very well characterized and selected for subsequent use.

In principle we could, as people talked earlier, disease resistance, flesh quality, other aspects. It is very compatible with other elements of genetic improvement of conventional selection process.

DR. MATHEW: Alan Mathew. To follow up on Kevin's question, if other genetics are going to be acquired or developed maybe even through classic cross-breeding with the recombinant DNA gene, does that require further review by the FDA?

DR. STOTISH: Again, I will defer to the Center.

DR. RUDENKO: In general, the Center does not regulate animal breeding. For these particular fish, if we -- we would ask that AquaBounty would come to us and consult with us on what their plans were as part of the overall product development and if we felt that there was any particular concern with respect to safety or effectiveness, we might ask them for additional data. But in general we do not regulate breeding.

DR. STOTISH: And I would only add -- and I think that is the appropriate answer -- but I would only add that the firm both has a commitment to product stewardship as well as durability, and remember that we are following these

animals and we are looking specifically for effects positive or negative, and we have a durability commitment so that there is a way to monitor our production and the introduction into other lines on a continuous basis. So this product will basically be in continuous regulatory lips.

DR. SENIOR: David Senior. Along those lines, is it the intention of the company to assist your ability throughout the production process? Is that something that would go on forever?

DR. STOTISH: It -- that is a part of our approval, and again as the Center talks about durability, and they will this afternoon, the firm has discussed with the Center how we will handle that. We have a detailed program in place so not only the production of the fish but also following the fish commercially after they are produced. So the durability program we believe is very robust and is unprecedented in -- out of aquaculture.

DR. SENIOR: But the monitoring would be forever?

DR. STOTISH: Yes -- right, at the moment. Perhaps at some time we could go to the Center and say we have been doing this for a lot of years.

But there is a reason that the company would want to maintain a strong, viable durability program along with the genetic improvement program. So that aspect will go on. Whether or not there will be a regulatory requirement, who

knows? That depends on our experience, that depends on the data that we can provide, and depends on, you know, whatever discussions we have with the FDA.

DR. RUDENKO: The regulatory program would go on for the life of the product ---

DR. SENIOR: So --

DR. STOTISH: There she goes again!

(Laughter)

DR. SENIOR: One more thing. Your grow-out studies presumably were done at PEI. How many grow-out studies with this particular construct are being done in Panama?

DR. STOTISH: Really, one. We did work in the hatchery, which is sub-optimal because the water temperatures there are cooler than optimum. But we are having a very positive experience in our trials in Panama right now.

DR. SENIOR: Is the Panama facility already -- was it used previously for some other ---

DR. STOTISH: That is a grassroots facility. It is on a location near another trout production facility. But I know this is a grassroots facility that we constructed. And there are a variety of reasons why we chose that location but it is ideal for growing Atlantic salmon.

DR. SENIOR: Thank you.

DR. THORGAARD: Are your -- I am curious about the origins of your stock. Are they Canadian, Canadian origin

salmon? And I was wondering whether there has ever been any head-to-head comparisons with the selectively bred Norwegian salmon versus the AquaBounty salmon?

DR. STOTISH: We have introduced the construct into a variety of different lines, Norwegian and Canadian. Most of the work that we have done is done with the St. John's River strain which is a popular commercial breed in Canada. But we have introduced this into a number of lines.

And the performance of the construct is very consistent. And the controls are built in. You have non-transgenic siblings that lack -- they differ only in one locus. And so you have a built-in control.

So the kind of question that was asked this morning about growth rates in freshwater or growth rates at all, we always have built-in controls. We have genetically identical controls that we can compare against. Whatever background we have introduced to this construct, then we have always seen that consistent -- rapid growth in early life stage.

DR. DUNHAM: That question was from Dr. Gary Thorgaard.

DR. STOTISH: Sorry?

DR. DUNHAM: I was just saying that question came from Dr. Gary Thorgaard, so they have it for the record.

DR. STOTISH: Yes, yes, yes.

DR. DUNHAM: Seeing no further questions, thank you

very, very much, Dr. Stotish.

DR. STOTISH: Thank you.

DR. DUNHAM: We appreciate that. All right. Now we will move forward and Dr. Larisa Rudenko is going to address regulation of GE animals at FDA.

Regulation of GE Animals at FDA

by Larisa Rudenko, Ph.D.

DR. RUDENKO: Hi. We have heard from our Center management, you have heard from the Commissioner's office, you have heard from the Office of New Animal Drug Evaluation, you have heard from our two distinguished speakers, you have heard from our sponsor. This has all been a big set-up for our presentation on how we are going through the process of evaluating the data and information that AquaBounty has submitted to us as part of their application.

For those of you who were here yesterday, this will be a little bit of a repeat, but repetition often breeds familiarity, if not understanding, so I am hoping that that will indeed be the case.

I will go through things relatively quickly because each of our experts who will be introducing themselves and their expertise will be able to tell you about the kinds of data and information that they have evaluated and the outcomes of their reviews. I am just here to give you a little bit of background to what we did and why we did it.

(Slide)

So, one more time, in case you haven't gotten it so far.

Why are we here today, okay?

Genetically engineered animals are reaching commercialization. You know we have already approved one; that was a biopharm animal. There are lots of other genetically engineered animals, including fish, in development, in various stages of research and development.

When applications come to CVM, we evaluate them. You have heard, of course, that we are regulating these fish under the New Animal Drug provisions of the Federal Food, Drug and Cosmetic Act and the applicable provisions of the National Environmental Policy Act as well.

Guidance 187, which has been posted on our website, clarified the statutory authority under which we are operating and provided some recommendations for industry as to how they can submit data for us to evaluate.

The AquaBounty Technologies has an application in front of us. They have completed their submission of the major components that have to do with the assessment of safety and effectiveness. The entire application is not yet complete. There are still some administrative things that need to be addressed, but the safety and effectiveness data has been submitted and has been evaluated.

My job here today is to refresh your memory about the methods for review that we have in place, introduce the scientists who are going to be talking about the data and our analyses of that, and to remind everybody one more time: FDA has not yet made a decision on the approvability of this application. We have not yet made a decision.

We are here to hear advice and recommendations from the Veterinary Medicine Advisory Council. We are looking forward to their discussion.

As I told you yesterday, we are not asking for a vote. We are specifically not asking for a vote. We are specifically asking for good, constructive conversation. We want to learn from you what you think about the way that we have reviewed this and if you have any additional ideas for us.

We are here to hear the same from the public. We want to know if there are any additional data that we have not evaluated that are pertinent to this particular application and any thoughts that the public may have as well.

(Slide)

So, you have all met the fish before, the genetically engineered Atlantic salmon. It is intended to grow faster and it is intended for food use. The fact that it is intended for food use makes this an historic event for the agencies agency [sic], the first time a genetically engineered

animal has been considered for approval.

(Slide)

Ron just told you a little bit about their production plan. The broodstock and triploidy induction will be performed in Canada. Eyed eggs will be shipped to Panama. Grow-out and slaughter of the animals will occur in Panama and food will be exported to other countries -- used in Panama, exported to other countries and possibly the United States.

(Slide)

So, just one more time. We regulate under the New Animal Drug provisions of the Act because the recombinant DNA construct meets the definition of a new animal drug because it is intended to alter the structure or function of the animal. That means it is -- the regulation must cover all of the same kinds of requirements that a conventional new animal drug must do.

This pre-market approval prior to introducing these animals into commerce in the U.S., it covers all of the GE animals that will be made in this process, and in general, it covers all GE animals that are made for any particular use.

We take this from pre- to post-market regulation, and as I so sadly informed Ron a little bit ago, all of our durability requirements go on for the entire life cycle of the product. While it is in commerce, we regulate it.

We use a risk-based approach to do this.

(Slide)

So, we use -- as I explained to you yesterday -- an event-based review to address risks. An event is the result of the incorporation of the rDNA construct into the genome of the animal. If you were to introduce the very same construct into an animal a second time and it ended up one nucleotide away from where it went in the first time, it would probably be considered a separate event.

Each GE animal rDNA construct product pair poses a unique risk because of the potential for insertional mutagenesis and any other downstream effects, so each one requires a specific set of risk questions and a specific set of data and information driven responses will come from the Agency for that specific set of risk questions.

We do case by case evaluations. We do not do programmatic assessments. We don’t do a programmatic environmental assessment on what would happen if Atlantic salmon were released in the Bay of Fundy if that is outside the scope and product definition of this particular application.

So therefore, the conditions of use as set forward in the product definitions are critical, and that is why I cautioned you folks that you may have all the conversations that you would like with AquaBounty about their future business plans but this application considers this set of

conditions of use and any other conditions of use would not be considered appropriate or lawful.

(Slide)

So let us talk a little bit more about hazard and risk.

Dr. Hallerman told you a lot about hazard and risk when he was talking about risk assessment that is associated with things. And again in the period of repetition aids in familiarity and understanding, let us very quickly review that harm is an adverse outcome, is defined as an adverse outcome. A hazard is a substance or an activity that has the potential to cause a harm. It is not the same thing as a risk. A risk is a conditional probability of an adverse outcome occurring provided that exposure of a receptor -- and a receptor is an individual or a population that might experience the adverse outcome-- has occurred.

This is the definition in the National Academy of Sciences report on animal biotechnology published in 2002.

So risk is some function of exposure and hazard together, or the likelihood of harm. It is a probability.

A receptor, as we said before, is the individual, or population, experiencing the risk.

So, one more time I am going to give you the same analogy that I gave you yesterday:

A harm is something that could cause an adverse

outcome to occur. If there is ice on the sidewalk in March, the hazard is the ice. The harm is that you might slip on it and break a leg, okay? The risk is if someone is walking down the street and slips on the ice and breaks their leg, that is the risk.

That risk can be mitigated in several ways. You could cross to the other side of the street and avoid the ice entirely. You could put rice or sand on the ice and therefore decrease the slipperiness of the ice and sort of decrease the severity of the harm.

But it is not actually a risk until a receptor -- klutzy me -- walks on the ice and falls and experiences the risk. The fact that there is ice on the sidewalk is not a risk. It is a hazard, okay? I want to make that very clear.

And our safety standards, as Laura Epstein told us yesterday, our food safety standard is reasonable certainty of no harm, which is our established, very high food safety standard. And for animal safety, it is a balance of risk and benefit for animal health.

(Slide)

So what do we mean by risk-based when we do an evaluation of one of these animals?

Well, the first thing is we clearly need to distinguish between hazard and risk.

I just bored you with telling you the difference

between somebody falling on a piece of ice and a piece of ice, okay? There is a difference. Some things are hazards, other things are risks. You have to have a hazard in order to have a risk. Not every hazard results in a risk.

We need to break the overall determination into steps to be able to consider it all individually and then wrap them all up again and look at it in its totality. We have to ask the appropriate risk questions for each step of the analysis, and each of the scientists will go through those with you.

And at the end of the day, we use a weight of evidence evaluation for both determining what the data and information tell us and to identify very clearly any uncertainties that remain.

(Slide)

So how does the review team look at data and information?

Well, for each GE animal and rDNA construct and for each product definition, we come up with a set of risk questions that are specific for that particular animal. And at about the 100,000-foot level, the questions are all the same: Is this safe? But for any particular application, we ask specifically: Is this construct in this animal in this location safe?

We look at study quality. Some of the studies that

are submitted by any sponsor are excellent. Some of the studies that are submitted are not, okay? Some of the studies that are published in the peer review literature are excellent. Some of them are not. It takes a lot of expertise to be able to tell the difference between the two.

So one of the things that we do is evaluate study quality as we move forward. We look for internal validity of individual studies and of the dataset in general.

For submissions that have come in from a sponsor, have they validated the kinds of systems that they use? For example, if they are doing a PCR analysis, have they validated that, that they are actually using the right primers and the primers work appropriately, and are the conditions appropriate for what is being done? If you are looking at a hormone binding assay, have they validated that? Is there a negative control and a positive control? That is what we mean by internal validity.

Statistical analyses are also important, but statistical significance is not necessarily a measure of biological relevance. So when we look at statistical correlations, the next question we ask next -- we say: "That is nice. What does that mean biologically?" And we look at that in the context of what we are looking at.

We look -- and we look to see if our conclusions are consistent and coherent with the data in the entire dataset.

I mentioned yesterday that we borrowed from the Bradford Hill criteria for causation to develop a set of criteria to look at all of our datasets together to see if they hang together, and more importantly, if there are datasets that do not hang together, which are the most important ones? If they are anomalous data, that means we need to look at them more carefully.

(Slide)

So here again, it is the weight of evidence determination. We consider all the data and information.

Dr. Vaughn talked to you earlier this morning about the kinds of studies that are used. Our approach is slightly different in that we don't consider any one study to be pivotal; we looked at all of the data together.

We use a system of deference where the best studies that are most well conducted and most directly address the question, those questions at hand, are given the greatest deference, sort of a qualitative weight.

If there are, for example, very interesting observations that are done in Coho salmon with a different promoter with a different construct, there is information to be gleaned from that, but that is not data that can be used to be evaluation of this particular construct, okay? That is a very important distinction to make. Just those studies will provide us useful information; they are not data that are used

in the evaluation of this particular study application, okay?

(Slide)

So, a little bit about nuts and bolts, about how we do this in house. I am sure some of you wonder: How does FDA actually do a data review? I mean, does a box of information arrive and somebody sits in a room and we slide them a tray under the door until they are done and come out? But that is the way we usually do it, but not in this particular case.

(Laughter)

DR. RUDENKO: What we do, and Steve alluded to this earlier this morning, is unlike a standard, conventional new animal drug review which is handled by the Office of New Animal Drug Evaluation, we decided to do a more matrixed approach to this.

And what we have done is to go around through the Center and identify all the subject matter experts that have particular expertise in the areas where we needed them and assembled them into a team. So some of them come from the Office of New Animal Drug Evaluation, some of them come from the Office of Research, some of them come from the Office of Surveillance and Compliance. It is not important where they come from; what matters is they have specific expertise that we need to be able to do this.

And then --- as we convened the review group, we broadly outlined what the issues were and then we assigned

two, at least two, in depth experts to each step of that hierarchical approach. Each of those two independent experts went and did an independent review of the dataset. They came back independently and presented the results of their analyses to the rest of the review group, which acted as a peer review group on their analysis. If they had further questions, the in depth reviewers were sent back to do more analysis. If the kinds of questions that were asked could not be resolved by further analysis, the call went in to Dr. Stotish and we said we need more data, and we would go and get more data and until we resolved the question that we needed to resolve.

And this happened for each step. We did not move beyond any particular step until there was unanimous consent from the in depth reviewers and the peer reviewers in the group.

So what you are looking at is not one reviewer's opinion; it is the opinion of the entire group of people who are sitting to my left.

(Slide)

So, here we are, finally. This is the methodology that we have employed to assess the risks associated with AquAdvantage's AquaBounty fish -- I am sorry; AquaBounty Technologies' AquAdvantage fish. Got that backwards.

You will notice that this particular diagram has got different colors on it. And the reason for that it goes back

directly to the point that I was making about hazard and risk.

The blue represents hazard identification and hazard characterization. In these steps, we take a very careful look at the data and information that have been presented and try to identify any hazards, things that could cause bad things to happen, and see if they are found in the construct, in the insertion of the construct, and if there is anything about the phenotypic characterization of the animal that could lead us believe that there is either a risk to the health of the animal or that those changes to the physiology of the animal itself could result in a human food consumption risk.

So you will notice the phenotypic characterization of the animal has got both colors in it because it both contains a hazard characterization and a risk assessment or safety assessment step.

Yellow are our safety assessment steps. And you will notice in the post-approval reporting component we have both a search for continued -- a continued search for additional hazards that may arise and an assessment of risk that occurs.

(Slide)

So I am not going to spend any more time talking to you about each of these individual steps, although those of you who were here yesterday have seen all of these slides before. The idea is that each of our experts are going to

take you through each of these steps.

So now what we are going to do is actually take you through the actual application.

Please remember that the subject of this particular meeting, the subject of this particular assessment, is the application that is in front of you which is very tightly constrained. We are not interested in other constructs and other fish. We are not interested in growing these in balloons on the moon. We are simply interested in the conditions of use and the product definition that was given to us here tightly constrained.

But before we do, one more reminder: We have not yet made an approval decision. We are presenting our methodology, the data and information that we have considered, our analyses and our conclusions from those analyses.

What we are asking for is comments from the VMAC, any additional data or information from the public and their comments, and we will think about all of these and take them all into consideration prior to issuing a decision.

So this is going to be hard work, what is coming up next. Please concentrate hard on the data. Most of it is -- almost all of it is in your briefing packs. There is nothing that is going to be presented that is not in your briefing packages already. If you have any questions of clarification, stop and ask the presenter right away so that you don’t get

confused. Thank you very much.

DR. DUNHAM: So on that note, does anyone have any questions for Dr. Rudenko? Yes?

DR. POPPENGA: I just have one question and it -- oh, Bob Poppenga -- one question regarding the weight of evidence scheme that is employed. I assume for most New Animal Drug Applications there is a range of -- you probably cover all four categories of data. Is it possible to have something approved without data in the top category, if all the other data was, say, in the second category?

DR. RUDENKO: I think that is a hypothetical question that I am not prepared to address right now. I think it would be difficult to do. But we can talk about that later, if you would like.

DR. DUNHAM: Okay, seeing no further questions, it is my pleasure to have Dr. Jeff Jones discuss molecular characterizations.

Molecular Characterization of AquAdvantage Salmon

by Jeff Jones, DVM, Ph.D.

DR. JONES: So I am Jeff Jones. My basic science expertise is in DNA damage repair, molecular virology and molecular biology. I am also a veterinarian. I am supposed to remind -- yes -- I was an in depth reviewer on both of the molecular characterization steps with my colleague Jay Cormier and I was also an in depth reviewer on the phenotypic

characterization.

Today, my job is to describe the molecular characterizations for you and I have already adjusted the microphone, so that is good. Okay. And if I fade in and out, somebody raise your hand so I realize I stepped away from the microphone.

(Slide)

The molecular characterizations are carried out in two phases. The first phase of the molecular characterization is evaluation of the construct in the test tube. So it is molecular characterization of the construct and there we are specifically focused on the rDNA construct in the test tube.

The second phase of the molecular characterization reviews is the molecular characterization of the GE animal lineage. There, we are focused on the specific rDNA construct as it is stabilized in the genetically engineered animal lineage that is under development.

(Slide)

The main goal of the molecular characterizations is to narrow the scope of the review from the universe of possible hazards to identify the specific hazards, or the potential hazards, if any, that are related to the specific rDNA construct again in the specific GE animal lineage under development. And we -- as we move forward, we confirm consistency with the product definition.

(Slide)

The overarching risk question that we asked for the molecular characterization of the construct is: Are there any sequences that are likely to contain potential hazards to the animal, humans or animals consuming food from that animal, or to the environment?

(Slide)

Practically, the questions that we are asking are: What is the rDNA construct? How is the rDNA construct made? Is the rDNA construct as it was intended to be? And, is there any additional useful information in the submissions or anywhere else that can help us evaluate the submission, or the construct?

(Slide)

Okay, so shown in the middle of this figure -- I am going to be using this little diagram a lot -- this is the actual -- or representation of the construct that was used in the AquAdvantage salmon. And what we want to know here is: What is the source and description of the DNA?

Probably of most interest is the growth hormone cDNA, that was derived from Chinook salmon. Regulatory control regions, or the 5 prime flank and 3 prime flank were derived from an antifreeze protein from ocean pout. There is a small synthetic linker that was used to assist in the construction that is shown here.

And finally, for the assembly and manipulation in bacteria to assemble the construct and to amplify it, a series of pUC, or “puck” family plasmids were used. In the final construct, it was pUC18, but there were several from that family. At the time that this construct was being made, these were widely used, very commonly used, molecular cloning plasmids.

So all of the components that were used to assemble the construct were very well described in a number of submissions.

(Slide)

So then the next step we explore is: What was the rDNA -- or how was the rDNA construct made? This is basically: We want to understand the steps that the sponsor went through in assembling their construct. What kind of methods were used and is it a plausible plan for assembly of the construct?

(Slide)

And basically -- this figure is way too small to see up here, but it is provided for you in the briefing packet on Page 12 -- a very straightforward, very standard assembly process was provided to us. The methods that were used were routine molecular biology methods. There was nothing exciting in there. And so we wound up with the final construct that was there, so the assembly process was very well described.

(Slide)

Is the rDNA construct as intended?

Basically, doesn’t really matter what the assembly process, or the molecular cloning scheme, says. We need to know what the final product really was. A variety of different data and information was provided on that, but the bottom line was that the plasmid was sent out to a contract research lab and DNA sequence analysis was conducted. The entire rDNA construct, the insert, was sequenced. The data and information was provided. We had the chromatograms, the alignments. The depth of sequencing was at least twofold and particularly in the section pertaining to the growth hormone gene, there was at least tenfold depth of sequencing. So we have really, really good understanding of what the final construct was.

(Slide)

So from molecular characterization of the construct, we also looked at any other additional useful information that was provided to us in the submissions or elsewhere in the literature.

One of the bits of information that is useful here, and will be relevant later when I talk about the construct in the GE animal, is that the ocean pout regulatory regions, these antifreeze protein regulatory regions, had been analyzed for their functionality in salmonid cells and it was

demonstrated that this was a reasonable promoter to use and that even very truncated promoters functioned in the salmonid cells.

And finally, the injection solution that was used to introduce the construct into the eggs, the salmon eggs, to make the initial transgenic animal was the DNA in a saline solution, so there was nothing exciting there.

(Slide)

So, in conclusion for the molecular characterization of the construct, the data information that was provided was substantial and acceptable. Standard rDNA components and methods were used to assemble the construct.

There were no known toxins, pathogens, oncogenes or tumor suppressor genes included in the construct. There were no viruses or mobilizable elements that were used or included in the final construct. And the construct is consistent with the product definition.

(Slide)

So now we turn to the molecular characterization of the GE animal lineage. Here, the overarching question is: Does the GE animal contain sequences likely to pose hazards to the animal, humans or animals consuming food or feed from the GE animal, or to the environment?

(Slide)

Okay, so we need to take a second just to talk about

what we mean when we are talking about insertion of the construct into the GE animal. Basically, what we are talking about here is using the rDNA construct, introducing it into the cell, or in this case into a fertilized egg, hoping for a recombination event between the rDNA construct and the chromosome of the fish. And, of course, the chromosomes are in the nucleus of the cells of the animal.

(Slide)

Okay, so what is the result of this rDNA construct insertion?

Well, the first thing we want to know is: What actually wound up in the animal? Was it just the rDNA construct or did the plasmid backbone go in? What is the final stabilized copy number? What are the location or locations? And what is the final stabilized structure of the rDNA construct?

Okay, so on the first two -- did the rDNA construct itself go in or did the plasmid backbone go in, or both? -- through a series of experiments, probably the easiest to describe is the southern analysis, we know that the rDNA construct went in and it actually went in in two locations. The plasmid backbone was not present in the -- any of the animals.

(Slide)

The next question in looking at the result of the

rDNA construct insertion is: What is the copy number?

And here I am representing the possibility of multiple insertions at a single location in various types of orientations. Turns out we didn't have multiple insertions at any given location.

(Slide)

The next question is: Do we have multiple locations?

And these different colored bars are representing different chromosomal locations. And in fact we did have multiple locations within the genome of the initial GE animal. What we had was two different locations. Two copies were in the initial GE animal, the EO1 as that fish was designated, the alpha location, which I will talk about more in a minute, and a beta, what was termed a beta location. The beta location appeared to have a partial insertion and appeared not to be functional.

Through selective breeding, just routine breeding, the sponsor eliminated the beta locus from the lineage that was being developed. There were a series of southern analysis and PCR analysis studies showing the -- that the beta locus was eliminated from the lineages.

(Slide)

Okay, so the last thing we need to know about is: What is the -- you know, what is the structure, the final

structure, at the stabilized locus? And so the first question here is, what is the stable -- what is the structure of the actual construct?

So, the top line here is what the original construct was designed to be, the 5 prime flanking ocean pout regions, the 3 prime flanking ocean pout regions, and the growth hormone cDNA.

The final stabilized structure actually had undergone a rearrangement. This is not a surprise. We were looking for a recombination event between the rDNA construct that was introduced into the GE animal and the chromosome of the GE animal, so it is not a surprise that we had additional recombination, or rearrangement. Happens all the time.

What happened here was that the part of the 5 prime region -- actually, part of the 5 prime region here was translocated to the 3 prime end. This was initially characterized using PCR analysis and then the complete sequence of the inserted construct was determined, again by a contract research lab, again with excellent quality of data, great over-sequencing of the various regions, high confidence data.

So we know what this structure is. Also, from that earlier data that I alluded to with the truncated promoter regions showing functionality in the salmonid cells, it is not surprising that this construct works with a somewhat truncated

promoter region.

(Slide)

Okay, so the structure of the actual construct was very well evaluated and described. We also need to know: What about the junction between the construct and the chromosomal flanking regions?

Here, we are interested in knowing if we have interrupted a chromosomal gene or if we have created a new fusion protein which could by itself present a hazard.

It turns out that the construct integrated into a 35 base pair repeat which is not a coding region, it is not a gene. So we don't have either of those problems.

(Slide)

Okay, so the last thing I need to talk about is the analysis of the data over the -- over multiple lineages. So this is just a -- one of the genealogy charts that is also provided in the packet.

The whole point here is that the stability of this construct as stabilized has been demonstrated over seven generations. Sequence analysis was also provided us, and actually I should say multiple methods were used -- DNA sequence analysis, southern analysis, and a lot of PCR analysis to both show that the construct as stabilized at the alpha locus is present over seven generations and the beta locus is bred out after the early generations. And I think I

am done.

(Slide)

Oh, conclusions -- yes. The data and information was substantial and acceptable. Again, there was a single copy in the line of GE salmon that is being produced. That single copy was well characterized. It is stable over seven generations. The -- let us see -- no hazards posed by the integration event were identified. And the construct as stabilized in the genome is consistent with the product definition. Now I go to questions.

DR. DUNHAM: Thanks, Dr. Jones. Does the VMAC have questions? And if so, please give your name before asking a question. Thank you.

Questions and Answers

DR. KANEENE: John Kaneene. I am curious on your conclusion. I will let you sit first -- stable of -- stability over seven generations. Was there any variation? How stable is stable? You said seven generations. Was there any variation at all?

DR. JONES: Well, so if I understand the question that you are asking me, is how do I know that this construct is stable in the genome over seven generations?

DR. KANEENE: Right.

DR. JONES: Okay. So, I guess the best way for me to answer that question is to describe some of the studies

that were done.

For example, there were a couple of fish that were sequenced over a number of generations, the sequence from those fish, and I think the two that I am thinking of from that particular study were the second and fourth generation. The sequence was identical in those two fish to the base.

There was also a huge -- well, I would consider it a pretty large study looking at about 70 or so fish by PCR analysis and the structure by PCR and then restriction mapping was stable in the -- was identical in the 6^{th} and 7^{th} generations.

There was a southern analysis that was done. Actually, there were multiple southern analysis studies, 1 in the early generations and then 1 over the 2^{nd}, 4^{th} and 6^{th} generations where we had again, you know, southern analysis with restriction analysis, where the structure was the same in all of those. So I think that gets at your question.

DR. KANEENE: I have a follow-up. Can you give me a sense as to what numbers we are talking about? How many fish who are involved in, you know, in all the studies? Are we talking about 10, 15, 20, what? Give me a sense of the numbers you are using.

DR. JONES: There was a number of different studies using a number of different fish in the studies. But the thing you have to remember about -- let us use the plasmid

backbone -- well, actually, let us look at the, you know, one of the late generation studies.

If I have a late generation fish that I characterize and it is identical and the data that is there applies to all the fish that preceded it because it is not going to like change and then go back.

DR. KANEENE: Okay.

DR. JONES: So some studies would have a dozen fish. Other studies would have more fish. But there were multiple studies that were done, you know. The one study that I can think of had, you know, 70 or 72 fish in it. It just -- it depends on how many different lines they are looking at it. It depends on the question you are asking, really, how many fish you include, and also the technique as well.

DR. APLEY: Good morning. Mike Apley. A question so that you make sure a clinical pharmacologist can understand.

As I understand the construct, there are two things we are changing in the animal. We are changing both the growth hormone gene coming from a Chinook salmon which would have different effects on rate of growth while it was on and working and then the ocean pout regulator changes the duration of activity that that has expressed?

DR. JONES: So I think that the intent in any time you change the promoter is to change when you are expressing

the given gene.

The other thing that I want to take a -- or move back to is the, you know, the growth hormone genes. They are really, really similar to each other. There is only a handful of base -- of amino acids that are different between these two salmon growth hormone genes.

DR. APLEY: This is a follow-up question. What happens with the original Atlantic salmon ---

DR. JONES: Oh, they are still there. When you do the -- the way they studied the PCR analysis, those actually act as internal controls for knowing that the reaction worked so that the standard salmon genes are still present.

DR. APLEY: So are they both on? Are they additive? Is that how it works?

DR. JONES: I don’t know that I can answer that question. I think we will -- there is analysis later on of the expression or the presence or --

DR. APLEY: Of the hormones?

DR. JONES: -- so maybe that is a better time to answer that question.

DR. APLEY: --- a regulatory clarification question from me. If 20 generations down the line we found a genetic drift and the company, the sponsor, wished to go back and essentially start anew with putting the construct in again and start anew with a different line, is that a whole new NADA?

Is it a supplemental NADA?

DR. RUDENKO: You betcha!

(Laughter)

DR. RUDENKO: That would be if they decided to go back in and use the same construct and reinsert it into a new fish egg, that is a whole new NADA.

DR. APLEY: Okay.

DR. RUDENKO: If in the course of the durability assessment there were some differences in the phenotype, those might be subject to a supplement, but that is a more complex issue and I am going to have Dr. Cormier talk about that in his part of it.

And to get at your question about whether or not two elements are being changed in this, remember yesterday we talked about when you make a construct, you have got to have a traffic signal, and the traffic signal is the ocean pout promoter. So it is hard to expect realistically that the insertion of just the coding sequence would get you expression, so you have got to put the both pieces in at the same time.

DR. APLEY: Well, as a follow-up. I guess we will talk -- maybe it will be clarified later. But I am just wondering if -- is the difference we are seeing phenotypically driven by a traffic signal being on all the time versus part of the time, or do we actually have a significantly different

growth hormone effect? Is it is time or magnitude, or both?

DR. JONES: So the point of the ocean pout promoter region, that antifreeze promoter region, is that it should be constitutively or all the time expressed so that -- and it would not be subject to the feedback regulation of the normal growth hormone gene. Having said all that, it is not expressed at very high levels. Anyway, so it will be talked about more. And I think that is that.

DR. ALTIER: Craig Altier. I have a question about the location of the insertion. This is insertion of 35 base pair repeat region. Is there more than 1 of these kinds of regions in a chromosome or is this unique, this region?

DR. JONES: So there are repeated regions in the -- in chromosomes in various species. And for our purposes analysis, the point was that this was just in a repeated region. Some people call it junk DNA. It is just -- it is not functional as far as a protein is there and there is no protein coding region there, so we don't have to worry about the knocking out of a gene, we don't have to worry about the generation of a fusion protein.

DR. ALTIER: Right. But if there was more than 1 35 base pair region on a chromosome, could you not have in fact a large deletion rather than insertion? You could have gotten a non-homologous recombination that included 2 disparate 35 base pair regions and lost intervening DNA.

DR. JONES: Yes, it is possible that you could have something like that.

DR. ALTIER: So have any studies been done to try to examine whether that is true or not?

DR. JONES: Well, I think that the way you would assess, if there are any deleterious effects from that sort of an event, is by looking at the phenotype of the animal in the characterization of the animal. So I think that the question there will be addressed more in a phenotypic characterization rather than a molecular.

DR. ALTIER: But you could do it genotypically in fact if you had the appropriate southern blots with flanking DNA known or sequencing methods. You could determine that.

DR. JONES: Actually, as you say that, yes, I mean there were southern analyses done and the southern analyses used unique restriction maps and so we don't see multiple copies.

DR. ALTIER: Right. But that would be, I think, a different question. And I think that is some other point I wanted to make here. In this first section, I thought we were quite sparse for preliminary data, primary data here. There are oftentimes when there were conclusions drawn and I asked myself: Can I support those conclusions? And the answer was, I don't know because I don't have the southern blots.

So I realize that these data -- there was a lot of

data produced and probably it is seen as being all pretty much well established. But for me, that question remained open. I think there is one that, if you did the appropriate southern blot, you could determine that. But if you do the southern blot simply asking whether using the construct itself as a probe, you are not going to know the answer as to whether you have a deletion or not.

DR. JONES: So I guess that there is -- one question is: Could you have had a deletion between 35 base pair repeats that occurred? And the next question is: If you did have a deletion like that that occurred, does it matter? And I think if it mattered, then you would have seen an effect on phenotype, and that is why I am deferring to the phenotype, because even if you did do a study hypothetically and found a deletion of a region, if it didn't affect the animal, what difference would it make?

DR. ALTIER: Well, that is certainly true. However, these animals do seem to have some phenotypes and we can do that a little bit later. But the question would be: Is the phenotype due to the growth hormone or some sort of a hypothetical deletion?

So it seems to me it is very important to know whether you actually have the construct that you think you have.

DR. CORMIER: The name is Jay Cormier. If the -- if

we did have a -- I think your question gets at the question of mechanism of action, of what is going on. And if the growth type phenotype were due to a large deletion and somehow that changed the growth rate phenotype of the fish and nothing else and the phenotype is otherwise okay, then the question from a regulatory point of view would be: Okay, so long as the -- that fish continues to exhibit that growth phenotype and we can confirm that those subsequent fish still are the same fish, then it comes back to the mechanism of action question is -- you know, reaches too far in that sense because it is less informative than asking the direct question as to whether or not there are impacts on the safety of the animal and the effect on this, based on the claim of the sponsor.

DR. ALTIER: Maybe we can discuss this later. I don't think that answers my question, but we will have time later and we will see.

DR. EENENNAAM: I guess I would just bring your attention to the paper in ***Transgenic Research*** 2006 by Yaskowiak -- oh, I am not sure that is pronounced correctly -- where they do do the complete phenotypic or genetic characterization of the construct and that -- all of those southern data is in there.

With regards to your question about repeats throughout the genome, they are very common and routinely will -- as there are hypervariable regions quite often and will

often have deletions and insertions just as a result of nature and because this is not a fully sequenced genome, I think it is a difficult question to say categorically whether or not there was any other changes that are associated with it.

DR. ALTIER: Right. But it is an easy experiment to do, to figure out whether you have a deletion or not. It is simply a matter of determining the flanking sequence and then southern blotting with those, determine whether they are adjacent or not. It is a simple experiment.

DR. SENIOR: I probably need to intervene here. Let me just say that the first comment was made by Alison Van Eenennaam and the second comment was made by Craig Altier. If we can direct our questions to the speakers, that would be great. We will have your discussion at a later time. Kevin?

DR. WELLS: Kevin Wells again. Jeff, if the mechanism of action were in question in that a gene were missing and that was providing the phenotype, would you expect to see the same phenotype in a hemizygous state and a homozygous state and would the gene be missing on both haploid genomes or just one?

DR. JONES: So I think, Kevin, that it is sort of a hypothetical question and we are sort of going out of the realm here, so I think I would like to defer that question to later. Thanks.

DR. SENIOR: I have a couple of questions. David

Senior. The -- what happened to the antifreeze protein gene, the ocean pout antifreeze protein? I mean that was not included in the construct somehow?

DR. JONES: No. The -- in the cloning scheme, the 5 prime and 3 prime regulatory regions were inserted separately and the antifreeze protein was -- the coding regions were not included.

DR. SENIOR: So if the 5 prime at the end -- it ended up at the end of the construct, the right hand side of the construct, that is very naïve genetics here. The -- if there was to be recombination of chromosomes, could that 5 prime end up next to something that it could activate, that there would be an open reading frame there that -- see, at the moment, it abuts something that can't be read, right?

DR. JONES: Well, I guess there are two answers to the question. One is, is it adjacent to something that is non-coding? And the answer to that is yes.

The second question is, is that the 5 prime region that was translocated to the 3 prime end, is that a functional promoter? And I think the answer -- well, the answer is no. It is just they took a longer region than they needed and so they -- it is -- they could have actually potentially made their construct with just a shorter construct all together and not included that at all, but they didn't, and that is how it wound up.

DR. SENIOR: So are you saying that if -- this would be non-functional just because it doesn't have the right coding sequence?

DR. JONES: It is certainly not a complete promoter by itself so that that piece by itself could not drive expression of a gene.

DR. SENIOR: Absolutely?

DR. JONES: That little -- that piece that is there and it moved downstream, that piece all by itself, it doesn't have the complete elements that would be necessary to drive expression of a gene.

DR. SENIOR: Okay, all right. Thank you. One thing. With the preservation of the model, the fish, is it preserved in the latest generation or do you somehow freeze the eyed eggs at F2 or something and hold them there? I mean, at what point do you -- can you only keep it alive in the current generation?

DR. JONES: I don't understand the question.

DR. SENIOR: Well, you have got to have broodstock and they have got to come from somewhere. And how do you -- can you maintain the broodstock from an early generation by some kind of freezing process or what have you, like you would do semen, or do you have to use the latest generation to create the current broodstock?

DR. JONES: I think that is more of a durability

question that Jay will address when he does the durability plan. Or do you want to do it now? Okay.

DR. CORMIER: I believe the production plan proposed by the sponsor is to have an ongoing broodstock development plan so that the broodstock would be more current generations.

But to get at a point that was also addressed earlier, I think that you are also hitting that one can store milt, and so in the event of a durability failure down the road, one can go back to an earlier generation and reconstitute the line at a time without having to reinsert DNA and have a whole new transformation event.

DR. McKEAN: Jim McKean. So that raises a question of what are the regulatory impacts of that going back that you just laid out?

(Laughter)

DR. McKEAN: Since you raised it, I am going to ask.

DR. CORMIER: This is the risk of answering that question. Sorry. If the -- may I ask your indulgence and can you please write that down and ask that question later because I don't want to start answering that and produce more questions. But, thank you. Please make sure I answer that question later.

DR. DUNHAM: Okay, thank you very much. No further questions. We will move on to Dr. Don Prater, who is going to discuss the phenotypic characterization. Don? Thank you.

Phenotypic Characterization of AquAdvantage Salmon

By Donald Prater, DVM

DR. PRATER: Good morning, by two minutes. The phenotypic characterization is one of our largest and most robust datasets. Consequently, it is also one of the most complicated datasets that we have to examine.

In addition to myself, there were three other in-depth reviewers. Drs. Eric Silberhorn, Jeff Jones and Jay Cormier were all in depth reviewers on this session.

I will just share with you that I am a veterinarian and an aquatic animal specialist at FDA. I did an in depth review in this section and also in the environmental assessment and participated in the team that did the inspection and site visit to the AquaBounty facilities.

So one point that I would like to make just before we get started, and I think we touched on this yesterday, is that the phenotype is very simply the expression of the genotype under a given set of environmental conditions. And this is a critical component to keep in mind when you are looking at the phenotypic characterization.

Much of the data, many of the animals that we are going to look at, are potentially outcrosses, and so these are data from the AquaBounty construct, or the AquAdvantage construct, on a genetic background of other animals, in some

cases. So let us take a look.

(Slide)

Okay, so just to recap quickly, what are we doing?

We are reviewing data and information from thousands of fish across multiple generations. We think of this as a mile-wide approach. Simultaneously, we are evaluating specific subsets of animals using very highly sensitive analysis, our mile-deep approach, and I will tell you a little bit more about those types of data and the particular studies.

Why are we doing this?

The phenotypic characterization of the GE animal allows us to do an assessment of direct and indirect toxicity to the animal and thereby assess animal safety. It also helps us to evaluate the fitness characteristics that might impact the environmental assessment and serves as a screen for unintended consequences for other steps of the hierarchical review such as the food safety.

(Slide)

Okay. In our information in our briefing packet on Table I, Page 3, we talked about our approach to weight of evidence.

In the phenotypic characterization, there are basically 4 categories of data that we examined -- controlled studies conducted on the specific animals being considered for approval; non-controlled studies and pilot work done by the

sponsor on these same animals; and we also looked at historical hatchery records and data for these animals quite extensively from the 2001 through 2007 year class; in addition, we looked at studies reported in the scientific literature investigating some of these same animals or their relatives. So there are 4 categories of data.

I will try to describe for each section what the conclusions rely on as we review the conclusions for each section of the phenotypic characterization. I will just mention that those are conclusions that are based on that particular dataset, and overall at the end, we will address the risk questions and the overall conclusions.

Also, just to remind you that each of the conclusions will rely more or less heavily on different datasets, so that is important to keep that in mind. Also, during the presentation, I will be reviewing specific tables in the briefing packet. I won't put them on the screen because they are very tiny, so have your briefing packets handy.

(Slide)

When we consider the phenotypic characterization -- sorry, I am searching for a slide; here we go -- we look at certain risk questions:

Is there direct or indirect toxicity for the animal?

Are there phenotypic characteristics that identify

hazards for other steps of this evaluation, as Dr. Rudenko described?

What are the risks to user safety?

What are the risks to the animal from any components of the biological containment strategy?

Also, when we approach the phenotypic characterization, we think about the potential for direct and indirect toxicity such as insertional mutagenesis. We are looking for carcinogenicity but also the effects of potentially over-expression of the gene construct.

We are also looking for the intended effect versus the unintended effect, and I mentioned before that this is our best screen for unintended consequences.

In addition, we are looking at the biology of the Atlantic salmon. We heard in some of the earlier presentations today that, in fact, wild Atlantic salmon, particularly males, undergo significant morphologic alterations as they move back and forth from freshwater to seawater and then return to freshwater to spawn.

Similarly, we considered the frequency and severity of skeletal anomalies in rapidly growing phenotypes of non-GE farmed salmon, along with the current literature that describes multi-factorial causes for these anomalies.

And then further we considered the reproductive biology of fishes and techniques, some of which have been used

for decades, to manipulate and alter their gender and fertility.

So those are some of the considerations that we have in mind when we approach the phenotypic characterization.

I am going to go back to the previous slide.

(Slide)

This is the organization of the general sections that are in your briefing packet. I am going to try to quickly, at this point, go through each of the sections and tell you a little bit about some of the important considerations. I won't take time to address each and every finding or each and every conclusion, but we are happy to answer questions, myself or any of the other in-depth reviewers, but I want to try to highlight some of the major findings in each section.

(Slide)

Okay, this is something that is important to understand, and as you look through your briefing packet for the phenotypic characterization, you found many references to the animal safety study. This is our most detailed and sensitive analysis, conducted necessarily in a smaller population of animals, but I think it is important for us to spend just a minute and take a look at the experimental design for these animals.

Okay. Initially, when this study was conducted,

there were populations of diploid and triploid transgenic animals as well as what we call “sponsor controls” and also a satellite group that had to be derived from the reference population. And so this diagram actually represents one particular group, and from the reference population we are ultimately going to end up with a total of 60 animals in the study.

From the reference population, we randomly selected -- this is by arbitrary dip netting -- to get down to a population of between 100 and 200 animals that is further randomly selected to end up with the actual animals that are used in the study.

Because this was a very highly controlled study, it was important for us to have animals of a very specific weight range and also a very specific gender because we wanted to have equal numbers of genders. Therefore, when they were filling the treatment groups, they had to exclude certain animals from the study in order to arrive at the particular ratio of genders at a particular size range.

A couple of important points to note that are described in the briefing packet had to do with culling.

This study was conducted in the broodstock facility at PEI. It is a constrained broodstock facility. This is not something that was conducted on a large scale. Culling ordinarily occurs at this facility, primarily to address space

limitations. As these animals mature, you have a limited amount of density at which you can maintain these animals, so there is a type of culling that is described as “ad hoc culling.” And when we did ad hoc culling at this point in the study, these animals received external examinations, and you will see the results from those later on.

In addition, there was something that occurred called “for cause culling,” and this would be animals that had low viability, morbid animals or mortalities. These animals got a little bit larger dataset of information and you will see these animals described as well in the study.

It is important when we look at the data tables later on to understand the source of these animals -- where these animals came from. And I hope that kind of gives you some idea of what the culling practices are. We will talk more about that when we look at the individual studies.

(Slide)

Okay, the first section addresses general husbandry conditions. Above, you will see the water quality parameters and general husbandry parameters for the Prince Edward Island facility. This is important. This is the environment. The expression of the phenotype relies on a given set of environmental conditions.

Our conclusion is that these environmental conditions don’t identify significant hazards and that they

are consistent with those in the aquaculture industry.

(Slide)

We looked at specific facility conditions. So the PEI facility where the eggs are developed is essentially a broodstock facility. We have talked about it at great length at this point. It is indoor, freshwater, recirculating.

The Panama facility is a grow-out facility. Again, it has got an indoor and anoutdoor component. To a certain extent, it is what we would consider to be a flow-through system.

And again, the parameters at which the facility is going to operate in terms of temperature, alkalinity, may be slightly different, but we determined that ultimately these are conditions that are consistent with commercial aquaculture and don’t raise any specific concerns with respect to animal health.

(Slide)

General observations. These are things that were described that are general health observations and things that help us assess the behavior. I am going to pick this back up in a later section. But these are feeding activity, behavior, posture, position in the water column, coloration, the general health parameters that were reviewed.

We looked at these specifically in a very sensitive way four times, or multiple times, throughout the study period

during the animal safety study.

But some of these are also types of parameters that are recorded in the hatchery data, and so we can look across multiple generations for many years and make an assessment of these types of parameters.

Our conclusions for the general observations were that the AquAdvantage salmon show no general health or behavioral abnormalities compared to the comparator fish.

(Slide)

Size, weight, related parameters. Now, contrary to what you think, this section of the phenotypic characterization doesn't deal with the rate at which the fish grew; that is in the claim validation. This section reviewed the statistical analysis of various growth parameters among the different groups in the animal safety study.

The only significant difference was that body weight of the diploid and triploid AquAdvantage salmon was greater than their age-matched controls, and this was consistent with the hatchery records and data from pilot studies.

And our conclusion was there were no adverse effects on the size, body weight or related parameters in AquAdvantage salmon relative to comparator fish.

(Slide)

Okay, physical examination. This is the meat of the matter.

So for these particular salmon and for farmed salmon in general, we are very interested in the external observations, their skeletal condition, jaw malformations, those type of things. This is what we began to assess in this section of the study.

So this section of the briefing package begins with a brief summary of behavioral observations that we covered previously. However, the focus of this section is on the results of the physical examination and physical abnormalities.

The data are derived primarily from the animal safety study -- you will see that in Tables II and III in your briefing packet -- and then compared to hatchery records described in the 2003 to 2006 year class, analysis of the data from the animal safety study with microscopic correlations found further in the briefing packet. Table II contains the results of those 60 animals that were enrolled in the animal safety study. 9 specific observations were recorded and a rank score was assigned to each fish.

The results appear to indicate that the occurrence of external abnormalities was similar, if not lower, in AquAdvantage salmon versus the comparator salmon and suggest that the induction of triploidy, not necessarily the introduction of the gene construct, accounts for the differences in these abnormal findings.

In Table III, we look at the results of the external examinations of all fish in the pre-qualification phase of the animal safety study. Those are the fish that were pulled out earlier, the 100 to 200 group. When you look among the fish in this group, the triploid AquAdvantage salmon have the lowest frequency of morphological changes -- 10.2 percent -- while triploid non-transgenic salmon had the highest total percentage of malformations.

As previously mentioned, culling of the fish due to space limitations at PEI was known to occur. In addition, early life stage removal of fish for reasons specific to failure to thrive and/or abnormal appearance is not an uncommon industry practice. And to the greatest extent possible, we tried to document those findings in Table III that describe the fish in the pre-qualification and enrollment phases of the animal safety study.

Nevertheless, there was a period of time between the time that fish hatched, swam up as swim-up fry, before we started external -- documented the external observations where culling also occurred.

Because of that period of time, there remains some uncertainty in this particular dataset, and as a result, we are going to try to deal with that in the terms of recording additional observations in a post-market surveillance program. And you will hear a little bit more about that further on.

Again, Table IV contains a summary of irregularities from the 2003 through 2007 year class.

(Slide)

Our evaluation discusses potential reasons and in addition, well-documented effects caused by triploid induction for various rates of irregularities seen in AquAdvantage salmon and comparator fish for different year classes. In fact, we come up with several different potential explanations, given that these types of irregularities have a multi-factorial etiology.

In particular, our report discusses an abnormally high rate of irregularities in AquAdvantage salmon relative to their non-GE comparators in the 2005 year class.

After examining the entire dataset and considering various explanations, we concluded, with the support of literature, that AquAdvantage salmon in this year class were likely to be an outlier among comparator fish and those of other year classes. Again, this section of the briefing package contains a brief summary of gross findings which we will take a look at later as well as some microscopic findings.

So our conclusion, based on -- with respect to physical abnormalities is that analyses of the behavior and gross external anomalies of market-size Atlantic salmon -- again, these are the fish that were targeted in the animal

safety study -- show no demonstrable differences from comparator fish when reared under growth conditions at ABT's PEI facility.

Although we have no reason to believe ABT's culling practices are inconsistent with the approach used for a broodstock development program in the commercial salmon industry, the culling procedures at PEI are not likely representative of those used in commercial production and grow-out settings.

Consequently, there is some uncertainty regarding the likelihood of incidence of abnormalities of AquAdvantage salmon under commercial rearing conditions. And to this end, the durability plan includes monitoring, data collection and reporting of abnormalities observed under the commercial production grow-out facilities in Panama where the AquAdvantage salmon will be reared.

(Slide)

In addition, we looked at overall mortality and morbidity. This section of the briefing packet looks at some of the animals that were culled for cause from the animal safety study. And in those animals, the veterinary diagnostician identified small inflammatory changes that were noted both in AquAdvantage and the comparator fish. Those were regarded as normal and typical findings.

We also looked at hatchery records -- and this is

where the bulk of this section comes from -- hatchery records on survival to first feeding from the 2001 to 2006 year classes and that information is contained in Page 5.

Although survival to first feeding varied significantly from year to year and was sometimes different among spawning crosses in the same year, in general, survival at this stage was similar on average between AquAdvantage and non-GE salmon.

(Slide)

Clinical pathology assessments. These results are taken entirely from the animal safety study. Important points to note for this section include the following:

Reference ranges. While quite extensive information is available for Atlantic salmon relative to other fish species, it is quite limited compared to terrestrial species. The ranges are wider and may be influenced by seasonality and other factors.

Effects of triploidy, including the increased cell size and resulting from -- resulting effects on other parameters, for example, erythrocyte counts are generally lower for triploid animals than for diploids and corresponding decreases in packed cell volume, hematocrit, and hemoglobin are reported.

In this particular study, we noticed a discrepancy between lymphocytes and neutrophils. In particular, we noted

a significantly increased number of lymphocytes and decreased number of neutrophils among the GE diploids.

However, we also noted that these values were consistent with the normal range and also nearly the same in terms of the comparable age-matched satellite controls.

We concluded that these differences were more likely the result of growth conditions at the time of enrollment rather than the result of the presence of the gene construct.

One thing I will mention, that when these fish were enrolled into the study, due to the rate at which they grew, there were actually three separate time points throughout the year where the fish were enrolled. So the transgenic diploids were enrolled first, in February, which is a very different season in photoperiod and potentially growth parameters. The transgenic triploids were enrolled in July, and then the comparator animals were enrolled in October, I believe. So, very different growthing conditions.

We tried to control for that using an age-matched control group and it actually came in handy with the clinical pathology assessments.

I apologize; I know I am out of time.

(Slide)

More specifically, we wanted to look at macroscopic and microscopic evaluation. The data from this section of the report again is derived entirely from the animal safety study.

Macroscopic findings included gill abnormalities, fin abnormalities and heart-shape abnormalities that were all generally attributed to the triploid induction.

One macroscopic finding, however, was present among AquAdvantage salmon exclusively, and that is jaw erosions, and those were noted in three of six males and one of six females.

Microscopic findings included an increased prevalence of focal inflammation that was higher in diploids than triploids and higher in AquAdvantage salmon and in size-matched or age-matched controls. Other microscopic findings included gill lesions, ectopic mineralization and hepatocellular vacuolization, all of which had higher prevalence in triploids than in diploids.

(Slide)

Our conclusion for the macroscopic observations were that the observations of the gill, fin and heart abnormalities were most likely due to the induction of triploidy rather than a result of the AquAdvantage construct. However, in this limited dataset, the most likely cause of the jaw erosions had to be ascribed to the presence of the AquAdvantage construct.

Microscopic evaluations of gills, gill lesions, ectopic mineralization, again most likely associated with the induction of triploidy. However, the increased prevalence of focal inflammation had to be ascribed to the presence of the AquAdvantage construct.

Again, these conclusions are conclusions derived specifically for this dataset.

Ultimately, our conclusion was that although the presence of the AquAdvantage construct appears to increase the prevalence of jaw erosions and focal inflammation in adult fish, these findings are of low magnitude and are not likely to be debilitating in a production setting.

(Slide)

Disease resistance. We looked at limited information on disease resistance and kept in mind that these fish are raised in biosecure facilities at Prince Edward Island and also with limited confinement at the Panama facility.

We looked at a pilot study that was conducted by AquaBounty where they challenged AquAdvantage and non-GE comparators with ***Aeromonas salmonicida*** (furunculosis), a very common disease in salmonids. We also looked at hatchery records.

Our conclusion was that limited information doesn't indicate a significant change in disease resistant relative to the non-GE comparators.

Smoltification and seawater. We also looked at smoltification and seawater survival based on pilot studies provided by AquAdvantage, or AquaBounty, and these studies indicated that the AquAdvantage salmon do undergo normal

smoltification probably and will likely survive if transferred from freshwater to seawater. Comparable data for the triploid AquAdvantage salmon were not available, but there are literature reports that indicate survival of triploids in saltwater is lower than that of diploids.

Our conclusion was that diploid AquAdvantage salmon of smolt size will survive and grow normally and indicate the basic aspects of the GE salmon has not been altered such that the presence of seawater would not act as a physical barrier to survival and establishment. This could have important implications for the environmental assessment.

(Slide)

Other phenotypic characterizations. This information comes primarily from our review of the scientific literature. We looked at literature -- sorry -- looking at a wide range of phenotypic characteristics, some of which have been mentioned by earlier presenters this morning.

We looked at high critical oxygen levels. We looked at cardiorespiratory physiology.

In our survey of the literature, our conclusions were that none of these changes would be expected to adversely affect the health of the animals under normal conditions of growth if adequate oxygen levels were maintained. And so therefore we provided a recommendation for the labeling that accompanies the animals for grow-out that they would need to

maintain certain levels of dissolved oxygen. Also, these reported changes would potentially make the animals less fit and less likely to survive if they were to escape.

(Slide)

We have already heard about gynogenesis, masculinization procedures, in addition to triploidy.

One thing that we wanted to make the Committee aware of. It is important to know that the studies that we have evaluated to date have included mixed populations of both males and females.

However, based on our understanding of the physiologic mechanisms of the gynogenesis process, we believe there is no reason to think that the phenotypic characterization of the mixed gender population would not adequately represent the range of phenotypic characteristics in the monosex population.

(Slide)

Okay, here are our risk questions. I will go through these quickly. These are in your briefing packet.

Basically we did identify some minimal direct effects and forms of the things that you see here. These effects are likely to impact the overall fitness of the AquAdvantage salmon. However, they are unlikely to provide consequences in a production setting, or else the consequences would likely be small.

(Slide)

Are the phenotypic characteristics -- are there any hazards identified for other steps of the phenotypic characterization?

We did not identify any further hazards for other steps of the phenotypic characterization.

However, with respect to environmental safety, some of the phenotypic changes that were described could result in decreased fitness. Also, these changes are expected to impact survival and establishment should any AquAdvantage salmon escape from commercial facilities.

There were no data in the file that suggest any additional risk to handlers of AquAdvantage salmon above those of commercially farmed salmon.

(Slide)

What is the risk to the animal from components of any biologic containment strategy?

Induction of triploidy certainly contains increased risk of gill, fin and heart abnormalities, perhaps also ectopic mineralization. The severity of these effects is generally minimal and is not expected to have a consequence in a production setting.

A reduction in growth characteristics often reported in the literature associated with the induction of -- has often been associated with the induction of triploidy. The

increased growth rate of AquAdvantage phenotype may mitigate some of the effects of this triploidy procedure.

And finally, the effects of triploidy on AquAdvantage salmon are no different than those observed with the comparator salmon. Triploidy is a common procedure in aquaculture regularly used today.

(Slide)

Okay, here are our conclusions. In the interest of time, I will allow you to look at your briefing package with respect to our conclusions. These are all the same conclusions that we have included there. Thank you very much.

DR. DUNHAM: Thank you very much, Dr. Prater. We will now open this up for questions from the VMAC.

Committee Questions and Answers

DR. ALTIER: Craig Altier. I have several questions, but in the interests of time, can I just ask one clarifying question? And that regards the fish that are shown in your tables as they relate to those that are culled.

So, for example, Table III which is on Page 27, there are groups by ploidy and, you know, GE and non-GE, and then you have “include,” “excluded.” Are there culled fish in this study or not, and which ones are they or aren’t they?

DR. PRATER: Absolutely. And if you will just pardon me, I am going to step to my table so I can look at what you are looking at.

Thank you very much. The fish that are in Table III are the results of the examinations for ones that were culled. Now, these were culled by design. These fish were supposed to be culled in the study design in order to arrive at the particular number of study fish in the final study population.

However, knowing that they would have to reduce the number of fish, we require the sponsor to collect external observations.

And so what you see here among these fish are ones that were included in the study versus ones that were excluded in the study from that population of 100 to 200 fish. So in other words, we required a collection of data parameters on fish that we knew would not, a priori, not end up in the final enrolled population.

DR. ALTIER: So am I to understand that the ones that are in the excluded group are actually the fish that were culled for cause?

DR. PRATER: No.

DR. ALTIER: Okay. Where are those fish?

DR. PRATER: Okay. The ones that were culled for cause are represented -- actually, let us see -- the ones that were culled for cause actually received a diagnostic work-up and are not contained in the table.

There were only about 25 fish that were culled for cause. And so those fish are not represented among these

tables. However, they were examined in a similar fashion, and if you will allow me some time, I can come up with the results of those examinations.

DR. ALTIER: I would like to see those.

DR. PRATER: Okay.

DR. ALTIER: Thank you.

DR. THORGAARD: Gary Thorgaard. I was interested that earlier this morning in the AquaBounty presentation that they described a growth trial using pooled sibs in which there was faster growth in the fish carrying the construct. And I was wondering if they had survival data in that trial that showed that the survival of the fish with the construct was comparable to the ones without the construct.

DR. PRATER: I apologize that I am not familiar with the specific study that was described this morning.

DR. THORGAARD: It just seemed like, you know, there hasn’t been a -- any experiment described that just had a simple survival comparison and that looked like an opportunity for that, you know?

DR. STROMBERG: With respect to the observation of focal inflammatory lesions and granulomas, the report states that no etiological agents were observed in those granulomas. Can you tell me how they were looked for, screened?

DR. PRATER: Yes, sir. That was -- that is a very good point and I appreciate you bringing that point out

because that is a point that I did not highlight in the presentation. But that is the case. So they were used -- they received a bacterial stain, a Gram-stain, and there was a look-at by the study pathologist for etiologic agents and those focal inflammatory lesions. That would have made a nice explanation. But the study pathologist did not find that on a Gram-stain.

DR. STROMBERG: Specifically, I would be interested to know if there were acid-fast stain and was there a consideration for microbacterial agents because they wouldn't likely show up on a gram stain.

DR. PRATER: That is correct. And I don't have that information, but I can find out.

DR. POPPENGA: Bob Poppenga. I guess this is a question that sort of goes to the decision making process when you are looking at these kind of studies. And I use by example the macroscopic and microscopic evaluation other than gross morphology study.

In the briefing packet, there is a statement: "Although this is not an adequate and well-controlled study due to a number of different factors, the information was considered as part of the weight of evidence evaluation." I guess my question is: When do you decide a study is not adequate, sufficiently adequate, to then require the company to go back and repeat that study?

DR. PRATER: That is a very good question, and I think that the most salient point is the first part of your question. How do you know when you have a sufficient body of information?

And so the weight of evidence approach is a little bit different in that we give deference to studies in a variety of ways in terms of their standard of conduct, the numbers of fish that were used, validation, that type of information.

For the phenotypic characterization, we are fortunate in that we often have independent substantiation of a variety of data points. And so that is -- when we have results in the animal safety study that are -- we can then take a look across multiple generations, across thousands of fish, then we have our best situation where we can provide independent substantiation.

DR. McKEAN: Perhaps a follow-on to that question. In the preceding page, it talks about glucose analysis and the fact that they were outside the norm and it seems that it simply says it is a factual anomaly and we will move on. And then I read the next section that Dr. Poppenga read, and I am wondering here of quality control of data in these parts of the analysis.

DR. PRATER: With respect to quality control, the animal safety study was probably our most tightly controlled

study and had quality assurance parameters around the study. In fact, many aspects of the study were conducted under Good Laboratory Practices Act.

With respect to the specific question about glucose, we did consider that to be an anomaly. However, we are dealing with a very wide reference range, and so we actually used several different values in the public literature for Atlantic salmon to ascertain whether something was in the references.

Another potential explanation for a glucose finding like that could have to do with lower metabolic scope as it has been described in some of the different studies in the literature. However, we didn't have information to substantiate that, so that was our best explanation on glucose.

DR. SENIOR: Okay, that question is -- all right, Dr. McKean.

DR. McKEAN: Yes, I am sorry. Jim McKean. I am at it again. Table 6, Gross Observations. The way I read that table, we have got less than 50 animals. And what was the population that that group was drawn from and why were not more gross examination? Appears to me to be a fairly simple procedure. Why were more not supplied?

DR. PRATER: Thank you very much. And I appreciate the question because it helps -- it will help clarify a point.

There are several points in the structure of this report where we look at the same dataset. The reason there are not more animals there is because these data were taken from the animal safety study in which there were a total of 60 animals. And so that is the dataset that this table is based on.

We looked at it again in this section so that we could actually look at a macroscopic/microscopic correlation. And that was part of the study report that is done.

Typically, one of the roles of the study pathologist is to look both at the macroscopic observations as well as the microscopic and perform a correlation. And this section of our review focused on that -- on the study pathologist's report and this table is derived from those 60 animals.

When we look across the different year classes, that is where we get our broadest look at the rate of irregularities, and that was done earlier in Table IV. But that is the reason why there are 60 animals here. And I appreciate that it may be a little bit confusing because it is the same dataset that we are looking at again, but in a different way.

DR. LAPIDUS: Jodi Lapidus. I have a couple of questions just again to clarify sample sizes in the different tables. You mentioned that there were 60 animals used in the safety study and that corresponds to a number of the graphs in

the appendix, is that correct?

DR. PRATER: That is correct.

DR. LAPIDUS: Okay. How were those 60 animals selected from those 100 to 200 per group?

DR. PRATER: Yes. And I can go back to the diagram if you would like, but what happens initially is you have a larger reference population of animals of both diploid transgenic, triploid transgenic, as well as sponsor-control animals, and those animals are winnowed, if you will, into a certain point in the study protocol. So the study protocol begins -- let me move to the board -- the study protocol really begins here, where we start to capture observations that will later be reported in the animal safety study.

So here we start with a group inventory, and then there is random selection from the reference population. So this would be the reference population perhaps of diploid transgenics.

And so there are 100 to 200 animals that receive a general health assessment, they get projections on their body weight, and then you have to winnow them down again to a smaller population that then get an intensive 2-week assessment. So this is where a lot of the general observations were acquired, from this population.

Then they come down through this pathway and they are screened for body weight to find out if they are in the

proper size range -- 1,000 to 1,500 grams -- and if they are not, they are excluded from the population that is finally enrolled.

Then they go down to get a gross external observation and you look for sex, to be able to fill the treatment groups with the right number of animals.

We recognize that this information is valuable, and so we required the sponsor to conduct external examinations and present that, and that is what you see in those tables. So there is a table that contains approximately twice as many animals -- I think that is Table 3.

DR. LAPIDUS: My question is, then, what opportunities for bias enters into that picture?

There are a couple of spots with random selection, but you are also using the term “winnowing down” and “appropriate sample sizes” that are unequal in the group. So what opportunities for bias other than the ones mentioned could enter into this picture for selection of the animals?

DR. PRATER: Excellent question. Here is my assessment. This is a random selection step, and the method of random selections, arbitrary dip netting from tanks, very commonly used in our aquaculture studies, so I think probably minimizes opportunity for bias. Again, this is a random selection, minimizes opportunities for bias.

These are areas where if there was a relationship

between body weight and sex, for example, potentially you could have a small amount of bias.

The largest area of uncertainty resides out here, and this is the area that I was trying to describe before.

So we are aware that there are culling procedures that happened before the animals reached this standpoint, and so we have data that talk about the survivability, the mortality, of fry, of smaller animals, but we have a gap there where we don't really have that information. That is what we are going to try to collect through the post-market surveillance.

We felt like we had enough information on the very early life stages. We have a very sensitive analysis, plus we have an ability to look back through multiple year classes, so we felt like we could go ahead and draw conclusions. But in any risk-based approach, it is very important to characterize what you know and what you don't know so that you can make further decisions.

DR. LAPIDUS: Are there any concerns remaining about the confounding between the time of enrollment between the transgenic and the comparator fish, given that they were enrolled at different times of the year?

DR. PRATER: Yes, there are potential confounders and I am not sure how we could get around them.

We tried to control by having this satellite group.

So this group of animals down here is actually the age-matched group and they were enrolled contemporaneously with the diploid transgenic animals. And so what we were trying to achieve there -- it is a tradeoff. So you can have age-matched fish or you can have size-matched fish.

Also, because these animals mature and reach this weight range, and we thought it was important to look at market-size fish, the resulting impact is that you are enrolling fish in February, July and October. We know that those animals experienced different photoperiods which can impact their clinical pathology parameters. It also influences potentially the micro burdens that you see in the water to a small extent, so there are some confounding factors. We tried our best to control for them.

DR. STROMBERG: Paul Stromberg. Is there a regulatory requirement for a carcinogenicity bioassay?

DR. PRATER: For animal safety study, for a target animal safety study, we would not do a bioassay such as a lifetime study like the 18-month mouse or 2-year rat study. So to answer that question, is there a bioassay for carcinogenicity? No, there is not.

What we do look for is we look for animals that are mature, market-size. It depends again on what the claim is. In this case, it is a little bit different. But we were looking for potential for carcinogenicity based on our a

priori knowledge that the insertion event could cause insertional mutagenesis. There was no evidence in tumors in these animals. These were sexually mature animals but they were not old or aged animals.

DR. STROMBERG: And how old were they when they were evaluated?

DR. PRATER: I would have to look to tell you exactly.

DR. STROMBERG: I mean, with respect to their normal lifetime?

DR. PRATER: With respect to their normal lifetime, these are animals that are just reaching sexual maturity.

DR. STROMBERG: So the concern really is over market -- really only market life, not lifetime?

DR. PRATER: We are looking primarily at market fish. That is correct.

DR. McKEAN: Jim McKean. As long as you have got that chart up there, perhaps you could explain the -- in safety data, the use of the number of animals that you used, the 60 animals. 6 animals, male and female, seems on its face to be fairly small for safety data.

DR. PRATER: The number is very small, and so to be able to derive inferential value, we need to look at other things.

However, I would note that it is a slightly larger

end than we look at for our conventional small molecules. Under our VICH guidance for a typical margin of safety study, we are looking at 32 animals.

DR. McKEAN: Okay. You are getting to where I want to go.

DR. PRATER: I see.

DR. McKEAN: I want to know what the criteria are for setting the numbers so that we can a feel for how you derive that this was the appropriate number of animals.

DR. PRATER: That is a very good question, it is a fair question. It also impinges on how we would do it for a conventional small molecule and I won't try to address that here.

But what I will try to address is: How did we arrive -- how do we know when we have enough information to demonstrate animal safety?

And in this case, we used the two different approaches. We used the mile-wide approach that allows us to look at certain parameters over multiple generations over thousands of fish, recognizing that those parameters aren't as numerous, and so we have morbidity and mortality -- those are very common and good parameters in fish for animal safety.

We also have external observations that we looked at across the different year classes, and specifically irregularities. And the irregularity would be a target

lesion, if you will. And so that was our best screen. So we used the mile-wide approach, multiple generations, thousands of animals, the mile-deep approach, the 60 animals, recognizing its limitation. We can look at a whole lot more parameters, clinical pathology, than we could ever look at across, you know, thousands of animals. And that is how we decided that we had a sufficient amount of information.

DR. McKEAN: Thank you.

DR. KANEENE: You mentioned a number of times -- oh, John Kaneene, I am sorry -- you mentioned a number of times that the opportunity for confounders in this particular phase of this study. I am wondering whether you had an opportunity to look at that during that analysis because you could do that in one or -- either in the design or the data analysis, and I am just wondering whether you are -- you had that opportunity to do that.

DR. PRATER: Yes, that is an excellent question, and it gets to the point, the expression of the phenotype being the genotype on the environment. And as we mentioned before, seasonality we did recognize as a potential confounder.

Places where it shows up that we can really demonstrate are in the clinical pathology parameters, and so if you look at some of those values, you can match up the age-match controls that were enrolled at the same time and see a very distinct correlation among numerous serum chemistry

values, and so that gave us some confidence that, yes, there probably is a confounder of seasonality.

Other places, we were less sure, but we suspected, like the focal inflammation. We had the same question. Could it be an etiologic agent? Are the micro burdens different in the water from the middle of winter to the end of the summer?

In almost every aquaculture system I have seen, they are. And we know that some of the causes for this, causes of morbidity in this hatchery, are the same as any other fish hatchery. They have fungal problems at different times of the year and sometimes those can manifest themselves in changes that we would see microscopically.

So, yes, we do recognize that there were some confounders in the study.

DR. LAPIDUS: You mentioned that, you know, these -- all of these animals are both -- from both genders; they are equally split between males and females. And given that the claims were around females only, what was the rationale for providing both genders in these analyses if these analyses were not necessarily stratified by gender?

DR. PRATER: At the time that this study was developed, and it was developed in consultation with FDA/CVM, we reviewed the protocol and gave the sponsor a feedback on the protocol. It was not determined with certainty at that time that it would be an all-female population. And so the

study was actually looked at, and I think even still it is a valid reason to do it that way to see if there are any gender effects.

So if, for example, you saw sexual dimorphism in the expression of the phenotype, you would want to know, and so you could potentially make an adjustment retrospectively on the product definition.

So I am glad we had this study that actually looked at males and females and in only very few occasions did we see any difference by gender in the effects.

DR. LAPIDUS: Were you powered to detect those differences -- any differences by gender, given the small sample size?

DR. PRATER: Admittedly, it is low power, and so the effects that we did notice, and I think they are documented in your briefing packet, would have to be with a caveat that they are likely to be powered in such a way that it would be essentially difficult to draw strong conclusions, so, acknowledge.

DR. GRIFFIN: Just quickly -- were these all done --

DR. SENIOR: Pardon me. That last comment was provided by Jodi Lapidus.

DR. GRIFFIN: Dicky Griffin. These were -- studies were conducted at Prince Edward Island or both Prince Edward Island and Panama?

DR. PRATER: These studies were conducted at the Prince Edward Island facility.

DR. APLEY: Mike Apley. Look at your clin path data, and you found a couple differences and explained them. I guess if you were doing -- I counted 21 different parameters across multiple groups. I would be surprised if you didn't find a difference by chance. What did you do statistically to assure me that those were real differences?

DR. PRATER: We did look at statistical analysis on these parameters, but again we suffer the same problem with power on those analyses.

The other factor is the reference range. So if you are going to make any conclusions, you need to have a strong reference range. For Atlantic salmon, we have had probably the best reference ranges published that we have for any fish but yet they are very wide when you look at them all together.

And so what we did was we had a map of the different -- probably generated tables like this as well where you list the different references and you list the values going down one side and you end up with a reference range if you look from the lowest value of one reference to the highest value of the other. It is a fairly wide range.

And so even though we could see apparent differences and that is why we wanted to graph the data and present them in that way in the appendix, it was difficult for us to be

able to say that these fell outside the normal reference range. In fact, I think some of the ability for us to utilize the satellite control groups and detect a potential seasonal effect still comes with the idea that those values probably all fall, or nearly all fall, within the reference range.

DR. SENIOR: David Senior. And that would include the lymphocytes and neutrophils, the percentages in the hemogram? That is within the reference range of --

DR. PRATER: I am going to turn to Dr. Jones and I believe that is what it says in the briefing packet. I think we described that point in the briefing packet. But again, it is important to look at the seasonal effects on those things, so -- but those are very wide. I would need to double-check. Maybe Dr. Jones would --

DR. SENIOR: So my question is: Has that kind of seasonal effect on the hemogram been recorded in Atlantic salmon?

DR. JONES: I don’t -- so I don’t think we have the data to say that there has been a report that -- of a seasonal effect like that. And I can’t actually say that that effect is a seasonal effect or an age effect.

But the fact that the clustering matches between the GE animals and the sponsor controls, we got a strong internal control in the study.

DR. KANEENE: John Kaneene again. I need to follow

up that comment. I thought you said you had age-matched pairs that you looked at.

DR. JONES: Exactly. The satellite controls are age.

So there are two things going on. We have got size-matched controls and we have got age-matched controls. The satellite controls, or SCs, are the age-matched.

And so I can't separate it between seasonality and age as having that difference that we are seeing, for example, in the lymphocytes on the top of Page 147. But you see that the SAT 2 ends and the TX 2 ends are both clustered and high, but equivalent. The satellite controls are the age-matched controls.

DR. DUNHAM: Well, thank you very much, Dr. Prater, and thank you for the VMAC engagement. That was excellent and what it is all about, is having your questions answered by our staff. So thank you so much.

That brings us now to lunch. Jodi is going to assist and walk our members of the VMAC down to lunch and I would like to ask that we try to be back here by 1:45. We will give you 45 minutes for lunch.

And this is actually planned because we did want to have this kind of Q&A with the VMAC members, which means we will probably be just, you know, going over it a little bit, but that is okay. It is more important that we have this

really good discussion.

So Jodi is standing over here for the VMAC members, and before we adjourn, Aleta has some additional comments.

Announcements

by Aleta Sindelar, RN

MS. SINDELAR: Hi. I just want to alert anyone who is parked in the bank spaces located outside, adjacent to the hotel, that for you to please move your car. They will tow your car. Parking is free, so please use the garage. Kelly Covington is at the desk. There are free parking tickets as well as if you have received a parking ticket to enter the garage, those should be validated at the front desk.

Second, I want to remind members of the Committee and the staff that we will not be conducting interviews with the press until the meeting has adjourned.

I would like to recognize our press officers once more -- Siobhan Delancey, Mike Herndon and Shannon Cameron. Do you want to stand, Shannon? You haven’t been recognized earlier. Great. Thank you.

Also, to remind everyone that the VMAC members are not permitted to discuss AquAdvantage with anyone prior to the deliberations of the Committee which are scheduled for later this afternoon.

And again, please remember to turn off your cell phones when you are in the meeting room and the meeting

discussion is occurring.

Thank you very much.

(Whereupon, a luncheon recess was taken.)

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

(12:53 p.m.)

DR. DUNHAM: If everyone can get seated, we will have a dual presentation on the food safety assessment. Dr. Greenlees? Thank you.

Food/Feed Safety Assessment of AquAdvantage Salmon

by Kevin Greenlees, Ph.D., DABT and Kathleen Jones, Ph.D.

DR. GREENLEES: Good afternoon. I suppose it is fitting that we come back from lunch to talk about an evaluation of the safety of AquAdvantage fish for food.

My name is Kevin Greenlees. I am a toxicologist and physiologist. Together with Kathleen Jones, I will be making this presentation. I also want to point out that while I was one of the in depth reviewers, as was Kathleen, the other two in depth reviewers were Larisa Rudenko and Karen Eckelman, who is also here. And as you have heard over and over again, in addition to the in depth reviewers, the entire team participates in the review.

(Slide)

All right. I know by now you are getting very tired of this pyramid, but it is really the basis of the way we go forward. And I know this is a little bit of repetition for

perhaps some of the people who are here, but I would like to again repeat the importance of the position where we are in this hierarchical review process when we are looking at food safety.

Food safety probably more than some of the other sections because it is a safety assessment; really depends on the previous steps of the hierarchical review to build that hazard identification to know what you are looking for in your hazard assessment and your risk assessment.

If this was a small chemical entity, a typical new animal drug, you would know what you would be testing for and what your concern is for. It is in the formulation. It is the API, it is the active pharmaceutical ingredient, it is the incipient. And you know what you should going off and doing your testing for.

When you have a genetically engineered animal, that is not necessarily that straightforward a question and you have to make sure that you know what you are looking for and what are those things that you should have concern for.

So in this case, we rely very heavily on the molecular characterization to say: What actually was entered into the animal? What kind of hazards might have come with that? What was actually in the animal once it has gone into the lineage - construct has been entered into the animal's DNA? And try and identify through that and through the

phenotypic examination what kind of hazards we might see.

We didn't find anything identified in the molecular characterization that added any additional concerns. There were no mobilizable elements. There were no other parts of the construct that you would have concern for consumption of. There was nothing that was identified in the insertion into the lineage that was of particular concern.

So at the end of the day, the hazard characterization really just boiled down to what was being expressed by the construct and the potential for, and indirect effects by, the insertion.

(Slide)

Again, you have heard this repeatedly, and it is very necessary that you understand: What is the standard to which you are doing your food assessment by? Our standard is a reasonable certainty of no harm. That is a very high standard. That is actually, when you are doing your risk assessment, that is the level of risk that you consider to be acceptable, reasonable certainty of not having that hazard have an effect.

(Slide)

We talk about a number of terms. Again, you have heard these through the previous presentations. I want to make sure that we are clear for this presentation. ABT can mean AquaBounty Technologies. We also talk about ABT salmon.

ABT salmon, or that larger group of the genetically engineered salmon that are the Atlantic salmon bearing a single copy of the alpha form of the construct at the alpha locus in the identified EO-1 alpha lineage. So this is that larger group of genetically engineered salmon in the lineage of concern. The AquAdvantage salmon is a subset of that. It is the triploid hemizygous, all female Atlantic salmon, again bearing a single copy of the same construct in the same location.

(Slide)

For this particular presentation, partly for the interests of time but also because of what is the actual focus of the approval, the briefing package presents all the data we looked at, so it presents the diploid and triploid salmon that are -- were looked at throughout for the evaluation. Only the triploid AquaBounty technology salmon, the ABT salmon, or the AquAdvantage salmon, are the actual subject of the approval of this application, should we get to an approval. So the presentation will focus primarily on the triploid ABT salmon.

(Slide)

The basic question we are asking is: Are there any differences between the food from the AquAdvantage salmon and other Atlantic salmon such that it poses a food consumption risk?

(Slide)

I do want to point out, and it was mentioned very

briefly, the Codex Alimentarius is an international standard setting body. The Codex Alimentarius Commission has set a guideline for the conduct of food safety assessments for -- they call it “recombinant DNA animals.” Those are what we are calling here “GE animals.” Our hierarchical review approach mirrors that approach very, very closely; it is essentially the same process. So we, in looking at how we built this together, we looked very carefully at the international standards that have been established.

(Slide)

Our basic approach is to identify and characterize the hazards based on direct effects, which is what I will be talking about; indirect effects, which is what Kathleen Jones will present, and then the analytical methods, which is something that Kathleen will also talk about.

(Slide)

So one question we could ask, to start with, is: Are these still Atlantic salmon? And there are a lot of ways you can look at that.

One way that we thought would be useful was to look at the standard that has already been established by the FDA. The FDA has a standard that has been established by the Regulatory Fish Encyclopedia. This is a database of some 1,700 finfish and shellfish that have been put together. The basis of that -- this database was originally to try and

prevent economic fraud, the sale of a cheap fish or fish product masquerading as a more expensive product.

The approach that is used is to use isoelectric focusing gel where you essentially produce a fingerprint of the protein patterns in the known fish species and then take your test species and say: Do these match? Do I still -- is it still the same fish I think it is?

(Slide)

We ran fish through this assay at our Office of Research. We got samples from AquaBounty Technologies. We got non-AquaBounty standards from retail in a store and tested them across, and the AquaBounty Technologies fish turned out to be – to meet the standard of identity of an Atlantic salmon.

(Slide)

Again, for direct consumption, we are talking about food consumption risks resulting from expression of the inserted construct.

If we find a hazard that needs additional characterization, then we can go through and do traditional toxicological testing for the potential hazard. This can include standard *in-vivo* and *in-vitro* tests, this could include allergenic testing of proteins new to food. It is whatever would be deemed appropriate depending on the hazard that has been identified.

(Slide)

One of the potential hazards we identified was whether or not the Chinook growth hormone itself could produce an allergenic concern. And the other one is the Chinook growth hormone as a potential hazard being expressed in the fish for consumption. Related to that, we looked at other hormones which might be impacted by the rapidly growing fish, so these were hormones of the somatatropic axis.

(Slide)

Again, this just repeats what I just mentioned to you.

(Slide)

The hormones we looked at were estradiol, growth hormone, IGF-1, 11-keto testosterone, T3, T4, and testosterone. We looked at them in ABT salmon, sponsor control salmon, and farm controlled salmon, and we looked in muscle with adhering skin.

(Slide)

When we looked across the board in the AquAdvantage salmon, there were no hormones that were statistically different from the comparator of the non-GE fish. The fish -- in addition, based on a literature search, it was -- we also looked at the potential impact of growth hormone just being in the food supply, and the literature has fairly strong evidence that fish growth hormone does not move up the phylogenetic

tree very well and it does not bind or react to the mammalian receptor, so it would have no impact even if it were present at any level of concern.

(Slide)

For the hormonal analysis there, neither the growth hormone nor the selected hormones were different in the AquAdvantage salmon compared to the non-GE Atlantic salmon.

(Slide)

We also looked at the potential allergenicity of the Chinook growth hormone by itself, the concern, potential concern, being that this is not normally present in Atlantic salmon although it is normally present in Chinook salmon. There is -- the Codex Alimentarius does recognize that if you have a source for your construct that is an allergenic source, you might want to look at the potential for allergenicity in your new genetically engineered animal.

(Slide)

We looked at sequence analysis using Allergen Online and Structural Database of Allergenic Proteins. There were no significant amino acid sequences that formed an identity with any known allergenic sequences. And at the end of the day we concluded that there were no new allergenic risks posed by the Chinook growth hormone itself.

(Slide)

For direct effects then, we found that there were no

biologically relevant changes in the hormones of the somatotropic axis and there were no new allergenic risks posed by the Chinook salmon growth hormone in the AquAdvantage salmon.

As we move to indirect effects, we are going to change hats and I will let Kathleen talk to you now.

Comments by Kathleen Jones, Ph.D.

DR. JONES: Thank you, Kevin. Good afternoon.

My name is Kathleen Jones and I am a trained immunologist and molecular biologist and I have spent the last 10 years with the Agency looking at the safety assessment of foods from genetically engineered organisms. My specific expertise is in allergenicity assessment. And I am going to be talking to you about indirect effects.

(Slide)

We talked a great deal about indirect effects yesterday but, as Larisa said earlier, we will reinforce.

With respect to food safety, indirect effects can be defined as food consumption risks resulting from the rDNA construct with a gene expression product perturbing the physiology of the animal. And indirect effects can include such things as insertional mutagenesis or the appearance of a new, unexpected open reading frame and that might be identified by our molecular biology steps.

(Slide)

Now, why not use a whole food feeding study to address indirect effects?

Well, toxicological studies in animals don't really work very well on whole foods because animal toxicological studies are designed for single, highly purified substances usually very well characterized and they are administered in highly exaggerated doses. This can't be done with whole foods. They are complex mixtures. They vary widely in composition. And when they are fed to test animals at high doses, they perturb the normal diet of the animal, and therefore you wind up with adverse effects that have nothing to do with the safety of the test subject. Also, high dose testing is really not possible.

FDA does not recommend the use of animal whole food feeding studies. We instead use a more focused approach that to the safety assessment of foods from GE organisms, and this approach that the FDA recommends is consistent with that recommended by the Codex Alimentarius Commission that was mentioned by Kevin previously.

(Slide)

So, how did we look at indirect effects in the AquAdvantage salmon?

Well, there is a comprehensive compositional analysis that was performed as well as a look at the allergenicity of the salmon.

Now, compositional analysis is a long-standing and well-established approach to look at the safety of food from -- safety of novel foods; typically involves looking at the levels of key nutrients and toxicants in a particular food relative to one or more appropriate comparators. And again, this approach is consistent with Codex.

(Slide)

For the AquAdvantage Salmon, ABT conducted a comprehensive compositional analysis. They measured a number of different compositional constituents, the data of which is present in great detail in the briefing packet, looking at the muscle and skin of ABT salmon, sponsor control salmon, and farm controlled salmon.

Sponsor control salmon is an appropriate comparator in this case because they are most closely genetically related to the AquaBounty Salmon and they are reared under near identical conditions.

Farm controlled salmon was obtained from commercial fisheries and this is an appropriate comparator because it represents the salmon that is commercially available today. It is what you can go to the store and buy or what you can order in a restaurant.

You will note that there was no wild caught salmon used as a comparator in this study. The composition of wild caught salmon is very different from the composition of farmed

salmon.

And as you heard earlier today, greater than 90 percent of the Atlantic salmon that is currently consumed in the U.S. is farmed salmon.

(Slide)

So the study looked at proximates, vitamins and minerals, amino acids, and fatty acids. Clearly I cannot go into the details of all this data with you here today. We do not have several days to really cover this in great detail.

I can talk a little bit about the approach we used. It was a heuristic one which is basically a fancy way of saying that as we were going through our analysis, we made changes to how we analyzed the data based on what we found. And so we improved our analysis as we went on.

We looked at the data in a number of different ways and that again is outlined in great detail in the briefing packet.

(Slide)

And what we found was the levels of all analytes in the AquAdvantage Salmon were similar to levels that were found in the non-GE comparator Atlantic salmon.

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I would like to talk to you in a little bit of depth about the fatty acid data given that salmon are fatty fish and this is an important consideration.

From the literature we know that total fatty acids are directly proportional to total lipid deposition in fish. And basically what this graph shows you is that relationship of total fatty acids to total lipids has been preserved in the ABT Salmon and it also shows you just how similar the ABT salmon are to the farmed control salmon.

(Slide)

In particular, salmon is a source of omega-3 and omega-6 fatty acids. Not only are the levels of omega-3, and we are talking not only about individual levels of the omega-3 and omega-6 fatty acids but also the totals of omega-3 and omega-6 fatty acids, similar in the ABT salmon and the comparator salmon but the relationship, the ratio between omega-3 and omega-6 fatty acids, is no different in the ABT salmon than in the farmed Atlantic salmon controls.

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So in terms of conclusions on the compositional analysis, based on all the data and information we had we saw that the levels of compositional analytes in the AquAdvantage Salmon, and that includes the omega-3 and the omega-6 fatty acids, were similar to levels in the non-GE Atlantic salmon comparators.

In addition, that important ratio of omega-3 to omega-6 fatty acids was also not different between the AquAdvantage Salmon and the other farmed Atlantic salmon.

(Slide)

Next, the allergenicity of the AquAdvantage Salmon was examined. Basically Atlantic salmon are fin-fish and fin-fish are one of the eight major allergenic food groups in the U.S.

(Slide)

And some individuals in the U.S. exhibit fin-fish allergies. AquAdvantage Salmon are fin-fish.

(Slide)

The question we asked was is food from AquAdvantage Salmon more allergenic than food from other Atlantic salmon?

(Slide)

Because fin-fish allergic individuals will avoid consumption of fin-fish, all fin-fish including Atlantic salmon, our real concern at this point was if there was a great increase in the allergenicity of the AquAdvantage Salmon.

And so the sponsor performed a study determining the allergenic potency of sponsor control salmon as well as ABT salmon. And they used an assay and they measured it by the inhibition of salmon-specific IgE binding to a commercially available salmon standard.

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We analyzed the data, both looking at the data of the individual fish as well as looking at the groups of fish,

and what we saw was that there is no difference in terms of allergenicity between the ABT triploid salmon or the AquAdvantage Salmon and the sponsor controls.

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And so the conclusion we drew was that in terms of -- based on this data, the allergenic potency of AquAdvantage Salmon is not significantly different from that of non-GE Atlantic salmon.

(Slide)

Based on the data and information, we concluded that there were no differences in the composition or allergenicity of AquAdvantage Salmon relevant to food safety to other Atlantic salmon.

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Now I am just going to touch briefly on analytical methods. And I went over this in some detail yesterday so I will try to keep it short.

Because no hazard was identified, no tolerance was set, and there is no need to have a method for tolerance. But because safety and effectiveness are established for a specific construct and a specific insertion event, it is important to have a method to show that this GE animal in commerce or the edible product from that GE animal in commerce is derived from the approved GE animal lineage. Therefore, all GE animal NADAs need to have a method for identity.

In this case the sponsor provided us with a PCR-based methodology. CVM's Office of Research took that method, further refined it, made it even more robust and practical for use in regulatory labs.

And this method can identify the presence of the AquAdvantage construct in food or in animals. It is also event-based, so it can distinguish between an AquAdvantage Salmon and other perhaps "me too"(sic) salmon that may show up eventually.

In addition, this method was validated in a single laboratory trial by the FDA. We found this method to be acceptable as a regulatory method should the AquAdvantage Salmon be approved.

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In conclusion, all of the data and information that we reviewed as part of this food safety assessment in addition to all the data and information that formed the previous reviews of the previous steps of this hierarchical weight of evidence approach, really drive us to the conclusion that AquAdvantage Salmon is Atlantic salmon and food from AquAdvantage Salmon is as safe as food from other Atlantic salmon.

And now it is time for questions.

DR. DUNHAM: Thank you both very much; Dr. Jones and Dr. Greenlees.

So to the VMAC, do you have questions for our two speakers?

Committee Questions and Answers

DR. VAN EENENNAAM: Yes, I would like to ask a question of Table 15. I am sorry, Alison Van Eenennaam. As I understand it, there were 73 animals involved in the food composition study but the numbers reported in the end here are substantially less and in some cases zero. I just want to clarify that in all cases that meant that the level of whatever was being measured was below the detectable limit of the assay?

DR. GREENLEES: Yes, that is correct.

DR. ALTIER: Craig Altier. I have a question about an assay that was done to look for the allergen parvalbumin. I do not think that was described in your talk; it is on 103 and 104 of the briefing packet. First I had a question about whether this was done on diploid or triploid animals. There is some question there later about which one the experiment had been done on but it did not state it in that. Can you tell me that?

DR. JONES: Yes I can. The study that was done looking at parvalbumin was done on the same fish that were used in the IgE binding assay for total allergen. So it was both diploids and triploids of ABT salmon and a set of sponsor control salmon.

DR. ALTIER: So it looks like from this briefing packet that that experiment was a real mess there. It says here that there were lack of controls, there was one case in which the gel appeared to be inverted in respect to the blot which means it is completely uninterpretable. But then there is the conclusion here that -- well for that that nothing reliable can be gained from this study. But it seems to be that if that is an important thing to study and the experiment was a bust, why hasn't it been done again?

DR. JONES: Parvalbumin is only one allergen in fish. And so, the other study that was performed also looked at not only parvalbumin but all the salmon allergens. And so it was not necessary to have a specific assay looking at the level of parvalbumin. But we had another study instead that looked at the total allergenic proteins that were present in the salmon.

DR. ALTIER: So your interpretation is that this second study superseded the parvalbumin study and made it irrelevant?

DR. JONES: Correct.

DR. POPPENGA: Bob Poppenga. With regard to the hormones, it is my understanding that if growth hormone or IGF is actually ingested as part of a fish meal, that is not absorbed; is that correct?

DR. GREENLEES: Two different issues. Growth

hormone is probably highly digested and so there is not much for absorption. In addition to that, there are fairly good data in the literature that would say that even if it were absorbed in mammals, it will not bind to the receptor.

IGF-1 on the other hand has some mixed data on its ability to be absorbed. When we had done the approval some years ago looking at rBST milk, it was a major source of investigation for that approval. And it was found that in the presence of milk, it could be protected in the gut and then be available for absorption. In the absence of milk which had the fat binding that protected it, in other foods it would more likely be digested. So it is possible to have some absorption of IGF-1. It is very unlikely, for the growth hormone and again for fish growth hormone, it would not make any biological impact.

DR. LAPIDUS: Jodi Lapidus. I had a couple of questions on the numbers as well. I noticed that on page 78 they mention that 144 fish were originally evaluated but 73 were analyzed in these tables. And I was wondering how those numbers were selected for tissue sampling?

DR. JONES: I am looking for the specific page where that --

DR. LAPIDUS: Page 78, right after the small italicized letter “i.”

DR. JONES: If you turn to page 87, under CVM’s

analysis, I believe that it has a discussion -- well actually I know we included a discussion of how the fish were selected. It is actually page 86 at the bottom of the page.

DR. LAPIDUS: So data was not available on 100 and -- so ABT did 144 and then they did their own internal analysis on 60 but then your analysis was on 73 so I was just trying to reconcile those three figures.

DR. JONES: We are currently trying to find that information but I know that 73 fish were analyzed for compositional analysis.

DR. LAPIDUS: Yes, I did see that. It appears that you compare just arithmetic means across the distributions whether or not the -- and omitting data where the assay values were below or above the limits of detection. And was there any -- for example looking at the carbohydrate values in Table 21, I noticed that the ABT salmon or treatment salmon were -- approximately half of them were actually missing that value. Were there ever any indications or methods done to explore the distributions of the different, either the proximate analytes or any of the other compositional measures in light of values above or below the limits of detection?

DR. : Can you repeat the second part of your question?

DR. LAPIDUS: Were there any methods used to evaluate the distributions of these analytes, either the

proximate analytes or any of the other compositional analytes, relative to whether they were above or below the limits of detection as opposed to just measures of central tendency when the measures were available.

DR. RUDENKO: Right, as part of the initial heuristic study, we sort of took a look at the data initially and tried to look to characterize it with respect to where we had information that was above the limit of detection, above the limit of quantitation, and then try to figure out what the best way to analyze those datasets would be.

And I think what we did was run a number of different statistical analyses in order to determine what would be the most appropriate ways to do it. What we have presented here was what we thought was the most appropriate method for analysis again stressing as we did earlier, that statistical significance, although an important indicator of potential tendency, needs to be considered in light of biological relevance.

DR. MATHEW: Alan Mathew. This question is for Dr. Jones. You mention on the validation of the PCR assay for detection of the construct that it was tested on a number of foods. Can you give us some idea of what type of foods were tested? Had any of them undergone any processing that may confound it?

DR. GREENLEES: We are changing hats just to keep

you confused; I'm sorry. You are talking about the regulatory analytical method, correct?

DR. MATHEW: Yes.

DR. GREENLEES: Okay, the method was tested on salmon. It was all raw tissue because that is the standard that we use. In part because when you start looking at cooked tissue, it is very difficult to assure that you are going to have a consistent standard once you go out past the laboratory. In other words if you get it from one source or another, it is all going to meet different cooking standards. And our standard normally for looking at foods for residues is raw tissue.

DR. APLEY: Mike Apley. A question on page 103, back to the mean allergenic potency. Could you put those allergenic potency values in context for me? What is it like for nuts and other commonly allergenic -- or is that even a fair comparison?

DR. JONES: That is not a fair comparison. Basically the assay was to determine if the allergenicity of this AquAdvantage Salmon had changed relative to other Atlantic salmon. Atlantic salmon are fin-fish. There are fin-fish allergic individuals and therefore we know that fin-fish are allergenic as a food. We were trying to determine if this was significantly more allergenic.

And so in terms of allergenic potency, it is a

relative -- they are relative units.

DR. APLEY: Looking at the design I see a uniform standard error so I consider this is basically an ANOVA or that type of analysis on it? And if that is the case, we are comparing the diploid which are not the product we are looking at the label for today. And by having that in the analysis, don't we start throwing degrees of freedom places we do not need them which decreases our ability to find a significant difference?

DR. JONES: I am not a statistician but we work with one of the best statisticians around and unfortunately she was not able to be here today. But she assures me that the statistics that she conducted for these were absolutely above board.

DR. APLEY: Oh I am sure. If it is the same one we are talking about, I have the same respect for her but --

(Laughter)

DR. APLEY: And I always ask questions at these looking around for maybe -- my question is not the statistical method. My question is if including the dataset you provided to her -- if including a treatment that represents something not of interest to us here today, that you diluted out the power of the study by including that in there; not whether the statistical method was correct.

DR. RUDENKO: There are two answers to that

question. And one of them -- they are both similar to answers you have heard before and others use.

When we first started doing this analysis, we were not entirely certain that the product definition would settle on triploid fish. So the appropriate analysis would have been to do both diploid and triploid animals; so it was entirely appropriate that way. And there was a pairwise comparison as well so that there was no loss of power.

DR. APLEY: Well okay, I will have to ask so when you do a pairwise in this, you still distribute power amongst -- it is not the same as just doing a pairwise of paired details(sic), correct?

DR. RUDENKO: That is correct. And you have reached the limit of my statistical knowledge.

DR. APLEY: So I would propose there is a loss of power by that. I will let someone more statistically accomplished on the panel --

DR. LAPIDUS: Jodi Lapidus. I was looking at that table as well. Not for the reasons that were just brought up although there is not any indication whether an adjustment for multiple comparisons has been made here. I had assumed there likely was.

What is interesting to me and I wonder if you could comment on why the sponsor control was all diploid and there was not a group of sponsor control triploid in this analysis?

So that for example, we are seeing the GE-triploid and the sponsor control diploid are not significantly different but we do not know if the -- but there is a difference between the sponsor control diploid and the ABT triploid. So we do not know if those differences would have been seen in the triploid group if you had that fourth group there.

So we have numbers in the diploid that go from 2 to 3 and then we have a triploid group from the GE group that is 2.6. We do not know if their corresponding sponsor control triploid would have had the same magnitude difference that those other two groups are seeing, the two diploid groups are seeing.

DR. JONES: My understanding of why the sponsor control was diploid is because that is the majority of Atlantic salmon that is consumed. And so because the way we asses for food safety is, is it as safe as what is on the market today. And at the time it was diploid Atlantic salmon.

DR. GREENLEES: I would also like to get back -- I'm sorry, I just want to respond to Dr. Lapidus's earlier question about the 144 and the 73 and the 60.

The sponsor actually originally had 144 fish. On the advice of their statistician they elected to actually do analysis on 60. The 73 I am very sure is a typographical error on the previous page because it is the same number that was used for the hormonal analysis and that was not the same.

DR. JAFFE: This is Greg Jaffe. For the hormonal analysis, the AquAdvantage Salmon produced both the Chinook growth hormone and they also produced the Atlantic salmon hormone. Are those biologically the same and was there any distinction made in the hormone analysis between them if there is any biological difference?

DR. GREENLEES: There should not be a biological difference between them and the analysis could not tell the difference. It would measure total growth hormone whether it was Chinook growth hormone or Atlantic salmon growth hormone.

DR. LAPIDUS: I have one more substantive question around the IGF analyses on pages 68 and 69 of the report. I see that the sponsor control on Table 15 is 11 salmon and the GE are 6. And I notice on the next page the sponsor control numbers are 7 and the GE salmon is 7. I think the increase in the GE salmon was due to replacing the lower limit of detection for one salmon with the limits of detection of the assay which is fine. But I was wondering how the 7 sponsor control salmon were selected for that particular Table 16.

And I ask because when you summarize the 11 versus the 7 sponsor control salmon, the means are different. And they are more similar in Table 16 than they are in Table 15.

DR. JONES: My recollection is that all of the fish that were in the compositional analysis were tested in the hormone analysis but not all of the fish showed hormone levels

that were detectable above the limit of detection in the hormone assays.

Now with respect to the IGF-1 levels, I believe that there was an elevation seen in diploid ABT salmon. And I think those were selected for further analysis and compared against similar diploid non-GE salmon.

DR. LAPIDUS: So those are matched. And then I also wonder, just in general, if we are talking about food safety here and being reasonably certain that these salmon are similar, I wondered if FDA ever considered running any of these analyses or requesting any of these analyses be conducted rather than looking for significant differences but instead formulated these studies as equivalent studies to show that they are truly the same or very similar within a certain margin of error as opposed to looking for statistically significant differences which with 7 fish would have to be quite large.

DR. GREENLEES: It is a good idea. I do not think we looked at it that way. What we did do to try -- because we did recognize the power was not very high, was to back off and take the very simple rule of thumb which basically said what do we have that is outside the range? And if it fell outside the range, we said well then we think we might have a difference and try to look at it further from there. But unless our statistician did that and I was not aware of it, I

do not know that we did try that particular approach.

DR. APLEY: Mike Apley again. I am not trying to just badger a point but I have to answer a pretty important question here in a few hours. Back to the allergenic potency; Dr. Rudenko made a very good point about biological significance as well as statistical significance.

So Dr. Jones in your experience with the years you have done allergenic work, what is a difference in those allergenic potencies that would make you go “wow, that is really significant.” Is that even fair between different tests?

DR. JONES: Well, let’s step back and look at this in a slightly different way. We know that fin-fish allergic individuals will react when they consume fin-fish. We know Atlantic salmon are fin-fish. And the way that most people who have food allergies, myself included, deal with their food allergies is to avoid consumption of the food they are allergic to. So these studies were not designed to see whether they would affect a change on a specific food allergic population.

We are really looking for an order of magnitude change that may affect other individuals in the population that may be genetically predisposed to have a fin-fish allergy but have not perhaps received a high enough dose to have a clinical response.

And so I think in this case when we analyzed the data and we plotted out the data for the individual fish and we looked at the ABT triploids, the AquAdvantage Salmon versus the controls, we said “there is no difference.” And just to be sure we asked our statistician to take a look and she said “statistically there is no difference.”

DR APLEY: As a follow up, I am struggling to remember my four types of hypersensitivity reactions. I have slept since that class. Are the common food allergy reaction types even dose dependent?

DR. JONES: I think we laid out pretty carefully in the briefing packet, there is a lot of uncertainties with respect to food allergies. What dose is needed to sensitize, what dose is needed to illicit a reaction once you have been sensitized, and frankly there is not any data out there about what is the range of allergen normally found in Atlantic salmon, it just does not exist, or other fin-fish for that matter; it does not exist. And so this would be a type 1 hypersensitivity, IgE mediated. Does that answer your question?

DR. APLEY: Thanks, yes.

DR. KANEENE: John Kaneene. I want to go back to the data analysis for a little bit and sample size. A number of comments have been made and I wish the statistician was here. But I wonder whether she approached the data this way.

I am just asking the question, because of the low numbers that have been mentioned in different experiments has, what we call backward power characterization(sic), been made? Backward power characterization(sic), has it been made?

DR. RUDENKO: That is a really good question and we do not know the answer to that. We could check with her and get back to you.

DR. POPPENGA: Just sort of a follow up. I thought you mentioned the Codex Alimentarius provides guidelines for -- does it provide guidelines for associating allergenicity?

DR. JONES: It provides detailed guidelines for assessing the allergenicity of proteins new to food, so that would be the Chinook salmon growth hormone. It does stipulate that you should look for endogenous allergens. It does not stipulate how to look for those.

DR. POPPENGA: That was my question; is are there specific tests that have been recommended to make that assessment. And it does look like this enzymatic immunoassay was designed specifically for this study. It really has not been used elsewhere to do the same thing.

DR. JONES: That is correct. The study was modified from -- well it is basically the clinical ImmunoCAP assay that is run to look for antibodies in people’s blood to help diagnose allergies. And so it was modified for this purpose,

for this study.

DR. VAN EENENNAAM: Alison Van Eenennaam. So with regard to that study, what level of endogenous allergens would have been unacceptable?

DR. JONES: Well when we looked at the data and we saw that there were differences or what appeared to be differences in the diploid ABT fish, we did not believe that we had sufficient information to say we think that difference is safe for people to consume. And so with respect to the diploid ABT fish, there was significant uncertainty in terms of the allergenicity. And that is not a great difference either. But we thought it would be best to err on the side of public health and to be conservative.

DR. SENIOR: David Senior. So if you selected a particular allergen that was in goat meat and another allergen that was in sheep meat and you compared the two and you found a significant difference but both of them were at irrelevantly low numbers, who cares? So my question to you, which everyone has been directing, is what number, because we are a little naïve relative to these numbers, what number do you -- let’s forget about the statistical significance between the two, what absolute number here would lead you to believe that there is an issue?

DR. JONES: I do not think I can answer that question directly. I think in terms of a public health

impact, we may need to see an order of magnitude difference. But we decided in this case to be conservative.

And seeing that there was a small difference between the ABT diploids and the sponsor controls and not having sufficient information on the natural variability of allergenicity of Atlantic salmon as in other farmed controlled salmon, we chose to tell everyone we were uncertain. And with respect to the diploids which are not the subject of this application, that we could not say based on our uncertainty that we had confidence to say that there was reasonable certainty of no harm for the diploids.

With respect to the triploids, there is no difference.

DR. WELLS: I suppose this is a related question. But we are thinking about the transgene and the transgene product essentially as a small molecule drug. Are there other drugs that one has ever asked this question of? I mean has anyone ever administered something to an animal to change a phenotype of the animal, growth promoting or something, and then turn around and ask is there a different allergenicity of the animal?

DR. GREENLEES: We have looked at some drug products looking for potential allergenicity to the residue of that drug product.

DR. WELLS: But not of the animal?

DR. GREENLEES: I am not aware of any time where we have gone back and said does that change the nature of the meat, not necessarily the residue itself. So no I do not think so.

DR. WELLS: So from a scientific point of view, excluding the Codex recommendations, is there a reason to ask this question?

DR. GREENLEES: I think that -- this was not looking to see is it allergenic. This was looking to see is it more allergenic.

DR. WELLS: I understand.

DR. GREENLEES: That actually was an abundance of caution on our part looking at a -- we already know it is allergenic. Could it be more allergenic? Well we could look and ask the sponsor to put together a study to try and do that. What we got were not very convincing results. Again if it had been orders of magnitude difference, then we would say yes it is more allergenic. It is something, just a little bit. And we were just not -- it does not say that there is a lot of concern. What we basically said was we could not prove reasonable certainty so let’s be cautious.

DR. WELLS: And in a related question, would there be a level of salmon IGF-1 that one should fear in their diet? I mean there seems to be a lot of concern about that number also. We are talking about fish IGF-1 in a mammal consuming

this IGF-1.

DR. GREENLEES: I do not believe there is any concern for consumption of IGF-1 in and of itself. It is the article which causes the IGF-1 response which you might have a concern for. I am sorry, I am thinking of IgE; I am thinking of allergenicity.

For IGF-1 there might be a level that would be of concern but this was not that high and it was well within -- in the briefing package we walked through a margin of exposure analysis that showed for the diploid, which is where this was elevated in one fish, that we had that difference but it was still well within the margin of exposure that you would see from other fish. I think it would be possible, but I could not give you a number.

DR. DUNHAM: Any further questions on that section?

DR. McKEAN: I am sorry; I am still -- Jim McKean. I am still a little confused about Tables 15 and 16 and the rationale for those differences.

DR. GREENLEES: It is probably more my fault than anyone else's. Table 15 shows the fish with ploidy not taken aside, so it is the diploid and triploid together. That is why the number comes out to be 11 for IGF-1.

Table 16 are only the diploid fish because when we went back and looked at it, we saw that the triploids simply did not contribute to that question so we just said well what

is the worst case? It clearly appeared to be diploids that were behaving differently so we presented it as diploid in Table 16.

DR. McKEAN: And the rise in 7 and 6 is, the difference there -- the rationale for that? I will try again.

Table 15, your controls have -- your GEs have got 6. In Table 16, there are 7. And I am still unclear as to where that extra sample came from? When you are dealing with 6 or 7, those start to be interesting.

DR. GREENLEES: I am sorry. We are trying to be very transparent and it made it non-transparent. In Table 16, the first row there shows the values and then it shows the value that is below the LOQ. In the next row where it has 7 and 7, we included that level at the LOQ for those numbers. That is why the numbers were 7 and 7 and it was 7 and 6 above that.

DR. McKEAN: So essentially in Table 15 you had a below the level of detection, one or more on the GEs, that simply were not recorded. It was a non-event.

DR. GREENLEES: Of triploids, yes.

DR. McKEAN: Okay, thank you. I am a little slow.

DR. GREENLEES: No, we made it confusing and I apologize for that.

DR. DUNHAM: Any additional questions?

(No response)

DR. DUNHAM: Well seeing none I thank you very, very much. I appreciate everybody's patience on working through this; it is important.

Now I am pleased to have Dr. Eric Silberhorn come and talk about the Environmental Safety Assessment.

Environmental Safety Assessment

by Eric M. Silberhorn, MPH, Ph.D., DABT

DR. SILBERHORN: Good afternoon. So I am Eric Silberhorn. I am an environmental scientist with training in fish biology, toxicology, and ecological risk assessment. Today I am going to present our findings with respect to the Environmental Safety Assessment.

I would like to reiterate that although I am the primary presenter here, I was not the only one that participated in this assessment. The other principal reviewers for this section were Dr. Don Prater and Dr. Barry Hooberman.

And in addition to this section I also participate as a primary reviewer on the phenotypic characterization. And I also was part of the inspection of the Prince Edward Island facility and the site inspection in Panama.

(Slide)

So again we are starting at a high level. I want to address the overarching risk question for the Environmental Safety Assessment and that is are there any direct or indirect

effects that might occur through introduction of a GE animal into the environment?

(Slide)

The things I am going to talk about in my talk today are the kind of information that we looked at, the regulatory context for the evaluation picking up on what I talked about yesterday, a risk model, and specific risk questions that we asked.

I am going to talk about the different types of containment that are applicable here, the physical containment, the geographical/geophysical containment, and the biological containment.

I will talk about our conclusions and also talk about our path forward.

(Slide)

So again it is important to -- I cannot overstress this as Larisa did earlier, that our evaluation is built into the context of the product definition and the conditions of use and I will repeat those in a minute or two.

The other information -- so that was part of the evaluation, our starting point for our evaluation. The other information that was included as part of the evaluation were an environmental assessment document that was prepared by the sponsor under the direction of FDA, also information from the site visit to Panama and an inspection of the PEI facility,

information from the sponsor's triploidy method validation study, and information on phenotypic characterization that Dr. Prater summarized this morning.

(Slide)

So again it is very important to understand the conditions of production and use. The production of eyed eggs is going to occur at a facility in Prince Edward Island. The eggs would then be shipped as eyed eggs to Panama and then transported to a location in the highlands of Panama where there will be grow-out to market size. The fish would then be slaughtered on that facility property and then transported to a local processing site and then from there processed fish could potentially be imported to the U.S.

(Slide)

So the regulatory context is again under NEPA, the National Environmental Policy Act. We have our regulations codified. I had a talk on this yesterday but in Part 21 CFR we have long in-depth regulations that talk about how FDA implements our Environmental Impact Regulations. But the important thing to know is that a new animal drug approval requires preparation of an environmental assessment unless it is categorically excluded. In this case this action is not categorically excluded so there as been an EA prepared.

And the outcome of this EA is going to be one of two things. It is either going to be a finding of no significant

impact which is a document that expresses the agency's decision that this finding would occur, that there would be no significant impact. Or we would decide to prepare an environmental impact statement which is, as probably some of you know, a very long and involved process with public comment.

The other thing that comes into play here, and this is also codified in our regulations, is Executive Order 12114 which requires agencies to consider the environmental effects abroad of any major action. So this is considered a major action.

So the types of things that we need to consider in this type of a situation are effects on foreign nations not participating in the action. So that in this case would be foreign nations contiguous with Canada and Panama. The other things we need to consider are the global commons, which are areas outside the jurisdiction of any particular nation, an example is the oceans or the upper atmosphere, and any resources of global importance.

(Slide)

So I wanted to come back -- this is the risk model I presented yesterday and I want to go back through it quickly. I realize we have limited time but this sets up the context for the risk assessment and the environmental assessment that was performed for AquAdvantage Salmon.

In this case we are starting with two facilities, one in Prince Edward Island and one in Panama. And we are concerned about direct and indirect effects that potentially could impact target resources.

To get from this facility down to these effects first requires a number of steps. That is either intentional release or escape of the fish from that facility intrinsic to an accessible environment and then the fish have to be able to survive in that environment or there has to be continual escape -- well there always has to be survival. And then from there if the animal is able to reproduce, there is the potential to have spread of the transgene to either wild species or feral relatives.

So all these pathways could potentially lead to direct or indirect effects.

(Slide)

I guess what I want to bring out is that you need to consider different types of containment when looking at this type of model. Physical containment comes into play here which is at the facility level which would prevent escape or release.

We have biological containment over here which affects if the animal is able to survive in the environment; it affects its ability to reproduce which then implies whether you can have establishment or spread of the transgene.

And then there is also geographical and geophysical containment which are things like environmental tolerances, say oxygen conditions, temperature, salinity, and things like that that affect the animal's ability to survive and its ability to disperse and move out from the initial accessible environment where the facility is located.

So all of these things come into play when trying to determine whether there could be these direct or indirect effects.

(Slide)

So I am going to step back and talk about the specific risk questions that we evaluated in this assessment and our conclusions regarding those.

The first one is what is the likelihood that the salmon would escape? And these are hopefully presented in the context of the risk model that I just presented. So what is the likelihood the AquAdvantage Salmon will escape the conditions of confinement? And then what is the likelihood that they would survive and disperse if they were able to escape?

(Slide)

And then number three, what is the likelihood that these salmon would reproduce and establish if they were to escape confinement? And finally what are the likely consequences? And the things here we are considering are

those that I described under the Executive Order 12114 earlier which are what are the likely consequences to the surrounding environment, foreign nations not a party to this action, in other words not directly involved in the approval, and to the global commons should there be escape of these animals.

(Slide)

So I am going to take the risk questions one-by-one and walk through those.

So the first question is what is the likelihood that AquAdvantage Salmon will escape the conditions of confinement? That depends on a number of things but it is a function of the extent and redundancy and adequacy of the physical, or usually termed mechanical, containment.

(Slide)

So that has been well described, this type of containment for both facilities. But for the Prince Edward Island facility which I will talk about first, it is described in Table 8 and in Figure 8 of the Environmental Assessment.

And the bottom line is, and I will show you a diagram on this coming up, but that all areas of the facility have at least three independent or redundant forms of physical containment. And that the egg incubation units in those areas of the plant that are holding the early life stages of the salmon have at least four levels of containment. And that is important because the early life stages are those that are the

smallest life stages and those that are potentially most able to get out into the environment sort of sight unseen.

(Slide)

I am not going to go into this in detail. This is in the EA, so you can see it. But this is the diagram for the Prince Edward Island facility. It just shows the different areas including the early-rearing area and the grow-out area. And you can go through here and you can see the different types of containment that are in the different flow systems that eventually result in discharge to a local river here. So all the effluents in the facility come together and go through a final screen here at the end of the facility. But this is an indoor facility, totally enclosed facility. There is no chance for predators or anything like that to come in.

(Slide)

So the types of physical containment include things like metal screens, tank covers, netting, floor drains, different types of filters, drum filters. And there is some chemical containment also particularly during times when there is spawning in the facility, there are chlorine pucks that are used in the water systems.

(Slide)

So here is a picture of some of the representative types of containment there from our inspection that we conducted. You can see here we have netting on the top of the

tanks; all the tanks have netting to prevent the fish from jumping out. Here is another example of that.

Here are catchment basins; these are perforated, metal, stainless steel baskets. Most of these are double, actually two layers of screening. So those are heavy duty, thick, stainless steel which all the areas -- part of the facility, the whole entire facility -- part of one facility would drain through these baskets. These are floor drains in the early-rearing section of the facility so that any water that would spill on to the floor cannot just go straight into the drain it has to go through these small filters first.

(Slide)

So I would like to reiterate that we verified the physical containment that is described in the Environmental Assessment; we verified it by inspection. And this facility has also been inspected multiple times by Canada's Department of Fisheries and Oceans which is typically referred to as DFO. And the DFO inspection reports have concluded that the facility is "as escape-proof as one can reasonably expect."

(Slide)

We have two facilities, the eggs have to get from Prince Edward Island down to Panama and that occurs by shipping, including transport by truck or car and transport by air.

That shipping is going to occur within hard -- would

occur if this proceeds, within a hard plastic cooler where there are egg incubation trays within that cooler. There would be physical strapping outside that cooler and then that cooler would be contained within another cardboard container.

There would be labeling associated with that shipment. We are not talking about that extensively today but their product, as all regulated drug products, would have labeling and the control would be under the control of both AquaBounty and the freight forwarder. And as I said earlier, transport will be by truck and airplane.

(Slide)

So in Panama, again we have the conditions of containment described in Table 9 and Figure 9 in the Environmental Assessment.

Again we have at least three different independent forms of physical or mechanical containment. In some flow patterns there are many more because there could be multiple, several redundant forms of the same type of containment. We have only considered that to be one form of containment if it essentially was the same type of screening.

The fish in the grow-out tanks are subject to at least four independent types of physical containment.

Again this was verified by a site visit by myself and others including a member from the National Oceanic and Atmospheric Administration.

(Slide)

So this is a schematic again. You can look at this and see the types of containment. There is screening, sock filters, there are again these heavy perforated types of screens, and then there are sedimentation basins before there is a discharge to a local river.

(Slide)

And again here are some pictures. This is for the early life stages, a very fine metal mesh screen here. This is a standpipe with -- they are so fine but there are slots in here that are roughly probably 2mm wide that would exclude passage of any early life stages. This is top netting. And there is side netting on these. These are the grow-out tanks here that were alluded to earlier this morning. So there is predator control there. All the effluent from the whole facility passes through a canal that goes through this basket right here. Again that is heavy stainless steel mesh. It goes through another series of stainless steel meshes and then further downstream there is even more screens and filters associated with those sedimentation ponds. So there are multiple redundant types of containment.

(Slide)

So in summary, we believe there is redundant containment measures in place at both of these facilities and for the shipment of eyed eggs.

We believe that the level of containment is consistent with recommendation in the USDA ABRAC Performance Standards. That is not something we really talked about today but there are people in the audience that are well aware of these performance standards and helped generate them. But they were specifically designed to cover investigational studies on genetically engineered fish or shellfish so they have particular relevance in this case. And in conclusion we believe there is a very low likelihood of escape for all stages of Atlantic salmon at both facilities.

(Slide)

So moving on to risk question two, what is the likelihood that AquAdvantage Salmon will survive and disperse if they escape confinement?

Well again it is going to be a function of the specific locations and the specific accessible surrounding environments at those locations.

It is going to depend on the phenotype or the fitness of the animal and we have talked a little bit about that today.

And it is going to depend on aspects of geographical and geophysical containment which again are related to the specific location.

(Slide)

So to talk about this for Prince Edward Island there

is a very high salinity in the local river that receives the discharge from this facility. It has a salinity of about 21 ppt which is just a little less than full-strength sea water. The water temperature there is around -2 to 2^{o} in winter and there is often ice on the surface of the water there.

So particularly during the time of year when they are spawning at that facility which is November/December timeframe, the conditions there are very inhospitable for early life stages and particularly for eggs and pre-smolt stages of salmon. So those salinity conditions would preclude survival for early life stages of salmon. Not just the water temperature but particularly the salinity.

It is possible that adults, we believe at this time, adults, post-smolts and broodstock that are at that facility potentially could survive that environment if they were to escape. So there is some question particularly about salmon if they are not transferred from fresh water to salt water, that they may lose their ability -- they do not actually go through the smoltification process, they might lose their ability to survive. But in this case we do not really know if that is the case or not. So we are making the conservative assumption that they could potentially survive at this time.

It is important to note that there are no populations in the immediate area of the facility of

naturally-reproducing Atlantic salmon. There has been stocking of Atlantic salmon using hatchery-reared fish in the area but there is not a naturally-reproducing population.

(Slide)

So in Panama the facility is at a high elevation, it is in the highlands. The drainage basin, the watershed, does drain to the Pacific Ocean but it is a fair distance let me say down to the Pacific Ocean. And the water temperature in most of that watershed is at 26^{o} or greater and also out in the Pacific Ocean where the watershed drains to.

The lethal range for salmon is 26 to 28^{o} so in most of that watershed the water temperature is at or above the lethal range for Atlantic salmon.

And the other thing, even at a lower temperature salmon stop feeding, so at about 23^{o}. So even -- and obviously you cannot go for too long a period without feeding and survive. But the lethal range is specifically under acute conditions, so a short-term exposure would be lethal.

The other thing about the watershed there is it has a significant number of water diversion structures such as dams and spillways which at certain times of the year move the water from the main watershed and drainage basin to hydroelectric plants that consume most of the water. So obviously that is not a situation that is optimal for survival of any fish including salmon.

(Slide)

So in summary for risk question two, we believe there are geographical and geophysical conditions present at both facilities that would limit the survival and spread of the fish to other locations.

The early life stages would not survive in Prince Edward Island. Any survival and dispersal in Panama would be greatly limited by these high water temperature conditions. And survival outside of Panama is essentially precluded also because of the high water temperature.

(Slide)

So moving on to question three, what is the likelihood that AquAdvantage Salmon will reproduce and establish if they were to escape?

Again this is a function of the extent and adequacy of the biological containment or bioconfinement.

(Slide)

And our information on biological containment applies essentially to Panama because that is where the triploided eyed eggs are shipped to.

We have a specific study that was conducted on triploidy that was carried out by the sponsor after extensive protocol review and comment including by our statistician from FDA prior to the study being conducted.

The study used 20 different batches of eggs which

were looked at. I think about 300 to 350 eggs per batch. And the average triploidy from that study was 99.8 percent. The range was 98.9 to 100 percent so it was a fairly small range. And out of the 20 batches of eggs that were triploided, 14 of those had 100 percent triploidy and so it was 100 percent of 350 eggs.

It is important to note that going forward if there is an approval, that all batches of eggs would be tested prior to shipment and that the minimum standard and essentially the release specifications would include a lower, a 95 percent lower confidence bound that would ensure that there is a minimum of 95 percent triploidy. So in other words we would be 95 percent confident that there would be no batches of eggs that had less than 95 percent triploidy.

So I think we have heard a little earlier today that triploidy usually is equated with sterility and that is essentially what we believe to be the case although it has not been specifically verified for these fish. Fertility is greatly reduced or negligible due to triploidy. And the other probably more important fact that I think is somewhat overlooked is that this is an all-female population. Just by that fact alone there is essentially reproductive containment. But when you put these two together, you really do have complete reproductive containment or effective sterility.

(Slide)

So in summary the fish in Panama will be effectively sterile and the likelihood that these fish could reproduce and permanently establish themselves if they were to escape is extremely small.

(Slide)

Moving to question four, what are the likely consequences should there be escape from confinement? Again this has to be looked at on a specific basis for each specific scenario and it would depend on the number of fish that were to escape or be released, the time of year, the location, and lots of different factors.

(Slide)

So it is a little bit hard to generalize but we believe that at Prince Edward Island because there are a limited number of broodstock in this facility -- and I think we heard things this morning about culling and other factors related to that because it is not a large facility in terms of hatcheries, it is not at all large so there is a limited number of broodstock there.

As I said there are no wild populations of Atlantic salmon there. We believe that it is highly unlikely that there would be establishment of adults in the environment if they were to escape. We know that the early life stages would not be able to survive the salinity conditions there.

Therefore we believe that effects on foreign nations

or on the global commons essentially would not be expected or are essentially precluded.

For Panama, again reproduction there -- all the eggs there would not be able to reproduce or the fish resulting from the eggs that were shipped there. There would be no survival or establishment outside of the local environment if they were to escape. And therefore, there could be no effects on the foreign nations or the global commons.

(Slide)

So to summarize the overall conclusions of our Environmental Assessment, we believe there are multiple, redundant forms of containment present at both facilities. There is a very low probability of escape for all stages of salmon present. The early life stages will not survive in Prince Edward Island. The fish in Panama will be effectively sterile.

(Slide)

And therefore under those conditions of use and based on all the available information that we have, we believe that the AquAdvantage Salmon are not expected to have a significant effect on the quality of the human environment including the United States, foreign nations not participating in this action, and on the global commons.

In addition we believe that there are no effects expected on stocks of Atlantic salmon.

(Slide)

So where do we go from here? Well at this point FDA is in consultation with the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, and with the Fish and Wildlife Service with respect to the Endangered Species Act. So that is a process that is playing out. We cannot talk about it right now really but that will affect our moving forward potentially.

At this point we have not made a decision. We have not made a determination of whether we are going to prepare a FONSI or whether we would prepare an Environmental Impact Statement. So that is going to be based on information we have received today, information and feedback, and part of this consultation with NOAA and the Fish and Wildlife Service and comments from the public.

And once we have receive those and move forward, then if we decide -- we are really going to have two pathways again as I talked about earlier. We either make a preliminary determination to prepare a FONSI and if we do, then there would be a public comment period associated with that and a Federal Register Notice and thirty days for comment.

The other option would be that we would decide to prepare an Environmental Impact Statement and that would go through the normal public participation and the normal process for doing that.

So with that, I am ready for questions.

DR. DUNHAM: Thank you very much Dr. Silberhorn. Questions from the VMAC?

Committee Questions and Answers

DR. THORGAARD: Gary Thorgaard. I was wondering if there are any native fish or amphibians in the watershed in Panama around the rearing site?

DR. SILBERHORN: Are there native fish? I would imagine there are. The company had a local biologist do some survey work and studies of the watershed. There is reported to be rainbow trout in the upper watershed from stocking that was conducted by the Panamanian government over a number of years. Now we were not able to verify that so it is just something that is reported to occur.

I did not show pictures of the actual river there but it is a high gradient river. I would not say that - its definitely not ideal conditions for salmonids but it would not preclude their survival.

DR. THORGAARD: But you do not have specific information on the native fish or amphibians?

DR. SILBERHORN: No I do not have information on native fish. Now one thing I want to bring into play, I don’t know whether I mentioned it, is that the assessment of the local environment is still under the jurisdiction of the

Panamanian government and the same thing would occur in Canada. The FDA's responsibility is the global commons and the foreign nations and the United States. The assessment of effects on the local environment still remains under the regulatory jurisdiction of the countries where those facilities are located.

DR. STROMBERG: By precedent, if the range of triploid production is essentially 99 to 100 percent, why did you set the lower limit at 95 percent?

DR. SILBERHORN: Well we did not set the lower limit at 95 percent. In reality that is the lower confidence limit for the statistical analysis. In other words, we have a 95 percent assurance that triploidy would never be less than that. In fact it is probably closer to 98 percent but we wanted to have a statistical basis for setting that.

DR. SENIOR: That question was posed by Dr. Stromberg.

DR. ALTIER: This is Craig Altier. This morning Dr. Hallerman said that operations management is critical in facilities like this and that the unauthorized entry of humans needs to be eliminated or liquidated or something like that.

(Laughter)

DR. ALTIER: I agree -- sorry, no you didn't. I agree this is obviously -- a fish is a valuable commodity here and somebody is going to want to steal it. So what safeguards

are being put into place to prevent that? Specifically Panama I think is important but maybe PEI is more important because that is where the fertile fish are.

DR. SILBERHORN: Right, that is absolutely true. PEI is the worst case scenario here. There are physical controls, physical security, that has been put into place and additional security has been put into place since we have even conducted the inspection. So there is fencing and surveillance and other things. Some of this is described in the environmental assessment and some of it might be things that we would add to this in another version or the next version of the Environmental Assessment if there is one. But there is physical security and frankly that would be our highest concern rather than through escape.

DR. ALTIER: Do we have a copy of that because it says here there was not a very adequate response; you asked for further information and the display copy of the EA has it but is that in our packet?

DR. SILBERHORN: Yes, the EA in your packet is the public display copy so it has the most recent available information on security.

DR. WELLS: Kevin Wells. As a similar question, what about employee theft?

DR. SILBERHORN: What about it?

DR. RUDENKO: Are you concerned about people

stealing the employees?

(Laughter)

DR. WELLS: No. I can see building systems to prevent break-in but I find it more difficult to identify how to prevent someone from walking off with something especially something as small as a salmon egg. And so I am just curious if that is in the plan somewhere to deal with potential employee theft.

DR. SILBERHORN: No it is not. Not at present it is not. I would just say that obviously the number, as I alluded to in the thing, the consequences depend on the number of animals that could be escaped or released. So you can only remove so many adult broodstock or something like that so that would be very limiting on the number of fish that could be taken out of the facility under that scenario.

It would be different for the eggs though obviously. You could have a substantial number of eggs in a fairly small volume.

DR. POPPENGA: Just one question here. Earlier there was the limitation of use where these eggs could be sold only to an FDA approved facility, correct?

(Panel nodding of heads)

DR. POPPENGA: Does that apply to, and not that it would happen, but ABT selling eggs to a third country where that fish would not be imported into this country?

DR. SILBERHORN: No it would not. If AquaBounty or another company were to decide to sell their eggs to another country or a facility in another country and if there was no intent to bring the fish from those eggs or food products from those eggs back into the United States, we would have no regulatory jurisdiction.

DR. SENIOR: That question was posed by Dr. Poppenga.

DR. JAFFE: This is Greg Jaffe. I guess I had a follow-up question. A little broader question about the operations management I guess. I know that FDA went down to the facilities and inspected them and I guess this relates again to the general physical containment requirements that you mentioned.

What kind of analysis is in the EA or what kind of analysis has FDA done to ensure that those are maintained? I mean, nets and all these kinds of filters and things like that, that they are maintained over time and that there are not escapes or problems because of what systems are in place within the company and how have those been analyzed to determine how robust those will be over time.

DR. SILBERHORN: Well there are SOPs already in place so I will just say that. So SOPs, there is training of employees and those kinds of things. But in addition to those which are generated from the company itself, there is going to

be continuous inspections and reassessment by FDA of all these facilities. In other words, these facilities are not going to be treated any different than other types of manufacturing facilities that we would inspect on an on-going basis. And Jay may talk a little bit more about this. Some of this comes into play in the Durability Plan which will be discussed next -- well upcoming.

DR. McKEAN: I want to follow up on Dr. Poppenga's question. In terms of -- you said that if it goes to a country where the product is not going to come back to the United States and my question is how would you know? This is Jim McKean asking the question. How would you know?

DR. SILBERHORN: Well we would know because of the analytical method that Kathleen talked about. I mean we can detect whether the specific fish -- we could detect if it was in the food supply because we have a regulatory method to do so.

DR. McKEAN: What percentage of the salmon do you test coming into the United States?

DR. SILBERHORN: A fairly low percentage.

DR. McKEAN: That is what I expected.

DR. SILBERHORN: I mean I will be honest with you; there are reports that there are transgenic shrimp in -- there are all kinds. But we won't go there.

DR. McKEAN: Don't muddy the water for me please.

My point I think is in dealing with your regulatory oversight and whether you really have oversight if you have an area where you say this can simply go to another country and we hope it does not come back here.

DR. RUDENKO: There are a couple of things that need to be clarified here. One of them is that this particular application is for a specific set of conditions that includes importation of food into the United States, that is our regulatory hook.

There may be an assumption that this is the only regulatory oversight that is being applied here and in point of fact, Canada and Panama, the last time I checked, were sovereign nations that had their own regulatory structures that still needed to be met. The fact that the U.S. may approve this fish has nothing to do with whether or not Canada or Panama will approve this fish for growth there as well.

So there are other -- whoever, AquaBounty in this particular case, must meet the federal and local jurisdictional requirements of the two countries in which they are operating. So the fact that the U.S. is saying these are okay conditions for us does not mean that we are making those decisions for Canada or for Panama.

DR. DUNHAM: Excellent, any further questions?

DR. GRIFFIN: Griffin here. It seems to me that there is a point at which if we -- maybe what we are thinking

about is if we turn them away, we lose oversight period. Is that part of what you have explained to us?

DR. SILBERHORN: I would just say that the company brought this product to us and we are evaluating it based on their desire to have us evaluate it. So it is not our decision to make whether or not we would want to turn them away or they would be turned away. We are required to evaluate this product because it is brought to us in a new animal drug application.

DR. GRIFFIN: But they really do not need us.

DR. RUDENKO: They do need to get a U.S. approval if they intend to sell the food in this country. There is another more arcane way that they can sell food in this country from a GE animal but we do not need to go there.

The regulatory reason for AquaBounty to come to the FDA right now and ask for approval of an animal that is going to be bred in Canada and grown-out in Panama is because they would like to sell the fish, the food from that animal, in the United States. In order to be able to sell food that has a new animal drug in it, you have to have an approval for the residues of that drug and so that is why we are going through this process right now. Laura, have I stated that correctly? Okay, counsel says yes.

DR. DUNHAM: Any additional questions?

(No response)

DR. DUNHAM: Seeing none on that session I thank you very much and we are now going to move into the claim validation with Dr. Evgenij Evdokimov.

Claim Validation

by Evgenij Evdokimov, Ph.D.

DR. EVDOKIMOV: Good afternoon, my name is Evgenij Evdokimov and I have training in molecular biology and analytical chemistry. Today I will be talking about the Claim Validation for AquAdvantage Salmon.

(Slide)

The previous steps of the hierarchical review process primarily address identity and the safety issues.

In this step of the pre-market review process, we evaluate whether the GE animal meets the claim established in the product definition. In other words, we need to find an answer to the question: does the GE animal do what the sponsor claims it does?

(Slide)

So the working product definition for AquAdvantage Salmon contains two claims. The first claim states that AquAdvantage Salmon grows to a mean body weight of at least 100g within 2700 ^{O}C-days.

The second claim states that when compared to conventionally raised Atlantic salmon, AquAdvantage Salmon

exhibit a significantly greater proportion of animals weighing 100g or more within 2700 ^{o}C-days.

(Slide)

Before we proceed with the presentation I would like to clarify a few terms that I used in the claim. So what does 2700 ^{o}C-days mean? Because the rate of the development of salmon as well as other fish depends on the water temperature, the aquaculture industry measures the age of salmon using degree days. So thus, for example, if a salmon aquaculture facility maintains the water temperature at 10^{o}C, so the age of the fish would correspond to 270 days.

(Slide)

The next question I would like to clarify is why we evaluate 100g. This size was chosen by the sponsor and in addition to that, 100g is a commercially relevant size at which salmon are capable of transitioning from fresh water to salt water.

As a part of our weight of the evidence review approach, when we evaluated the claim we reviewed information published in the scientific literature as well as historical studies provided by the sponsor. All this data and information supported the claim but the main focus of our review process was the study designed specifically to address the claim.

In this study we have 24 eggs from Atlantic salmon,

24 wildtype Atlantic salmon females, those eggs were fertilized by 10 AquAdvantage neomales. A portion of those eggs -- this number will give us approximately a similar ratio of GE and non-GE fish. A portion of those eggs were pressure treated to induce triploidy. And then all the eggs were allowed to grow out.

At the end of the study, the sponsor tested for the presence of the AquAdvantage construct and the weight of each fish was measured and those data were submitted to us for review.

(Slide)

So our review has shown that the mean body weight of the fish in the control salmon group was 72.6g. At the same time, the mean body weight of the fish in the AquAdvantage Salmon group was 261g. So if we look at the proportion of the fish weighing 100g or more, we see that only 4.9 percent of the fish in the control salmon group reached that size whereas in the AquAdvantage Salmon group this number was 98.6 percent.

(Slide)

So the graphic presentation of this data shows a dramatic difference between those two groups. Most fish in the control group are below 100g whereas the most fish in the AquAdvantage Salmon group are above 100g.

(Slide)

So thus, our review demonstrated that when compared

to the salmon sold today, the AquAdvantage Salmon grow to a mean body weight of at least 100g within 2700 ^{o}C-days. And also a greater proportion of AquAdvantage Salmon grow to at least 100g or more within 2700 ^{o}C-days.

(Slide)

So in other words, we can just summarize it as AquAdvantage Salmon grow faster.

DR. DUNHAM: Thank you very much.

DR. EVDOKIMOV: And now we can go to the questions.

DR. DUNHAM: Do we have any questions from the VMAC for Dr. Evdokimov?

Committee Questions and Answers

DR. VAN EENENNAAM: Alison Van Eenennaam. I just had a question. You have 24 full-sib families effectively and I was wondering if there was a sire effect in there, a family effect in other words, that would be playing out in that data? Clearly they grow faster but was there also a family effect?

DR. EVDOKIMOV: I do not believe we have any sire effect.

DR. RUDENKO: I think the answer is Alison we did not actually specifically look to see whether or not there is a sire effect. I think there are certainly non-GE fish that tend to grow very quickly so the possibility of a sire effect is present. I think none-the-less the question that was asked very simply and the one that we needed to answer was whether

or not AquAdvantage fish reach this particular growth goal faster than their non-GE counterparts and that question was answered in the affirmative I think fairly compellingly.

DR. DUNHAM: Any additional questions?

(No response)

DR. DUNHAM: Seeing none we move to the final presentation from CVM and that is with Dr. Jay Cormier and Dr. Barry Hooberman on the Durability Plan and post-marketing requirements. Thank you.

Durability and Post-Market Requirements

Comments

by Joseph W. (Jay) Cormier, JD, Ph.D.

DR. CORMIER: We are almost there. My name is Jay Cormier. My scientific background is in molecular pharmacology and biochemistry and together with my colleague, Dr. Barry Hooberman, we will be discussing durability as well as some post-market requirements.

(Slide)

As a general overview, I will be speaking to you today about the pre-market durability review whereas Dr. Hooberman will be focusing on the post-market requirements of the sponsor.

(Slide)

Before I begin, just some brief terminology that we have been using. We have been throwing around a lot of terms

today and I am going to use two of them quite frequently in my talk.

And just for clarification's sake for both the committee and the audience, when I use the word genotype what I am referring to is all the genetic information that defines a specific cell, specific tissue, or organism. It refers to individual genes or individuals within a certain population.

Phenotype on the other hand is the expression of an organism's genotype. It is the actual observed properties such as morphology, development, behavior, all of which derive from its genotype.

(Slide)

So the overarching question that we are asking, or questions, with respect to durability is whether the genotype or phenotype is changing over the product lifespan in a way that would affect the risks associated with that product?

And secondly is there a plan in place to monitor those changes if that product is granted approval?

(Slide)

So to hammer this home, what exactly is durability? The purpose of durability is to ensure that future animals in commerce are equivalent to those evaluated for safety and effectiveness during the pre-market review. And that those animals in future commerce introduce no new risk.

(Slide)

So how does durability fit within the larger context of an approval? There are both pre-market considerations and post-market considerations.

During the pre-market evaluation of durability we have both sort of a backward looking aspect of durability as well as a forward looking. Looking backwards we determine whether or not the sponsor can demonstrate that both the genotype and the phenotype of the animal are stable from one generation to the next. And then in the future whether or not the sponsor has a plan in place to affirmatively monitor and determine that both the genotype and phenotype are indeed stable after approval.

And then finally in the event of a durability failure, does the sponsor have in place specific procedures to deal with such an event?

With respect to post-market considerations, this primarily covers data collection and data reporting for the Durability Plan.

(Slide)

Genotypic durability evaluation of the AquAdvantage Salmon looked at seven generations and they were evaluated for consistency of the genotype of the AquAdvantage Salmon. That genotype was found to be stable through several methods including PCR, sequencing, as well as Southern analysis.

(Slide)

The phenotype of the AquAdvantage Salmon was evaluated over six generations for heritability of the phenotype and again the phenotype was found to be consistent.

(Slide)

The sponsor has provided us a Durability Plan. Again the goal of that plan is to ensure that future animals are equivalent to those evaluated during the pre-approval process. This plan contains specific testing, testing methods, specifications or acceptance criteria for those methods, as well as a specific testing schedule.

In the event that a given test is determined to be out of specification, there are procedures in place to deal with that. And then other additional sponsor commitments.

With respect to Dr. McKean’s question earlier regarding what would happen if there were a durability failure in the future, the sponsor is required to notify the agency of such a durability failure. And if they were to use frozen milt or other means to regenerate a line, that information would be provided to the agency at that time and we would be able to evaluate that information.

(Slide)

The Durability Plan considerations, we are concerned about the presence of the construct, continued stability of the construct, egg ploidy as well as post-market reporting.

(Slide)

Construct presence is being committed to be determined through the use of PCR. Internal primers have been designed to identify the coding region of the Chinook salmon growth hormone cDNA and that PCR method is adequate to determine the presence of the AquAdvantage construct in the future.

(Slide)

Construct stability is evaluated through a combination of PCR as well as Southern blots. Although my figure here does not show it, the PCR is looking at the junction region between the insert and the genomic DNA surrounding the AquAdvantage construct.

(Slide)

With respect to egg ploidy, every single batch of eggs that are produced by AquaBounty is proposed to be subjected to testing for egg ploidy and consistency of their triploidy process. Up to 900 eggs from each batch are tested for ploidy and are subject to specific acceptance criteria to verify that that batch meets the triploidy requirements. Failing lots are destroyed. They are never introduced into commerce and they are never introduced into food.

(Slide)

With respect to post-market durability reporting, genotypic verification will be conducted on all of the broodstock at the AquaBounty facility. Confirmation of

triploidy will be conducted on each of the batches of eggs. And phenotypic monitoring will be conducted on initial animals at the Panamanian facility to look at the effects of grow-out conditions and under commercial conditions in Panama with respect to mortality which is death, morbidity or illness, morphological changes, and early life stage culling rates.

(Slide)

In conclusion, the genotype and phenotype of AquAdvantage Salmon are stable. The testing methods and specifications presented are acceptable. The post-approval surveillance program for Panama is also acceptable. And the sponsor has in place appropriate procedures to insure that products marketed in the future are indeed equivalent to those evaluated for safety and effectiveness during the pre-approval evaluation. With that I will turn it over to Dr. Hooberman.

Comments

by Barry Hooberman, MPH, Ph.D., DABT

DR. HOOBERMAN: Hi I am Barry Hooberman. I have training in toxicology and I have fallen into the practice of risk assessment quite a bit. So I am going to talk to you a little bit about post-market requirements. Again a lot of the Durability Plan that Jay just outlined melds nicely into what we look at after approval should an approval happen.

(Slide)

These are kind of the principles that we are going

to go by. The first question is are there any data or information that would alter the agency's prior conclusions regarding the safety or effectiveness of this product?

The second question is what is the potential exposure of the approved product to the public, including consumption, or to the environment?

(Slide)

And there is one more, how does the sponsor demonstrate that the actual marketing and sales of the product are within the scope of the approval?

(Slide)

So let's go through the questions. For the first question really the overall goal is to confirm that animals in commerce are equivalent to those evaluated for safety and effectiveness during pre-market review, and to detect any new/unidentified risks. So you heard Eric talk about in the environmental review about redundant systems, this is kind of a redundant system for our review in that we are going to continue to collect data. We have systems in place to check to make sure that anything we found and all our conclusions are confirmed in ongoing studies and ongoing experiences. And we will have a system in place to detect any new or unidentified risks.

(Slide)

Just to -- when the information comes in as part of

our data or information collection, it will be evaluated very similarly to how we evaluated all the other data.

We are going to look at the genotype and see if it is as specified in the application, the same for the phenotype, and then we are also going to check whether the current conditions of use and production are as specified in the application. You have heard that we are being very specific should an approval occur.

(Slide)

So how is the information going to come in? There is mandatory periodic reporting that the sponsor must submit to FDA. You see up there that it has to be submitted after 6, 12, 18, and 24 months post-approval and then annually thereafter.

The types of information that will be submitted include Drug Experience Reports, that is anything that the sponsor has come upon. Adverse Drug Experiences, anybody can report an event that they think reflects on the product. The Durability Plan data that Jay just outlined. Minor Changes in Stability Report; those are things that may happen along the way of manufacturing and production. And then something called Distribution Data which is really “Quantity Marketed” and we will get back to that in a second.

(Slide)

One thing I wanted to cover is that if something

does change, if there is an intended change in the product, they can submit a supplemental application; you heard reference to this earlier. Again it gets reviewed as you see up there by genotype, phenotype, and whether they are equivalent to the approved product. That means whether the change that they are proposing will change the product in a significant manner. And then of course it would also change the conditions of use.

I am going fast, I'm sorry, I am trying to move quickly.

(Slide)

The second question we talked about was an exposure question and this basically gets at the Distribution Data or "Quantity Marketed." How much of this is getting out to the public or to the environment. And you see the eggs there. We are talking about information like how many eggs were shipped from the Canada facility and how many eggs were received in Panama and data like that which should be submitted to FDA.

(Slide)

Finally the third question, how does the sponsor demonstrate that the actual marketing and sales of the product are within the scope of approval? We have ongoing surveillance that we do of all advertising and promotional materials as well as other activities to ensure that no undue claims are being made or there are problems in marketing.

(Slide)

So finally just to kind of sum-up what you have heard today is that we received an application. We had a lot of questions too just like everybody out there had a lot of questions about this. We applied our risk-based system as outlined in Guidance 187.

(Slide)

And of course you cannot escape without seeing the framework once again, our pyramid. Just going through it again, the bottom levels are hazard identification moving up into the safety evaluation and answering risk questions and all the way up through post-approval reporting and that is our framework.

(Slide)

Again, I cannot emphasize it enough, we have not yet made an approval decision. You can see here as you have heard before, we are asking for comments from the VMAC. We are always looking for additional data or information from the public and we also welcome any comments from the public. And we will consider all of these before we make a decision.

DR. DUNHAM: Thank you very, very much Barry. So questions from the VMAC for Dr. Cormier and Dr. Hooberman?

Committee Questions and Answers

DR. WELLS: Kevin Wells. I have one for each. Again for clarity, Jay I think you said that up to 900 eggs

will be measured to assess ploidy?

DR. CORMIER: That is correct.

DR. WELLS: I am more interested in the “at least” number as opposed to the “up to” number.

DR. CORMIER: At least 200 from each batch and then depending on the statistical results from the initial 200, those data are pooled together with a secondary sampling of an additional 700 eggs.

DR. WELLS: And the second question which is for Barry is when you are assessing the number of eggs shipped, are those actually measured on a per-egg basis or will those all be estimates on some volume measurement?

DR. HOOBERMAN: The latter, they will be estimated on volumes based on shipping containers and things like that.

DR. LAPIDUS: What percentage, you gave us the actual numbers of the eggs that would be tested, but what percentage of that -- like how big is a batch if you are going to test up to 900.

DR. CORMIER: Each batch is somewhat variable. But the 23 liter up-welling chamber that these eggs will be sampled from is expected to contain anywhere from 100,000 to 200,000 eggs.

DR. SENIOR: That question was from Dr. Lapidus.

DR. DUNHAM: Any additional questions?

(No response)

DR. DUNHAM: Seeing none, I want to thank everybody for their patience as I know we have gone over time but I thought it was very important that the VMAC have the opportunity which they need to ask these questions and receive the answers and I thought we had a very good session with each presentation so I do thank you all.

Now I am very much looking forward to the public comments to the VMAC and I am going to ask Aleta Sindelar to come and organize that. So we will just take a minute to revamp this and get our first public speaker up. So anybody needing a break or "just restrooms," maybe you could slip out quickly and come right back. Thank you.

Public Comments to the VMAC

MS. SINDELAR: Thank you everyone. This is the Open Public Hearing portion of the meeting for all registered public comments. We have 13 registered speakers. Each has five minutes for their presentation. I will make a remark when you have one minute remaining. The microphones will diminish.

The VMAC members are permitted to ask all registered speakers questions after each presentation. The Chair of the committee at the close of presentations by the registered speakers may recognize comments from the floor, time permitting.

The VMAC members, you have two documents that have

been submitted today just in case I did not get to you sooner. You have one from the Center for Food Safety and one from Dr. Kapuscinski there at your desk.

At this time I ask Dr. Senior to read the conflict of interest statement for the Open Public Hearing as it relates to a Particular Matters Meeting; Dr. Senior.

DR. SENIOR: Thank you. Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the Open Public Hearing session of the Advisory Committee Meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the Open Public Hearing speaker, at the beginning of your written or oral statement to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or a group's payment of your travel, lodging, or other expenses in connection with your attendance at the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

MS. SINDELAR: Thank you Dr. Senior. Let's begin with our first registered Open Public Hearing speaker David Edwards from BIO.

Comments

by David Edwards, Biotechnology Industry Organization

DR. EDWARDS: Okay just to test the microphone, is it on, can people hear me?

MS. SINDELAR: Yes.

DR. EDWARDS: Members of the Veterinary Medical Advisory Committee, thank you for allowing us to have the public forum here. I wanted to provide comments on behalf of BIO the Biotechnology Industry Organization.

My name is Dr. David Edwards and I am the Director of Animal Biotechnology at BIO, the Biotechnology Industry Organization. We represent 1,100 member organizations that research, develop and produce innovative healthcare, agricultural, industrial, and environmental technologies.

The application of technology to animal agriculture is not something that is new. It has allowed us to more efficiently and sustainably produce food and fiber for a growing population. The application being considered today is an extension of technology that precisely applies our genomic knowledge to improve the rearing of salmon and the production of a high quality food.

The process of bringing this application through the regulatory system is based upon the rigorous process at the U.S. Food and Drug Administration, the FDA, for approval of a new animal drug which BIO supports as the most effective way to determine both the safety and efficacy of the recombinant DNA construct in the target animal and the safety of the food harvested from the animal for human consumption and enjoyment.

Many products of BIO member organizations go through reviews at the FDA and we appreciate and support the rigor of the process that was followed allowing for the application of existing FDA product requirements to the review of genetically engineered salmon.

FDA's statutory authority under the Federal Food, Drug and Cosmetic Act to evaluate articles intended to alter the structure or function of the body of an animal has allowed FDA to create a science-based method to evaluate these DNA constructs. FDA has assumed this responsibility by undertaking its own extensive and exhaustive review of the data in this application as well as by assembling outside experts on the subject matter to review the application through the public process currently taking place.

As a government agency in charge of protecting public health, the FDA experts performed this review process for all of us, for the public at large. The FDA requires scientific data to validate each step in the review process

outlined in the FDA Guidance 187 which has been articulated here today. This process was finalized in January, 2009 after extensive review of the underlying statutory authority, the existing and anticipated products poised to undergo the process and many public comments.

The FDA's regulatory process sets forth a cautious and scientifically sound approach to the application of well-established legal and scientific requirements to this new technology illustrating why the FDA maintains its position as the world leader in science-based reviews of products affecting human and animal health.

The technological advances in agriculture discovered by researchers have reduced environmental impacts of agriculture while continuing to feed a growing population. The use of these technologies and specifically the approval of this biotechnology in the U.S. would benefit American aquaculture and lead to more jobs being created here in the United States. Domestic production in approved well-regulated facilities would increase food safety and lessen the impact of trade disruptions on the available aquaculture supply.

FDA participated in the Codex Alimentarius Commission's ad hoc inter-governmental taskforce on foods derived from biotechnology and its working group that developed and adopted guidelines for assessing food safety of foods from recombinant DNA animals. Codex standards are

recognized as international food safety benchmarks and act as models for governments in the establishment of their own food safety policies.

The information needed to establish food safety for food from GE animals under a new animal drug application is --

MS. SINDELAR: You have one minute.

DR. EDWARDS: -- consistent with that described in the Codex guideline. Many other products from animal biotechnology have and will benefit consumers. Many of these products are in development and will undergo the same rigorous science-based review by the FDA.

BIO appreciates the opportunity to comment and looks forward to biotechnology helping to heal, fuel, and feed the world. Thank you very much.

MS. SINDELAR: Thank you Dr. Edwards. Our next speaker is Darrell Rogers, Alliance for Natural Health.

DR. : --- (Away from microphone)

MS. SINDELAR: Any questions you would like to pose to him from the VMAC?

(No response)

MS. SINDELAR: Thank you. Okay, Mr. Rogers.

Comments

by Darrell Rogers, ANH-USA

MR. ROGERS: Hello everyone. My name is Darrell Rogers. I am the Communications Director with the Alliance

for Natural Health in the United States. I have no financial relations with AquAdvantage and plan not to have any going forward which is another reason why we need labeling of GE products.

The Alliance for Natural Health is part of an international organization that is dedicated to promoting sustainable health and freedom of choice in healthcare through good science and good law.

We protect the right of natural health practitioners to practice and the right of consumers to choose the healthcare options they prefer. The ANH-USA is committed to sustainable health; the recognition that our environment and our physical health are inextricably related. Our dedication to sustainable health and choice through good science and good law directs our organization's actions.

We have opposed genetic engineering since our organization was founded. These untested, new to nature substances are hurried to market and consumed by live stock and unwitting consumers alike without any scientific proof of their long-term safety. Additionally the process the FDA uses to approve these substances lacks common sense and transparency. This is the antithesis with law.

In the case of the AquAdvantage Salmon, ANH-USA believes that neither the FDA nor AquaBounty Technologies has used good science in their safety assessment of this

genetically engineered fish. Scientific studies of the AquAdvantage Salmon have either not been released or have been released so late in the approval process that it is impossible for the public and experts to assess whether scientific burdens have been met. Current science suggests that health and safety implications of genetically modified organisms grow more pronounced over time. Due to the lack of data provided by AquaBounty Technologies, science cannot prove that this new gene spliced salmon is completely safe for human consumption over the long term since there are no carefully constructed double-blind studies of these fish being consumed over a long period of time.

Another serious concern regarding AquaBounty Technologies' genetically engineered salmon is that the FDA currently has no adequate means of assessing the fish as a GE animal intended for food by humans.

Rather than develop an appropriate evaluation method, the FDA is currently proceeding to approve the GE fish through a process of reviewing a new animal drug; clearly this is inappropriate. This process is meant for the review of new drugs used on animals not for the creation of new animals.

Such limited review by the FDA of the first ever GE animal for human consumption recklessly and needlessly endangers consumer health and is wholly inadequate to review potential public safety and environmental risks associated

with the consumption of genetically engineered animals. In short, ANH-USA believes that the FDA process to approve the AquaBounty Salmon stands in stark contrast to good law.

This genetically engineered salmon was filed for FDA approval many years ago but the FDA has withheld the application from public view until now. For an agency responsible for protecting and advancing public health, that action alone is contemptible. And given the gravity of the decision the FDA will soon make, we are shocked that the agency would even consider this new to nature bioengineered animal.

The lack of transparency by the FDA prevents the public and outside experts from submitting their comments. The absence of a public comment period on the approval of the GE salmon before this meeting prevents relevant scientific studies and data from ever reaching the FDA before this decision. Holding a comment period solely for labeling purposes presupposes that this AquAdvantage Salmon will be approved without proper public comment or solicitation.

I disagree with the AquaBounty CEO, Ronald Stotish, when he said in an interview with the Canadian Broadcasting Corporation that “this meeting will be subverted by a small but vocal group of people who are opposed to technology.” We are not opposed to technology but poor science. And our concerns are not the concerns of a small vocal group of people

but the concerns of Congressional offices, a coalition of over 30 organizations, and 160,000 individuals that have submitted public comments already. Thank you very much.

MS. SINDELAR: Thank you very much Mr. Rogers. Any questions from the VMAC?

(No response)

MS. SINDELAR: Then we will proceed to our third speaker Dr. Alejandro Rojas, Aquaculture Resource Management.

Comment

by Alejandro Rojas, Aquaculture Resource Management

DR. ROJAS: Hello, good afternoon. My name is Alejandro Rojas and I have been working in the commercial salmon industry for more than 20 years. During this time I have also been a consultant to many aquaculture companies, pharmaceutical companies, and also biotech companies including AquaBounty. I want to thank the committee for giving me the opportunity to speak.

(Slide)

Aquaculture is the fastest growing form of food production in the world. Nearly half of the fish consumed are produced by fish farms and this trend is expected to continue. However, fish farming is less ideal here in the U.S. where almost all of the fish and particularly salmon that we consume is imported. The domestic salmon farming industry in the U.S. is practically non-existent due to competitive economic

pressure from lower cost producers overseas and conflicts with environmentalists and regulators who do not favor salmon farming in sea cages.

I think that now with the potential introduction of AquAdvantage Salmon in land-based systems, I can see a fantastic opportunity to stimulate fish farming here in the U.S.

It has been well documented that genetically engineered crops such as corn, soybean, et cetera has been part of the American diet for years and I see a logical progression of genetic engineering from plants to animals.

Based on my years of experience in the commercial salmon industry, I sincerely believe that the introduction of AquAdvantage Salmon will revolutionize fish farming worldwide and particularly here in the U.S. in much the same way that genetically modified soybean has revolutionized soy production.

(Slide)

The economic benefits of this fish are several including faster growth up to harvest time, lower production risk, better feed and feeding control, and traceability.

It is also worth pointing out that farming this fish under the conditions presented and proposed by the FDA could actually be more environmentally friendly and sustainable than current methods of farming around the world.

Nevertheless, I also acknowledge that some people might be concerned about the potential environmental impact that deployment of this salmon might have. So I would like to address those concerns in my presentation.

(Slide)

As you can see in this slide, in commercial salmon farming there are two primary environmental risks. Mainly organic matter that can impact the water quality and seabed under and close to sea cages. This organic matter comes from feed and feces; mainly feed.

The second one is risk of fish escape and the potential for cross-breeding and perhaps disease transmission to wild fish populations.

How do we mitigate these problems? By following the strategy proposed by the FDA and AquaBounty in which sterile, all-female fish are stocked only in land-based contained systems. The risks associated have been controlled and removed.

(Slide)

Concerning the land-based contained systems, I have to say that these have been used already for more than three decades and have grown due to many benefits such as reduced environmental aspects, absolute control over fish escapes, better pollution performance, greater biosecurity, and site independency. We can locate this production site anywhere,

not necessarily close to coastal areas. We can locate these production systems in close proximity to local markets thus eliminating the high cost and carbon footprint associated with international freight. Of course there is a lower cost production and there is another important aspect which is less water usage and water footprint with low or even zero water discharge.

Many of these closed or contained systems already exist worldwide including in the U.S. and would only require minor modifications to be able to grow AquAdvantage fish.

MS. SINDELAR: One minute sir.

(Slide)

DR. ROJAS: The other important safeguard that AquaBounty has built into the fish is the production of sterile, all-female fish thus eliminating completely the possibility that GM salmon could establish breeding populations.

(Slide)

My conclusion is that the U.S. aquaculture industry and seafood consumers worldwide would greatly benefit from a healthy Atlantic salmon that can be produced almost anywhere in contained land-based systems that are more environmentally friendly and sustainable than the current method for salmon farming. Using sterile all-female that are confined in these systems eliminate any potential environmental impact. Thank

you.

MS. SINDELAR: Thank you very much. Do we have any questions from the Veterinary Medicine Advisory Committee?

(No response)

MS. SINDELAR: We will proceed with our next speaker. Would you like to come up? Michael Hansen, Consumers Union.

Comments

by Michael Hansen, Consumers Union

DR. HANSEN: Okay, my name is Michael Hansen. I am a Senior Scientist with Consumers Union. We are the publisher of *Consumer Reports* magazine. I submitted some fairly detailed comments just on the food safety assessment but in this short period of time, I would like to talk about some of the direct and indirect effects, the hormone and allergenicity concerns.

(Slide)

And I apologize, this was done on a Mac so you don't see the pictures but they are actually Tables from your briefing packet. So the first one is Table 12 and that is just for what the direct and indirect effects are and I will look at the Chinook salmon growth hormone levels, the IGF-1, and for indirect effect we will look at the endogenous allergenicity and the omega-3 to omega-6 ratio.

(Slide)

If you then look at Table 13, that looks at the first growth hormone level from the Du, et al. study and what you see there is if you look at that Table -- I am sorry it is Table 13. The sample size was so small that between -- there was a doubling in the growth hormone level from 20 to about 40 that was not statistically significant; that is one main point. The other point is when you look at the fish weights, the engineered fish were 47g and the controls were about 10g; those are not market-weight fish. So that they are so small that we do not think that they are relevant for food safety.

(Slide)

So if you then go to Table 15 which was the hormone analysis of the 73 fish, we see they used a detection limit for both the growth hormone and IGF-1 that was too high. So for the growth hormone if you look at that Table, you will see that none of the fish out of the 73 could they detect any growth hormone but yet they conclude “no biologically relevant differences were detected in the levels of the gene product.” Well of course, you have no data so the proper thing should have been to say use a more accurate method and come back with this.

(Slide)

If we look at the IGF-1 data as well, again only 17 out of 73 could they detect it in. And then if you go to Table 16 that is where it looks like there is -- in Table 15

there was a 40 percent difference in IGF-1 levels between the control and the engineered fish and that drops to 4 percent. We now know that that is because they only were looking at the diploid fish. So what that means is just like with the growth hormone for IGF-1 there is no data whatsoever on the levels in the triploid fish. So again you are making assumptions that IGF-1 is not a problem here in the absence of any data from the fish that you want approval from. Because they concluded that there did not appear to be a statistically significant difference.

(Slide)

What the next Table was is if you look at Figure 5 and Table 30, that is the allergenic potency data. What they dropped of there was the fact that with the diploids, the engineered diploid, that tells you that the process of genetic engineering led to an extremely highly statistically significant increase in allergenic potency. The p value was 0.0008. So the process of genetic engineering leads in this study to an increase in a potential allergenicity based on just six fish.

And what they do as well, the engineered fish are 52 percent higher but the triploids were only 20 percent higher and that is not statistically significant. The sample size is six.

We think they should redo this study using much

larger sample sizes. And it should also be pointed out that all the safety and nutritional data comes from fish that are raised in Prince Edward Island. They admit in the document that the rearing conditions will likely be significantly different in Panama and that the effect on the phenotype is unknown but they of course conclude that there is no problem when you have no data on fish from Panama. So at the very least this data should be re-looked at.

(Slide)

I am not going to look at the omega-3 to omega-6 ratios except to say that if you compare it to the wild fish, the wild fish are about three times higher so there is a difference.

(Slide)

So our summary is that if you ask do the data and information demonstrate a reasonable certainly of no harm from consumption of foods derived from AquAdvantage Salmon, I think the answer to that is no because of insufficient data of poor quality. We need more rigorous studies using better experimental design, more sophisticated or sensitive methodology with a large enough sample size to perform a power analysis to make adequate conclusions. There is a lot of technical details about how they did the allergenicity assessment, for example. They refer to Codex, they use eight amino acids rather than six --

(Microphone fades out)

MS. SINDELAR: Does the VMAC have any questions?

(No response)

MS. SINDELAR: Thank you very much.

Comments

by Wenonah Hauter, Food and Water Watch

MS. HAUTER: I am Wenonah Hauter. I am Executive Director of Food and Water Watch and I am delivering the last 7,500 comments from the 30 organization coalition that has been trying to alert the public about the GE salmon issue.

And most Americans agree with us. Today Food and Water Watch released the results of a poll conducted last week. Seventy-eight percent of adults surveyed oppose GE salmon's approval. That is across every demographic, age, gender, political affiliation.

We are concerned about the unintended consequences of this procedure. We are concerned that this is taking place under a veterinary drug process. And we think that the FDA needs to start over again, catch up with the science, and develop a real process for looking at genetically engineered meat.

At the very least, because we are so afraid that this approval process is moving forward, this salmon must be labeled. The FDA found significant differences between GE and non-GE salmon in Vitamin B6 and the hormone IGF-1. This

demonstrates a material difference in the nutritional composition and meets your standard for labeling.

What is most disconcerting, Michael Hansen has already talked about. It is the science that the FDA used in making its determination. The analysis of IGF levels looked at only two studies. A peer reviewed publication from 1992 and an AquaBounty study from 2004.

In its analysis of GE salmon's nutritional content, the FDA depended on one dataset supplied by AquaBounty from 2003. And the analysis of the GE salmon's allergenic potency focused on a 2006 study also furnished by the company.

These four studies, three of which are not even peer reviewed, formed almost the entire basis of the FDA's analysis of food safety issues. These studies exhibit great weaknesses in design as many of the critical datasets include only a handful of fish. For instance, there has been the discussion of the six triploid GE salmon that were used to determine the allergenic potency. This is a dangerously limited set of data. Even the FDA acknowledges problems with the sample size. What is the rush? Why can't this be done correctly?

AquaBounty's data collection is rife with potentially serious procedural errors. The company's primary investigator departed in the middle of his or her analysis. Company scientists unblinded the subjects of its study at one point disclosing the GE or non-GE identity of the salmon.

This is a serious violation of the fundamentals of scientific method.

A major concern with the safety of GE salmon is the enhanced hormonal activity that allows the fish to grow so rapidly and whether these hormones can be passed on to consumers. Data supplied by AquaBounty to the FDA showed that the GE salmon exhibited some increases in average concentrations of the hormone IGF-1. The FDA did not sufficiently investigate this --

MS. SINDELAR: One minute.

MS. HAUTER: -- from the toxicological basis. This approach fails to take into account a number of recent studies linking IGF-1 to cancer.

With all due respect, we do not believe that a veterinary advisory committee is the place to discuss these serious food safety issues. We should have a process developed by the Food and Drug Administration that really looks at all of the serious consequences of having genetically modified organisms in our diet.

I would like my full comments to be part of the record and I would also like the Lake Research Consumer National Survey to be part of the record. Thank you.

MS. SINDELAR: Thank you very much. Does the VMAC have any questions?

DR. KANEENE: John Kaneene. How many of you were

involved in your survey?

MS. HAUTER: Pardon?

DR. KANEENE: How many people were involved in the survey that you conducted? You said 75 percent of them agreed, what was your n?

MS. HAUTER: I can provide that to you. This was a large survey conducted by a company that does this and there were a couple of questions about this issue. I will provide this for you after the meeting.

DR. KANEENE: Okay, thanks.

MS. SINDELAR: Thank you. Our next speaker is Jaydee Hanson, Center for Food Safety.

MS. HAUTER: Actually can I answer that question because I just found the data. It was 1,000 adults, 18 years of age and older, living in private households in the continental United States. The interviews were conducted September 9 through 12 in 2010.

MS. SINDELAR: D. Griffin has a question Wenonah Hauter.

DR. GRIFFIN: Could I ask that since you are going to add that to the packet would you also add or site specifically the questions that were asked? Frequently questions paint the outcome so I would like to have those in the record as well.

MS. HAUTER: Okay.

DR. GRIFFIN: Thank you.

MS. SINDELAR: Thank you.

Comments

by Jaydee Hanson, Center for Food Safety

MR. HANSON: Yes, I am Jaydee Hanson from the Center for Food Safety. I am also representing our sister organization the International Center for Technology Assessment.

We have no conflict of interest -- it is a financial conflict of interest. Our funding comes from foundations and our 125,000 members. I do not invest in biotech stocks or nanotech stocks so my retirement is not dependent on the outcome of your deliberation.

I would note that you have received our comments electronically earlier but I have them printed out so you would have a hard copy as well.

I would also like to note that at the back of those comments are our comments on the guidance document that is governing our discussion here today. Please note that we believe this guidance misapplies the statute and the pathway created actually violates the law. That said, we are going to comment anyway.

But we believe that you have an impossible job.

That you are asked to work with a fiction that tries to fit a whole animal into a process developed for a drug. I do not think this is an intellectually honest approach and I have already said we do not think it is necessarily a legal approach.

We also note that we received these papers from the FDA only 10 days ago. And the most striking thing was how little data the company had produced over the last 15 years. Or at least how little data was being provided to us. We have discovered today that there is data that is not in this dataset.

You have discussed and others have discussed the limitations of the data. I would remind you that many of you supervise graduate students. I would hope that you insist that your graduate students would redo studies that are as statistically flawed as many that we have in this document.

I do not think you can really look at all of the data in this set of studies and say that adequate work has yet been done.

We have a number of specific questions; I will not go through all of those in my five minutes. I do urge you to at least look at the bold print in the comments that I have given to you.

And I would underscore that the FDA itself admits that there are design errors in these studies. Our

frustration is the FDA concludes that all these can be fixed after the fish is on the market, not before. We would urge that these be fixed before the fish goes to market, not afterwards.

One area that has not been discussed and that struck us as particularly interesting with its omission, there is no discussion on how much antibiotic was used with which fish when. Lots of discussion about weakness in the animals and problems in the animals but I would have liked to have seen data on antibiotics as well as the food that were provided to the animals and that is not there. Maybe it was identical, but it would be stronger if all of the inputs were included.

MS. SINDELAR: One minute sir.

MR. HANSON: Okay. I would urge you to look at our comments on the assumption that all of this DNA is generally regarded as safe. This is the first animal drug. It would be good to see data from animals that make clear why this is safe especially why it is safe with respect to new information we know about non-coding parts of the genome and I will underscore that.

I would also say that there is no discussion about what happens to animals when they are fed this product. Very often, defective animals are fed to other animals. So it would be nice to see that data as well.

We will have a chance to talk about the

Environmental Impact Statement in the next 30 day review.

MS. SINDELAR: Thank you Mr. Hanson.

MR. HANSON: Thank you very much. Let me give you, well somewhere here I promised to give you the 25,000 comments that have come --

(Microphone fades out)

MS. SINDELAR: That is fine. I must interrupt you for the fairness for all speakers that we have. I do want to thank you for addressing the conflict of interest as beginning statements for your presentation.

MR. HANSON: I think it should be required of all speakers and not just suggested.

MS. SINDELAR: Yes, also can I please ask you are the same comments that were distributed today the same that you sent to us prior to the meeting as we requested by Tuesday of last week?

MR. HANSON: Yes they were.

MS. SINDELAR: Thank you. Thank was very helpful and they were provided to the committee. And if you could leave that with me we will --

MR. HANSON: These are the 25,000 comments that --

MS. SINDELAR: Thank you. Our next speaker -- oh, I am sorry. Are there any questions from the VMAC?

(No response)

MS. SINDELAR: All right, let’s proceed with our

next speaker Nina Mak, American Anti-Vivisection Society.

Comments

by Nina Mak, American Anti-Vivisection Society

MS. MAK: Good afternoon and thank you for providing the opportunity to speak today. My name is Nina Mak and I am a Research Analyst at the American Anti-Vivisection Society.

AAVS was founded in 1883 and was the first non-profit organization in the U.S. established to monitor and expose problems with animal experimentation. I have no conflicts of interest to declare.

I am here today to oppose the approval of the new animal drug application for the AquAdvantage Salmon. I also have with me here a letter which I will leave with the committee signed by over a dozen other animal protection organizations representing millions of members and supporters who are also opposed to the approval of the AquAdvantage Salmon.

My focus today will be on animal health. Specifically as you heard, a NADA is required to show that the proposed drug, in this case the genetic modification, is safe for the animals involved. With all due respect, the data presented in the NADA falls so far short of meeting the animal safety requirement it is frankly shocking. There is no way the science here meets the standards for a new animal drug.

Given the short amount of time I have I will

highlight just a few of the most egregious problems with the data. For a more complete analysis I refer you to the written comments that were submitted last week from AAVS and Farm Sanctuary, another non-profit.

First according to the application, AquaBounty engaged in extensive culling of deformed, diseased, and dying fish before any of the data in the application were collected. In other words they excluded these fish from their studies and only looked at their healthiest fish. You cannot possibly evaluate the health impacts of a drug that way.

Second the FDA relied largely on only one animal safety study and in that study the sample size was just 12 fish. It is not possible to make meaningful comparisons between groups of just 12 individuals and many possible health effects would not even be caught by such a small sample size.

I want to emphasize no statistical analyses or tests of statistical power were performed for any of the animal health data.

Third the main study used fish from the 2007 year class which if you look at the historical data means that the most healthy AquAdvantage Salmon since 2003 were compared to the least healthy non-GE fish. Clearly this would skew the results in favor of the AquAdvantage Salmon.

Fourth despite these limitations the data provide indications that these salmon are unhealthy animals

experiencing high rates of abnormalities and mortality.

For example if you look at Table 4, more than 30 percent more AquAdvantage Salmon had slight to moderate abnormalities than non-GE salmon in three of the five years shown.

In Table 5 of the 15 averages provided for survival of AquAdvantage Salmon, 8 show survival rates of 50 percent or less and survival even dipped as low as 2 percent in one instance.

I can point to other data here but let me just leave at the studies were poorly designed, the data obviously slanted, and the conclusions of safety completely unfounded.

Switching now to the FDA's assessment of the NADA, let me highlight another set of problems.

First the FDA asserts that it will accept such limited and highly flawed data and instead rely on post-market surveillance to determine the rate of health problems. This is wholly unacceptable and inconsistent with standards for a normal drug approval process. The FDA is saying it will approve first and get the safety data later.

Second the FDA dismisses most adverse outcomes as being associated with fast growth or triploidy. But think about it, the drug is intended to produce the effect of fast growth and the side effects caused by inducing that effect cannot be dismissed as they are a direct consequence of

administering the drug. These fish would not exhibit these characteristics if they had not undergone procedures to produce the AquAdvantage Salmon under review.

Furthermore the fact that fish raised in aquaculture are often unhealthy and deformed should not justify producing a fish that will perpetuate this horrific state of affairs.

AquaBounty and FDA have selected a completely inappropriate reference point.

Third the data showed that genetics can greatly affect outcomes as certain genetic crosses led to 95 percent mortality. And we also know that husbandry conditions can impact health. If the FDA does not consider the impacts of genetic background or husbandry conditions on the drug's effect and in fact fails to specify any standards for how these fish should be raised to minimize adverse outcomes and promote health even though this too is standard procedure for a normal NADA.

Fourth and perhaps more importantly, the FDA only considered animal health in the context of how it would impact marketability and food safety. Therefore, animals who would likely be excluded from the food supply are considered inconsequential regardless of how many health problems they experience. But a new animal drug must be evaluated for any adverse outcomes it causes for any and all animals who receive the drug.

To summarize then, the FDA has not upheld the standards for a new animal drug review --

MS. SINDELAR: One minute remaining.

MS. MAK: -- and instead appears to work the process to fit with what seems to be a foregone conclusion to support approval.

Lastly let me quickly highlight a couple of problems inherent in regulating GE animals as new animal drugs. Genetic modification is simply conceptually different from a drug. And overall the drug model is just plain flawed and ill-suited for handling impacts to animal health and welfare. For example, using the drug model, lots that are found to be out of specification would be destroyed. That is one thing when you are talking about a batch of pills but quite another when you are talking about living animals.

In addition a drug is typically designed to provide some benefit to animal health against which the FDA would weigh potential risks. Genetic modifications, at least the kind we are talking about here, do not benefit the animal in any way. The FDA has not indicated how it can make approval decisions for a drug that has no benefit but does carry a risk of harm.

The AquAdvantage Salmon application sets a precedent for future reviews of other GE animals already in the pipeline. It should be held to the highest standards to

ensure that animal health, human health, and environment are maximally protected. The data in review presented here sets a dangerous precedent.

In conclusion, the AquAdvantage Salmon application fails to demonstrate animal safety and in fact is wholly lacking in scientific rigor --

MS. SINDELAR: Thank you for your comments. We need to move on to our next speaker; thank you.

MS. MAK: We all came a long way and I know that we have to move in time but we all came a long way to talk today and we have sat through and we have been delayed; this will just take 30 more seconds to finish, that's it.

MS. SINDELAR: Dr. Senior?

MS. MAK: In addition the FDA's approach to an analysis of the AquAdvantage application raises serious questions about the agency's commitment to protecting animal health.

DR. SENIOR: I'm sorry; I beg your pardon. Are there any questions? I am sorry, in fairness to everyone else. Are there any questions for this speaker?

(No response)

MS. SINDELAR: Thank you very much.

DR. SENIOR: Thanks so much.

MS. MAK: I would just like to say that the FDA has asked for $2 million dollars to approve GE drugs next year;

this is not what the public wants.

MS. SINDELAR: Our next speaker is Eric Hoffman, Friends of the Earth.

Comments

by Eric Hoffman, Friends of the Earth

MR. HOFFMAN: Thank you to the members of the VMAC for providing time for public comments today. My name is Eric Hoffman and I am with Friends of the Earth. I have no financial conflicts.

We have submitted full written comments to the committee that were signed by 21 environmental and public interest organizations representing millions of members across the country. And along with these comments we submitted letters from over 7,885 Friends of the Earth activists that I have included here but you also have an electronic copy. So we are indeed loud, we are not a minority.

AquaBounty's environmental assessment is flawed and fails to address the real threats posed by escaped transgenic salmon on wild salmon populations and local ecosystems or the environmental harm caused by raising and feeding these fish.

AquaBounty admits that the sterilization process does not work on up to 5 percent of all eggs. AquaBounty claims to have orders for 15 million eggs already so that means that right off the bat we may have up to 750,000 fertile

fish that can escape and wreak havoc on the environment.

Even more troubling is the fact that fertile males and females will both be needed to produce fertilized eggs on Prince Edward Island. This fact was ignored by AquaBounty's Environmental Assessment and needs to be included in any real assessment of environmental risk of this operation.

Hundreds of thousands of farmed salmon escape from contained systems every year due to damage by storms or wear and tear. AquaBounty's assessment admits that the facility in which the eggs will be fertilizes on PEI is surrounded by an abundance of favorable habitats for fish species including Atlantic salmon.

Studies have shown that if GE fish escape into natural populations, even if these fish were sterile they would lead to the extinction of both the wild and GE salmon populations. Faster growing GE fish that reach physical maturity faster will attract mates away from their natural counterparts which can affect the reproductive success of wild species.

AquaBounty claims that no significant natural disasters have occurred on Prince Edward Island. This ignores the fact that just this month the island was hit by a tropical storm and received upwards of 90mm of rain in a single day. That adds almost the same amount of rain AquaBounty reported as the average for the entire month of September in the

region. This tells that this EA is inaccurate, incomplete and that the risk of flooding leading to leaks in the containment system are in fact possible.

Climate change will only make the likelihood of serious environmental disasters such as hurricanes and flooding more likely. According to a report from the PEI Department of the Environment, the Island has been identified as one of the areas most vulnerable to sea-level rise in Canada. It will also experience increased storm events and an increased intensity of storms, extreme levels of precipitation and higher temperatures. Each of these points increases the likelihood that GE salmon will escape confinement.

AquaBounty also failed to consider the serious environmental impact of feeding farmed salmon an issue that is only exacerbated by the fact that AquAdvantage Salmon is engineered to be faster growing. Salmon farming already consumes an incredible amount of wild fish caught in the ocean and the AquAdvantage Salmon consumes up to five times more than its non-GE counterpart. This threatens many wild fish populations and the ecosystems in which they live.

Farmed salmon are very susceptible to disease and parasites and they will only become more vulnerable if genetically engineered grow faster. These GE farmed salmon will carry with them all the health hazards of other farmed salmon but may be more susceptible to disease and will

consequently need more antibiotics than fish currently grown in aquaculture facilities.

As AquaBounty admits, these transgenic salmon may be less fit than wild salmon and this increases the chances that the diseases and parasites may escape and enter into local waterways.

The only guarantee AquaBounty has provided that the fertilized eggs will not be raised outside of PEI and Panama, in open-ocean operations, in other in-land operations or even within the U.S. is a label on their plastic container saying not to do so.

There is no way AquaBounty or the FDA can guarantee that the eggs will only be raised in these specific contained facilities. And as AquaBounty has admitted this morning they do plan on raising their GE fish in America and other places. And it is irresponsible to ignore this fact and we need to look at these environmental impacts of full commercialization, not just the small research station in Panama that is currently under consideration.

AquaBounty is in the business of selling more fish and not less and they will do what is necessary to increase profits since they are a corporation even if it is at the expense of the environment, public health, and wild salmon populations.

It is incumbent upon the FDA to take these

environmental threats seriously and not just accept AquaBounty's flawed environmental assessment.

MS. SINDELAR: One minute remaining.

MR. HOFFMAN: Thank you. First a comprehensive and independent environmental impact statement must be completed and until then any decision on approval must be delayed. Even if an EIS is complete, the environmental and public health risks of GE salmon are simply too great to just fight FDA approval. Denial of AquaBounty's transgenic fish for consumption and commercial production would be in the best interest of the environment, public health, fishing communities across the country, and biodiversity around the world. Thank you for your time.

MS. SINDELAR: Thank you very much. VMAC questions, any one on the committee?

(No response)

MS. SINDELAR: Our next speaker is Anna Zivian, Ocean Conservancy.

Comments

by Anna Zivian, Ocean Conservancy

MS. ZIVIAN: Good afternoon and thank you for the opportunity to speak today on the important issue of permitting genetically modified Atlantic salmon to be raised for human consumption.

My name is Anna Zivian and I am a Senior Manager at

Ocean Conservancy. I have no financial conflicts.

While OC supports responsible aquaculture undertaken pursuant to appropriate environmental and safety standards, the FDA should deny the AquAdvantage Salmon petition at this stage and should undertake a more complete review of the issues raised by the company's ultimate plans as stated by its President to grow the fish not only in Panama but in areas closer to population centers.

As an initial matter, the FDA should not serve as lead agency under the coordinated framework for the regulation of biotechnology in considering this application as one for a new animal drug. The FDA process for approving new animal drugs allows for neither robust public participation nor thorough consideration of environmental hazards. Instead it protects confidential business information and looks at specific effects of drugs on human and animal health without examining the potentially wide range and serious consequences of environmental risks of transgenic salmon.

Until the release of the EA two weeks ago, the public has had no opportunity to learn more about, assess, or raise questions about potential impacts. In addition to problems with the process, the Environmental Assessment fails to address the full implications of the proposed action focusing instead on what are clearly the initial phases of a broader project. While the EA states that it addresses only

the risks of producing eyed eggs on Prince Edward Island, Canada and growing them out in Panama, it also asserts that the company is requesting approval in order to address an industry need for more rapidly growing Atlantic salmon broodstock. Under the conditions set forth in the EA, at most about 14,000 fish per year would be grown out in four tanks in Panama; hardly enough to provide seafood to a growing world population, service the aquaculture industry, or repay the $50 million investment made over the last 14 years.

Given that the production scenario as outlined does not represent the expected final production scenario for these fish, the EA cannot adequately address cumulative impacts to the environment including the global commons.

To do so the EA must consider issues related to realistic production scenarios including cases where containment strategies are far less rigorous and escape is probable. The EA fails to provide sufficient support for its conclusion that redundant containment measures render the probability of exposure close to zero and that therefore, there is little need to look at the hazard in any detail. The hazard section of the EA is wholly inadequate even for the production scenarios described in the application where many of the containment features are described only in qualitative not quantitative terms.

With respect to endangered species, the EA points

out that Atlantic salmon do occur in the vicinity of the production site on PEI and acknowledges that populations of wild Atlantic salmon have been declining without undertaking any significant analysis of the potential impact of the genetically modified salmon on endangered wild populations.

These considerations are significant and should be reflected as such pursuant to NEPA and if necessary the Endangered Species Act.

Similarly, while the analysis relies heavily on the supposed sterility of the genetically modified fish, it also admits that some of the fish may not in fact be sterile. These concerns are further amplified by the fact that there is a lack of effective monitoring and traceability in the global fish market.

MS. SINDELAR: One minute remaining.

MS. ZIVIAN: This poor quality control means that there can be no assurance that these fish and only these fish produced under the specified conditions of approval will reach the U.S. market. Lack of traceability and monitoring also raise concerns about potential economic effects for wild fish sellers and conventional aquaculture farmers who will have no guarantee that consumers will not mistake their product for the transgenic fish.

In conclusion, the EA is inadequate and does not justify a FONSI. The questions raised are substantial enough

to warrant the development of a full EIS that considers likely production scenarios in a transparent manner with provisions for robust public participation and additional publicly available input from other agencies with expertise in fisheries and ecological risk including NOAA, U.S. Fish and Wildlife and EPA.

If you fail to deny this application, any approval should make absolutely clear that that approval extends only to the limited circumstances set forth in the application with no implications for future activities on that scope.

MS. SINDELAR: Thank you for your comments.

MS. ZIVIAN: Thank you for the opportunity to speak and I would welcome any questions that you might have on this or my written comments earlier submitted.

MS. SINDELAR: Any comments from the VMAC?

(No response)

MS. SINDELAR: Our next speaker is William Muir, American Society of Animal Sciences.

Comments

by William Muir, American Society of Animal Sciences

DR. MUIR: I just want to say that I do not have any conflicts. The American Society of Animal Sciences sent me. I do in fact have an INAD to study transgenic tilapia for risk assessment purposes.

(Slide)

I wanted to bring to the attention of the FDA and the VMAC some additional data on risk assessment of these particular fish that actually Eric Hallerman and groups have collected.

My first several slides actually were already covered in detail by Larisa and Eric and Eric and so I am not going to go over risk assessment theory, all this other good stuff, because it has already been talked about in wonderful detail. And even the Trojan gene was talked about.

(Slide)

And what I wanted to look at was actually to talk about the data. Eric Hallerman's group had a very nice experiment federally funded by the Biotechnology Risk Assessment Program so it all came from public funding and the data provides us with an unbiased decision-making process.

(Slide)

So again this data was presented to the Transgene Conference at Lake Tahoe in 2009. And it was Ian Fleming and group, Garth Fletcher, Eric Hallerman and they were using these same Atlantic salmon that we are talking about here.

(Slide)

They had some empiric observations about for feed efficiency and things like that looking at viability and actually that data has already been talked about.

(Slide)

And as Eric has already said, the early findings were that the transgene would have a negative effect on fitness and the FDA has more or less approved -- found the same thing.

(Slide)

So in their experiments they use three lines. They had the wild Atlantic, the cultured stock, and the transgenic line of AquaBounty which was crossed too.

(Slide)

And the interesting thing I wanted to talk about is actually mating success, something that has not been talked about here in looking at male reproductive success. In the anadromous adult transgenics and control males, it was found in competition that they were competing for access to breeding females and the transgenic males were captive-reared and the control males were wild. So it was highly replicated with 11 replicates.

(Slide)

And the results, I am just going straight to results, is that the transgenic males were behaviorally outcompeted by the control males. In other words, the controls got more access to nest fidelity, quivering frequency and actually spawning participation.

So the data shows that the transgenic fish do not in fact have a mating advantage like I originally came out in my

paper in *PNAS*, I said all these larger fish are going to have a mating advantage. It turns out that females want more than size, I didn't believe that.

(Laughter)

DR. MUIR: And they also look at parr and they came up with exactly the same thing. These parr or these early maturing transgenic fish and came up with exactly the same conclusion.

(Slide)

So the results of the reproductive fitness conclusions were that transgenic males displayed reduced reproductive performance relative to control males.

(Slide)

So regarding the Trojan Gene Hypothesis, I want to clearly state that this only occurs as a result of a conflict between mating success and viability fitness. And the data conclusively shows that there is no Trojan Gene effect as expected. The data in fact suggest that the transgene will be purged by natural selection. In other words the risk of harm here is low.

(Slide)

And I just wanted to acknowledge my collaborators.

One of the other things that I did want to mention that I did not have in here is what would happen if the risk assessment -- if we talked about risk, what is going to happen

to the wild population if you have a transgenic male, let's say escapes, as opposed to a domestic male that escapes and interbreeds with the wild population, which has the greater impact?

Well it turns out because the transgene is inserted on a wild background, the offspring from a transgenic male will automatically produce half wildtype offspring which means that automatically adds to the wildtype population you have. The other half are naturally transgenic. It is dominant so it could easily get rid of it if nature decides to do that. But a domesticated salmon is polygenic and a polygenic offspring means that 100 percent of its offspring is going to contain 50 percent maladapted genes. So actually a domesticated salmon will have a much greater impact on the wild populations than the transgenic fish will. Thank you.

MS. SINDELAR: Any questions from the VMAC?

(No response)

MS. SINDELAR: Thank you very much. Our next speaker is Ann Kapuscinski, Dartmouth.

Comments

by Ann Kapuscinski, Dartmouth

DR. KAPUSCINSKI: Thank you. I am Ann Kapuscinski. I am a Professor of Sustainability Science from Dartmouth College with training and extensive research experience in fisheries and aquaculture science.

My comments that I am presenting today are on behalf on myself and also Frederik Sundstrom, Assistant Professor in the Department of Ecology and Genetics, Uppsala University. I have no financial relationships. Like Bill Muir, I also have an INAD for doing ecological risk assessment experiments with transgenic tilapia.

I thank you for the opportunity to comment on this application. Our comments focus on environmental risk assessment based on our two decades of experiments with transgenic fish and development of ecological risk assessment methodologies. Please see our written comments submitted September 16 for more extensive discussion of our points.

I would like to start by describing an elephant that I think is in the room. The company understandably wants to sell their eggs to many growers in order to be competitive in the global farmed salmon industry. So approval of this application will surely trigger other applications in the near future.

But the regulations that FDA is using do not require the agency to publicly release future environmental assessments for public review before their approval. Therefore, the environmental assessment procedure laid out in this case will be setting a precedent. And it is thus imperative that it follows high scientific standards and minimum scientific requirements.

I agree with what many people have been saying today; the multiple confinements of these transgenic fish is crucial to prevent environmental harm especially because of scientific uncertainty regarding their environmental risks.

If physical confinement fails and sterile transgenic fish regularly escape into environments where they can thrive, it is important to realize that they could still alter the environment.

We have two major concerns with the current application.

The first major concern, how will the FDA assure and verify that multiple confinement is continually achieved at the two facilities and in future facilities as farming of these fish proliferates? Confinement measures can appear rigorous but such complex safety systems are prone to human error and equipment failures. The FDA should require a quantitative failure mode analysis for all the confinement methods. Failure mode analysis is standard practice for technology assessment.

For example, failure analysis of the geographical confinement is missing but should be done and should include data on how AquAdvantage Salmon respond to changes in temperature and season.

The assessment suggests that water temperatures in the lower reaches of the Panamanian river and Pacific Ocean

will be lethal to these transgenic fish but has their thermal tolerance been measured? Published research on coho salmon shows an increased thermal tolerance resulted after growth-transgenesis. Are there data on how the transgenic Atlantic salmon will fair in the seasonal temperature ranges of this river in Panama?

As commercial production of these fish proliferates, an even greater challenge is how to assure multiple confinement at many larger facilities in different environments and nations. Does the FDA have the resources and sufficient overseas jurisdiction for adequate surveillance?

Our second major concern is that the Environmental Assessment does not give the full information needed to predict environmental effects of AquAdvantage Salmon. It stops at estimating that the likelihood of escape is "extremely small" due to multiple confinement at the two facilities. But this assumes 100 percent achievement of the confinement and even with actual exposure very close to zero, it is necessary to assess ecological consequences and then estimate the overall risk especially given the precedent set by this Environmental Assessment.

The assessment does not adequately address the major questions that should be asked about genetic and ecological risks. Empirical studies have shown there is high scientific uncertainty in predicting overall fitness and ecological

effects of growth enhanced transgenic fish because it is extremely challenging to extrapolate to nature from experiments using simulated natural conditions in the laboratory.

MS. SINDELAR: One minute.

DR. KAPUSCINSKI: As this published figure shows, and I will make a copy available to the committee, transgenic coho look very different when they are reared in a simulated natural environment, these fish here, the second from the bottom, to when they are reared in a domesticated environment here at the top making it therefore very hard to predict how transgenic fish will effect environments where they have not been studied.

I do not have time to give other examples, but overall the research shows that it could be very misleading to base an Environmental Risk Assessment on data for only a few traits that do not span the whole life cycle and are measured under a limited range of environmental conditions and time frames.

In short we are concerned about overly simplistic claims in the documents, of poor fitness of AquAdvantage Salmon without the scientific evidence to support this claim. The assessment relies on an outdated list of issues, a paper that I led in 1991 --

MS. SINDELAR: I am sorry, thank you very much for

your comments. Are there any questions from the VMAC members?

(No response)

MS. SINDELAR: With that we move on to our next speaker Jane Rissler, Union of Concerned Scientists.

Comments

by Jane Rissler, Union of Concerned Scientists

DR. RISSLER: Good afternoon and thank you for the opportunity to appear before this committee. I am Jane Rissler from the Union of Concerned Scientists and I have no financial conflicts.

This summary focuses on three recommendations and I refer you to our written comments for more detail.

We urge the VMAC to recommend that the FDA prepare an Environmental Impact Statement on the potential harms associated with the risk of AquAdvantage Salmon. Not only from the ABT facilities approved by the agency but other grow-out facilities likely to emerge in the wake of an approval of the GE fish.

The essence of FDA’s environmental argument is that simultaneous multiple redundant containment measures at each site reduce the possibility of release to zero. And since there is no possibility of escape there is no reason to assess the consequences. ABT has described biological, physical, and geographic measures complemented by management procedures that will greatly reduce the possibility of escape. However, while

these multiple redundant containment measures will substantially reduce the possibility, they cannot be counted on to reduce it to zero.

We need look no further than the failure of British Petroleum's Deep Water Horizon oil well. It was built and operated with multiple, redundant, technical, and management systems that should have prevented a spill yet they failed. Meanwhile the Interior Department had not produced a serious analysis of the consequences of a major oil spill apparently because it did not believe it could happen.

This committee should not allow the FDA to end up in the same position with GE fish. FDA's analysis also depends on the assumption that the two sites discussed in the briefing package are the only ones producing the salmon. If there are likely to be other sites, then the environmental impact is unlikely to be zero and a full environmental analysis would be required.

FDA should not avoid the obligation of assessing the risks to the global commons by assuming that these two facilities are the only likely sources of release.

Second we urge the VMAC to recommend that the FDA not approve the engineered salmon until it has determined the data requirements for a rigorous assessment of its food safety and environmental risks and the company has fulfilled the requirements.

In the current Food Safety Assessment, the FDA allowed the company to decide what food safety studies to perform and submit. Then the agency analyzed the studies, identifying and attempting to remedy the deficiencies it identified. This is not the role FDA should play. The FDA should be a neutral, objective evaluator of data submitted according to the agency's specifications. The agency should convene a puppet committee of experts to develop the data requirements for a rigorous food safety assessment.

The committee should also provide guidance on how tests should be conducted. The FDA should refuse to consider an application until the sponsor has submitted data in conformance with these requirements. It should do the same thing for the Environmental Risk Assessment.

Third the FDA review process starkly illustrates the inadequacy of using the drug laws for the oversight of GE animals. The drug provisions establish a system that is opaque to outsiders and hostile to public participation. Therefore we appreciated that the FDA has chosen in this case to share information with the public, convene this VMAC meeting --

MS. SINDELAR: One minute please.

DR. RISSLER: -- and allow public comment. However, we had hoped that the agency would have offered a more robust process for public participation. Instead it offered no

opportunity for the public to comment on scientific and legal issues except to this committee. The ten days available to read, analyze, and seek expert review on the diversity of issues raised by the fish were inadequate. The scope of the public comments was unclear because the agency did not announce the VMAC charge in advance of the due date for comments.

While composed of experts in fields pertinent to the typical review of new drugs, the makeup of this VMAC is inadequate for a comprehensive review of human and environmental risks and consumer concerns associated with the genetically engineered salmon. A properly constituted committee would include a number of fish -- oh you cut me off. Was my one minute up?

MS. SINDELAR: Your one minute is up.

DR. RISSLER: Too bad but thank you.

MS. SINDELAR: Thank you so much for your comments. Any questions from the VMAC members?

(No response)

MS. SINDELAR: Alexis Baden-Mayer, Organic Consumers Association is our last registered speaker for today.

Comments

by Alexis Baden-Mayer, Organic Consumers Association

MS. BADEN-MAYER: Hi I am Alexis Baden-Mayer. I am here on behalf of the 250,000 active members of the Organic

Consumers Association.

Genetically engineered salmon should not be approved for human consumption based on the data FDA has collected from AquaBounty. The data does not show that genetically engineered salmon is similar enough to normal salmon to be considered safe to eat.

The Organic Consumers Association has a number of concerns about the sufficiency, accuracy and interpretation of the data FDA used as the basis for its decision that genetically engineered salmon is safe to eat.

But first it is important to note that the FDA did not require food safety data on genetically engineered salmon DNA. The human health impacts of consuming the AquaBounty construct are unknown and are not being investigated.

Since 1992 the FDA has operated under the legal fiction that there is no risk associated with the consumption of genetically engineered DNA. As the FDA explains under this policy because DNA is generally recognized as safe, engineered DNA is considered safe as well.

But I would like to call your attention to a human study conducted by the UK’s Food Standards Agency that found that a single meal of genetically engineered soy can result in horizontal gene transfer where the bacteria of the gut takes up the soy’s modified DNA.

Research must be done to determine whether this

would happen to people who eat AquAdvantage Salmon and what the health implications would be.

The GRAS policy needs to be reevaluated now before the FDA approves the first genetically engineered animal for human consumption. As long as the GRAS policy is in effect, the FDA will not be researching the safety of consuming genetically engineered salmon DNA. Instead the current food safety review is a simple quacks like a duck style comparison of genetically engineered and normal salmon for hormone levels, nutrition, and allergenic potency.

Even accepting this elementary analysis, the data used to support the FDA's conclusion that genetically engineered salmon is similar enough to normal salmon to be considered safe is seriously flawed.

Number one, the FDA did not always segregate and sometimes did not even collect data from AquaBounty on the actual fish that people will be eating; the Panama raised triploid monosex AquAdvantage Salmon.

Number two, the FDA did not require AquaBounty to show that genetically engineered salmon is the same as normal salmon when raised under the same conditions. In addition to AquaBounty's control salmon, the FDA compared genetically engineered salmon to farmed salmon raised under unknown conditions and data from other salmon studies.

Number three, AquaBounty only tested a few fish

making it less likely that its food safety studies would reveal statistically significant differences between genetically engineered and normal salmon.

Number four, AquaBounty's detection levels were sometimes too low to produce food safety data for comparison.

Number five, AquaBounty selected which fish to test in unblinded samples which may have biased the food safety data.

But even with all the flaws and biases that are likely to have hidden the differences between genetically engineered and normal salmon, the evidence also showed that there were significant differences in hormone levels, nutrition, and allergenic potency.

Number one, genetically engineered salmon has 40 percent more IGF-1, a hormone linked to prostate, breast, and colon cancers in humans.

Second, genetically engineered salmon is less nutritious than normal salmon. It has the lowest omega-3 to omega-6 ratio of all the salmon in the studies FDA reviewed greatly reducing the health benefits associated with eating salmon.

MS. SINDELAR: One minute remaining.

MS. BADEN-MAYER: And third, genetically engineered salmon have mean allergenic potencies that are 20 percent and 52 percent higher than normal salmon increasing the risk of

potentially deadly allergic reactions.

With all that we know and all that we know that we don't know about genetically engineered salmon, there is no other way to protect the public health than to prevent genetically engineered salmon from entering the food supply.

Thank you for the opportunity to comment today. I hope you will read my written testimony including the 12,000 letters from individual members of the Organic Consumers Association.

MS. SINDELAR: Thank you very much. Any questions from the VMAC?

(No response)

MS. SINDELAR: I would like to applaud all of the registered speakers for their succinct presentations. Dr. Senior would you like to entertain any comments from the floor?

DR. SENIOR: Are there any comments from the floor?

Comments

by Leo Broderick, Resident Prince Edward Island

MR. BRODERICK: Thank you very much for the opportunity to speak to you. My name is Leo Broderick and I am Vice Chair of the Council of Canadians and I come from Prince Edward Island. And I am delighted to be here and to hear the proceedings. But I must say that Canadians are like most Americans, they do not have an appetite for the GE

salmon.

And I came to say to you that I think the process that has been established, and I am a guest in your country so I have to be careful, that the FDA is flawed. I believe the science has been sloppy, and I have been out to the facility on Prince Edward Island and I am not convinced that there is a 100 percent assurance that the fish, the eggs, the salmon will not escape. And so I urge you to reconsider and to look at this as a food not a drug. Because I always understood that if a drug were going to be given approval, it would go through animal and/or human trials and nothing like this has happened.

So I think on Prince Edward Island we do not want to become known as the home of the frankensalmon so please reject this proposal.

MS. SINDELAR: Thank you for your comments. Any other comments from the floor? If we can be brief, thank you. Your name?

Comments

by Lisa Weddig, National Fisheries Institute

MS. WEDDIG: My name is Lisa Weddig. I am the Director of Regulatory and Technical Affairs with the National Fisheries Institute.

I would like to thank you for the opportunity to address the Veterinary Medicine Advisory Committee with the views of the National Fisheries Institute.

NFI is a trade association representing all aspects of the seafood industry ranging from harvesters, processors, importers, and distributors to retail and food service operations.

Last year when FDA finalized Guidance 187 and with a decision on whether or not to approve AquAdvantage Salmon as the first genetically engineered animal intended for human food coming to completion, NFI's leadership felt it was important for the Association to develop a position on this important technological advancement and associated regulatory process.

After reviewing the benefits of biotechnology with respect to fish and FDA's determination that the rDNA construct would be regulated as a new animal drug, NFI leadership agreed to the following principles.

NFI supports the use of biotechnology in the production of genetically engineered fish that has the potential to enhance aquaculture capabilities.

NFI supports the FDA's regulating genetically engineered fish as a new animal drug because it provides a rigorous safety assessment prior to marketing the fish in the United States.

NFI supports requiring the aquaculture operations raising genetically engineered fish should adhere to good aquaculture management practices.

Aquaculture is the future of sustainable seafood. As we heard this morning, wild capture species alone cannot meet the needs of the growing global population.

MS. SINDELAR: Are you able to summarize?

MS. WEDDIG: Certainly. We appreciate the opportunity to provide these brief comments for the record and commend the committee on the challenge faced in advising FDA on this important landmark decision to approve the first genetically engineered animal intended for human consumption.

MS. SINDELAR: Thank you very much for your comments. This is a comment?

MS. CONLEY: Yes.

MS. SINDELAR: Yes sir. This will be the last comment from the floor.

MR. CONLEY: Very short.

MS. SINDELAR: Your name sir?

Comments

by Dave Conley, The Aquaculture Communications Group

MR. CONLEY: My name is Dave Conley from The Aquaculture Communications Group. I am Canadian and my colleague that just spoke does not represent all Canadians. Thank you.

(Laughter)

MS. SINDELAR: That was succinct. All right, any questions?

(No response)

MS. SINDELAR: All right, with that I am going to pass the baton to Larisa Rudenko so she can clarify the Charge to the Committee.

DR. DUNHAM: And before we do that, this is Dr. Dunham, I just want to say again thank you all so very much from the public for taking the time out to come and present your views and also to share with us your written comments; thank you.

Charge to VMAC

by Larisa Rudenko, Ph.D., DABT

DR. RUDENKO: Thank you very much. Dr. Senior, members of the Veterinary Medicine Advisory Committee, thank you so much for your kind attention, thoughtful questions, and what you are about to deliberate. We know this has been very hard work to sit and listen to various people for two days; I know how hard it is to sit and do that.

We very much look forward to what is going to happen next. I would also like to thank the members of the public for their written comments and for the oral comments that have been offered into the record here. The purpose of these VMAC meetings is to encourage transparency and we very much value the opportunity to do that.

(Slide)

Veterinary Medicine Advisory Committees are intended

to provide advice and recommendations to the agency. We hope that the questions that we have posed to the VMAC will serve as a framework for discussion among committee members and will allow for open but directed discussion of the four particular issues on which we seek advice. We will put up the charge one question at a time. Dr. Senior will direct the discussion for that.

To reiterate points that we made yesterday. What we are looking for is open and frank discussion. What we are not looking for right now is a discussion on whether or not the new animal drug provisions of the Act in part or in whole are appropriate to rDNA constructs. Whether there are components of the new animal drug provisions that may need to be amended. Or the labeling of food from GE animals which is the subject of tomorrow's Part 15 meeting.

Instead we ask you to address the questions that we have put forward to you and we look forward to your sincere and open comments and we wish to thank you one more time for coming; Dr. Senior, here is the first question.

Discussion among VMAC

by David Senior, VMAC Chair (Acting)

Question 1: Do the data and information demonstrate that the rDNA construct is safe to AquAdvantage Salmon?

DR. SENIOR: So I would like to open the discussion relative to the strengths and weaknesses pertaining to the

information presented demonstrating that the rDNA construct is safe to AquAdvantage Salmon. In opening it for discussion, I think I will just start with whoever wants to go first and we will work our way around from there; any takers?

Dr. Thorgaard.

DR. THORGAARD: I think the data that was presented is generally consistent with it being safe. I think the experiments certainly could have been better designed to look at survival. Another trait that I thought would have been good to measure was a trait called fluctuating asymmetry that measures degree of developmental disturbance in a comparison of fluctuating asymmetry of transgenic versus control fish within the same family as well as survival would have been optimal. But I am supportive based on what I saw.

DR. SENIOR: Any comments concerning the strengths or weaknesses of this?

DR. WELLS: Kevin Wells. When I consider this question, I am comparing the recombinant DNA to the same fish without the recombinant DNA. And I would argue that any sort of effect that we see with the recombinant DNA in and of itself is probably not greater than domestication itself. And the process of domestication and genetic selection is making much more profound changes to the genome and therefore any other genetic effects than the recombinant DNA alone. And so I would have to conclude at this point that the recombinant

DNA in and of itself, that construct, is as safe as domestication.

DR. SENIOR: Please state your name.

DR. JAFFE: This is Greg Jaffe. I guess to me I think that I would agree with the other two speakers that it looks like from the data that has been presented that there are not any particular concerns of the fish from the addition of the rDNA construct.

What I do think a weakness in the document is, is that it seems on the whole here over the last two days FDA has stated that we take what the -- the whole point of this application is not just the rDNA construct but the AquAdvantage Salmon itself and its use and its conditions around it which includes things like the triploidy of the genome. And it seems like in the Food Safety Analysis or the Environmental Analysis; we take those all together as one, the sex, how many chromosomes there are, and also the rDNA --

(Technical difficulty)

DR. JAFFE: -- the triploidy effects and I think that is an inconsistency or a weakness in the analysis because I don’t think you can sort of do it both ways. What we should be saying here is, is there harm from it being in an AquAdvantage Salmon, not just the rDNA construct, if FDA wants to be consistent with the other parts of its Risk Assessment, other parts of its analysis.

So although I do not see any data to suggest that the rDNA construct is problematic, there is some data that suggests that other aspects of the AquAdvantage Salmon do have some animal safety concerns. And I think that either needs to be included in the analysis or there has to be a better explanation by FDA about why they are not including that and why, in this instance, that is not relevant. I do not see that in the documentation, I think, sufficiently.

DR. POPPENGA: I think we were told that the culling rates -- Bob Poppenga. I think we were told earlier that the culling rates for the facility are about the same as industry-wide. So assuming that, I guess I do not see any particular problems. I agree with the previous speakers.

The one area that I think should probably be investigated more is there is very little with regard to disease resistance. And I understand that this is going to be a very controlled biosecure facility, there is not going to be -- there is going to be less chance of a disease being introduced to these fish but it seems to me that that is one safety area that has not been looked at.

DR. GRIFFIN: Dicky Griffin. To the previous two -- the construct itself is very common in agriculture and we know more about how those things have been done in plant science previously. Perhaps bacterial sciences have led the way which include the insertion of insulin production in *E. coli* of

which is used worldwide today.

The effect, however, adversely on an *E. coli* is quite different than an adverse effect on a salmon. And the AAVS questioned the survival data which gets at the disease resistance. In the location that I presently work, we look at disease resistance as part of our genetic groups and that is pretty unclear. So it seems to me like the construct is correct but the evaluation of the long-term effect is going to be open for a while.

And the geneticists will likely tackle disease resistance or perhaps modifications in the future.

MS. : Dr. Griffin, could you please speak into the microphone.

DR. GRIFFIN: I am sorry. Perhaps those things will be addressed in the future but currently the construct itself seems very standard and acceptable of what has been done out in front of us for a couple of decades in other living organism situations.

DR. WELLS: Dr. Griffin, do you see the construct's potential impact on disease resistance as being different than any other selection round? I mean essentially --

DR. GRIFFIN: No.

DR. WELLS: No, okay.

DR. GRIFFIN: No in fact we have a good deal of information in some of the domesticated livestock. And the

geneticists that I get to work with -- in fact a paper published in the last couple of years from animals at our location and I am at the U.S. Meat Animal Research Center, Gary Snowder, looking at bovine respiratory disease and genetic relationships to that.

We also looked at animal growth and other things that were -- and those are not independent.

DR. ALTIER: Craig Altier. I do have concerns about the studies that included culling of fish to quote the briefing packet here. It says that “culling procedures at the PEI facility are not likely representative of those used in commercial production and grow-out settings. Consequently there is some uncertainty regarding the likelihood or incidence of abnormalities in AquAdvantage Salmon under commercial rearing conditions.” The agency’s plan then is to address that in its Durability Plan. I think that is not appropriate. I think that if there are uncertainties and there are admitted uncertainties, that they should be addressed prior to this fish being allowed on the market.

DR. MATHEW: Alan Mathew. I generally agree that the animal safety data show that the construct is safe for the salmon at the market weight. Beyond that, there appears to be some health issues and I do not know whether it is possible then to look at longer-term health effects or if approval is granted, should the approval include slaughter at the age or

size that is relevant to the animal safety data; in other words, not growing the salmon out beyond a certain size or age to ensure that the animal health is not impacted down the road.

DR. McKEAN: Jim McKean. I came to this meeting with similar concerns to what Craig has in terms of the impact of culling. Those concerns are partially assuaged with the mile-wide analysis that we heard this morning, or this afternoon. They are not completely assuaged. So to answer the question directly, is the construct safe to the AquAdvantage Salmon, I think at this point based on what we have in front of us, is still somewhat of an open question. It appears to be safe but that loop has not, in my mind, been closed.

DR. LAPIDUS: Jodi Lapidus. I would have to agree with that sentiment exactly. I would categorize all the data that we have looked at thus far given its study design, sample sizes, and mixture of fish that are not necessarily representative of the salmon that will be marketed, I would have to characterize this body of work as potentially compelling preliminary work that would need to be validated and confirmed in other studies particularly on the population that would be marketed. Particularly removing the effects of the seasonality confounding and -- which we discussed and were not able to resolve in terms of explaining some of the

differences or lack of differences that were noted.

DR. APLEY: Mike Apley. My short answer to the question, do the data and information demonstrate that the construct is safe to AquAdvantage Salmon; the short answer is I don't know but being a Professor I have more.

All day the thing that has run through my mind about every two minutes is this is probably one of the most incredibly important precedents I have ever been involved in. I think as I sit here today, in a way, this is going to be evaluated and I struggle for the definition of safe.

The thing -- the word that has come into my mind all day is I think about the definition of safe for something that is designed for production is the impact of welfare to these animals for such things as jaw erosions or things like that; it keeps popping into my mind. And I am real -- I try to be real common sense about that but I just do not know how to put that into the definition of safe.

I do consider the whole of the label to include both the construct and triploidy. I think it was made very clear to us that we are here to evaluate the label today. And part of the label today is that triploidy will be induced in these fish. So to me, the issue of is this due to the construct or is it due to the triploidy may be moot in my evaluation of this which causes me to give the answer of I do not know.

I do get heartburn when we are going to allow post-

marketing surveillance to finalize our safety evaluation. I have issues with that. The disease resistance still remains a question to me. And again when we look at things like clinical pathology data where without a clinical -- we are unable to determine clinical relevance because we do not have the baselines for those. So I do not have adequate information to give an answer that -- to be able to answer that the data cause me to believe that it is safe.

DR. VAN EENENNAAM: I think some of my concerns here is I am not -- Alison Van Eenennaam. I am not sure I could answer this question for regularly produced triploids in aquacultural settings because I think you would see that sort of variability. And I think one of the things I am struggling with is that we are asking population-level questions, survivability, of a dataset that is really designed for a new animal drug application.

For example, we just take a sample 1 and an animal dies at, I don't know, 10 weeks; that is not telling you the longevity of that population. You need a broader number of samples in order to determine those sort of population characteristics and yet we are looking at much smaller sample sizes which is kind of the paradigm of a new animal drug approval where you are maybe treating with something, you know, six animals and seeing if there is an adverse impact on those animals. And asking of those six animals, does that

have this -- what is the population statistic of that animal. And so there are a lot of confounding effects; I agree with Jodi there.

And as a population geneticist, there is also the confounding effect of the actual genetics of the animals themselves. Are they full siblings as my question earlier alluded to because that will also affect the variability that might exist within the groups. And so with all that population stratification in the small size, I think it is difficult to answer these types of population questions. But I am not sure that that data exists for existing aquacultural practices.

DR. APLEY: Mike Apley again. I think you have helped me phrase mine a little different; it is beyond internal validity issues with some of the data. I think the external validity to the entire population that we are going to be dealing with is even more of a concern to me. How we go from what we have now, externalizing that to the populations that will exist, is what I am struggling with.

DR. WELLS: I am still struggling with the original -- Kevin Wells. We are asking a question of one gene, one of a minimum of 23,000 and this is the gene that we are considering and at the same time during normal breeding, normal selective pressure that would occur in any animal agriculture setting, there are tremendously more profound

effects that we do not regulate.

I grew up in Tennessee. They are famous for the Tennessee fainting goat. Every time it lightnings, they drop down on to the ground. Now if that same sort of animal were to come through this committee, I think we would band that entire breed.

So it is hard for me to get my mind around this one gene compared to the whole rest of the genome and why we are treating it differently simply because it was added through this technology as opposed to a breeding technology.

And so it makes it very difficult for me when I hear things like is there a health impact beyond the other genes? Then it gets to be a little bit difficult for me. Because I think amongst the rest of the genome this probably does not have a larger impact than many of the genes that are in the population right now.

I mean most of us in this room right now have probably 40 potentially lethal mutations, that is why we frown upon inbreeding.

(Laughter)

DR. WELLS: yet I do not think anyone wants to come in and regulate our ability to reproduce. I mean this is one more gene. It has characteristics. So thinking about it in the context of is this ultimately safe to the animal relative to the rest of the genome, I think that is a simple answer and

the answer would be yes. Relative to a drug, it is more difficult for me.

DR. McKEAN: Perhaps I am being too linear -- this is Jim McKean, I am sorry. Perhaps I am being too linear. I am looking at the construct and is the construct itself safe in its effects. I have not really looked at disease resistance or disease control because we do not have -- I have not seen any data that would lead me to make any of those decisions.

What I thought I was answering in this question was is the construct itself safe. Now the triploidy, the all-female, those are issues outside of the construct. Those are containment issues that we can talk about somewhere else I think if this is the linear question that we are being asked. If we are being asked to put all those things together, then I may have some different discussions so I would look to the Chair to help clarify really what our question is here.

DR. SENIOR: I believe the question relates to if the rDNA construct is introduced into the fish, is it safe for the fish? And so it implies is there any I guess anatomical, physiological changes that would be detrimentally introduced to the fish. It implies a phenotype and the overall behavior of that phenotype.

DR. McKEAN: Then I will stay with my original hand that I think the data is equivocal in that regard.

DR. SENIOR: Okay.

DR. McKEAN: The construct is safe I am convinced, but the ancillary data is equivocal.

DR. WELLS: Is it constructive at all to think about things like the jaw erosion data as a side effect?

DR. SENIOR: It is Dr. Wells and could you repeat that first sentence?

DR. WELLS: I am curious if it is useful at all to think about the situations where we may be considering a negative impact on animal health like the jaw erosions as a side effect in which case knowing its frequency becomes important. And I am not suggesting that; I am asking the question as part of a discussion.

DR. SENIOR: Any thoughts on that?

DR. APLEY: Mike Apley. Again back to the question if this is what we are supposed to answer. Asking that question, I think we were asked the incorrect question because no where else do we have a place to respond to the safety of the label. And it has been made clear to us about 28 times that we are here about the label. And so I will stay with my response --

(Off microphone discussion)

DR. APLEY: Then respond to that then please. What are we --

(Off microphone discussion)

DR. APLEY: I am not saying about whether we label it or not label but we are to evaluate -- in relation to the approval, correct? This specific approval.

DR. RUDENKO: The safety and effectiveness with respect to --

DR. APLEY: Of this, approval of this product.

DR. RUDENKO: Product.

DR. APLEY: That we are evaluating as a drug. And so I would maintain that this product contains both the construct and the other requirements which will require -- it may be semantics but anyway I will leave my input.

But back to the gene, comparing side effects or whatever, there are examples where aggressive breeding programs and fads of one particular bowl have led to things like pulmonary hypertension in one breed --

DR. WELLS: And curly calf.

DR. APLEY: Yes, and curly calf. All these things where we put in a widespread application of genes through selection of one genetic line and then later on we go, oops.

So the semantics of whether it is a side effect or not -- I appreciate your comment. Or a direct effect or side effect to me is -- I look at the whole of what we are going to do and thinking so is there some type of effect.

DR. SENIOR: No comment on that? I am surprised.

MS. EPSTEIN: I'm sorry, this is Laura Epstein. I

just wanted to clarify that we are not regulating the triploidy itself. We are talking about the construct. So when you talked about the label I assume you mean the claim that this sponsor is making and what the definition is on there of the claim that we will be looking at for the effectiveness piece of it and also what the product definition is; so what that construct is. And that is what we are talking about, is whether or not that construct is safe rather than the triploidy.

DR. APLEY: I will ask you before you leave, Mike Apley again, for one more clarification. My point is that the only way these fish will be able to go to market is that triploidy will be induced, correct? According to the --

MS. EPSTEIN: That is one of the conditions.

DR. APLEY: That is one of the conditions.

MS. EPSTEIN: Yes.

DR. APLEY: So maybe I am not using the right technology but I am saying that a condition of this if approved would be that the construct will be in there but they will also have to be triploid to be able to go to market.

MS. EPSTEIN: Yes, that is right.

DR. JAFFE: This is Greg Jaffe. I mean I agree with you Mike and that is what I was trying -- the point I was trying to make before was I thought the whole analysis that FDA is doing is not solely about the construct but about the

product and the product is defined not just by the construct but also by the triploidy and the all-female and so forth, and as well as the containment and the fact that it is only in two facilities. I thought that was the bound of their application. That there application was not just -- I understand the gene and the construct is the drug, but my understanding was that the assessment that FDA is doing is not solely of that construct but of the product which is both these other factors as well as how it is going to be used and that was the basis for the documents we got, the assessment.

MS. EPSTEIN: Yes, that is correct; it is the conditions of use that are under consideration.

DR. SENIOR: Then I need clarification. This is David Senior. So if I read the question now very carefully, I see that the question really did not include triploidy. And so any comments about evaluating the safety of is this safe for fish should avoid any discussion of triploidy. Because I do not think the committee was looking at it that way necessarily if we --

DR. McKEAN: Mr. Chair, this is exactly the question I was coming to. And in the four questions we have, I do not see any place where it asks --

DR. SENIOR: Your name Jim.

DR. McKEAN: Jim McKean, I am sorry. I keep forgetting who I am. That was my point about linearity. This

question is very specific and we have gotten, in some respects, we have gotten off. But I think part of the reason for that is of the four questions we have been asked to answer, there is none in here that asks us to evaluate the product, the final product, label, or however you want to describe that which would include the construct, the triploidy, the all-female, the durability, and the containment issues. And so we are trying to answer all those questions in the first one. When we get the first one done, I guess maybe we will be done. And if that is the case, then I will hold on to my answer to the first question as I think it is constituted.

I will say that in terms of the overall product that we have been discussing for the last two days, I would have similar concerns although again they have been somewhat assuaged by the CVM staff. But the issues related to smallness of numbers in a population, the size that I perceive a fish population to be, is difficult for me to get my head around and get complete confidence that I would have reproducible results at that level. When we get down to 60 animals to make a final safety decision in the population that I imagine to be aquaculture, that seems to be a pretty minor evaluation.

It reminds me a little of the vaccines that are put out after what are referred to as extensive testing and when

you get them out into the population, you find out that you, oops, you missed something. So that would be my comment about the full body of the work that we have discussed.

DR. SENIOR: I would like to summarize where we are so that we can maybe move forward.

What I have heard so far is that the opinion that this is probably generally safe and that there is no greater effect as a result of the incorporation of this construct than the normal selection process that takes place during domestication and improvement of domestic animals through selective breeding; so that this gene is no different from any other gene. And in fact the gene manipulation that takes place when people selectively breed for performance is actually even greater.

However, there are doubts and some of these doubts are raised because there are concerns that the small populations that were looked at makes it very difficult for conclusions to be made concerning whole populations. And so there is a serious concern there.

And in addition, specific studies, it was felt that the culling before evaluation of these animals, how that culling is done and how the culling is different in the unit where the culling took place from a production culling unit, throws some doubt on the situation.

Finally there was a comment about fluctuating

asymmetry and I will have to plead ignorance on that one but the comment was made.

There was a comment made concerning aspects of disease resistance; could that be looked at?

There was a comment concerning the evaluation of the long-term effects. Could we grow these animals out to an older age to see what happened or do we have to include in the approval process that they are killed at market weight so that we never find out what happens when they get older?

However, it was thought that we need to look at the actual aging process in full production up to the market weight because this has not yet been done. So that study is suggested as a thing that might be done as well.

And there was also the suggestion that some of these studies need to be done on the marketed product and again that is along the same lines, let’s see what it looks like when it is at the 4.5 pound level.

That is where we are so far. Does anyone have anything to add to that?

DR. WELLS: Maybe I have misunderstood something but it seems to me that the recombinant DNA is the drug. And I have been thinking of the animal as an animal treated with that drug with no withdrawal time. I have not been thinking of the animal as the product. And the triploidy is a condition of use. And the containment would be limitations of

use. But am I off? I mean is it a whole product or is the recombinant DNA the drug and we are thinking of it with no withdrawal time?

MS. EPSTEIN: You are exactly right.

DR. WELLS: So that is a little bit different than thinking of the product as a whole.

DR. McKEAN: This is Jim McKean. Which then goes back to my original -- that dealing with the construct, because of the issues related to culling and some of the data that seems to be floating around but it is really not in our materials, it leaves a cloud that is not -- it is not party sunny necessarily in the weather. That there are questions that have not been answered by the data that has been presented in the last two days.

DR. SENIOR: Well at this point -- this is David Senior. At this point it would be very good to give the FDA advice concerning what we believe they should come up with so that the floor is open to you Dr. McKean if you would wish to comment on where there is a deficiency and we need to do a better job here.

DR. McKEAN: I don’t know why I am the fall guy, but Jim McKean here. It seems like most of the data has been generated in an artificial environment relative to the way the product is going to be used.

My recollection on most of the drug trials I have

seen is that they had to be used in an environment that was fairly close to the final use. Therefore, I would make that analogy and I would say that this needs to go further and get into how the product is going to be commercially produced and make these evaluations, look at the culling, look at the changes that occur grossly in these animals, evaluate their health, and then we would know much more than we know today about how this product is going to operate.

DR. SENIOR: Alison -- excuse me Jodi.

DR. LAPIDUS: This is Jodi Lapidus. As a follow on to what has been said is that I do believe that just by employing somewhat more rigorous experimental design as well as applying rigorous epidemiologic principles, these studies can be designed and conducted in a way that would answer most of the questions that are being tossed around here today.

I just think, like I mentioned, I believe that this is early, fairly suggestive, preliminary data that actually could be used to inform those studies. And that with the appropriate guidance and expertise in study design and epidemiologic principles for populations, that these could be answered in a fairly straightforward fashion.

DR. SENIOR: I apologize for missing that in my summary. You did say that there was a compelling first start -- by the way I am David Senior.

(Laughter)

DR. SENIOR: Do you have a comment?

DR. VAN EENENNAAM: A couple of things on culling with fish is a little different to --

DR. SENIOR: This is Dr. Van Eenennaam.

DR. VAN EENENNAAM: Sorry. I think they probably know David but anyway. In terms of what is a normal cull rate in aquacultural production settings and just having worked with batches of fish and you can see the variability here, you will often only get, you know, 6 percent of a batch that hatch and you might have -- the rest will go to mold. There is a lot of variability there and it is a little bit different in concept then culling maybe deformed cattle or something. You do lose a lot of fish during aquacultural production.

I guess I am hearing a call for further studies and I guess my caution with that is what exactly is the intent of further studies and what is the appropriate number to do that?

Because I think you can call for further studies ad infinitum but do you call for further studies when you are genetically selecting enhanced growth fish using natural breeding? You are going to have quite often exactly the same phenotypic effects, the same risk effects, everything the same and those animals can go to market and this one has to go through another round of studies. It is more or less precluding the opportunity for this technology to go to market if you are ever forward calling for more studies. So I guess

I just would want to be very specific about what data is actually missing here and the size of studies that are being called for so we do not get just kind of we need more studies ad infinitum.

DR. SENIOR: Well is that enough input on question number 1?

DR. APLEY: One more before you run off; Mike Apley. External validity and then a context within which to put culling rates, jaw erosion; there were some references to it in there. But coming into this without an extensive aquaculture background, a context within which to put these occurrences in aquaculture production.

DR. SENIOR: Any further comments?

(No response)

DR. SENIOR: With that I would like to move on to question 2.

Question 2: Do the data and information demonstrate there is a reasonable certainty of no harm from consumption of foods derived from AquAdvantage Salmon?

DR. SENIOR: And the question 2 is we will discuss the strengths and weaknesses relative to data and information presented to demonstrate that there is a reasonable certainty of no harm from consumption of foods derived from AquAdvantage Salmon. Should I repeat that?

We are discussing the strengths and weaknesses relative to the information presented demonstrating that there

is a reasonable certainty of no harm from consumption of foods derived from AquAdvantage Salmon. Again I will open it up for comment.

DR. VAN EENENNAAM: I would like to talk about the issue of endogenous allergens. And I think that the experiment that was designed was designed with no idea of what the answer would actually mean. And I think that is particularly problematic when there is no consensus in the scientific and medical literature regarding the magnitude of increase in endogenous allergens in allergenic food that would present an additional risk to public health. Without that endpoint, I do not know how you design a study to see whether or not it is elevated significantly to -- what is the judgment for that?

And in the absence of knowing what is a significant increase, we were told it was one-fold, well we often have ten-fold difference just in variation within individuals within a species in levels of endogenous allergens. Different fish species vary by 100-fold. What were we looking for there that would have said, oh that is an unacceptably high increase when there is no agreed number in the medical and scientific literature?

And I draw your attention to a *Nature of Biology* article by Dr. Richard Goodman, et al. where he is discussing the fact that endogenous allergenicity testing is really an

area in the Codex that is not in full agreement with all the groups there because there is this variability in endogenous allergens and so without knowing what your endpoint is, I do not know how you design a study to test whether you are too high.

DR. POPPENGA: Well along those lines in looking at the --

DR. SENIOR: This is Dr. Poppenga.

DR. POPPENGA: Bob Poppenga. Looking at the data I think the allergenicity aspect of it is -- I don't think you can interpret the data that is there. Just looking at the enzymatic immunoassay, I think this is -- it looks like an assay that was sort of utilized because there was nothing else to use. And I do not even think that with the information that we got, we could say that is a valid assay to measure what they are trying to measure. And obviously the Western blot was discounted in terms of providing any useful information. So I do not think there is much that can be said with regard to the allergenicity in any sort of objective way based upon the studies that were done.

DR. VAN EENENNAAM: Having said that, I am not sure that there is an answer that a study could answer because there is no grade level as to what would be too high of a level of endogenous allergens or what would be an area that would cause concern. We do not know that for the foods we

currently eat, different varieties of peanuts, different species of fish. So in absence of that information, how can we ask the question of this particular product when we do not know that answer for the food we currently consume?

DR. WELLS: I think it would be safe to assume that there are no --

DR. SENIOR: Dr. Wells.

DR. WELLS: Yes, it is again. I think it would be safe to assume there is no novel allergenicity associated with this product. The salmon contains nothing that is not already in the human diet at all. We eat salmon from most salmon species. So I have a difficult time identifying what that question would be? Everything there is stuff we eat.

I can see asking the question about allergenicity when we bring in a novel protein or a non-food item protein but that is not the case here. They have brought forth food. I mean the product of the gene is an item that we normally consume. I do not understand how we could possibly get at the idea that somehow this is going to be more allergenic.

DR. POPPENGA: It seems to me in reading some of the -- Bob Poppenga. It seems to me in reading some of the public comments, there was a comment about the possibility of having proteins altered by a few amino acids and a conformation change and perhaps changing the antigenicity. And I guess that gets back to the question that as far as this

whole question goes, is using more powerful techniques like proteomics to help determine whether there are some differences in terms of the proteins in these fish versus other fish.

DR. WELLS: I will guarantee you there are differences in the proteins of every single fish that we have ever consumed. In fact, we have never consumed the same genotype twice. Excluding identical twins, we have never eaten the same combination of genes and alleles twice in human history. So I guarantee you there will be specific differences in those studies that would all fall within the realm of salmon.

DR. SENIOR: Any other comments on the allergenicity?

DR. GRIFFIN: Dicky Griffin. And I really wanted to move onto the issue of food. I buy the allergenicity issue question. But when I look at proteins and I look at the range of tests done, I frequently see this sort of thing from students when I ask them to take a blood sample down to the clin-path lab and they run it for $5,000 worth of tests on a dog that has hookworms. It seemed to be just a massive array of things that represent food.

And I recently, just for other reasons, looked at protein and fat structure of the common meats in the common diet and I used the USDA database for my references and when

you look at the grams percent protein which is what is listed here, it just looks like fish. And I saw some of the -- I mean earlier today when we had questions about well it did not add up to 76 and I am thinking about some concept I have had fun with with students when I ask them about things that they tested for that do not exist. And in this case, I am not really sure where a carbohydrate exists inside a piece of muscle other than as an error in testing.

And similar things could be said of some of the vitamin structures that we saw, that are analyzed. When you look at, for instance, Vitamin B1 which is off the charts for low, well that is historically since before I was born which is a long, long time ago we have known that did not exist in fish and there is a lot of dead meat to show for that.

It is a food. I am absolutely in Dr. McKean's camp that we ought to be looking at the final product not something out of a laboratory setting. But let's look at some animals out of Panama after we have raised them, we have fed them, and their structures, those numbers are going to look a little different.

I do also share Jim's concern about the 60 because that was a 2 x 6 x 2 factorial, that Rubik's Cube, that is where you are trying to find statistical significance in just a handful of numbers inside of a Rubik's Cube. And when you are running tests for a minimum number of 200 eggs tested to

see whether they took -- and if that did not work, we are going to go to 900 but we only did 60 animals. And when I look at the jaw erosions in some of those things that also apply to that same sample set, actually smaller sample set, those numbers were not different and they all showed up in the diploid side not -- which is where the gene was, not where that conditions for use showed up.

It is a food. The last thing that just makes me nuts a bit is when we start thinking about this -- what have we known about growth hormone for a long, long, long, long, time in food? I eat cows that have growth hormone and I do not get their growth hormone because it is very species specific. That is why, what's his face, that threw baseballs real hard -- that guy from Texas, what was his name? Not Nolan Ryan, the other guy.

DR. : Ross Perot.

DR. GRIFFIN: No, no he didn't throw --

(Laughter)

DR. GRIFFIN: No, the one that lied to Congress.

DR. : Roger Clemens.

DR. GRIFFIN: Clemens. I am sorry I should be a baseball fan right, but the Jets won yesterday okay.

(Laughter)

DR. GRIFFIN: But Clemens, he did not take a pill. Somebody bent him over and poked a needle in his butt. You do

not get this stuff orally. And do we transfer genes to bacteria? I don't know how many kazillion times a day does that happen in everybody's gut and uptake that protein. So those are almost scientifically silly discussions in my head.

And I agree with Dr. Apley which I will make -- I am going to say this and I will be run out of the room. I think the major point of interest here is that what we are in witness to or somehow part of is the framework with which GMOs may be approved or looked at around the world. So it is extremely important how this precedence gets set.

And it is not an economic issue. Well, it may be but it cannot be. Economics is the shovel with which we dig the grave to bury any piece of science. It is the truth. And nor should it be fear of world starvation because we are going to run out of protein. Or misuse of like nutrient dilution. Yes we have lower fatty acid ratios in farm-raised or any other cultured fish, also these fish under consideration, than wild fish but we also get three or four times more meat produced in that same nutrient flow that went through those fish. So the conservation of nutrients in Mother Nature's world has probably not changed. I am just guessing probably not.

Nor should we let the fear of, God I hate that the term came up, frankenfish, drive the fear of public acceptance. And it is so, so easy to drive fear in the public

and it is so, so irresponsible.

DR. SENIOR: Thank you for your comments. As Chairman I will have to please ask us to confine our comments to the questions at hand if we possibly can. And the question at hand is do we have any weaknesses or strengths in the material presented to us relative to the safety of AquAdvantage Salmon as food? Is there any reasonable certainly of no harm from consumption of foods derived from AquAdvantage Salmon?

DR. APLEY: Mike Apley. Dr. Wells' comments are well taken about the context; again that word context comes up. And what I struggle for is any changes that might be due to this specific group of proteins in construct related to routine differences that we see in animal to animal. And so what I struggled for is to be able to put any differences from this intervention in context with differences we see day to day. So that is how the agency could help me understand better, is to put that in context to other differences.

The other thing that came up in the public comments that I think is fair enough to ask and that I personally would be interested in seeing and I apologize if I have missed it in the briefing comments or something Aleta sent to us but the explanation of the original reason for declaring DNA or genetic material as GRAS and then the justification for carrying it on to here. I do not know if I agree or disagree,

but I would just be interested in finding out that reasoning and it would add to the information we consider.

DR. SENIOR: Do I hear any further comments concerning the food safety? So far we have discussed the safety of this product as a food for human consumption. So far I have heard comments concerning allergens. And we have had a brief mention of growth hormone. And would any member of the committee wish to expound further on the data presented, the strength of the data?

DR. LAPIDUS: Jodi Lapidus. I had mentioned it when I was asking questions of the FDA research team with regard to -- I have been struggling with when we think about reasonable certainty of no harm based on consumption of these animals, I wonder about the research paradigm that has actually been employed throughout the process here and whether it was a bit ad hoc. Although I think it was thoroughly reviewed, I think maybe some of the studies that were done could have been thought through, again, more carefully, employed more rigorous study design method.

And what I would suggest, if you are attempting to show that these salmon are equivalent let’s say to other similar sponsor controlled farm salmon or other similarly raised farm salmon, that the equivalence setting be used instead of testing for differences. As it has been noted, you may find some differences here or there just due to chance.

Small sample sizes will be definition only show the largest of differences.

I would encourage that the group consider testing these in terms of equivalence trials which is a common way to test therapeutics I know in human populations to show that one thing is similar to another, something is not different, as opposed to be largely different.

And I would also encourage consulting with appropriate experts because they exist in human nutrition and human nutrition requirements determine what those margins of equivalence would be. A priori, conduct statistical power and hypotheses to determine adequate sample sizes for equivalence tests. They will by definition normally require larger sample sizes than hypothesis tests designed to test for differences, by and large but not always.

In using that terminology, talking about reasonable certainty that no harm will come, and you wanting to show that something is really equivalent to the nutritional content of another animal that is out there, I really think that that setting needs to be applied.

DR. SENIOR: Greg.

DR. JAFFE: Yes, Greg Jaffe here. When I looked at the overall Food Safety Assessment in the document that FDA provided, I think that I can say that -- and I think they have asked the right questions about what they should be looking at

in terms of making sure that the food is safe. And I think the data that is there so far seems to support that view that these AquAdvantage Salmon are as safe as other Atlantic salmon.

But with that I think that -- and I work for a consumer organization and I know consumers are very concerned about growth hormone these days in food. Whether that is legitimate or based on science or not, they are very concerned in that. And I think that the explanation on some of the data on that area is less than convincing. And whether that reaches reasonable certainty of no harm, I am not sure whether it does, the legal standard. I am not sure I can judge that.

I think that there are a whole series of data points there where they are below the detection level and there were comments in the public comment. In other words that that may or may not show that there is not difference because there may be more sensitive tests or there may not be more sensitive tests and I do not know the answer to that.

But I think if there are more sensitive tests, then maybe that needs to be done. If there are not more sensitive tests or if those would not be persuasive or they would not be biologically relevant, then I think that needs a better explanation in the document and I think that is missing.

It seems to me there is an automatic conclusion right there that they are below -- they are the same, they are

equivalent, so that ensures the safety. That may be the case but I am not sure the public is going to understand why that is without a better explanation of whether anything -- because if it was anything below that level, it would not be biological relevant or other reasons. Or otherwise if there are, then you need to do those additional tests.

So I guess what I am saying is that I think that is going to be an important area for the public. Again whether science justifies it or not, it is going to be something that people will think about when they eat food, which is the hormones around it. And I think there needs to be a better analysis in the document.

DR. SENIOR: Dr. McKean, I said it for you.

DR. McKEAN: Pardon?

DR. SENIOR: I said Dr. McKean for you.

DR. McKEAN: Oh well thank you. Now I know who I am again. I am completely in agreement with Jodi in terms of looking at this in terms of equivalency rather than trying to sort out the differences. It is a different way of looking at perhaps the same data package.

What we are really discussing is if you put a drug into an animal with no withdrawal, do you end up from the use of that drug, do you end up with an equivalent product? Not what are the differences because that confuses. You can always look for another parameter to see if there is a

difference. But can we get reasonable equivalency in allergenicity? Can we get reasonable equivalency in protein, carbohydrate, vitamin, and mineral evluations? And if we can, then we go forward.

I suspect Dicky that out in the Great Plains that you probably have some constitutional differences in terms of meat products in your different genetic lines. We do not worry about that. But we also have not stuck a drug into those animals to make those genetic lines and that to me is the difference for this discussion today.

So I am all on taking the data and looking for equivalency and if there are, again I think Jodi is correct. This is good, really strong preliminary data and it may be all you need if you ask the question in that regard as opposed to trying to figure out what the differences are so I would encourage you to do that.

DR. APLEY: Mike Apley and I also would like to agree with Dr. Lapidus -- did I say that right?

I think that there were great comments and I agree with Jim. And again I am hung up on this context still. Perhaps the Agency's way to provide us a context for those equivalency type studies is to do studies across beef breeds, across swine breeds, across fish breeds and look at the variability in nutritive content.

Everything else just across -- it is going to be a

pretty big confidence interval but then the question is does this product fit within the variance of all the normal things? Give us a context and use that to derive the variability that we are inducing and you power the studies. What do I have to do to assure me there is no difference just like a bioequivalency study rather than go out and looking to try to show a certain difference but good comment.

DR. SENIOR: Oh, go ahead Gary.

DR. THORGAARD: It just sounds like people are feeling like there are no warning signs but if there is a need for further research to address these issues of strain specificity that might account for some of the differences. I mean I would not feel alarmed about eating this kind of fish certainly. I am not worried about it.

DR. SENIOR: There is one person. That comment was Dr. Thorgaard.

(Laughter)

DR. McKEAN: And I will put my vote in for that as well.

DR. VAN EENENNAAM: It is Alison Van Eenennaam. I just wanted to follow up with Jodi to understand. The conclusion of the FDA was that they are not materially different. And that as I understand it was it based in the context that you are looking for. They looked at studies outside to see if these ranges fell within normal, you know,

what is observed in other areas and there are a couple of tables that give that example.

For example Table 18 on the IGF-1 variances that exist in fish in different species. And I think the reason that the tolerances were set where they were was it is well below what is being found here. And so with regard to the biological relevance of levels of growth hormone for example that are below detection, I guess I would assume that the assay was developed based on biologically relevant data. And so if it is below that detection level, then it is not going to be biologically relevant.

But I want to ask you a question. What is the difference between not materially different and the term that you are using which is much the same or equivalence. What is the difference between those two?

DR. LAPIDUS: There are two different research paradigms. One where you start that with the hypothesis that there is no difference and you see to prove that there is a difference. And just because you do not find that difference does not mean that it is not truly there. You could be underpowered to detect it.

The equivalent study framework starts with the fact that -- flips it on its head and starts with the fact that let’s assume that they are different and would show with adequate samples and analysis that they are within some margin

of acceptability. That specified a priori, not after the fact do you look at the data and say I didn't find any differences so therefore they must not be there. This specifies the meaningful difference up front and shows that they are within that interval. And I think it is a stronger design.

And what I was wresting with, for me to conclusively say or feel more confident in saying that there would be -- for me to say that I have reasonable certainly that no harm could come from consuming that -- no additional harm would come from consuming that food that I would want to see that equivalence demonstrated more strongly than just by showing no difference with a sample size that is small enough without any real context for what could be detected.

I did a little -- because I am really a geeky number girl, I actually did a little power analysis on some of the data that was presented and the magnitude differences that would need to be presented for example on some of these analyses where there were just seven subjects; so if you look at the Table 16 and you have 8.89 to 9.26, the differences between the two groups. Actually the difference, if you take the 8.89 stationary, you would have to see differences on the magnitude of 16 in the other group to have called that statistically significant. Again that is the magnitude that you would need to detect and whether that is biologically relevant would need to be discussed.

To have a difference of the sort that is shown on the first table in Table 15 to be relevant, you are probably going to need on the order of magnitude of like 100 samples in each group to show those and it to be statistically significantly different.

So those are the kinds of things that I was wrestling with from a numbers perspective and then coming up with what would I feel comfortable saying reasonable certainty.

DR. SENIOR: Let me just summarize where we are on this question.

Relative to the allergens, there appears to be very little data to be able to judge what would be a significant increase in allergen levels in the food and so it is very difficult to interpret data there and in fact probably impossible to answer specific questions relative to antigenicity. However, it is safe to assume that there is no novel antigen in this rDNA animal. The thought was that the right questions were asked.

And relative to growth hormone it was noted that it was species specific but also recognized that there was great concern in the public relative to growth hormone.

With respect to how it could be done differently, the issue of proteomics was raised but a statement was made that the variation would be tremendous between individuals of

any species so that that might be a tough one. This is a very sensitive way to distinguish between animals.

The thought is that we should be analyzing the final product and these studies could be better designed and maybe work on the basis of equivalency trials rather than trying to determine differences.

A comment was made concerning using more sensitive tests but that was also if the ability of the test to detect something is still well below the biological important level, then maybe what we have there is good. But there was a comment relative we should use probably more sensitive tests if we want to get the facts straight on these tests.

Any further issues that we might --

DR. WELLS: I apologize -- Kevin Wells again. I have to make the comment that if we are going to suggest that something be measured, I think we also have to justify why we want to know what that value is. And so I personally would tend to have concerns about things that would be orally active. I would want to know if testosterone was 10 times higher than normal. I actually do not care of myoglobin is 10 times higher than normal. That is food. So we get nit-picky here.

If you go through and look at a steak cut out of a Holstein, an Angus and a Wagyu, you are going to see a minimum of a 15 fold difference in fat. The fact that they are

different does not make them unsafe.

So unless we are going to measure something that presents some sort of hazard, I am struggling as to why we are demanding that number. I don't know how to use that number.

DR. SENIOR: Any further comment from Dr. Wells' comment?

DR. VAN EENENNAAM: I guess that goes a little bit to study design with regard to experimental end as what difference is it important to detect that has a biological relevance because if there is no difference, you can have infinity and still not be able to get a statistically -- you know, a difference. And so in the absence of knowledge of what that critical level is, I do not think you can design appropriate studies to know what the appropriate size is to power your experiment.

DR. SENIOR: From now on, the lady with the Australian accent we will assume is Dr. Van Eenennaam.

(Laughter)

DR. APLEY: Mike Apley. It is exactly the point. If we cannot justify a biological relevance of something -- we should only measure stuff with biological relevance. And when we demonstrate a biological relevance, along with that has to come the fact that we have evidence that this much difference would be significant. Then we use that study design where we design it -- if we have this number of animals and we do not

see a difference, we are pretty sure there is no difference but it has to be something with biological relevance. And secondary to that has to be we have to have a difference then that we think makes sense. And without that, it is pointless to measure; you are exactly right.

DR. WELLS: Kevin Wells again. But do we care if they are different? The question is are they unsafe?

DR. APLEY: That is in my comment of biological relevance.

DR. LAPIDUS: Yes I think that would go into that study design to show -- your margin of equivalence would take into account issues of safety to be able to design such studies as I was suggesting. And I whole heartedly agree that you do not need to study every measure under the sun. The information that was provided to us was much more of an exploratory data analysis type of technique and I agree that certain measures should be focused on.

DR. GRIFFIN: And the biological relevance definitions need to come from a credible source.

DR. SENIOR: The last two comments were from Dr. Lapidus and Dr. Griffin. So are there any further comments on this issue?

DR. VAN EENENNAAM: Alison Van Eenennaam. I guess that begs the question then of what are the risks associated with this gene product that you would want to design a study

to look for biologically relevant concerns that would lead you to determine there is a reasonable certainty of no harm. What parameters should we be looking at?

DR. SENIOR: So I am just adding to the summary statement that we would need to define those things of importance. And when I say importance I mean biological importance. Determine what differences we believe would be important relative to these particular products in the animal. Define that ahead of time and then test to see if there are any differences that were significant. Does that capture it?

(Nodding of heads/thumbs up)

DR. SENIOR: Anything else? Okay, thank you. We are half way folks. You can tell this is a group of veterinarians, they just work all night.

Question 3: Do the data indicate that AquAdvantage Salmon grow faster than their conventional counterparts?

The next question, question 3, we need to discuss the strengths and weaknesses relative to data indicating that AquAdvantage Salmon grow faster than their conventional counterparts. I will entertain comments on that.

DR. GRIFFIN: Dicky Griffin here. I think the data is pretty straight up. If somebody says they are not the same and you have some nutrient density elutions associated with the final product, but they grow faster. The data is very clear I think from the stuff.

DR. SENIOR: Any further? It is Dr. Thorgaard.

DR. THORGAARD: It seems like a straight forward case.

DR. McKEAN: Jim McKean. It is okay. It is very clear that it makes them grow faster so with concurrence we will move on to question 4.

DR. SENIOR: Does anyone want to say yes in a different way?

DR. APLEY: Mike Apley. Yes.

(Laughter)

Question 4: Are any potential environmental impacts from AquAdvantage Salmon production adequately mitigated by AquaBounty Technologies' proposed conditions of use?

DR. SENIOR: Question 4, we need to discuss the strengths and weaknesses relative to any potential environmental impacts from AquAdvantage Salmon production. Are they adequately mitigated by the AquaBounty Technologies' proposed conditions of use?

So the question really presupposes that we know the environmental impacts, the potential environmental impacts, and the question addresses whether we believe that the strengths and weaknesses -- we need to define I guess and discuss the strengths and weaknesses relative to the mitigation efforts. So we are not here to define the environmental impacts so much as we are here to determine

whether the information that has been provided to us is strong or weak concerning mitigation of the environmental impact of the proposed conditions of use which are specifically relative to this discussion confined to the facilities at PEI and also in Panama. I will entertain comments relative to that.

DR. VAN EENENNAAM: Alison Van Eenennaam. I think the strengths are that they have followed the guidance of the animal biotechnology science-based concerns and have multiple redundant levels of physical, geophysical, and biological containment in place.

DR. JAFFE: Greg Jaffe speaking here. So I have a couple of comments. First I want to just say I appreciate that FDA said that this is the beginning of the public's involvement with the Environmental Assessment that is done around this product and that there will be a time in the future for public comment either with an EA and a FONSI or with an EIS and I think that is really important and I applaud the agency for doing that. I would hope that when they do that they will not just include their analysis but also some of the underlying documents or experiments that go with that. I think we have seen today that sometimes when you just get the summary data tables whether it is food safety or environmental, there are a lot of questions about where numbers come from and I think those would be alleviated and the public would feel more comfortable and we would have a

better public comment process and more transparency by including not just the final documents but having available those underlying documentation available for the public.

With that in mind, I think that this EA is very limited. It only addresses two facilities. I would agree with Alison that the sponsor here has put in multiple levels of containment. We have heard about the physical containment, the biological containment, the geographical containment and overall it seems like they have done a fairly good job of that. And so the likelihood of an escape and the animal getting into the population is very small. I do not think anybody said it is zero, but it is very small.

With that in mind though I still think there are some weaknesses in the Environmental Assessment I think that need to be looked at. And I mentioned one of them before when I was asking a question. I mean accidents happen and humans are fallible and some of those containment measures do hinge upon the humans at those facilities and how those facilities are run.

And there was a mention of Standard Operating Procedures, there was a mention of Operations Management, but I do not think those are really covered and those are not really assessed in the Environmental Assessment. How good those are in place, how they are going to be overseen, how they are going to be monitored, and what happens if -- what

are the risks associated around those activities. And I think that does need to be included in the Environmental Assessment.

The other thing I wanted to mention is an overarching concern I think and it was raised by some of the public comments and that is that this EA is very limited. It is limited to two facilities and there was discussion about supplemental applications if they have new facilities coming on and then new environmental assessments done there. And there is a concern here that there are going to be, at some point, there could be multiple, many, many little EAs each one about a new facility. And I worry that there is not a cumulative impacts analysis. And that this is a way to sort of get around doing an environmental impact statement about the fact that this salmon could be grown in multiple locations around the world in multiple facilities with different levels of control on them.

So I think there is a concern that these things are going to be very segmented to an EA for this facility, an EA for that facility, and each of them individually may look like a very good containment process. But the fact that you start flying these eggs to multiple places, many, many different places -- it is much easier to control things when it is two facilities that are very closely watched by AquaBounty. It will become less easy to control but each one individually may look like a good mouse trap. But when you look at

cumulatively the whole process and the ability to oversee and manage that, it becomes different.

And so I think there is a concern here that the process that is being set up may in fact avoid a full environmental impact statement or a full assessment under NEPA as this moves along, if this moves along, as the business plan of the sponsor suggests. I am not sure if I made myself clear but I think that is a concern going forward.

DR. SENIOR: Dr. Thorgaard.

DR. THORGAARD: Gary Thorgaard. To me this is the part of the project that has the most positive potential in many ways and also some of the most concern as well.

On the positive side I think the potential for controlling damage by escapes of farm fish is a very positive aspect of this proposal.

But I think the general kind of concern in the public of the potential impacts of escapes is real. And I think the containment on this project is excellent. It seems like it has been very well thought through at multiple levels. But I personally still feel like considering this issue in a comprehensive way, together with other agencies through an environmental impact statement, would be the best way to proceed.

DR. ALTIER: Craig Altier. I remain concerned about the potential theft and misuse of this product. I did read

the EA and there is a description there of security measures to be employed but this is a product that is going to be transported from one country to another so it remains a concern.

So the analogy I have been thinking of is a controlled drug. That if an application for a drug came before this committee, a drug that had high abuse potential, was perhaps a narcotic, we would be asking, I think we would be asking, that every dose of that drug be accounted for. Now I do not know if that is practical here with the millions of eggs we are potentially going to have but we are not even anywhere near that in terms of security. So I am quite worried about the security of this item.

DR. SENIOR: Yes, Dr. McKean.

DR. McKEAN: Jim McKean. And that raises an issue that I raised this afternoon and I was not really satisfied with the answer and so I queried farther.

I view this product, not the fish, but the construct since it is being treated as a drug, that that facility in PEI is a drug manufacturing facility. I queried and was told that FDA would consider that to be the case which means all the recordkeeping and all the things that go with producing a drug would be inherent in that discussion. That assuages my concerns about product disappearing to other places to be lost in the firmament substantially. So that part I am reasonably

good with.

The part about the facility in Panama specifically which you were talking about and the containment there, I think I am okay with most of that provided as Mr. Jaffe has said, that the human element is somehow maintained. And as you expand into new places, you have that issue of human element as well.

But I think that the part of where I am going with this is it appears to me that the drug manufacturing piece can already be handled under existing protocols. The containment issue between the genetic containment and the physical containment, if those things are maintained, even if you replicated it, you would have a pretty good system at hand. And so I am thinking that the answer is that it is probably adequately mitigated to the extent that we do with anything where we have humans doing the process not robots.

DR. GRIFFIN: Dicky Griffin. I looked at the 95 percent confidence interval around the -- in essence the sterile female production and those numbers, the worst that were listed in any of their replications, dropped that to 97 percent but those stood around 99.something to get in that 95 percent confidence interval. They are shipping sterile fish.

So the idea that they would escape to the environment and reproduce especially if the supplementals

maintain land-based production which is kind of where you live, salmon next to Jim McKean's farm, they are not going to escape. So I feel pretty comfortable about that; I think they have done a good job.

DR. APLEY: Mike Apley. I think within the conditions of use that are described, that the containment procedures were designed by the department of redundancy department. I think there is a lot of redundancy built in. And I do not believe in zero risk. I think that incremental increases in procedures would do little good.

The human elements involved -- the one thing I do still question in relation to the Environmental Assessment that I think needs to be further evaluated is I do think the question resides in case of an accidental or other type of release, exactly how these fish would behave in a natural environment and compete in there versus being extrapolated from the more artificial environments they have been in. I have any idea how to do those studies but that is the question that still remains in my mind.

DR. POPPENGA: I just want to reiterate what Mike just said. It seems to me the big data gap is, even though the chance may be small if these fish get out into the environment, what happens once they are out there and might be able to survive. And that goes back to some presentations earlier. I think the risk management here is adequate but I

think as these things go down the road the ecological risk assessment is I think a bigger question.

DR. SENIOR: That was Dr. Poppenga and I think that has been expressed several times but it is actually beyond the purview of our decision today. It is just that we are sending a bit of a message forward to further evaluation relative to if there are multiple sites, we multiply the risk because the risk remains. So I think we will leave discussion of multiple sites alone at this point. But any other comments on this issue before I sum up?

DR. WELLS: I would say that if one is considering --

DR. SENIOR: Dr. Wells.

DR. WELLS: I apologize. If one is considering the impact of release of the transgene, then they need to have a comparator which I think would be the non-transgenic counterpart. And so if you are looking at the environmental impact of release, that needs to be weighed against any other domestic salmon as opposed to the confounding transgene and domestication.

I have mentioned this before but I really do think the potential impact of the domesticated fish on the wild populations is much larger than the transgene itself. And if we are talking about the transgene getting out, in and of itself, I am worried about that much less than domesticated

fish. And I just think we need to balance that. And we are not here to question whether or not this particular strain of salmon gets out, we are here to address the transgene and it actually does not add much to the impact as compared to the domestic alone.

I mean a comparison might be, you know, if you were to cross a poodle with a wolf, I mean that is the sort of range, it is the same species.

DR. THORGAARD: Gary Thorgaard. I would argue that we do not know whether the transgene would be more harmful or less harmful than domesticated but the reality is we know that domesticated Atlantic salmon have had very negative effects on wild Atlantic salmon in a lot of parts of the world so that is a real problem and so I am not really disagreeing with you. I do not think we know that the transgene would be less harmful than the domesticated though.

DR. GRIFFIN: Dicky Griffin. But the part of the issue is that the constraints of this is that it would be land-based which really puts some boundaries around that escape.

DR. VAN EENENNAAM: Alison Van Eenennaam. I guess we had drilled into us that risk is the probability of that exposure and I do not know what else the company could have done to guarantee multiple redundant levels of containment. And I guess the thing it gets back again to let’s do an

environmental risk assessment, is okay where do we stop gathering data to know that we are satisfied that the questions have been answered.

I agree with Kevin's concerns that the current selection strategies in commercial salmon, that when those animals escape has environmental consequences. And if those traditional selection technologies can go forward and there is no regulatory paradigm and you put incredible burdens on this particular fish because it has one specific gene in it, it is more or less precluding that technology from ever going to market because the data demands are so expensive and so all encompassing that no one will ever be able to get through. And it seems like there is a very large hurdle that is put before this one specific gene that is not true of any other selective breeding practices even though the phenotypes may be the same and the environmental consequences may be the same.

DR. McKEAN: And that goes back partly to my comment about the rank order of risk is the broodfish. They are -- by the time you get them triploidy and you get all-female sterility issues, those offspring are fairly safe from everything I can figure out.

So the place that we have concerns is at the PEI facility and if that is going to be operated as a drug manufacturing facility, I have considerable less concerns about the risk at that point than I do about the downstream

operation because there are other levels of redundancy in that group. So that would be my -- in terms of the risks, that would be where I spend my time. And I would not be looking for a lot of extra risk protection other than what I foresee as FDA's Standard Operating Procedures for a drug manufacturing facility.

DR. SENIOR: That comment was made by Dr. McKean.

DR. McKEAN: I am sorry.

DR. WELLS: Kevin Wells. I think it is worth adding that there is some historical data here which does not necessarily read into the future in scale up. But these fish have been in existence for a very long time now. It is not as though someone is asking permission to make these fish for the very first time. They have been contained for years. Now that does not guarantee anything into the future but that facility is in place with fish in it.

DR. SENIOR: This is David Senior summarizing question 4. The AquaBounty appears to have followed the concerns and put into place multiple levels of containment.

There was extreme concern about release to the wild but the committee had no ability apparently to agree on the level of fitness of GE salmon in the wild. And in fact there was even an expression that there was less concern about the transgene versus release of domestic salmon. And there is proof that domestic salmon have affected many wild populations

worldwide.

There was also the point made that containment has existed at the PEI facility now for many years and so there is a history of success in containing it. I do not know how that was assessed but I will leave it at that.

The management of -- the risk is never zero but management of the SOPs on site is vital and keeping those up to date and crisp and right and tight would be very important.

The final thing is the potential for theft. The point was made that this is a valuable product so people would steal it. One would think that the company I suppose and this is my editorial comment, if they thought it was valuable they would protect it so I do not know how that works out.

There was a comment made, rather things to do, that the FDA was urged to include full datasets and results rather than just the results so that better interpretation could be made by the committee and by the public. And there was one suggestion regarding that maybe an EIS should be presented. And that about sums it up. Where there any other comments that I missed there?

(No response)

DR. SENIOR: Hearing none I would like to thank the committee for the tremendous amount of work they have put in and I would also like to thank the public for the massive amount of material that we received and the committee's

diligence in reviewing the massive amount of material that was received. And with that, do I adjourn the meeting? I would like to adjourn the VMAC.

DR. DUNHAM: And I would like to then thank everybody for staying with us and very specifically to thank the Veterinary Advisory Committee. On this particular issue you have really put a lot of very good thought into this and we appreciate the comments. That is what we needed, was your recommendations and advise and we will take those under consideration. And we will then review everything. We will also be announcing, if we do go forward with either a FONSI or an EA, there will be public comment. All of that will be announced at some later date. So we will go back and do our homework. And I want to thank everybody for staying so long and participating in a very important meeting and we really do thank you for your diligence. Have a very safe trip home and we appreciate it; bye-bye.

(Applause)

(Whereupon the meeting was adjourned at 6:58 p.m.)

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