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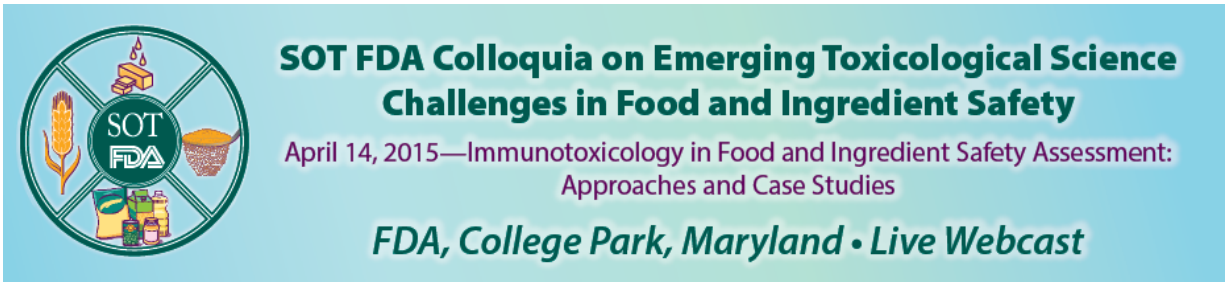

Immunotoxicology in Food and Ingredient Safety Assessment: Approaches and Case Studies

Schedule

8:15 am-8:25 am	**Welcome from FDA and Overview** Susan Mayne, Director, US FDA Center for Food Safety and Applied Nutrition, College Park, MD
8:25am-8:30 am	**Welcome from SOT** Peter Goering, SOT Vice-President, FDA, Silver Spring, MD
8:30 am-9:15 am	**Introduction to Immunology and Immunotoxicology** Dori Germolec, Colloquium Chair, Toxicology Branch, National Toxicology Program, NIEHS, Research Triangle Park, NC
9:15 am-10:00 am	**Immunomodulatory Effects of Perfluorinated Compounds in Rodents and Humans** Jamie DeWitt, East Carolina University, Greenville, NC
10:00 am-10:15 am	**Break**
10:15 am-11:00 am	**Toxicology and Food Allergy: Case Study of tBHQ** Cheryl Rockwell, Michigan State University, East Lansing, MI
11:00 am- 11:45 am	**Dietary Supplement Modulation of Autoimmune Disease** Prakash Nagarkatti, University of South Carolina School of Medicine, Columbia, SC
11:45 pm-12:30 pm	**Roundtable Discussion** Dori Germolec, Moderator All speakers

Welcome from FDA and Overview
Susan Mayne, Director, US FDA Center for Food Safety and Applied Nutrition, College Park, MD

I think we will get started. Welcome. My name is Susan Mayne and the director for the Center for food safety and applied nutrition. I am here no to welcome you all to the auditorium practice a group of people here in the auditorium this is also being webcast. Welcome to the people who are listening in from the outside. Of course, all of you are trained in toxicology know the name Harvey Wiley. Worked with the poison squad to be very crude approach for looking at the safety of food and ingredients and here we're celebrating the advances we've made since the early years of looking at food safety.

Today we're going to be talking about is this Colloquia emerging toxicological science challenges include an ingredient safety. It is parting with the Society of toxicology to provide a series of four half-day trading sessions based on a memorandum of understanding that FDA holds with SOT to foster our organizations shared interest in scientific aggressive disciplines it directly and indirectly affects human and animal health and medicine. Is my distinct pleasure to be here today and welcome you to participate to discuss the important topics of toxicological science? In CFSAN standards group with a standard of safety of reasonable certainty of no harm as was a standard of review a fair evaluation of all of the data. The standards are the same for food, color additives, graph substances in the context substances. The safety evaluation of subsistence attitude food intake is to count exposure as well as the chemical nature of the substance. Acted upon these considerations, a review scientist would expected it from toxicity testing that supports the safe use of a substance in food.

The guideline on toxicological principles for the safety assessment of direct food additives and color additives used in foods also known as the red, provides guidelines to assess petitioners and notifier's in developing and submitting the toxicological information that we need here within CFSAN. The paper describes how existing information is considered somehow the need for additional studies is assessed and provides a rigorous protocols for commonly used toxicology studies. Maybe some of you may know as a result of the review of our chemical risk and safety assessment program, where currently in the process of updating the Redbook. In fact some of you may have dissipated in a meeting that occurred last fall on that topic. In response to request with extended a comment period for input on the Redbook until the 11th of 2015. We're particularly interested in getting comments from people in the field about which parts of the Redbook should be updated and how FDA should prioritize those updates. The scope of the revised guidance of whether guidance documents could be addressed are incorporated by reference. New assays, test methods and endpoints it could be useful for safety assessment with justification for why and how these proposed new methods should be considered. Key studies and considerations for study interpretation for each of the regulatory categories of food and cosmetic ingredients and chemical contaminants overseen by CFSAN. Waste to make our guidance were useful to the stakeholders. Waste make our processes and criteria for safety assessment clear to the stakeholders. Many of you may have seen we issued a constituent update yesterday which is available on our website with information about how you can submit comments about updates to the Redbook. I'm very delighted to be here to welcome you here today on this rainy day here in College Park Maryland. These Colloquia provide a forum to engage with leading experts in toxicology to learn about the newest methodologies that informed the important work being done here within CFSAN. The next Colloquia is scheduled

for June 17 in the topic will be contemporary issues in risk assessment. Is dumb a pleasure to introduce Dr. Peter Goering SOT vice president of the President-elect for SOT was coming here to visit us today from FDA Center for devices and radiological health also known as CDRH. Dr. Gary.

Welcome from SOT
Peter Goering, SOT Vice-President, FDA, Silver Spring, MD

Thank you, Dr. Main, and welcome to the FDA began know you have a background in toxicology so I think we have a good future with the Society of toxicology working with you and your colleagues at the center for fruits and FDA in general in the future. On behalf of the Society of toxicology thank you for your support for the Colloquia series and this one in particular. We appreciate the commitment of resources both financial and human resource that it takes to pull the soft. I think these kinds of partnerships are important for several reasons. I think these partnerships are important and particularly this one because of the shared values of the society and the FDA specifically the center for fruits. We both have a common set of values, first of all our missions are very similar, the FDA's mission is to protect and promote the health of the public and the Society's mission is to create a healthier and safer worldwide dancing the science and increasing the impact of toxicology. The SOT just completed a new strategic plan this past year and the three pillars are highlighted here on this slide, strengthen the relevance and impact of toxicology. Develop and support toxicologist to capitalize on future opportunities and expand our reach and impact globally. I think these priorities and values are also shared by the FDA in the Center for food safety. Advancing the science, training and retooling our scientist then toxicologist to meet the do challenges in the science and to pursue public and environmental health goals. Then to share the information broadly, impact globally. I think these Colloquia are being recorded now and they're going to be available and they are available on the SOT website. This information, these advances in food science that we are hearing today for example can be shared throughout the world in the future. As scientist would always like to have evidence and I guess I'm calling this my evidence-based success approach for these Colloquia. The first Colloquia was held back in November, it was on a controversial topic regarding partially hydrogenated oils. We had 95 on-site participants and webcast did 239 participants. Society put up these recordings just less than two months ago it in that time we have had 140 video views, and these are people that are looking at a four-hour presentation of their making quite a commitment to look at this. We have also had almost 1300 downloads of the slides for the first Colloquia. In the second colloquium held in February we do not have the on-site data, download it here but we had almost 50 participants in almost 200 individuals on the webcast.

I am pleased that we have a great panel this morning. They will be introduced shortly. We have for well-qualified experts that will share about advances in food ingredient and food safety science this morning. Each speaker has about 45 minutes and will entertain questions after each presentation. An event like this morning does not happen in a vacuum and I would like to acknowledge the organizing committee that was a collaboration between FDA and the society in the society members of the organizing committee were Jim Petz guy, Dori [Indiscernible], Brian Twitty, [Indiscernible]. For the center of food safety, Alan Redman was the lead for the project. Suzy Fitzpatrick, Ron Chandra Baughn, Kristi Jacobs, Sabine Carol, Catherine Wayside period like to think this group are taking the initiative and planning today's event.

Is my pleasure to introduce the chairperson of this morning's session and first speaker Dr. Dori Germolec from the national toxicology program. From the and IE HS. Welcome to college park into the Colloquia series.

Introduction to Immunology and Immunotoxicology
Dori Germolec, Colloquium Chair, Toxicology Branch, National Toxicology Program, NIEHS, Research Triangle Park, NC

Thank you for attending and thank you to those on the web. I would actually like to thank Suzy Fitzpatrick because I was on SOT Council I was part of the FDA government liaison group and this was an idea that she actually generated that SOT and do some training sessions. Is much appreciated and I'm very gratified to be able to come and speak with you today it is my job to give you the introduction that will allow you to understand and know the players for the talks that come after me. Women have some title slides of the presentation for the second talk will be Dr. Jamie DeWitt will be speaking on the immunomodulatory compound spec Acer speaker will be Cheryl Rockwell from Michigan State is going to be talking about and preclude module and looking at the food additive, TP HQ as a bottle compound. Our final talk will be -- who believed it dietary supplements now they are autoimmune disease. Will close the session was one of the roundtable discussions would be taken additional questions from the audience as well as we hope to generate some discussion on critical food safety issues that we may not have covered in our talks. Again, your party or that the next Colloquia will be June 17, 2015 and will be on contemporary issues in risk assessment. I will touch on this a tiny bit at the end of my talk, hopefully I will have time. Again, thank you for your participation.

The immune system has many functions. It is really required to recognize self from nonself and to eliminate those things that it determines do not belong, bacteria, viruses, the pollen that is in the air currently neoplastic cells that arise due to exposure from carcinogens. We get a breakage of tolerance and you do not recognize South it can lead to autoimmunity and we will hear some examples of that later. It also is responsible for regulating the immune response ones is -- once has been initiated. And yet the response to the pollen in the air, you need something to take the response down.

Is a system in the balance. You will see this scale a lot of think in the talks today. When you tilt that one what you get immunosuppression or would like to call it immunomodulators in as well. You can get altered resistance to infectious disease or to neoplasia if you stimulate the immune system inappropriately it can lead to allergic disease or allergic a Spanish sensitivity as well as a lack of self recognition and autoimmunity.

Just to go through quickly the very basics of immunology. We refer to it as two separate types of communities so innate immunity is your first line of defense. Is what you see immediately when you have a wound, when you and help pollen. It is very rapid. Is very limited recognition for what it is seeing. It is really a rapid defender strikeforce. There is no self proliferation requires. It has very limited memory and you find innate immune responses in trial genetically low organisms like earthworms and lower forms of life. Again, just a very quick and rapid response.

This is in contrast to adaptive or acquired immunity. We refer to this generally as T cell immunity or -- B cell immunity and the generation of the antibody response. I will show you a bit later when an antibody molecule looks like. Because of the T cell receptor in the antigen binding

portion of the antibody molecule we can recognize an independent array of organisms or proteins that come into our body and respond to them. It does take some time to generate the response so delayed type hypersensitivity responses that are we mediated by T cells take between 42 and 78 hours. That is what you do not see poison ivy response right away when you get exposed to poison ivy. It requires proliferation and antigen specific differentiation that it is very long-lasting equity think about a vaccine response that you get, will you are dig is you are generating that memory response so then when you see that same pathogen or molecule again, you be able to much more rapidly generate on anagen response.

This is a quick anatomy lesson. For those of you that not very familiar with immunology. There are all kinds of lymphoid tissues all over the body. Primary lymphoid organs of the bone marrow and the thymus. The thymus is here in your chest comes a secondary lymphoid organs of the spleen, lymph nodes in the Pires patches and obviously I'm sure you all know lymph nodes are everywhere. There also tertiary lymphoid organs which are aggregates of tissues. The BLT, GAL T, MA Lieutenant, the tissues, the lymphoid tissues those are really clusters of lymph nodes in other tissue associated with specific organs.

If you are like me and you did not do very well in your pathology classes I urge you if you are interested to look at the NTP Alice of non-neoplastic lesions per the website is given here. It is a free resource and it is really excellent for being able to look at tissues. In pathology class people said you can clearly see and I could never clearly see what they were talking about but the Alice of non-neoplastic legions Julie does a good job of explaining and pointing out tissue architecture. This is the thymus. Is the source of naïve T cells through the body. There's a cortex in a medulla. It is very sensitive to certain and xenobiotics and drugs. It is very sensitive to acute toxicity and stress. In a toxicology study it is very frequent when customers aware of using very high doses to observe from a graphic a -- because response to steroid hormones. It is really difficult sometimes to discern whether you're having a true immunological effect or whether you're having a stress affect. That is something to be very careful when you are looking at it.

This is the spleen break the spleen is basically a giant filter. There are a lot of red blood cells in a. It also has lymphoid follicles and the PLS area which are T cells associated with the lymphoid follicles because were love antibody responses are generated to. The red pulp is large aggregate of blood cells. Lymph nodes look very much like a spleen again there is a follicular area and there are areas for anagen champ -- trapping, anagen presentation. We get exposed to something on the scanner when you inhale a particular particle, image in presenting cells take the -- they process the antigen which I will talk about more in a moment. They take it back to a training lymph node and they presented their to lymphocytes it to generate a specific response.

A lot of cells here. Really what the take-home message from this light is, there are a number of different types of cells that come out of the bone marrow. There are mildly type sellers, lymphoid cell lines, Ritz Freud cell lines, your red blood cells because of the granular sites Pritikin US have lymphocytes.

I want to introduce you to a few of the critical cells in the immune system. These are the neutrophils so they are commonly referred to as PMN. There very much the first responder, they [Indiscernible] bacteria they released different types of enzymes that induce bacteria killing. The release a lot of inflammatory mediators that promote inflammation and really recruit other cells to the site of injury. The essentials are very active and some of us currently as we are dealing

with the pollen. There very active in allergy. They secrete or a media the secretion of IGE. There also, the immediate the secretion the do not darkly secreted themselves. They are also responsible for killing parasite Lahr Viso again much of the response to parasite is IGE mediated period talk a little bit more about IGE later. Then Basophil which are circulating mast cells also important in allergy and particularly anaphylaxis. When you see something that you are allergic to, basophils the granulate and they release a lot of inflammatory mediators into the blood again recruiting a compound that help you get rid of the protein or pathogen

Macrophage and monocytes they are responsible for killing bacteria break their primary antigen processing and presenting cells and again release factors that mediate inflammation and this is not showing up very well on my screen. Maybe a little better on the screen that you can see here macro phage, Doshi bacteria because is how many bacteria are cleared from the body.

Of our dimension some of these inflammatory mediators. The eicosanoid so -- leukotrienes that are very important in the response to allergens. Hydrolytic enzyme such as mother proxies that are important killing bacteria, reactive oxygen reactive nitrogen species also important in bacterial killing. Adhesion molecules which are expressed on the cell surface and allow for the cells to go to the site of injury and then generate a specific inflammatory response there and then cytokines and chemo kinds of posts -- response attract other cells Tayside of injury.

There are more than 30 cytokines at this point in many chemo kinds. Would be much beyond this talk to discuss them all. I would like to mention a couple of the key players that you may hear of. Like IL-one and TNF are commonly generated in the for example the febrile response you get when you are exposed to a virus. IL-two and IL-four are very important in TNP cell differentiation and bullet -- proliferation. In fear and -- antiviral cytokines.

Just a couple more cells of the immune system. I mentioned the antigen presenting cells particularly in the skin take antigens back to training lymph nodes and the dendritic cell is what the most important of the cells. It is actually has some characteristics of myeloid cells and some character asked six of lymphoid cells is really an interaction between the adaptive in the innate immune response. It is very attuned to pattern recognition receptors on bacteria. These are very professional antigen presenting cells.

When we talk about antigen presentation what happens is, you will get a foreign antigen that enters the body, antigen presenting cells take those antigens. They process them through the use of some of the hydrolytic enzymes that we mentioned in things like life the sons. They attach itself recognition molecules to those proteins and then they go through the cellular [Indiscernible] and end up back on this service on the context of a self recognition peptide that allows other cells of the immune system to recognize the antigen and to respond to it to proliferate to generate the specific memory response.

This is an antibody molecule and I mentioned that I would show you how we have an infinite array of possibilities. This is the light chain, this is the heavy chain. Antigens bind here. There are multiple antigen winding sites and there are highly variable regions in both the light chain in the heavy chain and they allow you to generate all kinds of different molecular structures that recognize any type of antigen pic if you see a new pathogen if you see a novel molecules that you have never seen before because you go to a different place in the world to actually have the capability to generate a response to that. There are many different kinds of immunoglobulin response is primarily the primary response is IgM. Is very efficient in agglutinative soda tracks the PNM set have sites for binding this FC portion of the antibody molecule. I -- IgG is the

memory recall response. We get your blood taken it has the highest serum concentration. IGA culture mucosal surfaces. It is very important in resisting pathogens Points of entry. Then IGE again I've already mentioned is important in resistance to parasitic infections, very important allergy and it is the response that you get in anaphylaxis. Allergic to bee stings and you get stung it is an immediate IGE response.

Now we're switching to the adaptive immune system and we have tea lymphocytes. The CD4 positive cells OCD is clustered designation. It refers to molecules on the surface of the cells there are many different and changing every day types of T cells. Primarily revocable different populations, there are two cells that produce cytokines both similar -- stimulatory and regulatory that help another immune responses. There are regulatory T cells. Reese to say there were suppressor T cells. We do not call them that very much anymore now we call them cytotoxic sore regulatory T cells. Ologies cells can either directly affect killing in the case of the side of a toxic T cell or they assist in modulating other inflammatory responses.

There a number of factors that can affect your immune system. If you flew here on an airplane and the person sitting next to you was ill, if you have any and well in the last few days your age but the young and the old tend to be less Immunocompetence, if peaks in early after puberty and in the young adulthood particular gender, females are much more prone to autoimmune disease, we're not exactly certainly understand why but clearly there is some control over estrogen. Genotype is very important. Certainly, the underlying genetics particularly are important for allergy. Those with allergies know they tend to run in families. Similar for autoimmune diseases. There is genetic predisposition for at least some but very few of them, only one that I can think of really is about 95% predictive. We know there are clearly environmental influences on things like autoimmune disease. Then as well as lifestyle choices. Alcohol and drugs of abuse could also modulate immune function.

Quickly mechanisms of resistance to infectious agents. Again, I've already mentioned antibody is important. To some extracellular pathogen suspect is particularly parasites. Intracellular pathogens resistance to those is modulated by some mediated immunity things like Listeria which is a fairly common food pathogen. You will see that we actually use Listeria and some of our models. Susceptibility to infection is highly correlated with Immunocompetence so again the young and old tend to be more susceptible because of changes or lack of development or changes in immune function. If you have inborn errors of metabolism or genetic immune system defect, you will also have predisposition to susceptibility sister -- to certain infections.

There are nonimmune factors, things that the dose of the pathogen. Again, if somebody sneezes and you tend to get a higher dose then if you are just in a room with someone at the far end of the room. The various exhibit tequila pathogen some pathogens are very highly virulent sing -- things like Ebola. There are different products that the pathogens reduce. There are things that you produce, lysozyme in your tears, switch, the pH of your stomach. Some people are more susceptible to buy lower than others. Are many factors that influence disease resistance and when you encounter pathogens.

Hopefully that is giving enough of an overview that you will understand what follows and what the other speakers are going to cover in much more detail in their talks. Now I want to talk about what happens when something goes wrong with the immune response.

There are a few different types of adverse immune responses. We talk but immuno modulation and when I said that a mostly mean immunosuppression. We do look sometimes it immuno

stimulation where we're looking at something that is stimulates the antibody response unintentionally but we are not talking about allergic hypersensitivity. We also look at hypersensitivity as well as autoimmunity.

I'm going to focus first on immuno modulation. In the national talk -- toxicology program I'm responsible for the testing of environment a chemical sometimes things like food additives and dietary supplements store supplements of evaluating them for whether they adversely affect the immune system. We evaluate that using a number of different methods so we get some information from basic toxicology studies things like organ weights. We get some information from immune function testing and then if we see something in immune function testing will go into disease-resistant assays. In general for our studies we can use either adult or developmental exposures. In 1988 Mike Lester, had just started in my clusters lab at this point. Mike Lester and a number of very eminent colleagues, Al Munson, Jack Dean, Pete Thomas, that I be doing immunology testing in their labs for many years got together and analyze the results of their studies as they develop this tiered testing approach and this was one done in the US. There are several it came out of Europe from our IBM around the same time. This one is in mice comes when they came out of [Indiscernible] was in rats per the tiered testing screen was developed to evaluate the immune response. I have used colors here to shade in the observational endpoints of things like hematology, organ weights, simple cell counts, histopathology versus what we consider functional endpoints. Functional endpoints are we actually ask the immune system to do something like challenging it with a TD defendant RT independent antigen. We can ask you to proliferate in response to might agents. The natural killer cell assay, natural killer cells are part of the innate immune system. Or we have a second more comprehensive tear. I will go into that a little bit more in a moment.

This very complicated looking parallelogram came out of the paper. What I want you to notice is that along the diagonal axis here, some individual test are much more protective than others. If you look at things like T cell might agents, they are about 67% predictive which is not terribly high. Leukocyte counts which it of the basic toxicology study would you are doing into my path only about 43%. Again P value not very high there. There are a few things, body weight issues again -- suspect the antibody forming cell response which I highlight that appear natural killer cell activity. Some of these are highly predictive.

What is the antibody forming cell response so predictive? It is because it really involves a lot of players in the immune response so have already mentioned androgen presentation, macro phage take up antigen they presented on the cell surface. It is recognized by antigen specific B cell and seat -- T cell. They proliferate and provide help or they provide very antigen specific T cell responses or the be self look phrase and produces antibody that is antigen specific. When we talk about antibody -- immune responses we tend to compartmentalize that but in reality if you think about the response to something like influenza you get an initial cytokine response you get a response by the adaptive, sorry the innate immune cells things like natural killer cells then you get secretion of interferon gamma. To get a T cell response as well as an antibody response very far down the line.

I have to show the slide and actually have a beautiful photograph but is protected by copyrights I cannot show it but the antibody forming cell response when you quantitate this response is immunology and action. If you look at it under a microscope to meet is the coolest thing in the world because you immunize an animal with a T dependent antigen. In our case sheep red blood cells. Makes it with those sheep red blood cells. What you get when you finalize the response is a lot of sheep red blood cell you chart lymphocyte and you can actually see this

under the microscope. In the lymphocyte it in antibodies the complement the sheep blood cell all around it. It was called the plaque. You can actually visualize the lymphocyte under the microscope. What you cannot see the antibody the plaque shows you that it is there and it is very nice to actually see the system working.

This is the kinetics of the antibody response. I glossed over it in the tiered testing slide but you see that the IDM response comes early. You can also look at IgG response or do a secondary antigen challenge and then you get a more magnified IgG response but that is what happens when you get vaccinated. In general we have seen many of these antigens you give a vaccine which are mostly secondary antigen challenges with some altered form of the viral proteins that you see for example. You get the amplified IgG response it is a good memory response.

I show this again because, the secession of farewell, apologize for that. Which you can see is when you combine multiple assays for example the CTL in the antibody forming assay you have a very high level of predict devotee for looking at compounds that might affect the immune system. Multiple functional assays really allow us to be very protective of what may give in adverse response.

Based on we have learned since 1988, would currently revise the testing -- testing battery. We know really look more intently at immunopathology were using extended histopathology based on the parallelogram diagram that are Shoji were self quantification was very important. We move that up to tier 1. These assays remain the same although we do a different version now of the natural killer cell and CTL assay that actually involves using influenza as the challenge pathogen. That actually gives us a little bit of information about disease resistance without doing disease-resistant assays which is very helpful. We can measure more relevant endpoints then we used use with the traditional models for those tests. I will talk very brave thing about host resistance assays.

We used to do these a little more currently. We have refined many of the testing methods now quick look at things like colony forming units of bacteria in the spleen pickup already mentioned that we use the story which is a relatively common, not common but the pathogen progress use a Brogdon attenuated strep pneumonia a mimicking human disease. Plasmodium Yowell EI which gives us a handle on how impactful changes in humoral immunity are we use a Brogdon intended influenza virus and we have a number of other models as well. Will use these models if we find a defect in the immune response in the first-tier screening Super Bowl not generally see tier 2 type assays unless there is an effect. PC there something that impacts disease resistance and that flags there has been some significant change in immune function in the first tier of assays.

With implications of this for human health? When we do small studies and we see a 10% change in pathogen resistance, that is not very significant in a rodent city but if you think about a population of people and you see a 10% increase in the incidence of influenza that is a very significant impact. At the population level you can get small percentagewise but very significant increases in incidence or severity of disease which can result in a significant impact in terms of lost worker days, increased hospital visits, parental care if it is children. At the individual level of our to pointed out to that there are many factors that influence the response, your genotype, phenotype, and dose of infectious agent. The most likely consequence in people to exposure to immunomodulatory or -- is mild to moderate impacts of disease.

Just briefly assessment of Immunocompetence in humans. We tend to not do functional responses in humans. We have things like histories. However you can actually measure responses to vaccines and in some studies word is very important for example if there has been a significant chemical exposure you can give an official vaccine things like tetanus or other vaccines, you are not measuring a primary challenge or measuring the secondary challenge it does give you an idea what happens to the immune response. And if you think about for example in pins, if there immuno suppressed due to exposure to for example dietary PCBs and you alter their vaccine take they will have decreased resistance to things like measles or chickenpox which you can have a very significant public health impact.

I would be remiss if I did I mention that we really are trying to move some of these into alternative models. There are, there is an in vitro hunter part in many of the test we do. There often very good for providing mechanistic information can actually pull apart the antibody response and look at the effects on each of those individual cell types in vitro. There have been a number of efforts to validate in vitro endpoints with functional immune test however have to say we're not quite there yet in terms of immunosuppression. We're getting very close I think with the hypersensitivity endpoints.

I will switch briefly to hypersensitivity peculiar much more about this from Cheryl. I will just say that there are number of different hypersensitivity responses practices in ancient classification at this point. There been many suggestions to change it and increase it to a number of different types. What I want to point out to you is, there is immediate which is the IGE mediated what we tend to think of as antiflag. Responses and then there are more of the delay type responses. The immune complexes and ADC to to be more longer responses and they are more involved in things like autoimmunity. You can get immune disposition of the kitties and things like that which are type III hypersensitivity responses back to

We assess a journal -- dermal sensitization, guinea pig test of their several different kinds that mimic the exclusive patch tested the use in humans. They can do respiratory challenge but again we have very few really models for respiratory allergy. You see more about the Marine local SMX I. The mouse ear swelling test also gives us a handle on how much irritation is used. Sure will go into greater detail about the dermal sensitization. This is the local lymph node assay. I do not know why you cannot see the legends here. Essentially when you paint and antigen on the heirs of the mouse, you challenge for three days, you let the animal rest and then you inject it with something that will radio label peripheral eating says you can use -- DISA corporate into the DNA. You remove the lymph nodes and you look at the rapidly proliferating cells. You can either count them in a hammock counter or -- gamma counter or you can quantity down a flow cytometry.

Finally, I'm going to talk about autoimmunity quickly. Autoimmunity is in the inappropriate response again self antigens. The take a message from this very busy slide is that there is a spectrum of autoimmune diseases and associated auto antigens. It go away from organ specific so a good example is diabetes which targets the pancreas to not organ specific or systemic things like systemic lupus arithmetic is or scleroderma which affect large and multifactorial areas of the body so rheumatoid arthritis can target joints everywhere.

With a number of methods to assess autoimmunity. You can modulate genetically or experimentally induced models of autoimmune disease. In humans and experimental animals we invite with this by quantitative antibody levels. We can measure the inflammatory mediators and we can measure things like Sarah more urinary parameter so for example in kidney disease

we often look for protein or glucose in your in the example of diabetes. In experiment animals obviously we can look at histology. There are some measures of nonspecific proliferation such as the popliteal lymph node assay which measure very nonspecific immuno stimulation.

There a number of different types of animal models of autoimmune disease and I am running toward the end of my time here so I'm going to go through these fairly quickly. There are models where the animals have genetic predisposition so a specific gene has been either overexpressed or knocked out. There are models where you can autoimmune mice -- autoimmune your nice for multiple sclerosis you immunize with basic protein. That models called experimental autoimmune -- encephalitis. Will be hearing more about that from Dr. Nagarkatti. You can also induce in the disease by trading with specific chemicals are tracks things like Mercury, a penicillamine or the pain of my pics to make quickly had to reevaluate the data once we have obtained at?

It is a challenge because exposure to single agent or a single class of agents is very unlikely not only what people eat that what they are exposed to in the air, what they do with their lifestyle. There tends to be a long latency period between exposure and onset at least of some diseases such as neoplasia. No effects are tough to prove. Lots of toxic torts it is very difficult to distinguish no response on individual versus a large population of fact. Tend to have small numbers of exposed subjects and it is very difficult to determine dose often.

I will just say quickly that many of you may be familiar with NTP levels of evidence of criteria. They use these for their toxicology and carcinogen a stick studies with a look at some clear equivocal or no evidence. We have similar levels of evidence of criteria for immune function testing and this is the website where you can find the criteria you can go through them there. I will not do that today.

I promise you little segue into risk assessment and recently have been involved with a WHO and we published an I PCS document looking at risk assessment and hazard identification for immuno talks. This is the reference for the documents. This is a flowchart. We cannot read it would basically says is, is there evidence in humans that is your first question that goes through the functional test that we do and are their effects on functional test all the way down to our their effects on the observational endpoints like histopathology and this allows you to develop weight of evidence conclusions based on the data that you generate you can generate a point of departure from the data. If you actually look at, their number of case that is in there that will take you through it and there is one for all of the adverse immune endpoints that are discussed today.

With that come I will tell you that we have three case studies to real estate -- illustrate the different types of immunomodulatory effects that are discussed and our first or next speakers going to be Dr. Jamie DeWitt who is going to look at the immunomodulatory effects of perfluoroalkyl substances in rodents in humans for following that will have Dr. Cheryl Rockwell will be talking about case study with food preservative tBHQ and finally Dr. Prakash Nagarkatti who will discuss dietary supplement in modulation of our immune disease. I think you for your attention I will be glad to take questions. I think I have about four minutes.

[Applause]

Any questions? Since this is being webcast to be helpful if you would go to the microphone.

Audience Question: Thank you for the great introduction. I really enjoyed that. You mentioned that [Indiscernible] is very sensitive especially through stress. Would you please explain or make some recommendations regarding the review, certainly want to make sure that immuno respondents truly related to the test compound not stress or cow which you take that into consideration of the, for example [Indiscernible - low volume .

Germolec: Is a very good question. The question was really how do discern very specific effects from stress affects? In the NTP will redo our immunology testing were very fortunate because usually there have been other short-term toxicology test before. So often we will have a really good idea of the dose. I think that is really the key is that you need to look at dose. If you are looking at a toxicology test where you have testing at a maximum tolerated dose or half maximum tolerated dose NUC effects on the thymus are very often related to stress. What you want to be doing is looking at affects the do not generate any over toxicity. Then if you see things like thymic atrophy they truly are related to be the target of whatever the chemical is. A really good example of that are some of the poly-dated hydrocarbons things like TCDD where the thymus is very sensitive to those compounds and to get FX atrophy at very low doses where there are no changes in organ weights our body weights. Thank you.

Audience Question: Thank you for the presentation. It was very interesting. Is there a general change in prevalence of allergies to pedis or chemicals are two different components? Over a lifetime of an individual?

Germolec: It really varies actually. Some individuals become less sensitive to particular antigens that they are sensitive to as a child as they age out. We're finding actually now that there is an increasing prevalence of things like allergy and asthma now in older individuals there is some thought that, that may be due to the increased exposures that we face, increased level of pollutants and things like that where there are adjuvant effects for specific pollutants that may exacerbate the immune response. There are different prevalences of allergy in different places in the world and it has been explained by things that the hygiene hypothesis where if we beat a very clean life when we are young than we do not see as many things as we should to prime the immune response to appropriately respond to those pretty prevalence of allergy is changing, were not sure we really understand necessarily why and we are seeing an increase I think in allergy in older populations that we did not see before and particularly asthma I think. That is not good news for us alter people. That being said from a regulatory standpoint, to regulatory agencies, should they revisit evaluations of chemicals over the years? If the one your you pass a regulation on a chemical that is approved and all of a sudden there are some changes that go on that cause an increase in prevalence to a response to that particular chemical should the agency then changes regulatory decision?

I think maybe a better way to look at it is to look at it, the full spectrum of potential effects at the outset. One of the things that we do very poorly is look at susceptible subpopulations so when we evaluate FX we do not look at things in the very young. We very rarely look at things in older populations. I think if we are concerned about those types of changes that we need to probably need to do a better job on the front end rather than revisiting things. I think if we do a better job on the front end up looking at what the potential spectrum of activities is it we can get a handle on those types of changes later on.

We’re going to have an opportunity for more questions so I think I have to take Peter's question because he is the head of the society. But, then I think we will have opportunity for more discussion as well.

Goering: I wanted to get your perspective on an LL NASA. Think is been used as an alternative for maybe almost 15 years after the 1 refuted. I wondered your perspective and its predictability and usefulness for NTP and I know FDA product centers evaluated as well.

Germolec: I think it is great. We actually now have combined a local lymph node assay with the irritant assay because for us it allows us to reduce the number of animals that we use so we do not have to do a number of different tests. We can actually measure your swelling in the model that we use so that we get a handle on irritant see. I think it is highly predictive. It was compared to when it was validated. It was compared to the human response as the gold standard rather than looking at, although was also compared to the guinea pig response. I think it is highly predictive. It does miss some things. There is a problem still with things like mixtures which when you are looking at food safety or cosmetic ingredient safety you are looking at formulations so sometimes that changes absorption and maybe the potential for entered Excel to see. I have to say that think it has been a champ. I do think that it is very predictive and I will continue to use the but we do again hypersensitivity is the poster child for in vitro and we now have some really exciting in vitro methods and I think once we really gather all the tools in the toolbox we're going to be able to use some in vitro methods to do some good prediction for a terminal hypersensitivity. Stay tuned. I will still be doing the local lymph node assay for a long time.

With that I would like to close the questions for this point and we can have more questions at the discussion session later and I would like to introduce Dr. Jamie DeWitt from East Carolina University's want to talk about the immunomodulatory effects of intimate compounds.

Immunomodulatory Effects of Perfluorinated Compounds in Rodents and Humans

Jamie DeWitt, East Carolina University, Greenville, NC

Good morning everyone. Thank you very much to the FDA to the Society of toxicology for having this series. I should give a special thank you to Dr. [Indiscernible] for same and him onto the list of speakers. I appreciate the opportunity. I am from East Carolina University them in the Department of pharmacology and toxicology in the Brody school of medicine. Some of you may be wondering why in the context of food safety I am talking about an agent that is a synthetic compound that we do not typically associate with food or food contaminants and I want you to think about these compounds from the perspective of immuno modulation and from the perspective of the data on immuno modulation that we have been able to gather from these compounds over the years. The story is about how we have uncovered in the object is potential of these compounds not necessarily about the compound in the context of food although they can be food contaminants. I hope to educate you a little bit about these compounds but again I want to have the emphasis on the type of data that we generated from immuno toxicity studies to try to understand the immunomodulatory -- these compounds are for native compounds, the terminology is poly floor and -- PFASs prepare synthetic agents, it is a carbon backbone with thorns where the hydrogen would be in many of these compounds have functional groups attached to the end of them. This is a particular compound on as preflight -- is a Selleck acid. There really wonderful compounds for industrial purposes prefigures predominantly as surfactant so they are processing aids for creating other fluorinated compounds process used in aqueous film forming forms which are used quite a bit in the military the department of defense has some interest in studying these compounds because that used in a lot of training exercises and they are used most often in the extinguishment of highly flammable substances. For more

of a consumer perspective there is used as polymers and what is important is their oil stained Greeson water repellency so they're very valuable from a consumer perspective as far as providing materials that prevent rain so many of you today probably have umbrellas and nice raincoats that you are wearing burqas likely have some of these compounds in them to help to repel the water. From a food safety perspective, there also increase recent the contact paper so pet food packaging, fast food packaging, microwavable popcorn often have these compounds in the paper to prevent decrease from getting to the outside. They make our lives easier, they make it more convenient that as we know from many environmental contaminants they can become problematic when we start to learn about their toxicities. PFASs are considered emerging contaminants and even though we have known about some of their toxicity for several decades, they're still emerging because we don't have public health standards. Are some advisories on the environmental level for these compounds but not for all compounds that we would consider PFASs prepare emerging because we do not necessarily know all of the pathways. We do not really know exactly how humans are getting exposed. We do not necessarily know the concentrations to which we are getting exposed that would result in serum concentrations.

We also are costly developing new techniques for detecting these compounds in different environmental media. They are emerging because we do not have definitive information on what we should do in terms of setting safety standards. We're still trying to understand the toxicities of the storm going to toys about their immuno toxicity. I will focus on two particular PFASs. What is -- PFO way and this is a top compound appear with the Selleck acid tell. The second is overacting sulfonates. They have both been used industrially and in consumer products. PFO as was the predominant agent in Scotchgard so you could buy PFO lesson spread on your furniture so that your class of one would not soak into the couch. PFO if you are familiar with that you're probably heard it in the context of Teflon. It is used to make Teflon. It is not itself Teflon. In terms of food they can get into food through contact with packaging and through contact with cookware but from an environment of perspective they also can get into food from water and from soil contamination. If it, they are infidels have the potential to get into us.

To give a little history about these compounds in a regulatory context and this is the primary focus of this will be on PFOA. In terms of PFOA we have a memorandum of understanding between eight of the major US manufacturers, so they have agreed to a voluntary phaseout so by 2015, sometime this year we should have a reduction in some of these compounds in products and emissions. There's a commitment by a major US manufacturers to reduce the content of these compounds related to PFOA and in terms of PFOS, the major US manufacturer voluntarily terminate production in the late 1990s because of some of the data that were being generated about its potential toxicity. And about his persistence and presence in the environment.

As a mentioned these -- were so water are formed. The mantra to packaging and cookware. In terms of market basket surveys were different types of product for purchase from stores there was a 72,013 from four European countries. PFOA was the highest concentration in spinach from Italy so if you're going to Italy on vacation and have a big spinach salad that could be some PFOA in a. The FOA was the most abundant compound detected. PFOA was also the highest compound in intake estimation based on its presence in the different foods that were assessed thing the Czech Republic where in decreasing order in the terms of concentration to different compounds. Here in the US we have only to my knowledge had to similar studies done. One was done in 2010 and another was done by a corporation in the early 2000's. PFOA was one of

the predominant compounds in foodstuff in the US the particular study from 2010 did look at packaged foods, fresh food and prepared food. It appeared as if exposure to these compounds through food is pretty minimal, the concentrations of many of these compounds were below detect level so does not appear as if these compounds are pervasive in the US food supply at this point but again this is only from to individual studies that were published. As additional data become available we may find these compounds do occur with greater frequency in higher concentrations in some of the foods that we purchase that are fresher prepared.

These compounds are present in wildlife and the idea is not necessarily to show you the concentrations that are in wildlife because without understanding what is in the environment and what these concentrations mean in terms of their FX, they are meaningless. What I want you to take note of is that these compounds, like many synthetic compounds that we produce for the good of society often spread to there are no industries. Dolphins can live in contaminated areas but for the most part polar bears do not hang out around factory so they are in areas where the environment is pretty pristine practice demonstrates that these compounds are movable within the environment so they can persist and they can move from the place of production to a source of exposure that is distant from the place of production. We can have concentrations of these contaminants in wildlife that we would not anticipate having in exposure. Obviously, they're getting exposed by the food chain or by atmospheric deposition of these compounds.

Similarly we have got exposures are we have serum concentrations in humans and we do have occupational concentrations and these would be for individuals who work in the manufacturing facilities that make these compounds. There concentrations are relatively high but members of the general US population and these data from the national health survey. Members of the general population also have concentrations of these compounds in their CRM. All of us here because of what we do, what we eat, where we go, we get exposed to these compounds as well as some of us may get compounds to our food or the cookware that we use for cooking our food.

What does this all mean? Let's go through, Dr. Germolec mention some of the data front is the potential immunology -- combats which you mention some observational studies of you see changes in lymphoid organ way such as the spleen or the finest those can be suggested of potential immuno modulation. We can have changes in numbers of lymphocytes from the lymphoid organs but these can be suggestive and maybe a little bit protective if you have additional data to support those observational studies. We also can have functional data that tell us about the ability of the immune system to respond to a particular challenge as Dr. Germolec said you can ask the immune system to do something and you can determine the immune system is doing what it is supposed to be doing. We can look at changes in antibody concentrations after exposure to specific antigens. We can also look at the innate part of the immune system's we cannot functional test that allows to ask questions about the adaptive immune system and we can have functional test that allows to ask questions about innate immunity. Together, if we have a positive data for these types of test, we can start to make some predictions about whether or not exposure to these compounds can modulate the immune system. As Dr. Germolec said there's a weighted approach in these data are often prioritized in terms of their relevance to public health outcomes.

Let's look at some data for these two different compounds for PFOA M PFOS. These are not exhaustive of all of the data out there. I just highlighted some of the major studies of course I had to highlight one of my studies but these data for different types of mice tell us that when we expose mice to PFOA to the diet or through the skin or through drinking water, we can see

some pretty significant, the czarist statistically significant reductions in the wits of the spleen and thymus and Dr. Germolec mentioned that when we see reductions in the thymus that is a pretty significant outcome because the thymus is sensitive to exposures. You can see in these situations, thymus is decreased by 40% 80% relative to controls. That peaks our interest as Immunotoxicology's because it says, a sensitive organ is decreasing in weight. What does that mean about the capacity of the immune system to function the way it should?

Will also have similar findings for PFOS. One particular study we did not put the doses in this study they were very low. If we look at the data their reductions in lymphoid organ way so some suggestions that exposure to these compounds could modulate the immune system.

[Captioner's Transitioning] If we go back to the data, we can look at different doses and see that most of the time these decreases are occurring at the higher end of the dose range.

Said we have any other changes to accompany these alterations and organ weights? Do we have any data that are associated with the other changes? So let’s look at the cell count.

We’re looking at splenic and thymic lymphocytes from the spleen and the thymus. We can see in some instances we have decreases in T cells. We did not see any doses populations, but in earlier studies their word decreases in the T cells from the spleen and thymus. If we look at PFOA and PFOS by drinking water that are available in the spleen and thymus.

We do see these of relatively high doses. Do we have any other changes that accompany these reduction in cell numbers. So we have duction and T cell. Does the evidence suggest that this data allows us to say yes these are modulatory.

We have to look at the tier testing guideline and ask additional questions. These questions really start to come out in the TVA are. It is the T-cell dependent ends our response, where you expose a animal to her particular antigen that will a specific response and you can measure a specific amount from a specific amount time. It is ace unique study because you can look at a slide into the shield that has the blood cells on it and you can see a B cell of dead red blood cells around it. It is really cold as he under the microscope—microscope. It can give you a headache if you do not like to sit in front of a microscope for a long time.

And as Dr. Tran 25 it does require the different antibody responses. So if you see a difference in the PFASs nine that is very important because it can compromise the immune system.

If you any reduction and the antibody response can be considered biologically relevant. This because you don't see a statistical result of the response does not mean it lacks relevance. It is a very relevant test because when you see differences and control, that is a red flag of the immune response.

In the first PFASs nine and PFOA, it is diluted with a high particular plate. To take home with this, they used the FLA and they used course load cells and set of sheep blood cells and this dose was associated -- 30 megs per kid -- it was associated with organ weight and T cells. We have some offers facial data along with this this functional data which makes this weight of evidence stronger.

We also, through an additional study that I did and I did with my post dog -- postdoc, we knew that this 30 this 32 was a reduction in body weight. It is kind of hard to say that this particular concentration is immuno tocsin because of the systemic or possible systemic toxicity.

Soapy look at the low dose we went to 3.7 my -- 3.75 per gig and this dose was also not associated with reduction of body weight. It was also not responsive with T cell numbers. We have a reduction in the anti-body response and it does that is not accompanied by these observation responses.

And if you did a lower dose study are NOA EL which is different from a LOA EL, this was the highest dose that we could statistically get in the control group. We got a 3.75 in the LOA EL and that was the only change statistically. In both studies we do not see changes in the endpoint. So we see a functional change without associated changes in the lymphoid organ weight empty spots.

And the PF OS, with that limited dose of PF OS it is the amount that we see in adult humans. But anywhere within 100 to 1000 times that we see in humans. But even though we see a change in the PFASs nine and it is not relevant to humans. But with the Tran 30, it is relevant for the human exposure. And this particular study, -- just another metadata from a different study. We see the antibody response based on several different lab report that the functional immune response is modulated to this concentrate.

We have the TVA are with doses that are not impact organ weights or subsets. Is this sufficient for us to say that these companion -- compounds or do we need additional data to fully understand what is going on. Or can we use the TVA are and just use assessments based on these compounds.

We have done some studies were we looked at the adaptive cellular responsive and this is a sensitivity response. We can see at these doses of the PFO a, we did not see changes in the hypersensitivity. So and the data that I helped produced, it was in one but not in another response. So it tells us some specific duty of the exposure. And terms of the PF OS or the B though I said response we did see changes that were trying to kill the T cells.

Immuno modulation does not have to be suppression, but as Dr. Gamal and says when you see an increase or stimulation we do not exactly know what that means but the PF OS module is changing.

We have seen the impacts on side again production we have seen both increases and decreases after in vivo and the profile. PF OS might depress work PF 08 might enhance. We do not know if they are inflammatory but if these compounds were limited just to these markers of limitation because of the data from in vitro studies are contradictory, and there is no function data to receive with them -- we may not be able to come to it decision to modulate.

While these data are important to help understand mechanisms, they do not tell us anything about what the immune system is supposed to do.

Some disease-resistant studies have been done in the PFO a Japanese quail. It did increase but it was not significant so the quell were exposed to E. coli and their morbidity was tally aided afterwards. We did see an increase in mortality after an influenza challenge. This suggest that

the overall ability of the immune system to function could be challenged. Sans addition of changing the specific and points it could change the overall immune response.

So in summary, we have changes in TDA are. We have alteration of cytokine production. We have alteration of an eight immune function and we have possible impacts and disease resistance. So what does this mean towards humans?

Do we have immunological studies that can predict what's come out of the animal study.

So let's talk about mechanism before we talk about the human as far as the mechanism it is complex and I will not go over it in detail. But this is something that has been created from all of the different studies that have been put together out there. Sans terms of how these compounds may impact the compounds it includes in vitro -- in vivo -- and [Indiscernible]. If we just had impact -- impacts from PFO a it is from the receptor, if we did not have a [Indiscernible] change it would be harder for us to explain.

We also looked at developmental immuno toxicity. It can be challenging to use excess. There are multiple ways to expose to the impact and to the studies including the dams or through and gestured from adult hood. One thing to remember for developmental and Mena talk city is to ask your system to do something before it is reached maturity will not give you the best data. It may be best to wait for the immune system be fully matured for you ask it to do something.

We do have evidence that show the impact can suggest impact based on this particular response -- the TDA are a suppression on the highest dose but the concentrations are pretty low. We also see modulation of the natural killer cell response. You should note that at these particular dosage we see a suppression of the natural killer cell response. Sometimes you might see difference between adult organizes some and developing organizes them. How do you determine which is appropriate for protecting health the use and discernible studies? Which one do you use.

We have a couple of studies that suggest there are some modulatory events for children that have been exposed. So we did a study of some children from the Pharaoh Outland's and it is kind of getting to the bottom of the story. And the Pharaoh Islands, Marine meet is exposed and has a lot of contaminants in it. But it did find there was a association with serum PF OS serum was associated with a decrease vaccination response to depth area. So PF 0A and PF OS was a reduced response was not evaluated by the study but it is study because it is an out is to the TDA are. We asked the human immune system to do versus the animal immune system to do.

Again, damning city -- system was reduced from a paternal and trying to associate with what's going on with mom and the children is little more difficult. But two of these components were during episodes of the common cold. So is there an increase produced by this study. If your mom is exposed to this are you not able S children to fight off the cold.

So immunobiology told three. I am just an academic scientists but both suppress the TDA are. They've both change and the side a cane and they are associated with immune related changes in humans. So in terms of associating these data with evidence approach, we may have some positive answers to the important questions that Dr. Green Malik showed at the beginning of her talk. We did show from the epidemic knowledge you study and we do have mechanistic and in vitro data to support immuno to expose humans and animal models.

If we want to look at this in context for PF OA. This is associated with PFO a modulation. To take home from this is that concentration and all other organisms that have been evaluated, sea turtles, background of humans, this is higher than in the overall population. We have seen it in humans but rodents have a higher concentration.

When we see data like this we need to ask if there is a similar between humans and lab animals. So we need to ask if there are lab. It is well within the realm of concentration of both humans exposed from the general population. It tells us that not all PF OSs are not the same and they may not have the same set next mechanism. So for classes of compounds -- we may not be able to make a uniform decision on compounds and look at different compounds in a class to look at for human health. We have a way to look at the modulatory because they occurred at different concentration, we may have to evaluate and assess them separately to come up with a different compound. Not all the compounds and the different set will have the same data.

As far as the questions for future needs, I have been working with these compounds for the better part of my career. There are still some unanswered questions here the biggest is understanding how the replacement compounds are going to impact the immune system. We are getting ready of PFO a and PFA as but because they are so valuable there will be in veritable replacement compounds. I just said that PFO a NPF OS will change similar so that tells us that we need to understand these at a individual level II have the appropriate levels. They clearly have different mechanism of action depended upon the dosage and the species exposed.

We have serum concentration but we don't really know the major Ruth -- roots are the amount of exposure. If I have 9 ng because of my raincoat or is it because I fly to Italy and spinach all the time? I wish it was because of that. That we don't represent -- children represent the subpopulation. We has that these that suggest that it is more sensitive and we have the studies of the immunologic but we don't have a high end of studies to stay that we need to reduce and children. So how is this impact in safety and use this data to assess compounds that are being put into our food purposely.

That is the end of my talk and I have a few reference slides if you want to look at any of that. And I have about six minutes if you want to ask any questions. Please don't make me tell jokes for the next six minutes.

Audience Question: I can repeat the question. Just a quick question on the studies. Did you look at the [Indiscernible] and what you did didn't sustain or was it reversible

DeWitt: When we did that TDA are and the PFO a was for 15 days. Once we stopped the animal weight level did bounce back to the regular. Some of the other studies were 10 days of exposures and seven days. The PFO AES was a 28 days studies which was the guideline for toxicity for best aside regulations. Some of the tatties -- studies by Dang and John were 28 days studies, but 90 days 280 days there is some good data from the monkey points but there was not done any studies on animal. But it was low exposure and I don't think anyone has looked at longer term of exposure at lower concentrations.

Audience Question: As far as gestational explosion -- exposure -- is her problems with that. And it may not be relative -- but has there been developmental studies?

DeWitt: Yes. But the risk that these or assessments are driving those. There is some studies coming out a new labs that the EPA and the NGS that says destination all exposure can lead to lifelong -- lifelong persistent impacts. I think a recent paper that has come out from an HRIS -- we looked at the immune system and we received some animals that were exposed and they were city one mice here we monitored antibodies when they were one month of age and we did not see any changes here so the immune response in the strain of mice would did not see any changes. But taste on the studies would run to CD one mice they did not seem sensitive for the organ weight but we did see developmental issues. They do appear to exist with developmental from the studies that I have seen. That we have not actually done much studies after the ingestion during just station. Two minutes. Nice.

Audience Question: So given that the left ration and gene expression are so important here, is there any evidence of app and it change of PF a esses?

DeWitt: I do not know of any definitive RF budget genic studies. I do know that epigenetic can affect the immune system. It can impact the T cells for antibodies to get produced but for terms of specific effects of the PF O a of the PFA I am not aware of any studies so I cannot give you any good answers based on that question, except remind myself to look at the literature more thoroughly peer sorry, for the web office that I could not give you something more definitive from the data. And terms of the TDA are is to look at something or the TI TR or the independent response, this ask can be cells make antibodies without the T cells. And for both PF OA and PF OS we seen reduction and the T-cell dependent and the T-cell and dependent that tells us that the B cell as a likely target and signaling because we do not see the reduction of the signaling of the B cell is targeted. So the B cell is to secrete antibodies are oppose appropriately as somehow affected by exposure to these compounds we're still doing studies trying to understand this specific mechanism here and there has been some studies done on the gene mechanism.

Germolec: Thank you Jamie for getting us back on time. We now have a scheduled break. It is 10:10 AM and we would ask that you return at 10--10:15 so that we can stay on time. We will see you back here in about 15 minutes.

Toxicology and Food Allergy: Case Study of tBHQ
Cheryl Rockwell, Michigan State University, East Lansing, MI

Germolec: So while people are getting settled I will say that we appreciate -- I know that we haven't answered all the questions and if you will say those questions for the panel at the end. I'm going to go ahead and start the second part of our session and introduce Cheryl Rockwell who is with the Michigan State University and how food allergy relates to the study of the tBHQ. Thinking

Thank you. I am delighted to be here this morning to talk to you about toxicology. It is one of topical interest because it is an area that is recently emerged with the genetic organisms from the 1990s. I've been given the task of a general overview and a style of a continuing Ed top style. At the end I will talk specifically about a specific talk so college he of the food allergy of the study of tBHQ. I am going to start by giving you some of the trends in food allergy and are applicable to United States and other countries.

Then I will talk about the immune response during a reaction. Then I will talk briefly about how we are evaluating Allergan as it the of novel proteins and the lack of animal models. I will talk about a few of those because there are only a few them. And then I will end by talking about tBHQ .

Depending on upon what report you look at they can vary on the prevalence of food allergy. The most a set double food allergy is children. To have the highest privilege. Within that group, the children of age 5 have the highest prevalence. The numbers of prevalence and food allergy range from 4% to 8%. It is not showing aid by on gender that I do know that there is a gender bias that is being studied. But I do know that there is a study of white and Hispanics based on African-American but I do not know if that is accurate as of this study.

There is a rise of food allergy over the course of 10 years. We think this trend has gone on longer than that at least for couple decades the fastest growing group of Redlands is in children under the age of five. We also know that children who have been diagnosed with food allergy have a most higher likelihood of the and diagnosed with another allergic reaction at the time. Most of them have asthma because they can come from allergy and also a skin or respiratory allergy.

An addition to an increase in the prevalence of allergy -- of food allergy overtime -- we see an increase of hospitalization of food allergy. The increase is outpacing the increase of prevalence. That suggests that not only do we haven't increase of prevalence of food allergy we also have an increase of this severity of these type of allergic reactions. That is supported by evidence of other reports as well.

What is actually happening during the case of any mean response for a reaction? We know there are cells that are responsible and Dori did a wonderful example. The T cells play the role of leadership and have a lot of responsibility and recruiting specific immune types. They tailor an immune response against a specific immune response.

One way that T cells do this is a phenomenon which is called T-cell differentiation which is been touched upon with us some of the other speakers. Basically, T cells originally come from the bone marrow and they go through the thymus to become educated. This means they are learned to discern between thymus and non-thymus. When they emerge from the fine miss they emerge as non-mature T cells. I think of the cells as being analysis of high school graduates. They can get a job and have an apartment that they typically have not chosen a career track. T cells have a number of a potential career tracks available to them and for instance they can become ETH one course. It helps with fighting off intracellular pathogens. Or they could become it T H2 which helps with fighting off the helm and that's which is nematodes. That TAH 17 which is for extracellular pathogens. Or they can become a normal T cell which is an anomaly of all of these subsets of two selves because all of the other ones is a response, where the other ones are stopping or inhibiting a response. So I think of regulatory T cells is sort of a military police they are monitoring all the other sub types to make sure that they are not behaving inappropriately. If they are, they will not hesitate to arrest or detain for the T-cell that is exhibiting an appropriate behavior.

The different function of T cells can be different from cytokine profile in. So the T-1 cell is cytokine and T H2 which is IL-4 IL-5 and I'll 13. T8 17 which is L 17 a I L 17 F aisle 22 among others. The TR e.g. which is TG F beta and IL 10.

So what is happening on a cellular level on a mean response. So we have an individual that is allergic of peanuts. Ingesting peanuts will break down into proteins and the G.I. system. The protein can pass the gut and discernible. Dori gave you an overview of how the system works but essentially these were antigen presenting cells in the effect of that is they will present themselves of smudges of that protein to T cells the smudges are actually peptides that are between eight and 12 peptides long.

The T cells are going to ignore that pep tied. It is only going to be a subset of T cells that are responsive to that T-cell and sensitive individuals. Those T cells will become activated and they will differentiate between to make two cells which is IL-12 and I'll 13. This will be conducive to reacting be cells. It is not all of them but it is the one that will recognize the pep side -- pep tights.

This particular environment under the influence of these two cytokines will have these antibodies which will make the production of IGE and IgG. This production of IGE in the course of an allergic reaction is a bad thing because the downstream consequences of that on other immune cell types. Specifically math cells. So let's think of the T cells as the military as I do. It is a very dangerous cell because it is well armed. They granulate and if flame a torii mediate. They will release a whole host of the mast cell the granulation. Histamine, prostaglandin lens, look at trends and site toxins. It can be consequence that include swelling and itching on a local level. Talking of the G.I. tract can be nausea vomiting and Iran -- diarrhea. Some have a systemic response which could be hives and rash. Respiratory distress of swelling of the airway. There is a blood pressure dropping can go into shock. There is also a you and it can be severe enough to be fatal.

The first time someone is exposed to an hour Jen they are not going to have all of these happened there are two phases to this response. Since the Tatian and the elicitation. The first time they are exposed to an hour Jen third in a get part way through this response and then it's going to fizzle out. That is due to a number of different reasons.

One is the immune scene has first seen a reaction they are naïve. They like to think deeply before they become activated and their response is elicitation. Later they will respond much more quickly and more robust response. Then and the mast cells are loaded up with IGE and being exposed to the same protein that combined to these IGE receptors that are already in these mass selves cause cross-linking and revoke an immediate the granulation.

When that happens, you get this elicitation cell. It is basically taken a safety off a gun.
The majority of us do not respond to food antigen. So why do we not put respond to [Indiscernible]? It is based on oral tolerance. When it sees the protein coming into the gut really should not attack the protein like that. We still do not know how it works but it isn't phenomenon. I am giving you one hypothesis which is a piece of the potable -- puzzle but there are other parts that are also involved.

In this case, we have the protein coming from the gut of the of Gilliam and coming across the dendritic cells. They are not coming differentiated in the TV to cells they are becoming activated from the to did tricks sell and coming into the regulatory T cells. We know that regulatory T cells -- or have strong evidence -- claim a big part and mediating tolerance of this may be one way they are doing it. There is also evidence that show that T cells and this situation will become depressed and certain allergic meaning they become unresponsive and suicidal and commit a put ptosis. These are all different ways that oral intolerance is named.

So how are allergen assisted the of novel proteins evaluated. There are a number of groups that come up with evaluations and they create with allergens that we have identified. Food allergies -- 90% of the cases can be based on just a few foods. We have characterized a lot of protein and have mapped it down to specific epitopes and peptides that cause allergies and they then go to novel.

Then we look at digest and stability because our known allergen are stable and a digestive environment. Which is amazing because of the enzymes. But in some cases we have known that allergies that are not specific in the early digestive which we can simulate. That's operably because of the way we do the testing. And many cases it would become digested, even though it comes in the context of food it may be won't. You have to look at the complex of the food such as lifted and protein bodies and the way that the food is organ Ms. some. Some of the seeds which are storage vesicles and they have certain proteins that are here and some over here and they are more resistant to digestion then other areas. These all need to be taken in effect in trying to determine a gesture.

Another thing from testing is if you have a novel protein that has a sequence that resembles that of a known allergen, one thing you can do is take serum from a human that has an allergen to that food and you can test your novel protein against that protein. If it binds your novel protein then you can think maybe I have a problem because there is cross reactivity.

What is missing from this is validated animal models. This is been an ongoing area of research to develop animal models that can predict animal models in the city. I am not going to talk about all of the animal studies today but just talk about a few. Using a -- and atopic dog a neonatal swine.

I put the large mammals together because their cells are somewhat similar and in the interest of time. They are neonatal slime models and atypical dog models for allergy testing. Investigators that do these, studies typically are rating these and often begin exposure shortly after birth. So sensate station phase sometimes it is so cute intravenously or sometimes it is oral by feeding. Large animals it is easier to feed them things to.

The patient phase will go on for some phase. It seems to be shorter and the swine model than the atopic dog model. In order to get this to work, investigators have to overcome the barrier which is oral tolerance.

The way they do this is to include adjuvant. It is a chemical to promote an allergen response. We typically use these in vaccines to boost [Indiscernible]. You have to be very careful and select team because of many that may boost model or inhibit any immune response. That is why it is some important to try to create a DH to adjuvant.

After some certain time of agitation, that is followed by oral challenge. Because since the Tatian is happening early on -- right after birth -- they are nursing right after that. So we have to be cognizant of what the mother is being exposed to at this time as well.

Best to gators can monitor the immune response both through the [Indiscernible] face and -- used but a small allergen under the human skin. If it is sensitive you see a well that is red. You can also quantify specific antibodies and serum. For those species that the antibodies that are available to use for these type of assays. The anaphylaxis essay is something that is used in all

of the different animal models and will talk about that in a moment a nice feature of these large models is the ability to do endoscopic. I would imagine you could do endoscopic and rodents but it's very hard. But endoscopic is done in the large animals so that you can see when their scope ring they can add antigens and take biopsies of those areas.

Another advantage of the large animal modules is they tend to represent clinical as we saying humans. Swine, dog, can vomit just like humans. Another one is trying to describe children are neonatal and they are easier to work with as neonates. Mice are very small and very fragile and they cannot be -- you cannot get much tissue from them. The atopic dog model will naturally get food allergies and we are studying that right now. It is not as artificial as some of our other experimental module.

The downside is they are very expensive. For the average academic investigator these types of models are absolutely and completely out of the picture. So investigators that do these our wealth funded. All of these columns are not bred to everyone and you need to have a collaboration or some way of getting a hold of these clients. Is very possibly done is food our G. You need to take a food allergy from a dog that has Artie been sensitized you will take Sam from that animal and you will inject it to the dermis of your naïve. You will wait 2448 hrs. and you will use blue dye because it will make it more visible. You do not have to wait very long for you to see a response.

You can measure the response through inflammation and you can see this in error thickness and foot thickness dependent upon where you injected your rodents. Very often, investigators are measuring the size of the Legion. You can correlate the size of the lesion with the response. Histology is often done on this response as well. This assay can also be done to measure IgG. You can take blood from the an act of and you can inject both of those fractions into two separate animals and compare those. If your heat activated does not have a reaction but sure non-heat does because your eye GM is affected by heat where your IGE respectively is not.

Another study is with the Brown Norway rat. It is conducive to allergy testing because this particular strain of rat is predisposed to [Indiscernible]. What is interesting about what the Norway group has done is mimicked the way we think that humans are sensitized to allergens. What I mean, as they are doing daily oral sensitization besides injection.

They are able to show the raise of antibodies and the PCA test as well as the gut per ability. After the gut per meal be allergy they looked at the serum level of that bystander protein and the gut. They check the blood pressure in the represent Reppert's -- respiration rate which is an anaphylactic rate. We get a different range because some have in a minor response in some have a more severe response. There have been numerous mouse models that have been developed and I could've made an entire talk just on the mouse models alone. So I am going to break it down into two models.

First model was developed by Hugh Sampson's group and they are interested in the development of childhood allergies. So they are using very young mice many cases they are using three-week-old mice. They are sensitizing the mice to various allergens of milk and peanuts but they are using oral sensitization because they're interested in what is happening in the gut during the develop of food allergy. They have been very successful in raising severe allergic response in this model

The BL -- BAL the/C mice are used because they are predisposed to a T H2 response. They have done some interesting things with the mice because they have developed adjutant free sense of station and compared with multiple registration to try to get at what would be the best way of the Dick rank order allergen the city as well as different parameters at the end. What they found, if they used a highly allergenic protein are one of the proteins that cause an egg allergy and the potato extract which is minimally allergenic they could distinguish between those if they inject did my IP injection and they look at IG E response

If they look at total IgG response, all of the eight allergens would raise a response it was much less effective. They found IP injection to be twice as effect of as oral but potato protein extract which is minimal did create an oral provides where the IP injection was more discriminatory between these two studies. So it was a way for us to predict a novel protein.

The models that we use in my lab is a adjuvant free module and it was first developed by Dr. Danker. He could bypass the tolerance by the route of exposure. What he does is he takes and clips the further back of them mice and puts this ride on contact of the skin for 4 to 6 weeks. He typically observes a robust IGE and IgG1 and serum which are typically allergenic but proteins that are not usually allergenic. At the end of the adjuvant free sense of Tatian he sees a very severe allergenic response which can be fatal or near fatal.

He can quantify by going through a clinical system. He goes through a scoring system that worked and food allergy and models. So the mild system start by scratching or the rubbing of the nose and the mouth and then you see puffiness and you see labor respiration and no signs of the mucous membrane around the nose and the mouth

What is found is that he can see robust response of known response and hazelnut, sesame, cashew, and cows milk not foods that are not typically allergens such as vanilla. We perfected this module by doing this we could see pretty similar effects to what he's saying with other allergens which was a robust increased and IGE serum and four weeks after sensitization. We also see a robust increase in out the rhythms of IgG1.

Following oral challenge we see a significant decrease in body temperature almost 2°C. Also see an unresponsive stimulus. Most of them were in these top ranges. We were interested in creating this animal model because we are wanting to promote T H2 which are done in vitro which we show that TH TH2 as a decrease in the T age 1 gamma and the why tBHQ which is in the black box. One thing I did not tell you before between TA 20 H2 cells if they actually do not like each other. They antagonize each other. Of one goes down the other will go up if T cell different to his being impacted.

We also have an impossible that it was probably happened and that's why it was not included here because you will see that the tBHQ was not significantly impacted. It was significantly less in aisle for which is consistent promoting ATH to response than a TH1 response. And looking at IL-4 we found similar effects of IL-5 and IL 13 and the DH one and the tBHQ had very little.'s were all done in vitro and we wanted to know whether these in vitro effect would have the same in vivo.

So what DH one have a TH TH2 affected as why we wanted to create this model. So we put mice either that had no tBHQ or a small amount. It is different current -- difficult because the preservative is widely used for human food and adult food as well. So we had to have a special diet that did not have any tBHQ and then we put it back into the child

Will we found, -- one thing we should mention as we tweak the model a little bit. If you recall we were actually getting very severe response with a variant by itself. If we could increase the response we felt it was highly maxed out the response. We tweak it so that we could use a lower dose to generate a more mild to moderate hypersensitivity response.

Will we did -- when we did that we found that we could raise a significant about young and IGE but it was not robust and our previous model and we could also add in a specific and IgG1. We did not see a significant amount in body temperature and the clinical scores in the ODA we were getting ones and twos.

The tBHQ at a robust and were aspirated in the IGE along with the tBHQ we also found a robust increase in OPA and IgG1 and the mice that was on the diet and a decrease in body temperature after all challenge We found increase clinical scores as well. All of this was preliminary data and we have not repeated this we intend to go forward with it.

With that, I would like to acknowledge the people that did this work. Heather Dover has been the point person for all of this and she is remarkably organized. She got a lot of help from Jenna burst layer and David could as well as other members and the lab.

Also Dr. couple who I ended up Marian. Dr. anger who developed the model and Dr. Pesca who help us do the diet studies. Now I'll be happy to answer your questions.

Audience Question: People who are allergic to some allergens such as peanuts. Can you talk a little bit about the potential mechanisms for that ask --?

Rockwell: Immunotherapy is being -- that is without you. There a lot of people were going through immunotherapy. With that works is, low doses of the allergen, the basically become sensitized for desensitize actually is the way we think about it to the allergen. The mechanisms are not entirely -- but one thing we know, frequency of exposure and age of exposure all very important the previous guidelines as far as exposure of allergens to not do it essentially was to wait until they're not infants anymore that turns out to probably -- at least are increasing evidence to suggest that, that is not a helpful thing to do. I think you you are really doing is teaching the immune system that this is an innocuous food protein so I suspect -- immunotherapy are always if you teach it, actually is not really harmful, that over time you start to do pod and in Tenuate of response. There is a lot of the studies done to show that this does work.I see very busily writing a way. We have made one minute for questions. If you have one that is ready perhaps we can take it now and the other one we can do during the panel discussion.

Outstanding questions. It's a very intuitive question. Whether the two is something as dangerous as in many cases determined by what some of these bacteria in the gut and whether they will indicate -- part due to what part of it coming from the bacteria the same time the seafood antigen. It is reported there been a number studies to show specific strains of bacteria that are very important in the developing immune system of the gut and children. Is clearly a developing area but I think it has a [Indiscernible] it has been suggested that, that is risen where there's a higher incidence of food allergy in industrialized countries compared to non-industrialized countries that there is a difference in the micro bio that may be in response to that.

Dietary Supplement Modulation of Autoimmune Disease
Prakash Nagarkatti, University of South Carolina School of Medicine, Columbia, SC

Germolec: Will try to take some additional questions during the panel discussion. It is our final speaker this morning session Dr. Nagarkatti from the University of South Carolina is went to talk to us about dietary supplement modulation.
Thank you. I will try to focus my talk on tight supplements and how they can impact the autoimmune response. By now I think you'll have become expert immunologist, the last three lectures I really want to think and couple meant dory for doing such a great job because normally the University, [Indiscernible] condense it into 45 minutes. That is wonderful.

In any visual there has to be a balance between infection and immunity. When you get an infection, you have the innate immunity as well as the adaptive committee for the infection but at the same time you want to make sure that after the infection is clear the -- and attacked because that could cause severe damage to the cells and tissues. Therefore mother nature has created mechanisms to try to [Indiscernible] is cleared the what I call the regulatory mechanisms. These include the fact that our immunosuppressed [Indiscernible] that Jimmie and shall talked about are the [Indiscernible] immuno response that can immediately [Indiscernible].

What happens to an imbalance of this immune regulation is that effective mechanisms get into make you suffer from hypersensitivity actions, the allergies. You can also get autoimmune disease of my can also lead to chronic inflammation resulting in significant damage to your own cells and tissues. In such instances therefore we find that if we look at the regulatory T cells or other mechanisms that suppressed information if I may be the regular T cells are [Indiscernible] properly or sometimes genetic disorders that might lead to the regulatory mechanisms which lead to chronic inflammation or hypersensitivity as well as autoimmune diseases.

[Indiscernible] is considered to be the underlying cause of all major clinical disorders. Starting from cancer, clear that has been shown that certain types of cancers such as colon cancer, breast cancer and so on, chronic inflammation in the colon can trigger more susceptible to colon cancer. There are cardiovascular diseases which are shown to be having an imbalance component, also neurodegenerative diseases as well as obese -- obesity resulting from chronic inflammation. In addition there what are called autoimmune diseases. These are diseases in which your immune system goes haywire comes normally the function of the immune system is to discriminate [Indiscernible]. When it comes to our own molecules, them are not supposed to [Indiscernible] a strong reaction. This was we talked it up or concern individuals, the immune system is not able to make a distinction and as a result of the immune system starts to destroying in your own cells and tissues as if they are form. That triggers as Doretha to do really what I call as autoimmune diseases. There are over 18 different [Indiscernible] diagnosed and I estimate there about 22 million Americans who suffer from various autoimmune diseases or $120 billion annually in terms of healthcare cost.

For those of you hear from FDA, there is no need for me to introduce the dietary supplements. These are including vitamins, minerals, or biotics, herbs or botanicals, amino acids, anything that is marketable in the market as, excess, constitutes, tablets, capsules, softgels and so on. There has been some speculation six testing that -- suggesting that dietary supplements in the type of nutrition we take can change the epic genome affecting the epic genome. Semis dietary

components can alter your be genome which in turn can regulate the poor immuno response. In my talk I will try to give you some evidence for that.

Basically we are saying, there certain dietary supplements that can either suppress inflammation or increase the regulatory mechanisms such as the [Indiscernible] and other cells. Therefore try to balance in such a way that we and our -- the dietary supplements may be useful in terms of trying to suppress the chronic inflammation leading to a wide area of diseases.

Why data supplement research? 30% of -- 4% of children is some form of complementary and alternative medicine, there are [Indiscernible] out of the scene this is a region not have medications that can relieve you of the pain you are suffering from. In the US people spend about $33.9 million in out-of-pocket expenses to visit CAM practitioners and these include [Indiscernible] medicine, herbal medicines and other types of acupuncture, yoga and so on and so forth.

Clearly the dietary supplements pose but the risk and benefits which is what we need to be really careful about. Specifically the dietary supplements from the consumer spend about $20 billion a year and in the US again, coming from the FDA you will notice that dietary supplements are regulated as drugs therefrom all the drugs are approved for safety by the FDA, FDA is not directly responsible for evaluating the safety or efficacy of the dietary supplements unless somebody wants to introduce a new dietary supplement. Or, if the dietary supplement has certain contaminants or they are shown to be toxic and not safe then FDA can make a recommendation that these are not sold in the market. That happened with ephedra which is a compound that is used for reducing the weight, can be how the toxic so the FDA made the recommendation that this be removed.

There are a large number of herbal products are very few of them have been shown to boost the immune response, that means enhance the immune response. Some people take these compounds before the disease shows the for simply try to take these because they feel like it will help the response. Majority of the compounds of the products that have been [Indiscernible], marijuana, thunder god vine, reserve hotel has been shown for people who suffer from inflammation or inflammatory disease or autoimmune disease they tried to take these compounds.

As I said before, some of this compounds, they -- though there are available readily they have been shown to have [Indiscernible]. For example, let's look here which is used for the way to certain types of [Indiscernible] antianxiety agent has been shown to cause hepatic failure. Ephedra which as I told you is committed by FDA to be taken off the shelf, the weight loss effects have been struck. Saint. John's wort which is used for depression is done to increase the cytochrome oxidase is which interferes with a large number of drugs that you take.

Therefore, as I'm coming from medical school, then we teach the medical students we try to give a few lectures about documentary medicine and make them aware of the dietary supplements of that when they become aware of the fact that these dietary supplements can have significant impact on the conventional medicines so therefore the make it a point to ask the patients before they give any treatment to see whether they are taking some dietary supplements. Also, it is necessary to educate the patients to make sure that if you are taking dietary supplements and you disclose that to the doctors, to the physician so they know to make sure that the drugs that they are prescribing to you will not interfere with the [Indiscernible]

example I gave you is the Saint. John's wort which has been tried to increase the size, oxidase is which is one of the drugs that are available right now.

Complement remiss; practiced in India as well as in China, the herbal medicines which have been practiced for thousands of years, use pretty much botanicals. They are herbal products. If you look at how the drug is been introduced, you'll find that almost 25 best-selling pharmaceuticals in 1991 were either natural products or derivatives. There is tremendous science research that are over 250,000 species on earth, only 6% of been screened for biological activity in only about 15% have been screen for pharmacologically activity. Therefore return to discover new drugs that are potentially products with natural compounds.

Therefore research and botanicals can also direct the study as well. Again these are some of the examples, this products from morphing to digitalis to Taxol in Aspen. -- Aspirin from botanicals or trees. Also what it does is, a lot of people can put [Indiscernible] clearly more research needs to be done, more clinical trials need to be done in this particular area.

Before move on to the research that we are doing in the level want to introduce you to our receptor. This receptor was discovered by the toxicologist in that a lot of these poly cyclones including dioxin are [Indiscernible] hydrocarbon receptor, this is actually compound binding to the age receptor, buys two other [Indiscernible]. That complex in turn binds to the promoter of regional genes which have the I auxin response element. It expresses the dioxin the present which after exposure [Indiscernible] activation of age receptor can lead to addiction of CYP one. That causes chemical some of the metabolites that because of the toxic city of these compounds correlates with the ability to activate the age receptor. Also is important to remember that a large number of genes also extract some responsive elements that lead to induction of these genes in the products as well.

Interestingly more studies have shown that [Indiscernible] a crucial role in the regulation of the immune response as the previous speakers talked about. Is a inflammatory [Indiscernible] to sell such as the [Indiscernible] cells and there's -- there are these regular Teresa also try to suppress the to 17 cells. Reduce researcher recent research has shown that activation of age receptors can down regulate PTH 17 and use expression of regulatory T cells. There is more research going on into looking at [Indiscernible] for the aged receptors and analysis endogenous as well as [Indiscernible] or how we can treat the immune response using the [Indiscernible] for the receptor.

In that context it is interesting to note that a significant number of dietary lichens or significant number of the dietary supplements particularly the Bottalico's can act as lichens for the receptor and they can actually [Indiscernible] to the scepter which means that [Indiscernible] the questions are these through activation of age receptor ligands.

In today's talk alternative focus on to compounds, [Indiscernible] Redgrave's. Must've heard about the publications in science. Because it induces a gene called a certain one which is responsible for longevity. They find that reserve a troll has shown the increased lifespan in Nice, worms, flies in mice. The excitement to see whether it can also increase the [Indiscernible] in humans. What we are interested in is whether the Resveratrol can [Indiscernible] and whether the Resveratrol can therefore the to suppression of inflammation through activation of these anti-inflammatory mechanisms. Again, those of you who drink red wine must have known about this that Resveratrol is found in the red grape skins and seeds so therefore red wine has small amounts of Resveratrol [Indiscernible] bench paradox of people who drink red wine have less

cardiovascular disease. With a shows that way to inject [Indiscernible] Resveratrol to see the effect if you want to see the same effect in one you may have to drink for 500 bottles of wine. In order to avoid that, this compound from the red skin seeds and seeds there are throwing all of that the note is becoming lucrative in the industry that extracting the [Indiscernible] thousand milligrams of Resveratrol in a single capsule.

I will also talk a little bit about the plan to [Indiscernible] which is found in the citrus Festival such as the broccoli. As I said before, there over 18 chronic inflammatory autoimmune diseases. There is no cure. Some of the [Indiscernible] make them susceptible to infection, cancer, such as room for [Indiscernible]. There are drugs that which are not talks up a can also suppress chronic inflammation that may help us prevent to helping large number of chronic diseases from cardiovascular neurodegenerative to obesity and someone.

The first type of autoimmune disease that we try to study is what is called multiple sclerosis which is an automated season the system in the brain and spinal cord. There we start recognizing Mylan basic protein and as a result these have severely damaged myelinated nerve cells because of [Indiscernible]. To study that we use what is called the SF Follies are EAE. What we do is we inject an antigen or peptide derived from protein and we follow that with injection of [Indiscernible] and works the [Indiscernible] does is get the blood ready, see to the site that are actuated in the [Indiscernible] such as lymph nodes and so on those can then migrate into the central nervous system and there they go after these [Indiscernible] fibers. This can cause implementation. In these ways to find that they developed severe paralysis particularly the hind legs.

We scored them with no symptoms as [Indiscernible] you find in between a radius of science based on the severity. You find it up to immunize the mice, are on day 10 they start showing signs of paralysis. It peaks on day 15 you could almost 4 425 and that it gradually -- four, two, 5% it gradually goes down the kids as we say because of the anti-inflammatory methods that are triggered slowly the paralysis goes down. Then after some time, you start seeing severe signs of paralysis.

In those nice dash in those mice, will treat them with 100 to 200 mg of [Indiscernible]. You will find that there is significant delay in the onset, the suppression of the critical science such as the paralysis particularly at higher dose you find everything you must less than one. [Indiscernible] intelligent that my sitters suffering from EAE you could take a section of the brain, or the spinal cord you find there is dramatic inflammation triggered by TH1 cells then macro flashes and [Indiscernible] us -- also the types of [Indiscernible] Fisher that the Mylan is completely destroyed in these mice. As you can see here, treatment with the call -- Resveratrol correlating with the clinical science of these mice.

Looked extensively at the mechanisms and one of the things you can look at is the production. Entreated me to consider high levels of induced significant compression. Interestingly, if you look at the regulatory T cells these are the T cells that suppress the inflammation and if you look at the molecule called FO XP three, essential for the generation of the function of the regulatory T cells that gentrify seems to be regulated with Resveratrol. Regularly looks like Resveratrol is suppressing the bad guys that is the IL 17 and promoting the good guy, the regulatory T cells as part of the autoimmunity. The autoimmune response as you know the TH 117 -- TH17 cells the bad guys and [Indiscernible] are the good guys. It is the other way around.

Whether Resveratrol can activate age receptor would been able to show that because I chose subsequently that to take cells from HF [Indiscernible] Maisel more resistant to the immunosuppression caused by Resveratrol. As the T cells get activated, after they do their function such as production of cytokine and all of that, the [Indiscernible] into a process which is called apoptosis in robbing the receptor and a molecule called fast like impregnable easy T cells suppress fast but they also start expressing fast like an. Binding of the past liking or the FAS through [Indiscernible] would lead to the cells committing suicide or the cells dying through a process. This is critical so that the response when it is activated it comes back to normal levels that you do not have the activated T cells in your system for a very long period of time. We know that because individuals who have mutations in the past, are fast like and they develop a severe autoimmune disease. There spleens are large, there lymph nodes are enlarged in the same thing also see in the mice which are deficient in FAS and FAS like an. These molecules into play a critical role in making sure that once the T cells our activated they die otherwise it can cause severe autoimmune disease.

Interest in what we have been able to show is that FAS expresses the dioxin expression elements because of which when the surgical binds the base of the receptor and increases the receptor triggers the inception of the FAS molecule and also triggers the expression of FAS lagan [Indiscernible] but ultimate outcome is that the T cells now hyper express FAS lagan and because of each now they grow rapidly -- rapidly because of which they do not have time to stay in the system and cause chronic inflammation.

This is one of the slides which shows that age receptor cells are much more resistant to induction of apoptosis through Resveratrol. With an extensive studies with the multiple sclerosis and we also look at several other autoimmune disease models and one eventually is inflammatory bowel disease which is founded two forms, ulcerative colitis and Crohn's disease. 1.4 million people in the US over from IBD. There are various animal models of colitis and one of them is what we call dextran sodium sulfate. Because it is a strong chemical irritant it should trigger severe inflammation and that triggers colitis because you can see here, this is the control: and because of inflammation, you can come the reduction in the size of the: this is truck in trouble have to treat the mice with Resveratrol the length of the colon which you can also see an [Indiscernible] you can see here inflammation triggered by [Indiscernible] indicating colitis after treatment with Resveratrol a pretty much completely get rid of inflammation.

We have used so many other models [Indiscernible] mice also develop light is because of the [Indiscernible]. APC men mice also develop not only colon inflammation in the colon also: my cancer and the Resveratrol can significantly suppress it as well as the development of the number of polyps in the colon.

We have done extensive studies looking into -- all of these seem to be significantly [Indiscernible]. Natural indole such as ITC anti-I am can also act dads likens for age receptor. We use them against extra mental autoimmune and [Indiscernible] the experimental model for multiple sclerosis we been able to show the truth is developed the disease if you treat them they can [Indiscernible]. It is unbelievable how these compounds go. You can also clearly demonstrate that these compounds can also surprised inflammation. The new inflammation in the brain and spinal cord.

In summary, therefore what we believe is happening, it induces the expression of FAS, FAS lagan to increase levels of kurtosis. [Indiscernible] different unity 17 cells these compounds can suppress [Indiscernible] to 17 cells as well as increase the induction of TR e.g. of the

[Indiscernible] Foxp3 in inflammation. Next wanted to find out what are some of the mechanisms by which Resveratrol other compounds might be suppressing the to 17 cells in suppressing the regular cells. In order to investigate that we looked at Epigenome X empathy talked all about micro RNA. As no the micro RNA are small non-coding molecules and the silence the transcription and post-transcription regulation of gene expression. Typically, you find that there is down regulation or that it is fresh and of expression of the particular gene but the micro levels decrease more induction of the gene expression. There is [Indiscernible] regulation.

We take this out of the colon in mice for example and we [Indiscernible] looked at profiles, so 700 [Indiscernible] through hydro-screening this is the heat map. It shows they down regulated and upregulated. You can see here clearly based on the colors that there are some microliter clearly induced and some that are clearly [Indiscernible] and here are a few of those which are highly upregulated in a few of them which are highly downgraded.

White area for dietary supplements and the ability to activate these various [Indiscernible] compared to the [Indiscernible] controls. You can see that each of those seem to exhibit some similarities are some differences as well. Which is summarized over here in the wind diagram that there are about 136 µ index that seem to be commonly induced or altered by all of these five different compounds which are acting as a AHR ligands. All of those can alter the suppression of certain types of [Indiscernible] pretty goes to demonstrate that therefore you could have a wide array of age receptor likens but some of them seem to acted a unique way in terms of regulating the expression of the micron.

Without showing too much data, what we have been able to shows that we have been able to identify certain micron, M IR-190, MI are to 17, MI R-490 which seem to be [Indiscernible] AHR ligands and these are involved in the Foxp3. They are downgraded and that leads to use the civic targeting of Foxp3 and that leads to action of Dori Germolec five because of which you find it has increased regulatory T cells. We also found that some of these age receptor ligands can increase the expression of microns for, but seem to selectively target IL 17 we find that it is down regulation of IL 17 caused by the expression of -- CUSP activation of these age receptors.

Therefore, what we find is that likens, even as you -- ligands, even as you eat grapes or that shuffles that you take that are small amounts of AHR ligands that are found which can find to the receptor and modulate. You find that one of the mechanisms by which [Indiscernible] dietary supplements, about [Indiscernible] like 500 mg or 1000 mg then we find that at least in the experimental market they seem to induce and activate T cells in the expression of FAS, FAS lagan -- Ligon [Indiscernible] pathways increased induction of have ptosis of these cells are [Indiscernible] rapidly. In addition also find that the age receptor ligands, seem to alter the [Indiscernible] altering the expression of these micro to try to suppress information and suppress the bad guys and try to make sure that it suppresses chronic inflammation caused by IL 17 cells. What I'm not showing you is that some of these dietary compounds, HL ligands -- can also cause hyper modulation as well as IL 17 promoter hyper methylation. All of these together lead to more of these guys as well as the autoimmune disease, they suppress the IL 17 cells [Indiscernible] and that is how dietary supplements might help in venting inflammation and therefore you would [Indiscernible] as well as autoimmune disease.

Dearly in summary dietary supplements have both risks and benefits which is truly exciting. Dietary supplements can modulate the micro RNA as was the Epigenome and thereby try to alter the inflammatory pathways. They may play a role in the prevention and treatment of inflammatory and autoimmune diseases and understanding the mechanisms of action of dietary

AHR ligands which could lead to novel -- chronic information in a might also talk about how nutrition can impact Epigenome as well as prevent chronic inflammation there for preventing chronic disease as well.

Went to think Dr. Missy [Indiscernible], -- Mitzi [Indiscernible] long time collaborator who are responsible for generating all of this. Also did think the support of NIH for funding of the research. With that I complete my talk about be happy to answer any questions.

Audience Question: Thank you for the interesting top. My question is on the effect of Resveratrol in new generated diseases. Especially related to inflammation. Considering that Resveratrol has anti-inflammatory effects as you have shown, do you think the side of the action is more [Indiscernible] because I am thinking it could be an interact [Indiscernible] orderly could somehow have the effect which would induce the [Indiscernible] and other pro-inflammatory factors.

Nagarkatti: That is a great question. Question was whether Resveratrol was acting [Indiscernible] to suppress inflammation particularly the macro T cells in the spleens and lymph nodes or whether it is acting in the central nervous system. That is a great question. Clearly there showing that Resveratrol can enter the central nervous system so that it can cross the. Confined levels of Resveratrol but in this model we feel that it is in [Indiscernible] because what happens is, you immunize the mice which is an antigen, sub -- and that activates the T cells that are found in the lymph nodes in the spleen and these cells, because of [Indiscernible] tocsin their damage the cells migrate into the central nervous system and then cause new information. We feel like, we feel it is causing suppression of the site and activation in the [Indiscernible] and they're not able to be generated in enough numbers that they can cause, go to the central nervous system and cause damage. The way we know this is also that if you take the lymph nodes or spleen in [Indiscernible] the T cells that you take the treatment of Resveratrol and activate them with culture you can clearly show that they a dramatic decrease in the responsiveness so it shows atrophy as well.

Audience Question: I have got five questions. Have there been any studies looking at the effect of Resveratrol administration and response to infectious disease?

Nagarkatti: Is interesting, that is a great question. Question was, has anybody looked at effectiveness of Resveratrol in infectious disease? The land seem to [Indiscernible] Resveratrol response to certain infections. In humans, we still do not know because the nice part about some of the dietary supplements is that I do not think they are used in doses that cost severe immunosuppression. In other words if we inject Resveratrol in normal mice and you look at the time [Indiscernible] talked about looking at various assays to look at spleens and lymph nodes in terms of the super reality are looking at various functions you do it seem to see the effect. Which goes to demonstrate that Resveratrol and have shown that in our own cities that Resveratrol does not seem to act on naïve cells but they seem to act on two sites or other cells which are getting activated. The reason for that may be because, as I said, once the T cells get activated, there [Indiscernible] are triggered. The increases the [Indiscernible] expression of likens that may be the time when Resveratrol if it is president might increase [Indiscernible] or we also have shown that if you give Resveratrol normal mice and look at the macro changes they're not as dramatic as in a mouse that has been injected with DSS or other antigens. Therefore, immunized mice might have a different type of micron profile which might make it more targetable by Resveratrol. That may explain as to why, if you take Resveratrol amid not compromise your immune system to the extent that it would be more susceptible to infections.

Germolec: These are long questions. Maybe we can discuss these at the [Indiscernible] level. One of the questions was, what testing which you show would achieve the balance without causing immunosuppression?

Nagarkatti: That is a great question. I think I already answered some of that, is that looking at the effect of the dietary supplements in not only normal mice but also in mice that have been immunized as it was antigens or mice that have been undergoing infections or subjected to cancer and so on because what I believe is that some of these dietary components unlike dioxin that Dori talked about may not be such potent activators of age receptors that might cause severe immunodeficiency but clearly, what Dori talked about , the various tiers looking at the immune functions and the use for these dietary supplements as well to see whether there are significant modulations are going on in and in setting as well.

Roundtable Discussion
Dori Germolec, Moderator
All speakers

Germolec: You want to think you'll for your attention. I'm going to ask all of the speakers to come up to the podium at the front and we have about 45 minutes for a roundtable discussion. Will use that opportunity to take any additional questions from the audience as well as to try and address some of the questions that we did not get to at the end of the individual talks. Man by the speakers to the front. Perhaps I can ask first of there any additional questions from the audience?

Audience Question: I have been fascinated by the idea in the context of food ingredients setting aside the dietary supplement case and talking more generally about food ingredients. We have the assumption if an accused by broad populations overtime whether or how likely is it that you can get free lunch in terms of preventing the immune system on a population level? How SERS they should we take the concern that in any case where you made a biologically meaningful [Indiscernible] through chronic consumption of some food ingredient whether there is the intended benefit whether there could also be risk because of the shift in immuno homeostasis. I be interested in your the panel's view on that.

Response: It is a great question and I think the whole as you know, the epidemics is emerging in a big way and it is becoming very clear that the Titan the nutrition particularly during pregnancy can impact the fetus. Therefore later on in the adult stage of life, based on the nutritional exposure during pregnancy can have a significant impact in an adult stage of life. These are significant and relevant questions that need to be addressed. We really do not know because as I said, Resveratrol which was earlier sold in 100 mg, you can buy 10,000 mg, 2000 mg capsules. Dietary supplements are being introduced into the market without proper studies on risks and benefits. It is a concern that needs to be addressed in terms of long-term use in high doses, with a have a long-term effect particularly if they are taken during pregnancy how is going to impact the fetus in subsequent stages of life as well.

I passed that a number of questions and perhaps in the absence of additional questions from the audience if you guys have questions, please,. Otherwise we can also address the questions that perhaps we can take a turn it doing that. Cheryl I will ask you to go next.

Rockwell: The first question I have is an order to assess food allergies you look at sensitization or two allergic events?

Response: That is now standing question and it is one that is still being tackled I think by basic researchers. I think both approaches are being investigated so the advantages of using sensitization would be that it would be faster and easier if we could make them work. Rebecca and Kemper have been working on that. It be great if they could validate that model to make that work because that would be a nice way. Ultimately we hope to develop another tear even after that were we actually want to do an oral challenge and then test the allergenicity afterward. It would be great if we could do that.

Audience Question: Have a question about whether or not we have done particular experiments with PFASs will we have administered by food or beverage such as milk and then looked at impact on immune function ex vivo. The vast majority of these have been the oral exposure either through diet, through water ingestion or through oral gavage. The majority of tests that are not in vivo are indeed ex vivo said the majority of data suggest that dietary exposure or ingestion exposure to these compounds likely have an immuno modular effect.

Audience Question: I had a question which I would also be interested in hearing the other panelists thoughts on. This was, very few immuno toxicity and point to the drivers of risk assessment. Is that likely to change with new testing methods? I will say that I think the answer to that is yes. As we develop points that are even more translatable and more relevant to human methodologies that it is likely that we will use immuno talk state as a point of departure. I think that some toxicity data or departure [Indiscernible] nonspecific things like increased liver weight and I think it is much more relevant to actually use endpoints as we do increase testing that we will indeed drive risk assessment with immune endpoints. It is starting to increase and I believe that it will continue to and I look forward to any of your thoughts.

I wanted to point out that from our last speakers, majority of chronic diseases are associated with some sort of an inflammatory response. That suggested that the immune system is involved in a great many of diseases that are problematic for society. It also indicates that we really do not know a whole lot about how chronic inflammation leads to disease and in fact for diseases like Alzheimer's disease there is some emerging data to suggest that pro-inflammatory events might exert some beneficial effects. I think while it is important that we develop appropriate immune function test that we can use to evaluate compounds for risk assessment, until we really understand how the immune system is involved in chronic diseases, it will be particularly challenging.

I think that the immuno toxicity and risk assessment, we have developed a tiered system to assess immuno toxic effects that was based on what we knew about the immune system at that time. A lot of it was skewed towards trying to detect immuno suppression. Now have a more understanding of the problems that incur with overstimulation of the immune system. Unfortunately I think rather than streamlining we are probably going to start getting more complicated and how we do these types of assessments.

Go ahead and follow up actually was what Cheryl said. We have had a couple of relevant questions, testing strategy is designed to look at immunosuppression and as Jamie said in her talk we did not have a great idea of what that means when a particular endpoints -- we don't have what you would call it good screening strategy for things that help regulate the immune

system. We can look at the allergic response if in the tiered testing we get a signal for a particular cell type we can perhaps done look in autoimmune models and see if it is truly enhance the response but we do not have a really good way of screening for immunomodulatory effects. When something up regulates one of the standard tier testing panel assays we really do not have a lot of information on what that means.

Germolec: We have a question from the audience.

Audience Question: I just want to donate broad sweeping thought of the impact if any that toxicant in food will have on the severity of for susceptibility to communicatable diseases and special some of the reemerging diseases that were seeing like [Indiscernible] around the world.

Response: That is a really hard question. I think if we look at diseases that are reemerging and diseases that are increasing drastically improvement such as autism spectrum disorder and Alzheimer disease, is a toxicologist I cannot help but ask what role do toxicants play and how can we possibly manage all of our exposures? I think in today's world where exposed to so many different compounds that the question we has toxicologist have to ask is, how can we with the impacts on public health by looking at mixtures of toxicants and really continues a mixtures is pretty dismal. We still really do not know how to evaluate mixtures from a basic laboratory perspective other than throwing a bunch of compounds together in addition tried to figure out what is going on but from a risk assessment perspective, we do not have any appropriate models for evaluating mixtures and their potential impacts on public health. My answer to that is, we need more funding to look at effects of mixtures but we need appropriate models to understand mixtures and until we understand basic mechanisms of many of these reemerging and increasing diseases, we are struggling.

I'm going to follow that up with a little commercial. The project leader for these efforts is Dr. Cynthia writer in the NDP and she's actually leaving a study of mixtures and one of the idea of the project is to actually develop a rapid screening panel for things like the old oil spill so when we haven't unknown mixture that we would be able to take that then rapidly run it through some number of tests. I'm very excited to say that, because things like gold foil are [Indiscernible] hydrocarbons in the immune system is a big part of that and we are actually participating in that aware doing studies which are scheduled to study -- start next month. We're going to look at immune effects. The idea is, I'm probably stealing some of Cynthia's terms here but we are populating space. We are looking at a number of different chemicals in a number of different assays and we are populating space with data and we haven't unknown then we can test that unknown using similar or we can maybe use to SF that is possible depending on how we can take our individual test article and look at the mixture and see where falls within the space so we do not have to do all of the test but we can predict and based on what it does in one or two maybe where will react in the rest of the responses. I think there's some really exciting work being done but we still have a long way to go. And try to sort the questions and looking at the ones are easy to answer.

Audience Question: There a lot of questions here on the dose of Resveratrol or die tailored dietary supplements that we use but things like [Indiscernible] or after, we have done all of the studies and basically we find that if you immunize or if you administer these dietary supplements before you [Indiscernible] them with image and much more significant suppression of inflammation because I think these compounds [Indiscernible] even at the time of the antigen being [Indiscernible] sales presented to the T cells all of the things that are happening. Therefore clearly there much more affect if Burkett is relevant because these people who go

through multiple sclerosis to go through phases where they have major symptoms in new information and then after some time they are fine and after a few weeks or months they again get aggravated symptoms and they come to the clinic. Therefore if these were to help them prevent that which is great but we find that after you come after the disease onset if you administer these compounds it can also show significant decrease but not able to completely suppressed so therefore aping competition with certain other immunosuppressant drugs or lower levels of some of the [Indiscernible] drugs may be a defector as well. That is about the dose. I do not think the doses that we find like anywhere from 50 to 200 mg which translates to about 500 mg a day which is available in the market, you are able to achieve that in your diet because however much you eat in terms of red grapes or whatever do not think you'll be able to accomplish that. I think these are the type of compounds that might help us lead to new discoveries on drugs and pathways and things like that.

As wanted to thank that the bioavailability of these Botanicals is extremely difficult, Resveratrol just to give such high doses because it is not easily available. That is true at the [Indiscernible] so therefore there's also a lot of research going on in which there trying to use nanoparticles to try to see whether they can [Indiscernible]. The sites where they are needed or increase the availability or sustained in the system for longer time. There's a lot of research going on. That is with respect to the questions on those and when to give and things like that.

Have calendar which is promoted actual markers [Indiscernible]? That becomes particularly important when you are doing a long oral sensitization followed by an oral challenge. I distinguish between sensitization and [Indiscernible] burqas in the gold standard is probably [Indiscernible] which can be number of different ways. You can do that by measuring histamine release of that would be a serum marker for through histology pic if you can actually catch them in the act and see them actually the granulating that is a really good marker of solicitation. What is interesting is, when these responses become systemic, there is sales to creditors and tissues that would not be expecting it to have public cardiac tissues and other places like that.

Audience Question: I have an additional question and that is, is there any difference in toxicity of the compounds among various animal species?

Response: The answer to that is yes. That is an easy question to answer. In terms of differences in toxicity, one of the hallmarks of exposure that is perhaps controversially being used to drive forward a risk assessment is liver weights. Most organisms that are exposed to these compounds have an increased and never waits. In fact I might say all tested species have an increase in liver weight. That is a common sign of toxicity or a common sign caliber rephrase that, common sign of exposure to these compounds whether or not it is a sign of toxicity is being debated within the risk assessment community. In terms of the different types of toxicities that are observed there are strain differences. Imagined there were differences in different strains of mice in terms of their sensitivity towards them intermodulation praetor differences in strains of mice in terms of develop mental toxicity. On-Q seem to be pretty susceptible to the endocrine disrupting affect so they are changes in the sunroom addition to social progress are so prone to developing a tumor Triad cities are pancreatic tumors, testicular tumors in liver tumors period these tumors are elicited among the species in is not a common mechanism of action Burkett is not that -- separator Alpha [Indiscernible] is a mechanism of action that we to come different species of different levels of this receptor and that seems to be to differences in toxicity as a mentioned we do not have a clear idea of the mechanism of action for the different types of toxicities Brixton for ability among species makes it even more challenging to uncover.

Audience Question: I have a question, the studies, took this one even that was really for Dr. Trevor timber the studies presented [Indiscernible] were the effects of short-term exposures and have the effects of long-term chronic exposures to Resveratrol such as a two-year bioassay being done. I get to put my NTP had on and say yes, we have very recently completed a two-year bioassay of Resveratrol. That study has not been peer-reviewed so I will not comment on the results but stay tuned, there will be data. We would also actually done a routine immunology screen on transept -- Resveratrol and as [Indiscernible] admitted that sitting in the baseline immunology studies except for a decrease in weight at a very high doses and we know those animals were very stressed period netted over to Prakash and have you comment on any long-term studies that you know of.

Nagarkatti: Thank you for saving me. I do not know Yorty did a two-year study. We have been using it only for trying to treat a wide array of inflammatory diseases. We have done this in hepatitis colitis, MS, lupus, you pretty much find amazing results but it does not mean that these results will also see it in humans because clearly clinical trials have not been done and it remains to be seen whether they're going to be affected in humans. It is good to know that long-term toxicity studies have already been done so to be good to see how [Indiscernible] actually.

There was a question about what kind of in vitro assays are available to screen a large number of these natural compounds. What we have been doing is an in vitro assay to look at expression of Foxp3. As I said the Foxp3 expression is an indicator of induction of regulatory T cells that suppress the inflammation of the autoimmune disease. We're been looking at using a cell line-based assay looking at 10,000 naturally compounds are 100,000 compound library to look at rapidly induction of Foxp3 to see which of the compounds can be used and also whether they're acting through each receptor. We're in the process of looking at that. We have identified a few of those compounds but we need to know to studies to see whether after you administer these compounds in vivo can they really up regulate the [Indiscernible] and thereby suppress the information because experimentally there showing that in a mouse undergoing autoimmune disease, even if you transfer as little as [Indiscernible] they can completely suppress the fish inflammation of the autoimmune disease. Therefore we feel it is going to be exciting to see whether we can use this in vitro assays initially to screen these and identify compounds that can potentially induce Foxp3 and therefore in vivo can hopefully induce T Rex.

Audience Question: I have a question for Dr. Rockwell. Is included the questions that I was have about food allergy is, is it really a food allergy or suggest a food intolerance? Is it possible that some of the increase in prevalence in food allergy is an increase in through tolerance? That is question a. With the models that are being developed for food Arjun SRTR is a possible to distinguish between true allergenicity and intolerance?

Rockwell: Yes. Exit is very complicated. There is food intolerance which can be really not remediated all to be due to a lack of an enzyme and then there are multiple types of food allergy some of which are not IGE mediated sorely presented IGE mediated food allergy today but there are other types of allergy that are IgG mediated for instance. It gets very complicated than their other things like celiac disease which actually are more -- I've talked about type I hypersensitivity type for hypersensitivity and all these other diseases are in the two and three range. You have multiple different types of immune responses that are basically causing similar symptoms actually in many cases but because of the mechanisms are so radically different, you basically would need a different model for each one. And tolerance due to lack of enzyme or something like that would be a completely different mechanism for causing problems with food. Yes, I think these can be distinguished through different models of food allergy and then this

question of whether there is an increase in prevalence due to an increase in food intolerance, that is a very fair question because that weight that goes food allergy is complicated. Some people are self diagnosing themselves so some people probably have a food sensitivity that may not be an actual IGE or IgG mediated hypersensitivity reaction period the data is collected makes a huge difference in how the report comes out. I think that is a fair point however, the given if he's relatively conservative reporting the guessing to be an increase in prevalence of IGE mediated food allergy responses.

I think this is a million-dollar or billion-dollar question. Is a really important question which may be I think everybody can participate in answering this. Had to strike a balance between some chemical or dietary supplement or whatever component environment, had to strike a balance between things that help suppress the immune response to prevent chronic inflammation versus causing undue immunosuppression becoming more susceptible to infections are cancers. Where do you draw the line, how do you make those decisions? Dori to the grid expert in this.

Germolec: I will set is a particularly relevant question here the FDA and it is from. We have lots of people for example they are taking things like statins which are anti-inflammatory. Again, goes back to the, do we know -- we know what is happening on the individual level. We are reducing clearly beneficial, we are reducing cardiovascular disease. On the population level, do we know if we are modulating infectious disease? Think the answer to that is we do not know because it can we do not really tracked on a population level. We track it more on an individual level. I think it is, if you think about cancer drugs, the suppressed immune response but they are critical against cancer. It comes to that other part of the risk assessment process where really you have to assess cost versus benefit and that is going to be different I think for each individual disease. May be there for each individual.

Response: I just want to follow up on that. Basically my view is that, we should know what environmental chemicals or dietary supplements that we are exposed to on a working bases which of the basic understanding as to whether these compounds can alter the immune response or not. At least if we know that, we can always consider the population level what is happening to see whether those changes are going to impact the human health and product context are not work not being able to screen that are not having the technology to do that are not doing that would cause potentially other problems so my goal is to make sure that at least my view is that we have to make sure that at least we do that kind of screening so that we have a basic understanding and then we can always look at the population level to see what impact it has.

Audience Question: I have a question this is an outstanding question which is how much cross-reactivity antibody binding to proteins could actually protect a possible allergic event? That is an outstanding question because this person nicked my jugular a little bit here. We don't know a lot of our ability to evaluate allergenicity is based on cross-react unity so this is a really intuitive question because plenty of free make a protein that is completely novel is way to mediate allergenicity and is not cross-reactivity can we protect that right now with our current testing? I do not know the answer to that question. We do have certain things we can do. We can look at digestion stability, that is helpful but if we generated something that was completely novel on its way to cause allergenicity of not sure if we would be able to detect that without an in vivo model. Fortunately the likelihood of that happening is particular because there really only a few foods that are causing really problem does prevalent allergy it significant percentages in the populations of these types of reactions are limited to if you proteins that can do this. If we did develop what I'm not sure that we would be able to protect to.

Response: Shell cannot follow that question. Touched upon this a little bit in your talk. There are fairly conservative number of foods that we know are allergenic and we still allergy is increasing -- and we know allergy is increasing. Or the number of compounds identified as allergens increasing or is it just the individuals that are reactive that is increasing? I don't think that the number of foods that are causing allergenicity or at least for 90% of the cases it is increasing. We're developing increased understanding that there are these other foods that have caused relatively rare [Indiscernible] specific populations but generally speaking, the prevalence seems to be increasing into allergens that are already known, things like peanut allergy we know is increasing and other allergens like that.

Audience Question: As a follow-up question, does anyone know why they are increasing?

Response: That is an outstanding question. There is a number of different hypothesis one of which is the hygiene hypothesis was related to the what other allergic conditions are increasing at the same time. That is that we are to clean and when I getting our immune systems enough to do and not giving them enough busywork that will come up with creative things to do on their own. Like attack food. There may be something to that. There is, that gets back to the question about the micro-biome pick the hygiene hypothesis would affect our micro-biome potentially as well this: the of friendly critters inside of as these bacteria are really important in how the immune system develops. That may be in part why we are increasing in prevalence but it is not really clear.

Audience Question: I'm not an Immunotoxicology's been really interested in the different routes of sensitization. You describe a little bit about pros and cons about those different routes?

Response: That is outstanding question. Is one that we have dealt with before an Immunotoxicology. We have known for a long time that the route of administration of different chemicals in different whatever we're looking it makes a huge difference in the type of immune response that we get. Chemical sensitizers we know is virtue with this as well. With food allergens, we thought for a long time that the only way, the most relevant way to to the sensitization phases through oral sensitization because we stop for a long time that was the only way that humans were sensitized. Now there is research coming out that humans are actually sensitized to food allergens potentially in multiple routes so through the [Indiscernible] wrote even through the terminal route. We are exploring new different routes of administration for the sensitization pays that seems to be really important bases are relevant for a number of different ways. The immune system anticipates problems of innocuous proteins coming to the cuts. It is designed to deal with that. When you get exposed to proteins through routes word is not developed the system it is really a problem. For instance if you have journal exposure to an antigen, the immune system doesn't have the same system set up for oral tolerance for that type of exposure. We think this might be a particularly relevant say to perhaps infants who are wearing diapers. They are exposed to food antigens potentially to the skin through their diapers. There are multiple routes of administration for sensitization. We think it makes a big difference as far as health food allergens may develop.

I will also add to that, clearly there is a respiratory component, it is one reason why they stopped serving peanuts on airlines because in many school systems have banned peanut butter in classrooms because there clearly are people, whether that is a necessarily a remediated response or whether there are some other components to that response, if you are peanut

allergic and there is peanut in the air, can be a problem. Latex sensitization is a very similar, there's a lot of cross-reactivity with latex and a number of actually food allergens to people that are sensitized to some food allergens will actually cross-react to latex. There people that walk into a 10,000 foot grocery store and be able to tell of there are latex balloons in the store without seeing them because they have respiratory sensitization. It is really an exquisitely complicated and sensitive response. I see Betty busily writing away so I believe we have another question from the web.

Germolec: This question is, how should homeostasis be defined? It would seem that normal ranges need to be defined so that all [Indiscernible] observed in a study -- all that is observed in a study that differs from concurrent controls are automatically considered adverse. What we're really talking about is translating this into the human population in the range of normal in the human population is, the bell-shaped curve really is indeed truly reflect the above the human population. What is interesting is that what is normal for you today may not necessarily be normal for you tomorrow. It changes it lifestages and clearly there are people on either side of that bell-shaped curve and they can be shifted into that in balance quite easily depending upon pathogenic variants and things like that. I think homeostasis should be defined as mounting an immune response that reacts appropriately to what you see. It doesn't overreact, it doesn't under react. It really keeps you in a place where you can fight off the things that you are exposed to and at the same time, do not overreact to things that you do not need to overreact. I will open it up to the panel to comment on it as well.

I just wanted to take the opportunity to remind everybody who might still be listening that this gets into the question of appropriate study design, appropriate numbers of subjects, appropriate controls and by appropriate study design also mean appropriate statistical power and appropriate statistical methods. You could have the most glorious study design in the entire world with an appropriate number of organisms and you might get a response of that is statistically significant that makes you scratch your head and go this is not biologically relevant. On top of an appropriate study design with great statistics, would all still have to use our knowledge a scientist to interpret results so they are meaningful for the greater public health context. I mentioned that any suppression in antibody responses that are different from controller consider biologically relevant but if I see a change of less than 5% and it is statistically different and I have to question whether or not there really is going to have any adverse outcome for the organism that is exposed. We can't only use subjective rules and we cannot only use objective guidelines. We have to incorporate both to make appropriate decisions that are defensible in a greater context. That was a little bit so boxy I apologize.

May I also add that, that is what we do tear to screening so we can see changes and functional endpoints. We tried very hard to relate them to disease resistance on a more global scale but if we see a small change in a functional response, that is not necessarily considered adverse. What we do then is really try and take that to the next level and look at whether that impacts resistance to disease, development of autoimmune disease, resistance to neoplasia sewer really trying to translate that funding in an observational or functional event into what impacts the whole organism.

I think critically there is clear evidence to show that when there is significant immunosuppression you suffer from consequence of infection and cancer. With that isn't HIV-AIDS infection were the cells are completely destroy to become susceptible to a wide array of infections and cancer. In transplantation immunology were people used to take very high doses of immunosuppressive drugs to make them more susceptible to infections and certain types of

cancers. If use antibodies with too many process factors to try to treat rheumatoid arthritis, you may become more susceptible to some infections like [Indiscernible] infections per clearly you have a human population level it there is clear evidence to show that significant suppression of immunosuppression commissary significant suppression it to me at all response can trigger preceptor to infection, cancer and all of that. As I said, the study said that we do in animals and in vitro studies can speculate and say that, these potentially might have some immunological effects but again as they relate to human studies they need to be further extended.

I'm looking at the clock and with the end of our time period do not know if Betty to have any more ask

If the audience is anymore burning questions I urge you to come to the microphone otherwise we will take this last question. The last question is, when you speak of oral exposure and immune response, how does the extraction of tonsils play into the possible increase in allergic responses? So past that right over to you Cheryl.

Rockwell: What a fascinating question. I hadn't thought of that. I don't know. We typically think of the immuno response happening downstream of the tonsils but that is not to say, they are right in there. I have no idea. That is absolutely fascinating.

Germolec: One question from the web audience was, will the presentations be posted on the SOT website? The answer is an antithetic yes. The slides and the video as well as the captioning will be posted on the website. The slides soon, others later. The second announcement is that, FDA employees, I do not hope this is all or just select are invited to join the speakers for lunch in the training rooms.

[Applause]

Thank you very much for sticking with us and for your attention.

[Applause]

[Event Concluded]