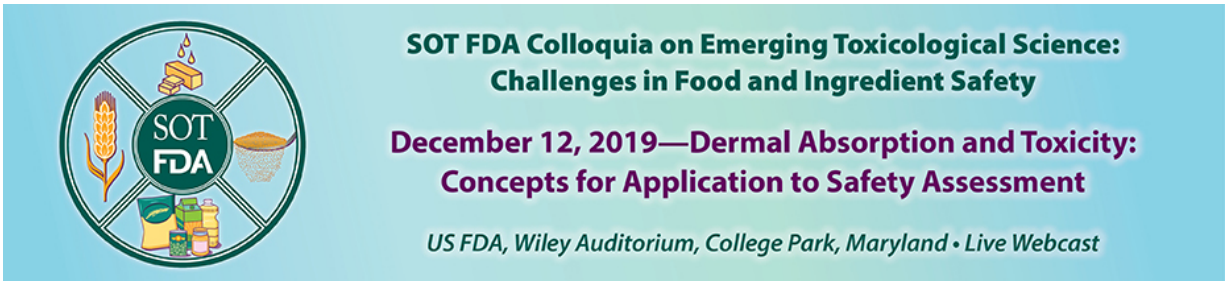

Real-Time Captioning

Note: This is not a transcript.

December 12, 2019

8:30 AM-8:40 AM	Welcome and Speaker Introductions Jeffery Yourick, US FDA, College Park, MD
8:40 AM–9:15 AM	Introduction to the Comparative Anatomical Factors Affecting Topical Skin Delivery Nancy Monteiro-Riviere, North Carolina State University, Raleigh, NC, and Kansas State University, Manhattan, KS
9:15 AM-9:50 AM	Simulation and Modeling of Dermal Absorption Kinetics: What Level of Detail Is Needed? Gerald Kasting, University of Cincinnati, Cincinnati, OH
9:50 AM–10:25 AM	Assessing Mixture and Formulation Influence on Skin Absorption Ronald Baynes, North Carolina State University, Raleigh, NC
10:25 AM–10:40 AM	Break
10:40 AM–11:15 AM	Cutaneous Metabolism and Its Importance for Skin Permeation and Toxicity Simon Charles Wilkinson, Newcastle University, Newcastle upon Tyne, United Kingdom
11:15 AM–11: 50 PM	Practical Considerations for Incorporating Skin Penetration Data into a Risk Assessment for a Consumer Product Launch Tim McCarthy, Johnson and Johnson, Skillman, NJ
11:50 AM–12:50 AM	Roundtable Discussion Moderator: Nancy Monteiro-Riviere, North Carolina State University, Raleigh, NC, and Kansas State University, Manhattan, KS All Speakers Nakissa Sadrieh, US FDA, College Park, MD

Welcome and Speaker Introductions

Jeffery Yourick, US FDA, College Park, MD

Good morning and welcome to today's colloquium on Dermal Absorption and Toxicity. This is a joint effort between SOT and FDA CFSAN. I'm going to be giving the welcome and overview and speaker introductions, and then we'll get on to the speaker talks for this morning.

Like I said, this is a partnership between the Society of Toxicology and FDA's Center for Food Safety and Applied Nutrition. And we have partnered for four to five years now to provide these colloquia. And we have tried to keep these focuses on high-quality, cutting-edge, future-oriented toxicology. And it's really set up as training for our FDA staff here, but we've been lucky to partner with SOT to give these colloquia. They are open to the public to attend in person, and we have a significant number of people who attend via webcast. And just a reminder, these are not a public forum. We are here to discuss science and hopefully learn something today.

Just to give you some background on the colloquium series. This is a composite of all five years, up until last October. I think the Committee of FDA and SOT is pretty proud of the participation we've had in this colloquium series. I think today is the 20th colloquium that we have sponsored. So, you can see a composite here of the overall webcast attendance, which is almost 5.000 and then our on-site participation. I think we're pretty happy that these are being accepted broadly.

Also, these colloquia are recorded and available on the SOT colloquia website, so you are free to go back, download the slides, download the recorded session, and view those later. And you can see from the page views, there are plenty of people who are going back to access these to refresh their memories or use them as training.

These webcast participants are worldwide. You can see this is a composite of all the countries that actually have attended via webcast. So, we're not just looking at a national crowd; we're happy to welcome the international community to participate in these.

Just to give you a couple of slides on our FDA toxicology program. I'm not sure if you're familiar with it, but it's another training experience. FDA released a Predictive Toxicology Roadmap about a year and a half, two years ago, and this is an overall agency initiative to develop models and technologies in toxicological methods and new technologies. We are looking at some of the basic model development with an ultimate goal, would be nice at some point to use these, focus more on regulatory review. But this is one of our guiding documents right now for toxicological research at FDA, and here at the Center for Food Safety and Applied Nutrition, we have our own strategic plan for at least our toxicology work in this center is guided by this strategic plan and the overall agency Predictive Roadmap. And I included the link on the bottom so that later you can click on that.

I'm in the Division of Toxicology here in CFSAN and we're very focused on in vitro alternative method development *in silico* to be used for prediction of toxicity potential. And we're always trying to balance between *in vitro* testing and in silico prediction and can we use that to help predict or screen what we need to test. And I just listed some of our division key research initiatives. We are looking at stem cell models for toxicity testing, dermal and buccal absorption, interested in *in vitro* renal and gastrointestinal models, we also have a program now in liver organ chip hepatotoxicity. We are starting to delve into bioprinting and are also looking at various *in silico* models for toxicity testing, and we're also looking at other alternative animal models for toxicity testing.

So, today's colloquium is on dermal absorption and toxicity. These are some of the objectives for today. We'll look at how different factors involved in absorption affect the exposure assessment and potential safety evaluation of chemicals of interest.

This is our agenda for today. I'm going to give you the speaker bios.

The first speaker is Dr. Nancy Monteiro-Riviere. She is our colloquium chair today, so you're going to be hearing the introductory talk from her right after I'm done. Dr. Monteiro-Riviere is a university distinguished professor emeritus and former director of the Nanotechnology Innovation Center of Kansas State. She is an emeritus professor of investigative dermatology and toxicology at North Carolina State University and a professor in the joint department of biomedical engineering at UNC Chapel Hill.

Following her presentation, we are going to hear from Dr. Kasting on simulation and modeling dermal absorption. Dr. Kasting is a professor of pharmaceutic and cosmetic science at the University of Cincinnati's College of Pharmacy. He teaches their graduate and professional program and has been director of the graduate studies and chair of the division of pharmaceutical sciences. He is currently the skin health adviser for the UC Research Institute for Skin Science and Technology Collaborative.

Next up on the agenda is Dr. Ronald Baynes. He will be looking at mixtures and formulations. Dr. Baynes is a professor of Pharmacology at North Carolina State University College of Veterinary Medicine. His primary responsibility for the past 21 years has included teaching and research in two areas of quantitative pharmacology and toxicology, and he is mainly focused on formulation and mixture effects and looking at pharmacokinetics and pharmacology of contaminant and veterinary drug residues.

Then we have a break and after the break we are going to hear from Dr. Simon Wilkinson. He is senior lecturer in pharmacology in the School of Biomedical, Nutritional and Sports Medicine at Newcastle University. He is the chair of the pharmacology curriculum as well as a leader on the pharmacology degree program. He is responsible for their collaborative research in dermal absorption and metabolism, and his projects are funded by the UK National Institute of Health Research.

And then we will hear from Dr. Timothy McCarthy, he is director of toxicology and a fellow at Johnson & Johnson consumer products, and he was previously a senior principal scientist at Schering-Plough and has over 20 years of experience in consumer products, looking at and supporting dermal OTC drugs and cosmetics and adjustable

OTC drugs and other things. He has provided technical support for regulatory toxicology and product stewardship and looking at professional communications both for Johnson & Johnson and through the Personal Care Products Council.

So, when it comes up to the roundtable discussion, Dr. Nakissa Sadrieh will be joining the panel. Dr. Sadrieh is in the Center for Food Safety and Applied Nutrition and is the director of the cosmetics division in the Office of Cosmetics and Colors. She is responsible for the oversight of FDA's cosmetic program, both from a scientific and regulatory perspective. And then before she came to CFSAN Office of Cosmetics, she had many years of experience over at Center for Drugs as expert reviewer and associate direct pharmaceutics over there.

So, I think with that I need to move on. If you have a further interest in dermal toxicology please consider looking ta the Dermal Toxicology Specialty Section at SOT and that is where a lot of the dermal work is kind of formulated and this is a great time to look at membership since SOT is asking for their renewals right now. So consider joining the Dermal Toxicology Specialty Section. And also, if you're a graduate student, please consider joining the chapter. We really have an interest in spring student graduate education. And you can find more information on the link that I listed.

Now I would like to welcome our colloquium chair for today, Dr. Nancy Monteiro-Riviere, and enjoy today's presentations.

Introduction to the Comparative Anatomical Factors Affecting Topical Skin Delivery

Nancy Monteiro-Riviere, North Carolina State University, Raleigh, NC, and Kansas State University, Manhattan, KS

Thank you for the introduction. Today, I would like to talk about how anatomical factors—they're loading my slides. Thank you. Now that my slides are loaded, I would like to talk about the comparative anatomical factors affecting topical skin delivery.

I have no conflict of interest. But I will speak today about how important anatomy is and how that can affect topical absorption of chemicals, drugs, and nanoparticles. I'm also going to talk about potential pathways for absorption, species differences, regional differences, experimental model systems, and disease and alteration of the barrier. Someone's moving my pointe; I am not.

Skin is a very complex and dynamic organ that has several functions besides acting just as a barrier to the environment. It can function in thermoregulation and function in mechanical support with a well-developed stroma, it can function as an endocrine organ, which can synthesize with UV lighting, cholesterol, 24,25-Dihydroxyvitamin D and make it [inaudible] 1,25 vitamin D. It functions in neurosensory reception [inaudible] and pre-nerve endings in the skin and also your [inaudible] cells that are on the base of the [inaudible], they function as mechanical receptors. The keratinocytes and Langerhans cells participate of the immunological area of the skin, and Langerhans cells help to process antigens and dendritic cells and keratinocytes [inaudible]. Skin also

functions in metabolism and we will hear from some other speakers, and biotransformation, and of course if you've got UV light exposure to skin, you will get sunburnt.

So this is a schematic of skin and what I want to point out to you is to the right of the hair follicles, to the right of the hair follicle, I had this diagram made to show that this is human skin and to the left of the follicle represents [inaudible] species. Human skin has primarily [inaudible] glands that open to the surface, and animal have [inaudible] sweat glands [inaudible].

Skin is composed of an epidermis, a dermis, and a subcutaneous layer. When you topically apply a substance to the surface of the stratum corneum, the uppermost layer of skin, it can go between the cells, through the cells, through an opening in of hair follicles or an opening of the sweat ducts. In addition to the cells in the epidermis they're known as keratinocytes, and they undergo differentiation and proliferation, differentiation, then exfoliation. This is all called [inaudible] and it's very much a cascading event.

In addition to these epidermal cells, which you have the first layer of stratum [inaudible], stratum corneum, you have non-keratinocytes, your Langerhans cells, you have melanin pigment cells, and you also have [inaudible] cells, which are your mechanical receptors. But if you look at this special stained section here of Langerhans cells, they're like macrofibers of the skin. And this is a concern when you talk about nanoparticles, because nanoparticles have, through the stratum corneum, the particle gets through the epidermis, the dendritic process of Langerhans cells [inaudible] particles, they go migrating out and cause systemic effects elsewhere, especially immunological effects.

So, let's take a look at the very top later, the stratum corneum. The stratum corneum is made up of enucleated and dead keratinocytes, and what you see here is just the membrane, but the principal route of penetration, and absorption primarily, is through the intracellular pathway. Ultrastructurally by transmission electron microscopy, the pathway where the lipids are between the two cells have electron [inaudible] areas, which represent [inaudible] and lipid domains.

So why is it important to know the lipid biochemistry? Well, if you remove that rate-limiting barrier with the stratum corneum and intracellular lipids, you will increase the rate of absorption. We know that the intracellular lipid pathway, which is a very tortuous pathway, is the primary pathway, and that consists of about 50% [inaudible]. And this all exists in a liquid matrix and the fluidity which is related to the permeability of hydrophilic drugs. But if you increase the temperature, you increase hydration, or you use chemical penetration enhancers, you increase the fluidity, and thus, the permeability.

So, this is a transmission electron micrograph, and where do these lipids come from? Deep down in the upper stratum [inaudible], you'll have these granules and it moves up to the stratum granulosum, at the point here you will see I've caught them fusing with the basement membrane of the [inaudible] and the granulosum area. And this releases the lipid [inaudible] and they also contain [inaudible]. So, this barrier here is not just

physical or chemical barriers but also antimicrobial barrier. So, if you look at the ultrastructure again, you'll see [inaudible].

So, if the composition or the orientation of those lipids changes some way or is damaged, it will alter the barrier function. And the schematic here shows the head and tail regions, but if you introduce it to solvents or vehicles, and this is how you try to get drugs through skin, you play around and manipulate that lipid. So, you pretty much get anything through by the way you manipulate the lipids or prevent stuff from going through. But you can get lipids [inaudible] that would increase the fluid [inaudible]. You can do extraction technique methods where you extract the lipid, leaving [inaudible] allow for penetration or changing the polarity or [inaudible]. In addition, there's enzymes that will break down [inaudible] junction and that will call fissures [inaudible]. Then, again, you would have cracks in the skin that would increase the rate of absorption or somehow denaturing the keratinocytes, the keratin within that [inaudible] and that would large holes in vesicles that would alter penetration.

To give you an overview of this schematic, the distribution of enzymes and transporter proteins. And this is to show you how complex the skin is, and I believe that Dr. Wilkinson will speak on the P450 activity and metabolism, but this will show us where are the cytochrome P450 and [inaudible] are within the skin. We know that P450 activity does occur in the skin, less than the liver, but it does have a very profound effect on bioavailability. You have Phase I and Phase II that can occur in the basal layer. You look at prodrugs' conversion of lipid ester to free drug, you get detoxification of pesticides, and bioactivation of toxicants such as benzo(a)pyrene. And there's always ongoing research trying to find out species differences in absorption related to biotransformation and this is what you have to remember is that you just don't look for parent compounds when you [inaudible]. The skin does metabolize [inaudible].

So talk about absorption and penetration, two words used very loosely in the field, and we should clarify this to be on the same [inaudible] that absorption relates to the amount of chemical that penetrates the skin and goes through the stratum corneum, the granulosum, the [inaudible], passes the epidermal-dermal junction to get to the capillaries and upper dermal papillary layer to be able to get withdrawn for systemic effect. And you can [inaudible] by perfusate in *in vitro* models, or blood by *in vivo*.

And penetration relates to the amount of chemical that gets to specific target organs within the skin that could be available for local cutaneous activity, and that can be detected by different microscopy [inaudible].

Another area of importance is the epidermal-dermal junction. And this is very big in human bolus diseases and the blistering diseases. We worked with this because we were looking to study the mechanisms of chemical warfare agents, which are the [inaudible], which is sulfur mustard. And this basement membrane is consisted of the basal layer of the basal cell, along with its [inaudible]. It has a [inaudible] area [inaudible].

And what we showed on this schematic here is that there are quite a few epitopes, and we spent quite a few months looking at immune-electro-microscopy and doing labeling

techniques to try to identify where is the cleavage plate, so we can understand the mechanism of this [inaudible], and that was up in the upper lamina lucida, just below the BPA epitope, that we found the cleavage occurring with vesicants.

Another pathway would be, probably, the hair follicle. And this is an old slide of mine, but it's to illustrate something that we're seeing now with nanoparticles. This is a pig skin where we had used electrically assisted drug delivery with ionophoresis of [inaudible] chloride, and if we expose it to ammonium sulfide and then we look for [inaudible] chloride precipitants. And what we found here, we measured it with [inaudible], but if you look at the surface, like if you just poked your finger here and form an indentation, and that's where the hair follicle starts growing. It's [inaudible] epidermis and the stratum corneum with it. And in this case, you can see the mercury precipitants, the ions—this is ions now, not nanoparticles—was binding all to this upper layer, which was actually stratum corneum. It's really not the hair—the hair is further down here. So, just to give you an idea. And this was thought that, years ago, when they did this on rats and mice, it was all on hair follicles, and it gave the illusion that everything came down through hair follicles.

We talked about experimental model systems and we will talk about *in vitro* and *in vivo*, and do you do one, or do you do the other? And do you do human or animal? Well, you can't always use humans for everything, but that would be ideal.

For practicality, animal models are sufficient. If you do absorption studies for toxicology, you want to use something that's very like a worst-case scenario, that's permeable, such as rats, mice, and rabbits. And if you do a pharmacological type of study that are similar to absorption in man, you want to use pigs, primates, and hairless rodents. Toxicity studies, rabbits, guinea pigs, and rodents are better for the immunological consideration. But you've got to realize that species differences occur with different mechanisms and they're very independent and that one species is not the best model for all endpoints in man. And because there are species differences, of course, in thickness, adnexial structures, there's biochemical differences, different receptors for immunological and pharmacological agents, and there's also physiological differences in blood flow.

So, as Churchill said, pigs treat us as equals. Yes, our skin is equal with pigs', practically. It has very large surface mass, and it's great for doing transdermal drug studies. It has areas of skin that are free of skin, similar to human skin, ten to eleven hair follicles per centimeter squared. But there are also differences. And when I was a graduate student, I discovered this little muscle. I realized it was opposite the [inaudible] that holds the hairs to cause the hair to be erect but this muscle ran opposite and it was a smooth muscle, and when it contracted it rotated the outer two follicles and plays a role in thermoregulation. Pigs now have apocrine sweat glands over most of the body [inaudible] in the snout, while humans have eccrine sweat glands all over their body and apocrine in special areas, like the [inaudible]. Immunological and drug metabolism differences are trying to be characterized. And this [inaudible] that shows that when pigs are young, their skin occurs on a triad, this is showing how the three hair follicles look.

Again, porcine skin is very similar to human skin. You have a nice epidermis, fortified cell layers, you don't see many hair follicles in these areas, and a nice, compact stratum corneum. Mouse skin has a lot of hair follicles, you can see some sebaceous glands, and mouse skin has something like 689 hair follicles per centimeter squared, and nude mouse may look nude but they have a lot of hair follicles, about 75 hair follicles per centimeter squared. Rat skin is a little thicker than the mouse but not quite as thick as the pig and the rabbit skin is very similar to dog and cat, where you have a primary follicle surrounded by clusters of secondary follicles.

So, the model, again, pig is very similar to human skin. But also, there are regional differences between these species. Number of cell layers, thickness, blood flow, and hair density. We compared—this is to show the different models that people use in skin absorption studies. If you look at the pig compared to the other animals here, it has a very thick epidermis and its corneum about 12.28. And usually we say epidermis of pig and man is about 45 to 60 roughly, again they have about four to five cell layers and the skin of the pig is very similar to man.

Blood flow measurements, we also used [inaudible], and we measured areas in the buttocks, the ear, the humeroscapular joint, the thoracolumbar junction, and abdominal area. And to look at blood flow because we know the blood flow plays a very important role in absorption. And what we see here if you look at the mouse, the mouse abdomen's got a very high blood flow, so most probably that would be a good area to test to see how much comes through for absorption studies. Now, hair follicles density was first conducted by Bronaugh here at FDA, where he showed that pig and human were very similar and of course the rat had a number of hair follicles 289 and the mouse 658 and again, the hairless mouse is hairless from the top, no hair shafts, but it does have follicles in the deep dermis.

When we conducted studies in younger pigs earlier with some nanoparticles, we happened to see some penetration occur. However, in our adult pigs, when we had around 8.4 hair follicles per centimeter squared that we did not see as much penetration because there was less hair follicles there.

So, if you look at body site differences, [inaudible] so if you look at that [inaudible] the rate of penetration and absorption [inaudible] and studies have shown that scrotum is greater than the forehead which is later than the axillary which is greater than the scalp [inaudible] plantar surfaces [inaudible] and this makes route to route extrapolation interesting [inaudible] realize and thickness [inaudible] now your skin is very different [inaudible] because however the animals are not [inaudible] sole environment electrocute the animals before putting them down [inaudible]. The ear is very thick on the outside and has a lot of glands and concave has of course [inaudible] the glandular density is very different between services in different blood flow paths [inaudible] run through the cartilage. That is to be taken into consideration.

[Inaudible] and kind of look like little [inaudible], so how does that affect blood flow? We are also interested in the lipid and we did lipid extractions [inaudible] and some of this work was [inaudible] what we see here is that when we looked at three different body sites, the back in the abdomen and the [inaudible] area we have the same size of lipids

relative proportion of lipids for similar cross body types [inaudible] shows tape stripping which removed most of the corneum [inaudible] histological section, never, never remove all of the [inaudible] but it does help to loosen the layers below. And sometimes you get the [inaudible]? And fissures [inaudible] devoid areas of lipids [inaudible] pathway absorption.

Now we show that pig and humans have similar biochemical composition and lipids, chemical properties and [inaudible] lipid traction [inaudible] waterlog and a proportion [inaudible] but the different models, body regions, and a lot of these models [inaudible] in diseases. When you conduct dermal absorption systems [inaudible] available for absorption and penetration studies and this goes back because there is a lot of controversy on the types you use, do you use the [inaudible] or do you use the static [inaudible] and what we want to tell you is you first test the animals usually cute dermatome the skin [inaudible] you put the skin and measure [inaudible] and then you measure [inaudible] maybe eight hours, 12 hours [inaudible] maybe longer because it is 48 [inaudible] but if you measure [inaudible]. Everybody has a different type of [inaudible] others have [inaudible] which is a surprise and then you take excise on the static confusion and the only difference is that here and at the bottom and you are not using a continuous flow [inaudible] circulates around here and this is the actual apparatus and we have both in the [inaudible] and conduct studies showing which is better than the other.

What came out here was UNIX six model chemicals is that the flow-throughs represented by the purple antistatic sales grade as you can see there was not really much difference. So, I guess it may be a matter of opinion of which method you prefer to use, the flow-through or static. Another thing I told you about was the vasculature was important and [inaudible] nonsteroidal drug in which we took the cranial and cuddle site of the pagan [inaudible] put it on diffusion cells [inaudible] and it was quite similar. However, when we did the placebo study, the mean penetration profiles of radiolabeled piroxicam in the cranial site was much greater than the caudal site and the result was the animal anatomy and you had direct cutaneous arteries here but upper thoracic area you had musculocutaneous vessels and in this case they acted as a convective force that penetrated deep into the muscle and then carried the drug through.

Now we have also played around with 3-D engineered models and right now it seems like it is coming around another big topic but in the 90s this was really hot topic and everybody was doing it and making 3-D interface models and this is to show you have to test these models [inaudible] zero very thickness difference and sometimes you don't even get epithelium and sometimes a whole skin wraps around itself. Some are grown on basement membranes and others are grown on nylon mesh. Just to be wise they do not have all the cell types and no hair follicles and sweat glands, and some are [inaudible] so forth. So, since we knew that the vascular was pivotal played a role in absorption, we developed the isolated provincial [inaudible] we will be talking about.

And this will be for the have a direct cutaneous artery [inaudible] and which week too we did [inaudible] geography studies and wholesale environment with the plastic surgeon and put in the chamber with under temperature pressure PHN humidity control and it has [inaudible] you can hook it up and you can do injections which we did with

nanoparticles into the arterial [inaudible] measured what came through and we pump it with oxygen and CO2 and you can also not only measure what comes through but look for metabolites and biopsies. [Inaudible] procedure we talk several contract labs how to do this and we have several patents under market with the drugs by using this [inaudible].

And when we compared this Ronald Wester's lap and [inaudible] versus *in vivo* human studies we found linear regression and you have [inaudible] pig fat was very similar in absorption to *in vivo* human studies. So, there are advantages of *in vitro* models for [inaudible] that would be unethical to use in animals and you could use and be chosen also helps to reduce the 3R, reduction and refinement and replacement. The disadvantages [inaudible] entering into lot variability and subjectivity recording techniques and absorption of compounds unusually much higher mathematical modeling greatly increases the ability to use *in vitro* models to study mechanisms and predict human absorption and again Dr. Baynes and Dr. Kasting will be talking about this.

The last 15 years of my life I have been looking at nanoparticles [inaudible] cosmetic stuff in the past. And we worked with many different [inaudible] nanoparticles and safety valuables but nanoparticles is greater than atomic molecular dimension but less than 100 nanometers which has very specific physical, chemical biological characteristics that is associated with its nanostructures in these physical chemical properties such as size distribution, agglomeration state, [inaudible] and ferocity are very important parameters because it determines how the nanoparticles will get up taken by the cell and how it will affect protein bindings in translocate and interact with the cells in your body fluids and all your proteins and there's been a lot of work done with approaching Corona in different cell types and how it could increase or decrease absorption and certain particles and its ability to migrate throughout the body.

When he first studied nanoparticles, we had keratinocytes in the lab and we [inaudible] we characterized it well and we thought you had to put lichens and conjugate them in some way to get them as cells. Not true, nanoparticles can easily get up to [inaudible] all types of nanoparticles if they are solid enough. If you look at the higher magnification here after 24 hours [inaudible] by the next day at the 84% of the cells [inaudible] nanotubes and also looked at quantum dots because they naturally fluoresce [inaudible] at Rice University, and they had these different nail shaped particles that [inaudible] had an effect and go into the skin and we did flow-through diffusion cells and what we saw is as I go back here I want to highlight the cross-section of the follicle. If you see most of it sits on the surface of the *in vivo* studies but around the hair follicles specially the [inaudible] you tend to see it light up. And the flow-through you see nanoparticles go in the intracellular pathway and remember pathway is very absorption and hear the nails [inaudible].

We also worked with smaller particles and this is a Buckley ball [inaudible] amino acid sequence with nuclear localization signal and we did this under repetitive movements, occupational workers, repetitive movements causing increase in flexion and being in by it—biomedical engineering which Julia rigged up a rotating motor [inaudible] 45 degree angle at [inaudible] cycles a minute and we looked at it every 15 minutes and normally

at 60 minutes to receive the penetrate and 90 minutes it penetrated even further and we did both eight and 24 hours. We also looked at rat skin with the quantum dots because there is a lot of controversy from yes, it penetrates and some [inaudible].

But if you look at all the rat skin all these have 24 hours prior to [inaudible] and then the particle when you are working, they just bind to the here and do not actually touch the skin surface. Flexion helps to spread it out more evenly but when you use tape stripping 10 times to remove all the or most of the here and some of the cornea but when you abraded with sandpaper for about 60 times and that is how much [inaudible] the [inaudible] them is off.

What we got in a nutshell we did this with human skin and pigskin and rat skin, and we also did it with three different types of negative, positive and neutral charges on these quantum dots and what we could see at eight and 24 hours is what you would expect with abrasion because you have the removal left epithelium and yet so will come through but [inaudible] voice tends to [inaudible] is it still stuck there in the stratum corneum cross-section? If you look at human skin, for eight hours again this is just imagination of the hair follicle as you see the CIC is florescent and floors and overlay is stays right there into the stratum corneum and does not really penetrate into the beginning of a follicle.

You may be interested in sunscreens because everyone should be wearing sunscreens that is over 1 million cases every year [inaudible] I have had four basal cells [inaudible] on the face and mainly I had because I was always in the water as a kid and as a lifeguard so we do not have sunscreen and in this case the benefits outweigh the whisk and I urge everyone not to listen to what you see and that or you may get nanoparticles in the clear sunscreen containing nanoparticles so we just did some studies with CIO two in different types of motion and oil and water and oil and also zinc and zinc being coded and zinc not coded and we did this to sunburned skin and the sunburned skin that is in red does that the [inaudible] if you're like me at the beach for the week you will not get in the sun or not get in the sun [inaudible].

What we did is we looked at [inaudible] treated skin and look at what TIO 2 did and we [inaudible] we did LM and TEM and toxin analysis, but we also put these on diffusion cells and actually [inaudible] analysis I look for the [inaudible] in sync and what we showed here when we do [inaudible] if it loses the layers a little bit and you know it is been inflamed then it penetrates a little bit in the upper layers, upper layers now with titanium and zinc is a different story and zinc [inaudible] always stay pretty superficial on top of the stratum corneum even with some of the [inaudible] but I want to point out is that a lot of people says they did not see it and [inaudible] look in detail and to look and realize that these particles, chemicals and drugs or whatever when they go through this [inaudible] matrix torturous pathway and depending on where you section the tissue makes a great field and we always do lots of material sections and some of her early work with it quantum dock stuff we showed 40 sections we did and we captured that and that we see some hot spots.

But again this is just to look at one section and I see just one particle and I am not a believer and I have seen many particles and a lot of people use cadaver skin and the

cadaver skilled, skin is very [inaudible] breast reduction. In consideration for assessing absorption penetration, this overview of stuff that maybe a lot of you have no but you have to know nanoparticles type in the species and the skin treatment and other mechanical actions [inaudible] and abrasions and all very important parameters and very critical to absorption. Size and distribution in shape and surface coating and PHN vehicle and suggest the literature right now is that minimal penetration for nanoparticles with Intech skin and of course of several factors I just talked about that I have recorded here all of this can increase absorption including metabolism. So, if you look at the schematic here it shows you how anatomical factors are important, and you take those into consideration when conducting absorption studies. Thank you. I have provided references.

We have time? No? Okay. You will introduce the next speaker? You want me to?

I need my glasses. The next speaker will be talking about simulation and modeling of dermal absorption kinetics and what level of detail is needed, Dr. Gerald Kasting.

Simulation and Modeling of Dermal Absorption Kinetics: What Level of Detail Is Needed?
Gerald Kasting, University of Cincinnati, Cincinnati, OH

Thank you, Nancy.

I have a microphone.

All right, welcome, I am pleased to be here and would like to thank the organizers for inviting me to speak with this very important group of skin scientists both here and online. I'm going to switch gears quite a bit from where the previous speaker was talking and talk simulation and one of the reasons is I teach in a cosmetic science program and we really do not use animal models in cosmetic science anymore although you can use pigskin from the slaughter house and that is sometimes a substrate of choice but by and large, cosmetics are out and they been out for most of the 21st century as a result of European regulation particular but that is been picked up worldwide.

We will move on with the simulation. I have no conflict of interest in this subject. They are my own opinions, okay? Not to be comprehensive but I will talk really about three things, what is the motivation for the modeling and simulation? And in this case on which it is done talk about or spend some time on simple dermal absorption models which pretty much everybody can do and widely used in regulatory and safety assessment and I will spend the last part of the talk on mechanistic dermal absorption models because I have a limited amount of time and I will not talk about everybody's but we will talk about ours and that is really where a research has been focus for number of years.

The title of the talk was What Level of Detail Is Needed and my thesis to you is that it really depends on the audience that you are speaking with. Regulators have important considerations of validated methods and uniformity across the world. Risk assessors

also focus on validated methods, something that is very defensible. But they need more detail and often toxicologists and basic scientists may need a great deal of detail and interested. As an and how are materials getting [inaudible] so a great deal more information and interestingly product development people at least in bigger companies get very interested in this because they are trying to improve products and they have to know a lot about mechanism and delivery and in the early part of our modeling work we did a lot of work in risk assessment and now it has really moved in with development people who want models that will help models to deliver benefits.

This diagram I powered from an engineering presentation. And it just showed the scale over which modeling is done in other areas and it really applies to any area of research in the 21st century. The level of detail varies from quantum, which is very small and very short timescale, normally up to decision-making which is at the end of the chain. And all of these areas are important, they are important in skin science in particular. A lot of the work these days starts with molecular dynamics type of modeling we are actually using [inaudible] and the solution chemistry associate with delivery systems so the commercial quantum programs help you with solution chemistry but there is molecular dynamics, there is him is a scale and component analysis and that is really where are mechanistic models are is in the second box. And those have been used by risk assessors that make quantitative predictions of the process and in this case in skin absorption or metabolism and finally decisions made on go ahead or not to go on product development which is the safety picture for a different material so modeling plays a role in policies, levels in every area of research and in particular in the 21st century in skin science.

I am going to focus on the thermal risk assessment aspects of the work for this body because this is what is important and I think many of you in the audience, and this is an objective or hypothesis that was written for one of the projects that we have conducted in our research and I will not read it to you but it gives you an idea of how we would frame a project proposal aimed at improving models for risk assessment.

Basically possible to run an assessment directly from molecular structure as well as in a higher tier where some experimental data will be fed into the framework and that was pretty ambitious goal and I cannot say we are able to do that yet but that is the direction that the business was going.

So, what do we put in the way of details? We have seen from our previous speaker there's a lot of details that was put in the wreck but what we put in your model? So, think about what factors determine the fate of topically applied compounds? This is a simplified diagram of what might happen and many of you have seen that. You can apply the dose, some of it will evaporate and the exfoliate, stays on the skin for a while, wrapped up in the vehicle and it is combined to the skin and you have the stratum corneum or lower and it can be metabolized and then finally it is clear different rates depending on the sites and the conditions of the exposure.

Here is the factors that ate at least partial list of factors that are clearly going to be important if you are looking at different chemicals and exposure scenarios. Fair number of properties associated with the physical chemistry of the material. There will be

environmental factors, what is the skin hydration state? What is the temperature? Wind velocity for volatile materials or if you're trying to decide how quickly your vehicle evaporates, volatile vehicle and finally big factor, what is the dose, what is the load on the skin and what kind of formulation, is it rubbed off or washed off after certain period of time and all important in the exposure assessment and looking at risk analysis.

So, for a comprehensive solution, you have to include these and other things connected with metabolism and biochemical interactions in the skin.

Let us look at the skin component and say what can you do with simple skin models? Here is a simple skin model and it is a slab, okay? That is a one layer, lipid membrane, tie the connectivity to it and there is a very well-known relationship, associated with that model. And it is useful. It was published in 1992, from pots and guy and what can you do? You can calculate steady-state and permeability from [inaudible] solution for moderately [inaudible] I did not read that on the slide and collect KP. You can multiply that by the concentration in an aqueous vehicle and get a steady-state flux and you can take the [inaudible] water solubility of the material and get a maximum flux which to some extent is considered to be independent of the vehicle because all materials have the same at [inaudible] of saturation, however [inaudible] that the only number that can be calculated but it is a lot and finally you can calculate at least in the most simple way calculate total absorption, in the area of application, times the maximum flux time and exposure time. That is pretty straightforward process if you look at it you need the [inaudible] the molecular weight and the water solubility to do that.

Okay, let us get fancy here. We will put on another layer. You have very lipophilic compound and you may get hung up with a viable tissues rather than the stratum corneum and look at the lipid pretty well but delayed by the aqueous layer. That was addressed, EPA was interested in this in the early 90s and they sponsored work that we can Bundy did in the Colorado School of Mines which is actually discussed in some detail in the interim guidance they put out in 1992, the EPA, and just added a second layer and parameterize it per appropriately in a two layer model and you still do not need any more properties to do this, okay? So, what can you do with it you can calculate the modified permeability coefficient and estimate or now it extends and slows down the penetration of highly lipophilic materials and you can accelerate eczema flux as before and absorption as before so that helps moderate the delivery and the predictions for highly lipophilic compounds like [inaudible] pesticides and fluorinated and chlorinated [inaudible] carbons that are interested in an viral—hydrocarbons that are interested in [inaudible] research. [inaudible] polar pathway, follicles, sweat glands or some combination of that lipid effect. That was at least sort of summarized by Annette will shut and other coworkers and a risk assessment article at Kings for your article in 1995.

And there were a number of models discussed and one that was recommended in that particular paper was called modified Robinson which has this kind of format here with formulas for each one and it extends now, aqueous layer but not we can leak small amounts of hydrophilic materials through the skin if we parameterize it right and calculate maximum flux and absorption through that, okay? The second of those methods, the we can Bundy method was recommended again, recently 2016 regulatory

toxicology and pharmacology article and you will see that our chairman is or chair person is one of the authors on that paper so that is still used and recommended for risk assessment and it is a two layer model and connected with that was a decision tree analysis which makes him a lot [inaudible] where in the risk assessment business have some variation of that diagram in terms of how the process chemicals through their system. So very useful models in risk assessment still and in regulatory thinking.

Okay. What if the questions that are asked are more details? These are some of the questions I get from product development groups particularly, how much of a fragrance will evaporate and what is the rate at which the high notes in the low notes go off? What is absorption from a rinse of product that context the skin for two minutes in the shower? And a number of things, concentration of the Langerhans cell surface if you're interested in the binding that happens to proteins on the Langerhans cell, so hydration in particular, or sensitive skin like babies, under her diaper, some work there, and targeting for the hair follicle, which could be for hair growth, for some of us, he could before hair coloration, or it could be for other reasons, to treat acne or see it associated with the [inaudible].

So here my contention is you need more complex models than the simple ones I just went through to cover those questions and that is where we focus our energy. Over a period of years now I have been working with a chemical engineering professor at the University of Buffalo, [inaudible] on developing microscopic models for the different skin layers and we in fact have microscopic models of one form or another for 4 different, three layers and here follow, okay? They are not all implemented in the software we use that what we do in our group is we map those models onto a slept type of geometry and to do that we use what is called effective medium theory, and we map the complex three-dimensional or actually two dimensional models in most cases onto a slept type model and then we can follow that slept type model with the numerical scheme. And in our group, we have done this for number of years on a spreadsheet using Excel with the Visual Basic at in. There is available, sometimes, a job aversion that on the [inaudible] it is more often not available then it is available and you can check out the URL there so it is still listed but it is not accessible and it has migrated platforms a number of times and it really has not been updated since 2012 saw not the most correct version of what we do either.

But it was available for a period of time or you can also have our spreadsheet if you want to try it. So, what does that model do? If we implement the same factors that I showed on the earlier slide in this three or one-dimensional slept type form with—slept type form with a detail associate with environmental factors and the dose. The most recent summary of that was actually put out in 2013 and advanced delivery review article so that is available and some erasers where we were at that time. That is available on a spreadsheet if you want to try it.

So, what have we done since and what didn't that model include? That model had very simple vehicle options and some complex vehicles were really not colored and had two skin hydration [inaudible] and it did not allowed polar compounds to the skin which is really the last thing we have been addressing so I will talk a little bit about these three areas as to where we have gone since 2013. Development.

So first in the area of complex vehicles, the earlier model had two types, it had a volatile vehicle and immobile vehicle which is kind of a two limits and volatile vehicle would be like at the no water solution, deposits the material in the skin and quickly evaporates, you have to consider it is a deposition mechanism. And immobile vehicle [inaudible] triglyceride oil which sits on the skin it does not evaporate and eventually gets sloughed off or rubbed off but does not get penetrated very well and essentially in that model it does not move at all so those are simple limits and it allowed us to do the calculations very easily. But to get to move beyond that, we took as to extend the model to follow the disposition of more than one ingredient in fact for simple oppositions, all the ingredients in the vehicle, and that allows the vehicle to gradually write down on the skin and the concentrations of everything change on the time and it was more realistic. It enables the interaction of ingredients both with each other and with the skin. Which is very important, present model does not include interactions with the skin, but it has the potential for including skin permeability enhancement by that addition of the capacity. There is an article associated with that which you are welcome to read on at your leisure from three or four years ago. [inaudible] article.

How about hydration? Those are the two states that are available in the 2013 model and there's a partially hydrated which is air dried skin, about 30% average water content and of course it has a gradient but that gradient is not part of the model and then there is a fully hydrated skin exposure which is [inaudible] solution or I call it the swimming pool exposure scenario and that is where the maximum flux calculation is relative the when I discussed, right? So that was the limits of that earlier model but there is static, and it could not change during the simulation and that model. So what really happens is you plight [inaudible] but this could be true with other solids, the skin swells and absorbs [inaudible] and swells and then gradually D swells or D absorbs the water so you have a dynamic process and not a static process. And when it does that, convective flow so [inaudible] in the vehicle can be carried into the upper layers of the skin and deposited and that is the basis for solving deposition but this is what we are now thinking of an explosive wait [inaudible] deposition. And that is an important part to initiate the penetration process.

We have an article associated with that and that is now or because it is a dynamic model, there is a moving boundary associated with it, it gets more complex mathematically and we did this with some help from engineers at one of our industrial partners. And so, it is done on engineering platform called G prop and is not publicly available, but the articles discuss, and the algorithms are discussed in the paper. The transient swelling process. We have done several articles associated with that and we did a study with the diapering group at consumer-products company looking at the swelling and de-swelling and skin hydration changes in a diapering environment so this is some work that was published couple of years ago in skin research and technology, the research scientist that led the [inaudible]. And I will not go through the whole thing but there is a lot of detail but there were different diaper scenarios with a relative humidity was buried with him for our cycles during the day an eight hour cycles during the night where the diaper might be left on overnight and then you could either have the skin gets wet from urination at the end of the cycle or not and for how long so we did some simulations of that nature and this is relative humidity and these are some of the

skins thickness, and measurements associated with the calculations, the spikes are when the urine hits the skin and it isn't absorbed by the diaper and begins to absorb into the skin.

Okay, more could be said about that but let me finish up with the last subject that we have been covering which is polar compounds, and like I say this model up until last year did not handle highly polar [inaudible] now water is a polar material but it is a very small one. And it actually penetrates the lipids pretty well, okay? That was not the problem but if you're trying to do charged substances or quantum area ammonium ions or things or hair ties that have [inaudible] contempt unique materials or a picture that will absorb some polar material so we started with [inaudible] brick-and-mortar picture and considered that there are some connections between the cells which could be associated with [inaudible] cells, could be associated with lipid defects, and our picture is different from document here [inaudible] in our models and it is more like a Windows and mortar model and not a bricks and mortar model in this or I will not discuss that here but there is some nice work [inaudible] and modeling of the situation from a few years ago and very important and a model work at [inaudible] on polar compound transport in skin but Annette Bundy and coworkers that we will not talk about today. In any case we now add riches between the cornea sites for the polar materials and think about [inaudible] when you wash your hand you expect [inaudible] that is easily shown. [inaudible] the method [inaudible] breakdown and small amino acids and other products and they are pulled up by just handwashing, okay? [Inaudible] sites are not impermeable.

I think I skipped one. Sorry. Going back. Yes, okay. But that is not the whole picture. We have these appendages and it could be hair follicles [inaudible] so we added up in [inaudible] we prefer the follicle are themselves microporous or their microporous and they have microporous in them. This is a picture of a follicle model in a sweat duck model and we think for path [inaudible] based on a lot of experimental work certainly what Dr. Nancy showed is the hair follicle is more important for passive diffusion but for [inaudible] at least in human skin it is not so clear. Sweat ducts may be more important. So, we have now a distributed component for the polar pathway and append the Gille component and I will show you some of the evidence and then wrap up quickly.

For the distributed component if you take XI struck, excise stratum corneum and soak it in [inaudible] and then it de-towards it very slowly so with these material [inaudible] and mannitol's the partition coefficients between the vehicle and the tissue are very high, they are one or larger for these sighs and a lot of them get into the skin. That has to be within cornea sites because they are not going into the lipid phase. The [inaudible] a fast stage and slow stage. The fast stage we believe to be associated with surface absorbed material and [inaudible] layer and the slow stage can be very slow. Fast part was more important, the more hydrophilic material, very hydrophilic materials have a lot of this surface absorbed material. [Inaudible] materials including testosterone which was one of the studies, really do not have appreciable amounts of surface absorbed materials, all interior. Where did my pointer go? Here we are.

Okay, absorption from the surface and desk were meeting measures from the fast stage. You look [inaudible] the rate depends very much on the polarity of the material,

on the left hydrophilic material, takes up to eight days to absorb, de-absorbed from [inaudible] stratum corneum whereas [inaudible] and about 4 hours, very different timescale so that hydrophilic materials are in there and they slowly leak out. This pathway is size selective based on the solids we studied so the microporous whatever is leaking out of the cornea site is another size selective process and not just [inaudible] and if you do a cylindrical poor model around that you do get a small radius of about average of eight Ångströms 6-19, not a lot of data there but the whole science selective process and not aqueous macro leakage the appendageal component we follow a lot from the a lot of in group [inaudible] and Juergen Letterman who have done a great deal of research on the morphology and pathways in hair follicles and we borrowed from them the concept of the lower part of the and for Debbie Lim, more permeable because it is not of the outer route sheet is not [inaudible] in the lower part of the [inaudible] so art leakage is confined to the lower half of the [inaudible] but also microporous or akin to the diffusion type of process.

And based on a number of studies and I will show a couple of them, we consider a pore [inaudible] if you want to call it a cylindrical court and there was no reason we have to have cylindrical pore but you have to choose a model so the last thing we did with the model was we have to be able to also explain in vitro data, some large hydrophilic materials based on *in vitro* studies get through the skin but the amounts are erratic and we associate that with a damaged mechanism and particularly in excise tissue where you might cut some of these follicles and allow leakage pathways. You can accommodate the *in vitro* data that has been published in a couple of large databases that allows one or 2% of follicles to be open to the receptor face. I do not know that that is going to happen *in vivo*, okay? So, you can turn it on or off depending on whether you believe it or not, right? The risk assessment probably have to let them leak [inaudible] risk yourself if you are trying to count on that route.

There are some of the studies and I will not go into them but they are all either passive diffusion of polar permits and let me know 11 [inaudible] Utah McRoberts and coworkers have done [inaudible] work and the last study was from our lab from or where we did iontophoresis in the skin and layout and all of them have 4 radii on the order of 15, 25 Ångströms, the harder you hit the tissues with [inaudible] like or Anna Freese is the larger the affected pore site is.

Finally, these are some results of the compilation that when we published this earlier this year [inaudible] the appendageal pathway, [inaudible] is very important for hydrophilic materials and less important for lipophilic materials it is a sigmoidal type of relationship. And here is a look at one of the large databases, steady-state permeabilities. Absorbed versus calculated permeability and you can now because we conclude ionization you come model permeation of the [inaudible] ionize is a function of pH and here is some comparisons with experimental data on three acids and three bases and it is better for acids than [inaudible] more easily and not just neutral compounds that penetrate.

So, we have been building support over the last few years for this model and we are continuing to work on that and right now we are making a transient version of that steady-state model. So, if I want to wrap up what I have to say, think simple skin models

for risk assessment. If you could get away with using maximum flex calculations, think the [inaudible] 30,000 chemicals for reach, right? We cannot do the detail and we certainly cannot do experiment so think simple if you are or if your questions are addressed that way. Mechanistic model can help you by answering more detail questions and a I do not expect revelatory acceptance in the next few years, but it is a direction the field is moving. We are looking to provide better answers to both safety and product development questions in a cost-effective manner. So, I would like to thank sponsors of our work over a number of years. And I don't know if we have time for questions, but I will finish there.

Are we taking a break?

Nancy Monteiro-Riviere: No.

Gerald Kasting: I will let you do that since you are already up here.

Nancy Monteiro-Riviere: The next speaker is Ronald Baynes. [Inaudible.] Dr. Baynes will be discussing skin absorption.

Assessing Mixture and Formulation Influence on Skin Absorption
Ronald Baynes, North Carolina State University, Raleigh, NC

Thank you very much, Nancy. And Gerry, thank you very much for your brief discussion and some of which Jerry spoke about this morning, I will be discussing [inaudible] go back [inaudible] both of you who laid the foundation which I will talk about which is primarily looking at how do we assess mixtures, formulations, and come up with nice, smart ways from a pharmaceutical approach and from prior *in vitro* approach in animal studies and ideally to validate all [inaudible] models and how can we go about assessing mixtures and what is going on here and [inaudible] great job in trying to delineate mechanistic and what is going on at Lisette the study level and micro level and try to put some numbers behind it and predicted some nice models to go forward. So, I will deal today with real world mixtures. Going forward.

There you go, no conflict of interest here [inaudible]. So, my objectives here, how do formations additives within those formulations alter skin permeability and we just had a lovely visitation [inaudible]. Formation effects on pesticides and metalworking fluids, MWF, [inaudible] trying to estimate effects of these [inaudible] mixtures [inaudible] across the skin. An example of [inaudible] focus primarily on bias sites [inaudible] as preservatives and then also looking [inaudible] another performance additives in these [inaudible] formulations and the question we have after all of that work, *in vitro* and *in silico*, can we develop predictive models and quantitative [inaudible] relationships thank you as PR's [inaudible] Q as PR's are derivative [inaudible] pretty predictive mixer formulation [inaudible] simple model that was generated back in 1992 by EPA Potts & Guy model pretty robust and still the model of the day the default model of the day but we decided to look at the effect of mixtures [inaudible] and nice summary at the end.

So, this I think captures in one might basically what was being said it some extent [inaudible] hydration, [inaudible] I love this presentation and talking about how the stratum corneum swells. And moves and shrinks and so on and absorption aspect that we often ignore and fascinating stuff and this was going on within that schematic to your far right of the last model the brick-and-mortar model of bricks being the [inaudible] and the stratum corneum and then looking at the effects on the additives, [inaudible] additives on the lipid matrix and that sort of paraded the glue and submit with the mortar of those bricks and of course the [inaudible] liberalization, and protein saturation. And water [inaudible] hydration water any solvent, okay? As was described [inaudible] but it is not a static thing, it is kinetic because solvents can evaporate and absorb quickly but then lipids itself, lipids can [inaudible] also evaporate and beautiful stuff. Now the solvent can also cause protein desaturation and it can change the biochemistry of these proteins, fantastic. And so, can other additives which are supposed to be non-active, non-toxic but I will use the word inert people use that word, but we can also [inaudible] as well.

As Dr. Monteiro described briefly, we are several model systems available and the ones that we like to use in a mixture research, pharmacy research, for assessing in vitro absorption, across the skin, would be of course the [inaudible] flow-through system models or [inaudible] model at the bottom there both as Nancy indicated earlier [inaudible] depending on your output and what you want to generate from it. In the view of these model systems as you can quickly, very quickly, very quickly compare formulations and mixtures all within a defined system and the systems can vary as Nancy indicated, the immediate perfusion can changing conditions can change and evaporation can change and you can see in all those things can change but you can define in a very complete across [inaudible] if that is the objective you can go ahead and do that with anyone of these two systems, okay? And of course [inaudible] did quite nicely, describe objective of courses looking at what is absorbed and also determine accumulative amount [inaudible] and all that fun stuff okay and we will talk more about that.

Let us look at formulation effects on pesticide absorption. We have a formulation, [inaudible] or any submarket for that matter on your far right and you will see active ingredients and there. That can be organophosphate's or [inaudible] focusing on pesticides today. And these pesticides can have [inaudible] ranging from 103 which ideally suit some level of absorption across the skin. Okay? Often refer to them as log P values and we also have inert ingredients [inaudible] some more inert than others but when it comes to permeability, they are not in our mind not necessarily inert, okay? A lot of information in there is proprietary and it might not be useful [inaudible] those additives can have on the at degree of absorption. One product for example [inaudible] product, contain [inaudible] as active ingredient [inaudible] and 50% [inaudible] inert ingredients. Informative, [inaudible] information. But expect those and other ingredients to have some effect maybe water and you might want to dilute that concentrate and there may be multiply [inaudible] non-ionic and ionic [inaudible] and by diluting this concentrate, are you more likely to get increase in absorption? Or decrease in absorption and the question is often asked, that question [inaudible] are you going to [inaudible] the question is what do you mean by absorption? And Nancy spoke about nutrition forces absorption and are you looking at the percentage [inaudible] total amount over

[inaudible] 48 hours and two different questions you have to ask, okay? We do a dilution of that concentrate and 02 product labels are always the same. In any risk assessment.

Some examples, hopefully I do not saturate you with too much this morning and I have provided some work that we have done over the last 20 years [inaudible] with looking at a substance that is a human. Call it cosmetic or pharmaceutical [inaudible] acetone and [inaudible] solvents, acetone, DMSO, ethanol on the absorption of DEET in three species. Mice, rats and pigs. Okay, the solid line is going to be defective of a substance [inaudible] and the dotted line acetone, okay? And compare rats and mice you can see there is a difference. Dr. Monteiro indicated the species differences and keep an eye on key Y axis, and you can see [inaudible] some steady-state condensation over it to our period about two hours there [inaudible] but they are different. The amount in the cross of the skin into a different species, a lot of hashmarks on the bottom, at the bottom there were two profiles and depicts ethanol and 50% ethanol which is how it is formulated and sold to the public, 50% DEET so we can see that DEET has effective I'm sorry ethanol has effective absorption as DEET and who cares about rats and mice? Real-world scenario and that is why we use rats and mites and as [inaudible] for various reasons [inaudible] but for pigs which is going to be model for humans you can see again focus on Y axis skin Y axis you see that the absorption is significantly less if you like to compare it to mice, right?

That is now focusing on DEET and look at another insecticide Carrboro. Quite often especially [inaudible] species that we work with and we look at this now in this light [inaudible] effective various elements in acetone and DMSO and acetone is going to be in [inaudible] blackline and the [inaudible] profile I look at the top when starting the top when you will see that acetone you'll get a nice peak and then it drops off and the reasons of these explained beautifully [inaudible] earlier that you do get evaporation at the same time [inaudible] and that is what you're seeing there to some extent and acetone is of course the driving of the carbon [inaudible] DMS not so, so now we say that is take it one step further and let others go ahead and add a bit more of solvent or a bit more [inaudible] and let us go in at the surfactant, 40% acetone or DMS and how do you see the slight change in profile and are you going to see [inaudible] DMSO there? And that again could be [inaudible] mice sales and maturation [inaudible] epidermis.

Here is the crux, what if we started adding more solvent? If you go to the left, you will see where we went from 40% solvent [inaudible] DSM [inaudible] keep an eye on the y-axis please, Y axis, he will see that we get the significant decrease in the absorption of carbaryl. Again, explanation as was explained based on [inaudible] interaction between carbaryl and the solvent and the acetone itself, okay? [inaudible] most of the carbaryl at the skin surface and released at higher concentrations of the solvent, okay? And of course, with a combination EC no improvement when you at surfactant to this.

In the interests of DEET on carbaryl, the effect of solvents on DEET and the effect of solvents on carbaryl, different concentrations, look at different concentrations, right? Of the solvents. On carbaryl but with DEET present and without going through every slide here, [inaudible] of the presence of DEET can be depicted here by the solid lights here on the bottom especially when focusing on acetone, right? So carbaryl absorption was decreased significantly on DEET and you expect DEET to be when enhancer [inaudible]

I don't have here and we are [inaudible] absorption across skin to be increased with DEET and you see it with other pesticides but with [inaudible] absorption was diminished and so was carbaryl, okay? Not so much so when we had DMS on the solvent. The chemistry is fascinating, right?

Let us look at another insecticide, on-site not insecticide but [inaudible] and [inaudible] family of acetone set we used to treat [inaudible] I like to paraphrase and agriculture and IVERMECTIN [inaudible] and minister to the skin in several species [inaudible] skin of pigs and we were looking at the stratum corneum penetration to the stratum corneum and we looked at two formulations on the market abamectin approved for cattle and up next approved also for cattle here [inaudible] and we just simply spiked Abamectin into these formulations [inaudible] how are those formulations aided?

We also took isopropanol hundred percent isopropanol because Abamectin contains isopropanol, okay? Upper neck is predominantly very early concentration and take-home from this, folks, kind of shocking to me [inaudible] close to seven or eight [inaudible] mostly Abamectin art and did a better job [inaudible] stratum corneum and the [inaudible] hold on to this very the [inaudible] drug so nice table and we go further and this is a cross species and have an interest in the substance penetration across multiple species and the take-home from this light I'm going to very big detail here, yes, date or species differences [inaudible] no surprise based on what Dr. Monteiro said, [inaudible] physiology with simple kinetics. I will not spend much more time on the slight because we spent a lot more time on this light and show differences am showing this slide in place and it has in another place and it is a slider want to throw in here [inaudible].

I want to talk to in the and before I get to metalworking fluids and that is, we often concern occupational exposure to substances, repeatedly. What we did here was one of the former grad students was with us for several years [inaudible] worked very hard in what he did basically was to expose the back of the pig skin, dorsal side of the skin, with a fabric soaked in jet fuel and for [inaudible] and then did it for four days in the one-day exposure so [inaudible] repeated exposures have in effect on increase absorption of several substances of concern and so you can see aromatic substances here and you can see various reasons that I will not get into, you could get to a full, to folder a fourfold increase with some of the substances, [inaudible] substances [inaudible] you want to address.

Now getting onto area I have been working on the last seems like my whole life or 10 years of my life looking at effective formulation effects on metalworking fluids and a focus would be eventually getting to or focusing on strategic bio sites and I [inaudible] which has occupational concern for those folks who are working in the metalworking fluid industry or metal fabrication industry [inaudible] as well. So, in metalworking fluids there is combination multiple mixture that can result in [inaudible] and other adverse health outcomes such as cancer and other things, if the substances are absorbed, okay? What are these formulations? Okay? These formulations can be soluble oil formulations or synthetic formulations, two brought categories. And they can consist of multiple hundreds of different additives but the majority of them are performance additives. To increase [inaudible] metal the fabrication of metal and the granting of

metal and you can see with the right hand side there is schematic, workers can be exposed and hands especially the arms can be exposed to the substances that it been associated with various irritated dermatitis and allergic dermatitis. Try using gloves, and they use plastic gloves, slippery and you get injury. They use cloth, gloves, they can get saturated and cause nasty effects. A lot of things and they're making a lot of substances in there and I'm just looking at examples of a surfactant of [inaudible] and those formulations so lubricants an example of lubricants like [inaudible] acid [inaudible] triazine and some phenols. [Inaudible] we will talk a bit about them going forward.

Can these additives influence dermal absorption of other additives? This was an extensive bit of work. And the answer straight to the answer, to the question, simply this, yes, bio sites, focusing on the triazine's and so on and some of the corrosive inhibitors that we have minimal effects associated with lubricants and surfactants to be expected because we see first of all *in vitro* models [inaudible] absorption with minimal less than 1% for the most part. But we set aside to focus on the bio sites, and this will be our tracer, right? Substances that we will monitor to do our mixture study.

We also looked at the influence of contaminants and system apply to whether you are looking at a cosmetic or industrial formulation or pesticide in technical grade substance products of course they are going to be contaminants in a good example of contaminants in working fluids as we consider witches [inaudible] which is nitro [inaudible] which can be present some potential carcinogenic [inaudible] good example to pick up and you looking at TCE, [inaudible] what is at there? [Inaudible] it used to be one of those degreasers and solvents for cleaning the metal after the metal was fabricated so workers were exposed to metalworking fluid and [inaudible] we do a number of test on them and take-home from this slide basically pics with a combination of these, contaminants, you can actually get a significant increase in the absorption in this case the bio site triazine.

Let us look at the synodic [inaudible] working fluids and we decided to spike the formulations on some of them [inaudible] good example [inaudible] and then compare that with a straight ethanol and water and of course with water as a [inaudible] you will get partition of the old BP of the phenyl phenyl and the simple formulation across the stratum corneum and you get the lovely increased absorption compared to other formulations present there and followed by that of course you will get the [inaudible] more on that coming back shortly and we also then decided to do some simple correlation analysis and can we actually estimate predictive if you like the [inaudible] biocides and these 4 mixers and did not do [inaudible] and we start looking at two classes of amines, beautiful take-home from a lot of this work was that it is consistent with their previous speaker and these hydrophilic [inaudible] log P range of -1, to what I have here -1 or 105, and you will see here absorption was going to be greater in the soluble oils, [inaudible] soluble oil compared to the state of synthetic formulation and no surprise there and these up and coming down the activity occurring on the surface of the skin and the lipophilic amines, the lipophilic amines, one example is the CHA [inaudible] you sort of see the reverse absorption more so with synthetic compared to soluble, okay? So thermodynamic activity of these amines and various relations can explain or in further theory explained different [inaudible] across the border.

That a summarize the metalworking fluid mixture effects and bio sites and [inaudible] amines readily absorbed [inaudible] of the 10% you expect to see from the pharmaceutical drugs and so 1-4% of dose more than expected than say a surfactant like [inaudible] 0.3 percent [inaudible] acid as I said before and this is why we focus on these two additives on these formulations as an example mixture effects and several differences were observed between these two brought [inaudible] soluble oil and synthetic fluids, right? Those are predominant ones up there and moving more toward synthetic fluids, but people still use [inaudible]. He was a take-home coming up formulation and contaminant-induced changes in additive absorption associated with the presence of single or combination of additives. These observations may be associated with changes in partitioning and formulation, skin, i.e. stratum corneum depending on whether you [inaudible].

So, let us wrap up here on the last thing focusing on last three or four years, what does all this mean? Can we do something a little different to what Potts & Guy did back in 1992 almost 30 years back when that came up? Can we develop models that capture these interactions, and can we develop some Q SR models [inaudible] models that can be predictive of these what we are seen as pedestrian effects, okay? So, what is the question?

Can tell is SER such as QS PR's predict skin absorption of complex mixtures and formulations? As I said before, [inaudible] formulations consist of hundreds of performance additives, okay? Different concentration and I hope I got it do to you on the early sleight that concentrations are important, right? Percentage of water, percentage of solvent and that is important. Very, very important and several if not all of these effect on skin pin [inaudible] and first time you see the term LSC art which is a linear energy relationship developed by Abraham [inaudible] Abraham [inaudible] SPR's. Simply we can estimate or try to predict permeability between those or if we know something about is this a chemical characteristic of those substances? That we want to assess. So we decided to target on Abraham's [inaudible] I will not go through all of them but the five of them are listed right here and the idea was also making sure we pretty much capture and appreciate the [inaudible] associated with each of those chemical parameters and we could look at 100 or 200 molecular descriptors and focus on the five.

Let us go back to our amines and develop QS PR [inaudible] metalworking fluids and using the simple model I spoke to you before, Abraham model, here aggression model and resulted in a very poor [inaudible] values here. And we added a vehicle indicator to this model and somewhat improved art R squared, predictability and that is well and good, and I think we take one step back and let us go see if we can develop some training sets. Okay? Quality approved [inaudible] solid training set and a validation set so we went ahead and one of my former graduate students who is now at the FDA went and exposed the skin of course, 25 diverse solutes and then we are going to be looking as I said at those three different mixtures and free Esther cut methanol and I spoke about that previously before and no surprise the water [inaudible] similar around here and the other two commercial formulations will be somewhere around the Macon's price the water is behaving it should [inaudible] variability so we have a training set and I would just read the top line there and pretty difficult to follow sometimes if you do not know what you're doing here so panel on the left-hand side, 2020 sets solutes and the

right-hand the test solutes that phenols, focusing on in those two metalworking fluid formulations and these are commercial working formulations, okay? Different chemical properties, one soluble and one is oil-based, right?

So, we said okay you know Abraham's model simple, simple model five descriptors did a very good job in predict even with small training set of 20, very small use up to 100, 150 to get a trading set and we were able to predict how good of estimate and what it should be based on an what we would [inaudible] I highlighted and put in a box those relationships. These are my box [inaudible] one is in the color red, what is in green and those transcribers are telling you something about the physiochemical properties on which property it is and it has an effect on other negatively or positively on the permeability of these substances. Within that defined class, okay? Better rate that chemical space which I will say is very, very narrow all, right? And you can say [inaudible] is a positive but the molar volume [inaudible] factor we have a negative effect on the permeability of the substances as a whole.

Just to summarize, bit of what we discussed so far, large and hydrophobic biocides, [inaudible] for example tend to be retained in commercial metalworking fluids especially some of the synthetic oil simulations and the more basic biocides based on the [inaudible] we were able to identify and the most basic biocides will tend to permeate the skin and some ingredients in metalworking fluids can limit biocides in [inaudible] and everything is not increasing absorption, right? And everything does not decrease absorption and again it all depends on the chemistry you are dealing with. Formulations can alter skin permeability. Formulations can increase or decrease pesticide absorption. Formulations can increase or decrease metalworking fluid absorption. Has taken by these two classes we focused on in some detail the bio sites and the amines. QS PR's can be predicted, mixture formulation effects and provide some mechanistic understanding as to why we are seeing absorption increased absorption decrease. Instead [inaudible] will finish off on this I could spend another 30 minutes talking about other models we have developed and recently published but I only have three minutes.

Pretty exciting what we have done in the last year or two and one simple Abraham model and these experiment conditions we develop expanded version of the Abraham LSER model to adjust for heterogeneity, [inaudible] solution concentration, right? All the things you see in future sites or *in vivo* sites for that matter so adjust for the heterogeneity [inaudible] and more of that on the paper you will see we put up late last year. We also in the middle of finally getting the paper on the *in vitro in vitro* comparison for these formulations and three major formulations we spoke about [inaudible] and semi synthetic. And that is in progress and it looks pretty nice and we have to select the permeability *in vitro* and we saw the variations and publications that we [inaudible] you can look at these references and get more details and I barely touched on some of the work we have done in the last 20 years and these references pretty much capture everything I have been talking about. I am grateful to all of these individuals, graduate students and collaboratives, [inaudible] and Dr. Hughes Oliver, statistics every here and other funding [inaudible] several companies and of course federal government.

That is it, two minutes to spare.

Nancy Monteiro-Riviere: We will have a short break, about 10 to 15 minutes. We've got until 10:40.

Break

Nancy Monteiro-Riviere: Welcome back after the break. We will have speaker Simon Charles Wilkinson from Newcastle University from the UK and he will speak about cutaneous metabolism and its importance or skin permeation and toxicity.

Cutaneous Metabolism and Its Importance for Skin Permeation and Toxicity
Simon Charles Wilkinson, Newcastle University, Newcastle upon Tyne, United Kingdom

Thank you very much and for using my Sunday name and I usually just go by Simon. If people call me Simon Charles, it's usually in court, usually when my ex-wife wants more money. And I'm sorry, I don't have an ex-wife, in case my wife is actually following this on the internet.

I have just one conflict of interest and it does to do with consultant work with a firm because I'm worth it and some of that data will be presented. All of the other opinions in my own do not reflect any policy or opinions of any of the funding bodies.

I will talk about my skin metabolism is important and talk about expressions and metabolism enzymes and some functional measures of metabolic enzyme activity and how they contribute to absorption toxicity both real and imagined and I will impact that as I go along and I will talk about how effect of *in vitro* models that we are having to rely on in Europe for predicting effects on the immune system and general toxicities that may be mediated by skin metabolism. We know from history and from what more recent research activation of chemicals by the skin can result in toxic outcomes and the classic example one back to the 1790 and I'm sure there are members of the audience remember this skin scrotal cancer and chimney sweeps Professor we'll talk about I believe it was in London and started to notice chimney sweeps with scrotal cancer and what was happening was the chimney sweep would do his job of sweeping chimneys and whip his face with a rag and stick the rag in his pocket and the carbons in the soot would defuse on the scrotum and also for those who are familiar with this particular part of the anatomy directly or indirectly there are an awful lot of hair follicles in the scrotum and around the hair follicles extensive activities of such [inaudible] especially when 100 and these are induced by the chemicals in the soot and it was not the soot chemicals themselves causing the skin cancer but the activation by skin enzymes.

We are also pretty concerned about the involvement of enzymes in the generations from [inaudible] and molecules which may trigger allergic reactions in the skin. Generally it is agreed the dermal tissue is a detoxifying organ and most scientists agree the amount of detoxifying activity phase 2 enzymes outweigh status phase 1 but very often some phase 2 metabolites are also reactive and we have pretty good evidence of enhancement of absorption by hydrolysis will, Esther .

We know metabolism can influence delivery and enhance activity of follicles linked to Esther and we also note you can deactivate therapeutic drugs as a cutaneous metabolism and I am sorry to keep going back to the scrotum but again is a good example of this testosterone was used a few years ago to treat hypogonadism and the treatment for this was a patch which for some strange reason was applied to the scrotum and two reasons this was strange, one wife you have hypogonadism to have an anatomical site then normal and watches the scrotum at all when you have to take a patch off and the main disadvantage of this was, thank you very much, I'm here all week.

Again the enzymes in the hair follicles were hydroxylated with the tax Ostrom and effectively abolished the pharmacological activity and there is the potential for drug interactions or drug-drug interactions and there is very little in the open literature and looking online it is pretty clear the skin does have the potential to cause drug-drug interactions as a result of the metabolism and it is very important for the whole physiology of skin both of equality, a couple of the previous speakers have talked about the squash mission and for legroom and to leave the skin surface and that causes these little reinforcing rods fit between whatever you like call them for bricks or windows may have to be broken down by enzymes in the skin and Filaggrin as was mentioned the process is at least two not natural moisturizing factor that requires enzyme interpretation but my colleagues at Newcastle especially Nick Reynolds one of the dermatologist is showing how important Filaggrin is as a result of mutations in the gene and it is to dermatitis and other substances as well.

Here's a development of some of the digressive scene in we have either bricks and mortar or windows and mortal for the stratum corneum and here's the compounds which are applied and we are able to detect at least in the spaces around the cells there are Printy come aplenty especially in the basal layer but also another Module 8 may be present in the aspect of the skin and as you will see shortly six may be involved in [inaudible] so basal keratinocyte differentiates the spiny sale and into a granular cell and finally into a corner site and it is possible they are involved in that and then the literature, it also is present in the dermis.

Especially around the appendages, around the hair follicle sale or the sebaceous gland and many of these activities have been identified using techniques and to a lesser extent also the sweat gland.

What of such CYP are expressed in *in vivo* and do we find messenger RA, yes we do so skin biopsies from the group at the hospital we found one B1, one A1, two S1 consistently expressed but very different levels into the genes in terms of messenger RNA and two S1 was expressed at a higher level and liver than other organs and one A1 is very basal level and less you are to cigarette smoke or the [inaudible] through the coal tar and two S1 also highly induced with coal tar and we have quite similar results from another study from Ynke in 2003 so we find *in vivo* at least messenger RNA .

Two S1 and extrahepatic express in lung tissue which is unusual for 450 strongly expressed throughout the epidermis especially around the epithelial cells and the

appendages and like one A1 and one B1 under control of the [inaudible] not regulated by a different ration of *in vitro* and we not believe two S1 has an endogenous role and it has a role-playing in the metabolism and the skin and in a separate study 2006 with the group of work they have looked at what happens to activity and expression when you change the differentiation state of Keras and the site and keratinocyte from the foreskin manipulated the culture conditions to change the differentiation status and looked at how different were expressed and found mass differences and mass increases and expression of some of the enzymes in the genes and no change in 2S1 and one A1 and one B1 and a decrease in the theory is they are involved in the metabolism of [inaudible] which in turn regulates causes quantification and again endogenous roles on some of these compounds in these are some of these data that we generated from the group in Paris and they are a comparison of normal human skin of NHS with the reconstructed skin model routinely use and testing by L'Oreal and these are originate from oriole research and what we found is very low levels of MRNA for some of these regular that you find in liver even one A1 have low-level across-the-board and to see nine and with Ester activity we do not find mRNA for CS one which is strange as you will see shortly and we do find quite a lot of expression for some of the enzymes especially in the NAT. Again, pretty similar between normal human skin when we constructed skin models.

Again, UGT not much detection here and one a six and normal human skin and some sofa translate is detected and again one A1 and 2B1 and certainly messenger RNA and slightly less for the UGT compared to normal human skin and what you have to be very careful of here is the origin of the skin and very often things like IQ skin can originate from the foreskin and normal human skin I don't know if you've seen that but there's usually not very much of it and I'm trying to fit it into a diffusion sale is a challenge we tend to go for things as Nancy mentioned the breast reduction or abdomen reduction for drastic weight loss.

We find measurable levels of, we used three different pro substrates for one A1 and one a two and one B1 and quite a lot of estimated activities despite the fact we did not detect any messenger RNA soap note messenger RNA but plenty of catalytic activity so therefore ladies and gentlemen, the central dogma of molecular biology is all BS. I'm sorry. Joe Marcin, if you are listening, I did not mean that. There is obviously no reasons why we are not detecting that which we need to look at and also measurable levels of NAT and GST and UGT slightly more usually in the 3-D model than human skin and the difficulty with human skin it needs to be fresh, especially for the [Indiscernible] and the issue we found historically in our research group is, is, not so much the enzymes are not working is they're not getting recycled and they're not getting reduced again to recycle so they can carry on their job. We find those activities towards phenyl and again that's a surprise and we have found these in previous papers.

A little about the parabens and friends of ours from long ago this is a paper published with faith Williams with a group and comparing metabolism and absorption between human and guinea pig and this is [inaudible] in the darker green is the compound and the lighter green is the Pro hydroxy metabolite and in this particular model we found results in guinea pig and human skin in metabolism and absorption.

It was pretty similar levels in terms of absorption into receptive medium and at the skin that the mini pig was much more capable of metabolizing [inaudible] in the human skin and very similar levels hardly any metabolite made in the skin itself. Broadly similar amounts metabolized in human and mini pig skin. What we did find is the two species human and mini pig showed quite different sensitivity to inhibitors and human skin showed very classical inhibition of hydrolysis. Been by a pair might wear as pigskin did not.

What we also found when we look, the protein, these are native gelled and strained activity in these are the human microsomes and this is the mini pig microsomes in cytosol and we find clear vans are clear bands and we do not find such clear equivalents in the skin so they are constantly very similar in terms of activity and maybe the protein involved in generating them are not the same. Another comparison between species published by Bay Don and toxicology *in vitro* looking different and different inhibitors and products and in DPF and inhibit almost all enzymes present and the substrate is [inaudible] and the inhibitor mentioned we find quite different sensitivities to inhibitors across the different species and again it's a just even for a protein they may be very have distinct differences in expression across different species.

Why is this important to us? As toxicologist, the main area to concentrate is the generation of Hamptons and pro-captains and also genotoxins very much like they hydrocarbons I mentioned earlier so good the skin metabolic activity and generate allergic reaction from general topic compounds previously genotoxins but there's more literature which I'm afraid I don't have time to cover and concentrating on skin sensitization these are the main steps in the pathway and first of all the compound has to get through the brick-and-mortar and it has to be electrophilic and combined nucleophilic and acid residues this changes the structure of proteins in the skin and this then attracts keratinocytes by dendritic cell and ultimately further down the line and increased population of T cells to that protein and we are interested in whether metabolic activation generates hapten from non-cabbage and it compounds.

I will do a [inaudible] study here and we have regarded some alcohol here is [inaudible] hapten which is not itself and we acted with proteins and following the metabolic activity for a few years now and we thought this was the scene of the crime and alcohol in formaldehyde and it is been converted into [inaudible] acid and it was from this protein generating the allergic response all well and good however we get more frequent positive patch test for the alcohol until the formaldehyde in the same concentration and conversion of cinema out alcohol to the acid reduction have all been observed and actually the skin models but the formation of cinnamyl alcohol has not been definitively demonstrated. Why not? Because it is reactive with proteins and binds to proteins and we are not going to see it in our observation we don't see any formaldehyde conclusion therefore it must be [inaudible] hapten and the problem here we are not seeing anything therefore it must be happening and they will almost certainly not diffuse away from skin tissue informed however more recently a group working with [inaudible] at Sharpsburg University used a wonderful chemical technique called high-resolution major ankle, angle spending where they look to in situ noninvasively with reactions between chemicals and nucleophilic residues on amino acids and the long or the short story of the long story is they found absolutely no conversion of cinnamyl alcohol to somehow

the had even though they found both of these activities presented skin so they were unable to detect the formation of some aldehyde and there was a research group that took a different approach to this. You will notice this is alcohol incubated with liver microsomes and they generated a number of metabolites and we have to bear in mind the microsomes and may not find the same metabolites in the skin but they found new proxy versions Ensign Amick had in concentrations and quite short order as well 13 or 30 and 60 minutes.

When they tested the reactivity of the compounds they compared how quickly the compounds are able to deplete proteins and they found both of these caused quite reductions in the availability of peptides so these are reactive compounds and maybe cinema alcohol is [inaudible], pro-hapten but again we have to be careful because these are compounds formed by liver metabolism. The way the first line measurement of how a compound is a hapten is the direct peptide derivative essay in this is a similar force method take model peptides that contain either sustain or lysine residues and mix them with a compound of interest which is a chemical method and no biological materials or cells and we do a single 24 hour exposure and look at what percentage of those groups are reacted with and we can then compare that to well-known classical compounds and give a productivity of how reactive the compounds are. We pretty much agree this test alone is not effective at predicting pro-hapten as the compounds have to be reactive to start with for this to happen.

One proposal that has been generated through a group and others is to use a surrogate for that metabolic activity which is [inaudible] which is to better identify pre-M Pro haptens and pre-haptens confusingly are compounds which are not reactive until you expose them to the air and the compounds naturally as a result of exposure to the environment and perhaps have a couple, classic example is hair dyes which are in contact with the air these products are then more reactive than the compounds or the starting compound. This is the method and it's a problem because people possibly these may generate false positives and the horseradish approach may actually generate positive signals from compounds which are not really Pro haptens because you do not get the same reaction occurring with biological tissue.

The second line method identifying whether a compound is a hapten is the Tara Tennyson's assay which has recently been adopted by the guidelines and this relies on a detector within the cell to keep one receptor and again how this works there are some groups and if they are intact because there are no compounds present ARF to will stay with its dance partner and keep one you will find residues and its exit for destruction so it is prepared by the cell is a constant level and slowly destroyed and if the hydraulic compounds are oxidized by an electric file then NRF to leads keep one and heads for the nucleus and triggers a number of genes linked to the response element in this particular sale model is a luminescent model and has an enzyme suck together with the reporter element so you get more luminosity if you have a greater level of [inaudible] and is regarded as metabolically competent and by the originators but how competent is it really and when you measure the cell line which is [inaudible] someone we detect FMO and ADH and UGT activities but they are very low. There is measurable activity and measure measurable NAT one and we find mRNA but no activity that we do find also messenger RNA high one and UGT and the answer seems to be not terribly

metabolically competent and [inaudible] looked at the keratinosens with the S9 fractions and they look that 10 compounds looked at Pro haptens and they showed an enhanced signal with the presence of S9 but only for non-Pro haptens were reclassified with the new test and they look also at inhibitors that had quite verbal results and what we have, this is a full induction in terms of how much luminescence we detect in the open triangles are not induced in these are induced levels of what we see is quite a substantial increase in the presence of the S9 for this compound we also measure cell viability at the same time to make sure the compound is not telling the site.

We had similar results with [inaudible] which is a more conduction for the presence of S9 and we may be able to use some of the methods to identify pro-hapten.

For genotoxins about six years ago they looked at the activity toward benzo pyrene in the X benzos skin and got 2-D and 3-D engineered models and they did two things they looked at the four made, formation of the metabolite and how do you toxics the metabolites were in the skin models using and assaying for those of you not familiar with the essay is a method of measuring the general toxicity on the basis is you expose your cells to particular chemicals and fix them in electro thesis gel and electro freeze them and the idea is the DNA is nice and intact and it will stay in a nice tight nucleus and it will look nice and spherical and if there is been DNA damage the DNA will unwind as a result of damage when you expose it to the electric current or move out of the nucleus and have eight nice comment tell Street so the word COMET is usually is not an acronym for the shape and the more detail you get the more DNA damage.

What they found, looking at a number of different models, epidermal and the epidermal thickness and the ex vivo skin was very comparable levels of formation of the different Ben Sawyer pyrene tablets from 15 animals in an acetone vehicle. We can demonstrate the formation and they also found the metabolites were damaging but only when you added and inhibitor of DNA repair and when it was exposed to skin with no DNA repair inhibitor there was virtually no COMET tell detected where If you: Q bait with a repel you cDNA forming in the tail with damaged DNA and absolutely nothing of the control with the DNA repair so this gives me hope for two reason and we can detect be general toxicity but also in the models it seems DNA repair is going on as well which I'm quite happy about.

To summarize wooden a considerable amount of research on mainly driven by legislation changes in Europe that were mentioned earlier, and we are now at Reliant of *ex vivo* and *in vitro* can no longer use the local assay. What we are missing is the metabolic equivalent of the skin integrity test and what we need is some measure of metabolic competency cells that were going to use that leads a certain standard so we are sure there present and that still is in the pipeline. We are confident although there still much basics has to be done, we are moving forward, and a lot of this work has come about because of the cosmetic companies especially have gotten together to share information and data and share research efforts.

Here are some of my references.

I would like to acknowledge my old boss Faith Williams and my colleagues at Newcastle and Joan Eilstein and colleagues at L'Oreal research innovation and everyone's contributions to this field past present and future and thank you much for your attention.

Nancy Monteiro-Riviere: We have one more speaker and that is Timothy McCarthy who will talk about practical considerations for incorporating skin penetration data into a risk assessment for a consumer product launch.

Practical Considerations for Incorporating Skin Penetration Data into a Risk Assessment for a Consumer Product Launch
Tim McCarthy, Johnson and Johnson, Skillman, NJ

Hello. I know I am between you and much, much. I'm with Johnson & Johnson Consumer Products.

I am the one with the conflict of interest and I have employment and research was funded by Johnson & Johnson consumer products and I will say we hope to show data are agnostic.

The outline what I want to talk about is typical consumer ingredient in weather exposure and safety assessments and the dedicated on how the intact skin with robust prayer function and that is what cosmetics are intended to be used for and if they were intentionally damage skin particularly significant damage skin you would start talking about applications and in fact the guidelines we referred to in our industry the 428 and NCCS is the European safety authority and they have a review article in 2010 how to conduct a concentration study they conduct the analysis first to show the prayer function is robust and throughout the samples from the assays, if you don't have to pass that interior function then yes there are no standards today and there are no models today for assessing penetration less than robust skin that's important to us and potentially compromise scanned like diaper rash and excellent acne and psoriasis and all are in the United States and there are drug products but there's or directly to the consumer and not prescription drugs. We still need to be able to address those to ensure consumer safety.

I would say lack of data is the devils workbench when it comes to when we deal with regulatory authorities, this was a major part of the practical application and without data and understanding is a difference and what's the magnitude of the difference, additional factors may be considered for the risk assessment for these populations and again whether diaper rash or eczema or acne and again the consumer should be considered sensitive subpopulation but the specific values what you tag onto the risk assessment could drastically change an arbitrary among regulatory authorities to put yourself in my position I'm trying to do a global launch one formulation and one safety review and if a French regulator wants to put on a 3X safety factor and the German says it's five eczema Danish this Tenex and ages it is 100% into usually otherwise how do I do a global launch in these, let's get the idea, let's put some data on the points and understand is it a big deal with the magnitude of the differences and that is exactly what

it is going to be and that was a business as well as the science driver behind the problem research that will be discussed today.

First of all everything I do needs to be tethered to what is clinically known in there's a lot research at my company on human skin as well as my peers have done a lot of work but the clinical work is typically done with noninvasive measures as burial functions but there are also at using the literature and you will see in the data I will present they are directional and they are not exact proxies for chemical penetration but again practical consideration is you cannot take a human subject to a French chamber study and a person walks away. Let's be realistic we have to use noninvasive methods dealing with clinical studies like this and what is typically done is trends abnormal water loss and essentially the diffusion water through the skin it is the and the consumer clinical realm is the most common told used to look at barrier function. Also research has done looking at skin and psoriasis the clinical measure of copper mind compromise scanned increased internally in healthy skin and what I thought was interesting about that is the biology of the different skin pathologies differ but the outcome on TEWL is pretty consistent between the three and four range so that was my moment to try and tether this research to get through the three and four time higher penetration value.

This was developed into *ex vivo* models to address chemical penetration and again TEWL is water loss so try to tether Tran 08 what is already known for the clinical literature and penetration across the less than robust barriers to refine the assessment and encouragement in these and in both cases we use the static friends chamber for the pigskin from animals euthanized for other purposes where the tissue as mentioned by multiple speakers and consumer really could not do animal studies on cosmetics and there are legal bans starting in California and New York are the first ones and we just cannot do animal studies so the idea is we are getting animals living euthanized for other purposes I was the food consumption and will take advantage of harvesting the skin and the same thing a quick tangent, an example of this was for eye irritation using the eyeball a new slowed up or showed up at a slaughterhouse with a bucket and it's a valuable assay and back to this talk, for the new ones can model we do what a pigskin treated intact so it is Intech skin with you present pigskin from the same colony and human cadaver skin. Another research group in the UK for the compromise can model we used suckling pig and was paired with untreated versus tape stripped skin groups and I'll show you what if I forget later I will show you why we made sure it was the same plank being used as the own control .

Burst markers a barrier function and I will get into some of these later and some were great and some not so great and we will get into the discussion but TEWL is a mention, electrical impedance and again I have the emphasis when things increase or decrease with the barrier damaging. There listed on the table or on the slide.

For the newborns can model the penetration of caffeine, this was caffeine and a simple solution at this point and it was compared with multiple markers in the barrier function among the skin sources and for the compromise scanned those similar markers and barrier function were assessed intact versus the paired skin with various numbers of tape stripping to assess the change in barrier function and this was actually for the second assay learned lessons and we put a representative of emotion so it came in

from some of the presentations earlier and it's very important in my sick or, you use a representative vehicle for the many reasons that were discussed by the presenters here. The same reason my pigskin and while we use pigskin because first of all you have to use skin source that is used for food production but as we saw in the rat and rabbit skin, it is just not I would say not predictive human skin and if you want to overexpress or overestimate absolutely for an pesticide absolutely use on the skins for the bear functions but in the consumer sector we are supposed to be using human skin or the pigskin. In the second model the penetration was caffeine three Ameena phenol, sucrose and benzoic acid and we first set studies and caffeine and three Ameena phenol, my mouth is not working well but these were my Goldilocks molecules and we wanted something that was representative of the consumer sector and they were not successfully [inaudible] or oil soluble like to start, testosterone which was great information but irrelevant for a sector but caffeine, both were very relevant for the consumer sector and slightly water-soluble or all soluble and roughly the same molecular weight so they gave a nice in between balance as representative molecules and later we added sucrose and benzoic acid and we are still using in the consumer sector.

For the first model [inaudible] is a great reference if you want to get a quick primer on the changes of the skin and early development and how that or how we formulate our products. It's in the reference section and what I want to say here is the barrier function of a newborn skin full-term human skin is sufficient and still developed in the first year of life but sufficient and let's face it as the species, it was not that long ago that we popped out so if the barrier function was not sufficient we would not be here today but how it does show up you will see in the baby skin on this side the cells are stacking and thinner so there are less stacking and the cells are thinner and less number and it is also a lot more water in the skin versus the adult and what happens with that you see in the paper there is a lot more water loss and there's more water in the baby skin but more likely to lose the water because of the normal structure but all the structures are there in the full-term and frankly in some of the other papers a reference that I have the cosmetic ingredient review did an assessment on baby skin and you will see in preterm which is not my world that is the drug world but in preterm how weekly the barrier function develops once the baby is born.

This is barrier integrity test and not it's just the upfront barrier integrity test before we did the caffeine for the neonatal skin. We look at a created water and water loss and impedance and you can look back and forth but we did the neonatal porcine skin from the same breeding colony and human cadaver skin as mentioned so when you think about it from the baby skin there are two extrapolations deliberately to tribulation that we have to take it since we did not have one, one is newborn pig to pubescent pig which is a robust barrier but the pig versus the human and again as we mentioned pig is the best target for the human but not a perfect surrogate and you see two leaps here but bottom line from the table, some of these, at least in our hands and we did the assay, the created water was not as consistent and often all wasn't experimented that we added and you will not find it in the guidelines but the research we're working with wanted to try octant on we put it in and we abandon pretty quickly and it was not that helpful but the water loss and impedance in this assay were the consistent changes across the groups were again not so much now we're getting into the caffeine and as you can see it is

caffeine in a solution this is not representative a consumer product but at least it was standard across all the tissues that we are using.

The bioavailability and total recovery, I am doing really, here we go, the viability and total recovery I would say this was okay and it was not, upfront it was not ideal but we are just at the cusp but the bio ability was adjusted based on the recovery and what we did see is the neonatal skin versus pubescent skin there was an increase viability of the neonatal skin versus the pubescent skin but really was not that much and it was between the neonatal skin and the human skin and it is there and it's not that big of a change. Now this was, I actually like this graph better than the table before so this is the French number study and what I will point out, there was a repeat and we have a neonatal porcine and that was one day in that's the first time we did the experiment and they say that's why we call we did research because we have to redo your work sometimes and this was, because it was pretty robust and comparable to the others but we found out because they newborn and one Dale skin was put on the French team for thickness because it was a thinner slab delivered and they put it on and they said you are not supposed to do that because of the common thickness but what's more important we had to redo this one, this was an interesting phenomenon and again the one Dale skin was pretty robust but then it just started to breach and the caffeine flashed through and one day old skin may not be, when you think about it, it was in shipment on the study take Cheryl then more than, the one day old was look at too quickly so it was not a good idea so they redid with the for day old skin and we got much better results with more predictable so the Sibley triangle Park so yes it was higher and it mapped the same but the magnitude was higher but did not act the same in you did get a spike that pretty much is dropping down pretty rapidly.

No are moving to the other side of the compromise skin. This is published and there are two publications developed on the method as well is only actually put the caffeine in the molecules in the reference section and one thing again practical consideration is what I want to say, this is comparing electrical resistance versus TEWL we have not manipulated the skin this is not intact skin so there is inherently scattering missed betas when you look at the publication and start manipulating the sales are the tissues you're going to introduce more variability so you will have to use a lot of samples and again the whole reason why we want to use hair samples, the intact versus tape strip from the same plank because we already saw a huge variability and just without even touching innocently without touching materials so it is farther explained in the publications.

One of the premise that I went into this research, I had seen other works during tape strip as a your function but to me, I hope I don't annoy people here but I saw my list tape stripping 20 times in 30 times I said the whole premise of this, this research, tape strip until you get up to three or four times the control in the nontreated and then you stop and that was again the issue using the same flank as its own control and for, I went into this thinking from sample to sample there actually may be a different number of tapes upsetting to begin and again this is practical consideration and what ended up happening it was not tape stripping this particular sample six times and this one I had this naïve idea it would be that granular and it is not because of the variability and data and again practical considerations that we're trying but what it did come down to is this is on the left electrical resistance with various tape stripping and this is looking at

numbers of tape strips and again we have individual tape strips as well but this is more clumping and you have the five and 10 and 15 and 20 and this is [inaudible] so you did see a response to the number of tape strips or electrical resistance goes down and the water loss goes up but again you see there is error bars in the Stata and on the right side you see this is when you completely remove the corneum but this shows the explosive difference between taking the entire [inaudible] off of the tape stripping's but again I want to emphasize what we're trying to do is show diaper rash and eczema and we're not trying to do third-grade burns in our product line, 3rd degree burns in our product line but what we clearly saw, and this is for taking the molecule as well the benzoic acid you will see intact over the time course you can definitely see an increase with the compromise skin versus the intact skin and you will see the magnitude did differ between them and as you can see with caffeine you have a big change and three Ameena phenom not so much and you did it with the gross but noticed, sucrose but notice the bars some of the take-home messages was in the grand scheme of things you see an increase and I think we have valuable information and on the microlevel age molecule is its own molecule and it also has error bars between the different molecules but overall message was the data confirms the noninvasive measures and barrier functions are indicative the TEWL is indicative but does not replace looking at the molecules themselves.

Also I would say the current 10 next safety factor and risk factor is to come it at a quickly captures the neonatal barrier function as an adult full term newborns have sufficient barrier functions and the ER was robust enough to discriminate between the barrier property changes in tape stripping lobby TEWL and the [inaudible] proved to be unsuitable and there was something that was for me as you tape strip the skin it loses or loses because it's a tissue and has water inside and you're looking at me and like okay you knew this but it was [inaudible] but after we did this it made all the sense in the world TEWL was looking at the diffusion of water coming out of the skin but if you do tape skip, tape stripping the berries of the cells are moving and we had to tape strip and let it equilibrate and do the TEWL reading and do a couple more tape strips, and then wait and then do the reading and you cannot do an essay since these are *ex vivo* skin so the ER it was totally agnostic to the factory tissues were using and we put the pro out of the scan and you had an instantaneous rating which practical consideration difference between theory and practice it should have been great but with the using it made a completely impractical.

The analysis that they tend tape strips, and again I thought this is going to be granular between sample to sample and it really was not all examples we looked at roughly 10 tape strips provided the loss of barrier function that approximated 3 to 4 times fold increase in the Tran 08 which again which is atopic dermatitis or diaper rash and in the final thing the individual molecules and different chemical properties had higher penetration compromise skin and the they were different but in the grand scheme the magnitude changed but not, it was not that drastic.

This is the work in the UK and the two Davies papers and one was developed in method and one was putting molecules in and the baby skin I never got this to publication and I think there's more work we need to do and that was with Fargo and [inaudible] who is at FDA he is an expert on *in vitro* and among you.

Some background papers and again I mentioned the to lap ski paper for infant skin which explain what goes on the dynamic changes in the baby skin and how it impacts our formulation and the cosmetic ingredient review 2014 did a comprehensive review and also looking at the metabolism going on a brave New World and all the things going on they were extrapolating from the liver because just the data is not there for the scan. It was different and again something briefly on that it depends on which enzyme system you're talking about. You can't make broad stroke but I will leave it at that point so any questions and hear more papers. And I'm sorry here are my acknowledgments: Neena Tierney and Catherine Mack at J&J, Jon Heylings and Diane Davies at Dermal Technology, and again Paul Lehman and then Sam who is with you now he does a lot of *in vivo* and *in vitro* correlations, and Sinclair Research was the source of the scan from the breeding colony for the neonatal study. Thank you.

Nancy Monteiro-Riviere:
This concludes the session as far as the speakers and I think they did an excellent job and now we will go to the panel where we will be glad to answer your questions or concerns and it will take place right away there will not be a break so don't go away.

Roundtable Discussion
Moderator: Nancy Monteiro-Riviere, North Carolina State University, Raleigh, NC, and Kansas State University, Manhattan, KS
All Speakers
Nakissa Sadrieh, US FDA, College Park, MD

Nancy Monteiro-Riviere: Do we have any questions from the audience? No? Yes, we do. Could you state your name please? Can you please go to the microphone?

Audience Question: Thank you for the very nice talk today, this question is for Johnson & Johnson and what are the major challenges in dermal risk assessment? Do you have any recommendations for moving forward? And also have another question I would like to ask so that information presented today be applied to further [inaudible] of the two ingredients. Thank you.

Timothy McCarthy: Thank you for the question and the challenges we're facing right now, I would argue regulatory acceptance of some of the models and for example what was presented on the mathematical modeling rather than doing *in vitro* modeling is that going to be accepted and in certain regulatory arenas? And if I didn't do an *in vitro* model and that is the basis of my acceptance will that be accepted by regulatory authority or will they say that's all well and good, but I want to see *in vivo* data? So, I guess that are some of the challenges we're facing now as an industry. For the consumer sector where we have legal mandates where we cannot do animal studies so we're going to get into an impasse or a regulatory authority in one region demands we have to do animal research, first we have to decide are we going to do it and secondly we then cannot reuse the data in regulatory regions, so that is the major disconnect.

One academic one I thought was interesting fairly recently and again on *in vitro* versus *in vivo*, the European authority look at retinol and retinol [inaudible] and the industry several years ago did a great study and women of child bearing age, oral and dermal retinal at the plasma levels from whether oral administration versus dermal administration, and they saw no plasma levels from the dermal route but plenty from the oral route, and it was showing *in vivo* there are circumstances where a [inaudible] study did not reflect reality and the European authority dismissed the human data and wanted to use the French chamber data because they say they want to see the worst case and the answer is worst case is not reality and they want to say worst case. So, again, sometimes it is regulatory dissonance that we are facing as an industry we face is a bit of a challenge.

Jeffrey Yourick: Did anyone want to address a second question, which is risk assessment of dermal injections?

Gerald Kasting: This is Gerald Kasting and I can address part of that, I've been working for several years with a research group at one of the pharmaceutical companies that is developing peptides and oligonucleotides that are injected intradermally and they have been able to use part of the modeling approach that we use for the dermal clearance part with a subcutaneous injection or intradermal injection, they are comparing the two, as the initiation of the process rather than topically applied, so some of the components of what we do in dermal delivery can be applied to intradermal or subcutaneous but it lacks the stratum corneum barrier but the physiology for clearance and Dr. Monteiro talked about this, is very similar and it depends on exactly the environment where the injection is made whether it is in the skin or below the skin and at what site so there are components of what we do that apply to other delivery routes.

Ronald Baynes: If I may add, because there is a concern I sense from your question, the acceptance by the regulatory authorities for a lot of the models that are being generated over the last 20 or 30 years, I am very proud to say and I think Dr. Kasting was the first to mention this morning the model was accepted by EPA but again this is EPA chemical toxicology versus FDA and that kind of thing and drugs and so on in the models have a role to play in models have a role to play in a lot we do helps refine in the studies before we get to the animal studies and reduce the animals that we need and models also help us ask questions that we normally don't have time or money to ask those kind of questions without an *in vivo* study and ultimately we need to, on my last slide, we need to do a [inaudible] and have room to do that kind of work and this is probably what has been catching up with within our skin and skin absorption work but models do play a critical role in early development and as you are well aware, but it is not to be discredited but has limitations.

Yourick: When you say models, it is not just computational models, as Dr. Wilkinson was talking about, models that have been really effective preclinical models have been simple chemical or cellular assays. So, it is not animal work or *in vivo* work but not computational either it is chemical reactivity or biological activity and activation so those are models as well and they are a lot simpler to run than *in vivo* studies in many cases,

a lot less expensive, and have gained acceptance in the regulatory world to some extent. You want to build on that, Simon?

Simon Wilkinson: Yes, certainly. One thing about the prediction of allergenicity in the scan sensitization was time was a huge pressure because of the changes in legislation. And quite a lot of the activity came in the last quarter of the football game and quite frankly the local lymph node assay was a very widely accepted assay. There was an animal-based assay in which the compound was painted on the ears of mice and it was a very simple method of measuring cell proliferation [inaudible] and that assay can no longer be used for cosmetics. We need now several different approaches which start with a simple *in chemico* method that was talked about which are economical to run and easy to run and fail early right through to more cell-based methods and detecting the other parts of the adverse outcome pathway for the allergic sensitization process and putting all the steps together has been a challenge getting regulatory acceptance, it is work.

Nakissa Sadreih: I want to maybe clarify the question that was being asked and I think for the intradermal injection was directed toward products such as tattooing so I don't know whether anyone addressed how one would determine the safety and the risk assessment for the intradermally injected tattooings which are composed of pigments as well as [inaudible] and they are not soluble, they are particulates for the most part, so I think my colleague may have been asking about that so maybe the panel can talk about potentially models that may be able to try to help determine by distribution as well as possibly toxicity both short-term sensitization as well as long-term carcinogenicity, depending on the pigment and the hazard posed by the chemical characteristics of the pigment.

Kasting: I don't think I have a complete answer to that but the process, once the pigments are injected, they pigments are solid but they gradually dissolve and the [inaudible] in many cases are more quickly distributed so I do not think you can do this in the absence of experimental data on how the compounds distribute, but if you gain some experience with selected ingredients you can measure dissolution rate of ingredients in a simulated dermal environment and you could do that *in vitro* and you could use a dermal clearance model to understand the distribution in the system and I think combinations of chemical assays and PBPK or other types of clearance models could get at that but it will take more than one technique and will take experimental data and you will not predict as an issue.

Monteiro-Riviere: I was going to comment on that because it’s very complex what you ask and not one assay will be able to tell you everything and if I can remember correctly with tattoos, the dendritic cells can pick up the pigment and goes to the lymph nodes and pretty much resides through life so how can you find a model system to mimic that? There are some things you have to realize that *in vivo* is really important. I know the trend is to go *in vitro* but certain things like that you may want to scale down and lower-level species, but I think *in vivo* would be necessary. And long-term effects.

Sadreih: I think it is helpful to know *in vitro* and with modeling one would not be able to risk from tattoo inks and the *in vivo* study will probably be necessary. And the tattooing,

they do migrate the lymph nodes, but a lot stay of them stay there because their purpose is to stay there, but the fact that the pigments are aromatic amines and you have metabolic capabilities in the skin to what extent you think that may impact potential sort of long-term term influences, such as cancer development? Is there any data, does anybody know?

McCarthy: We do not have alternative methods for the chronic endpoints. What we have is eye irritation, skin irritation in the newly formality and that's it. If you're going to do that invasive injections, there it is not an *in vitro* model currently and by the way it [inaudible] would also be needed in this battery.

Audience Question: I would like to thank all the speakers today for an excellent presentations and today's session covered a variety of different topics and gave a really good background and update for what is going on in the field. I'm a risk assessor but I am new in dermal tox area so I have two questions and the first one may be a little maybe everyone knows this, but I will take the liberty to ask. Recently I have been starting to read articles to try and find out what would be the best way to get information and quantify the skin absorptions and I found the literature that records those data has been separated into two different units. Some articles sort the absorption by the percentage applied versus the rest of them usually describe microgram per centimeter squared. What would be better, and which one is the last influenced by the amount that is put into the path?

Kasting: I cannot give you the exact page number reference and there is a nice review article that was led by Fred [inaudible] and it was I think *Environmental Engineer* or *Exposure Science* from about 2016 or '17 and I was part of it but was not the major leader on it but Fred does a really nice job and John Kissel was very much a part of this too in discussing dermal load and the question that you ask is commonly misapplied in terms of how does dose affect percent absorption and I can give you looking back on my computer the exact reference from that or send it out as part of the notes of this meeting but Simon is nodding his head, so this is where I would go for some guidance and a nice review that is quite recent.

Audience Question: Thank you and I feel like this is a basic question but based on my experience I think it is all case by case scenario and sometimes it has pros and cons and some of the study provided the number but if the study is not done scientifically the number may not be really reflecting the real case and that's just my take and thank you I would like to get that reference later. My second question is regarding sort of related to an earlier question for the *in vitro* models, absorption models, what would be the recommended validation process? For regulatory use, any recommendations?

Wilkinson: I would say it depends on which regulation authority are trying to convince. Most people either follow the OECD guideline TG 428 which has been in place since 2004 and was in review in 2010 and I still have no idea whether the recommendations of that review were completely implemented and possibly not yet. It was to fine-tune some of the aspects around that and I think if you stick to 428 you cannot go too far wrong. The problem with 428 is that it's a guideline and it tries to be all things to all people and sometimes some of the requirements may not be applicable to your

particular area of interest. I'm really pleased at the top of this whole show, someone defined the terms absorption and penetration clearly for us because these terms are used interchangeably in the literature along with permeation, whatever that means, and you need to be absolutely clear when interpreting the literature and even in the guidelines the OECD 428 says at the top of the document absorption reflects systemic exposure unless the chemical resides and [inaudible] in the skin, in which case, so you have about one paragraph into TG 428 and you are already in trouble. So, 428 is a pretty good place to start. Yes, please.

McCarthy: The European Safety Authority for Cosmetics has a guidance document first in 2003 and 2006 most recent 2010 and it is built on OECD 428 and there's a lot more homework you have to do up front and their documenting compatibility of your reservoir to the incoming test article, the number of samples are increased, and that is what we tend to follow because it starts at 428 and puts more requirements on it. To your point as well in the guidance when we determine what is bioavailable, it is not just what is in the reservoir; it's also what's in the dermis and the viable epidermis because those are considered reservoirs for potentially later getting in the reservoir but the tape strips and that is research we did as well, the tape strips you definitely need to do for mass balance accounting but what is in the tape strips and what is in the wash is not considered bioavailable. But what is in the viable epidermis and the dermis is.

Baynes: One of my recommendations, and this is what I told some students even this week while rotating through my lab, read the papers and read the materials and methods and assess the dose, the area, and all of those things because there will be considerable variability across the board even in peer review publications and we have guidance and we do have guidance but I'm sure in some of your preliminary assessments you will read papers for various substances that were tested. Check the calculations and how is permeability calculated and under what conditions and what was the dose or what was the dose volume, was it clipped or was it shaved, and Nancy and I used to fight over this all the time. People use the word shaved so do they really shave it, or do they clip it? Clip the hair. How old? So all of those need to be taken into the risk assessment conditions and I am sure you got that from all of us today and that is why the interlaboratory variability publication came out in 86 or 96 or whatever it was and it was significant. People are using different things to define absorption, so be very careful.

Monteiro-Riviere: Yeah, and I want to point out one thing, too. When people say they're tape stripping; there are so many different ways to tape strip, and there's a lot of variability on that. And there was a big talk at one of the Gordon Conferences, and I think it was one of Richard [inaudible] groups that had students, rolling, you have a rolling pin, almost like you're rolling pizza. And then you would roll the tape and strip it off. We have tried all of these methods 20 years ago, electrical tape, masking tape, and it should be 3M scotch tape is what works the best. But what size are you using? How do you spread it over? How do you roll it? And how do you pick it up. And sometimes you need to go 60 times to get tape stips. And a lot of people don't correlate that to histological samples and do the biopsy they can see that [inaudible] tape strip has not removed anything. And then they'll wonder why [inaudible] anything.

So, you need to do some backups. You need to look at a lot of papers. And I think one of the problems in the younger generation, you tend to Google or try to get things online, but a lot of the old classical papers, like compromised skin, we have books on it. And there's all this neonatal work, and we've done neonatal foreskin work with pesticide absorption. I think you need to go way back, and students are coming up with the same ideas and oh, this happened 25 years ago. So, go trace down the literature. Do not just depend on the last five or six years.

Yourick: We have a question online. And the question is, why does any regulatory agency choose to accept data, from *in vivo* animal OECD guidelines, but chooses to ignore or not accept human in vitro data, which has been through vigorous validation. The OECD test guidelines should be instantly acceptable to any authority. I am assuming the basis of this question is, why are the regulatory agencies accepting animal *in vivo* data but not human *in vitro* data, with a validated guideline? So, let's say for skin absorption, in regulatory agencies, I guess, is accepting *in vivo* data but not the human *in vitro* data as described in 428. I do not know if anyone has had any experience. Maybe this goes back to your comment that it really is a guideline. Different agencies have different regulatory requirements.

Wilkinson: I want to play devil's advocate because I do not necessarily agree with that interpretation. One issue with the *in vitro* models is that the skin papillary layer, apart from specialist ones like Ron Baynes skin flap model, is that the papillary layer, the capillary layer, which extends right under the epidermis, is not perfused in an *in vitro* model. So, that is a major concern between *in vitro* and *in vivo* for some people. And that may be one of the reasons why these data are not accepted. I can't think of any other.

McCarthy: The one that I mentioned with the retinol earlier, that it's an [inaudible] molecule, so there is a homeostasis mechanism obviously going on in the skin that is evident *in vivo*, but no *in vitro*, because it's *ex vivo* skin at this point. Again, it is saying that you are not having the, you have metabolism in fresh *ex vivo* skin but it's not *in vivo* because you don't have circulation there, you don't have the feedback loops. And the answer is, it depends, you have to understand the basic science of your molecule first and foremost. There are opportunities where, yes, 428 is legitimate in the right hands. That's the other thing I wanted to say when the other question was going on. Just practically, lots of labs say they do it. I would argue with the [inaudible], but there's so much art in that, and practicality, you want to use the labs, and I will be very biased and I won't say the labs by name, but you want to look at the labs who actually show up on these scientific meetings where they're talking about this stuff and presenting. There are lots of labs that say they do it, the assay, you want to focus on the literature from the people who publish regularly and present at these scientific meetings. They're the ones who actually do [inaudible] this art form with the tape stripping that goes on.

Kasting: I would like to add one comment. There are many in this audience who probably know more about this than I do. But in the FDA dermal area, they have the Office of Generic Drugs has been working quite hard in the last ten years or so to offer alternatives for bioequivalence testing for different classes or different particular topical drugs. But I understand that it's a one at a time approval proves, but there is guidance

for use of *in vitro* data for bioequivalence testing in the topical drug area that have, there have been quite a number in the last half dozen years and there is a direction going there, or a movement in the direction that you are talking about but it is not a blank approval process. Maybe someone who knows more details, maybe Jeff does, can anyone comment on that?

Yourick: I am so sorry, but I was reading a comment that came online, so if anybody else can respond, I was not paying attention. But I will go back to your comment. I am so sorry about that. This gets back to, I think, Janet's question. I'm going to read this comment and the audience can listen. You should always use micrograms per centimeters squared, as we were doing toxicology, we were looking at milligrams per kilogram per day dose, percent is interesting to make some basic simplifications, but you must never compare percent changes between different concentrations. For example, the [inaudible] and suggests that absorption is inversely proportional to concentration, which is completely the reverse of [inaudible] Law. And the reason they get this wrong is because they are looking only at percent changes. Percent of, I guess, applied dose. Everybody agree? Any other comments? Good comment. Thank you, whoever submitted that.

Wilkinson: Maybe I could add just one thing to that. When you talk about percentage, you have to have an element of time as well. 30% absorption in 24 hours is not the same as 30% absorption in 24 minutes. You almost need an element of time as well as a percentage. But the basic truth of that comment from the online commenter is absolutely right.

Yourick: Let me loop back to another comment that came in related to the *in vitro* versus *in vivo* acceptance. The LLNA, which were not validated, I'm sorry, the LLNA, the only *in vivo* assay that has gone through full validation yet not accepted by FDA. FDA should be commenting on the *in vitro* assays for regulatory acceptance. I mean we are not—

Sadreih: We don't require any testing pre-market, There are no test, whether *in vivo* or *in vitro*, that are required by the FDA for cosmetics. But for drugs, it's different. However, for cosmetics, no tests are required, therefore people can do what they want. That [inaudible] never comes to the FDA anyway, we never get to see it. Companies market their drugs.

Yourick: I guess another comment, too, is that we do participate in the skin sensitization working group through ICCVAM, so we are part of that group. A lot of that group is pushing validation of sensitization assays. And FDA is part of that effort.

Sadreih: I have a question. I appreciate the discussion today about the factors that can influence dermal penetration as well as absorption and I appreciate the distinction between the two. I want to go back to this, Tim mentioned CIR, the Cosmetic Ingredient Review. The industry's own group, which assesses the safety of ingredients in cosmetics. And they also look at individual ingredients and they look at size. They have this 500 Dalton rule, and pretty much, that's what determines whether something is absorbed or not, in their opinion, which would then impact the toxicity. I am curious to

know what the panel thinks about this 500 Dalton rule, and given the fact that all these other factors were discussed today that would impact absorption and knowing that large molecules can get into the skin depending on the formulation and that you cannot evaluate the safety of an ingredient in the absence of the formulation, what type of credibility do you give to the CIR's process for trying to evaluate the safety of ingredients used in cosmetics when it's done in such an artificial way?

Kasting: I'm going to answer your question indirectly, just it's interesting to note that the two most recent additions to the armory in atopic dermatitis have been molecules [inaudible] that have molecular weights of 700 to 800, and they're quite effective topically, thank you, so that doesn't fully answer your question, but there is no magic cutoff at 500 clearly.

Baynes: So, I did present to you the substance [inaudible], which belongs to the class of the [inaudible], which veterinarians use the [inaudible] class to treat topicals, to treat topically, to work topically, and also systemically, to treat for endoparasites. Their molecular weights are 800 and above, they work very well. I will stop there.

Wilkinson: We've looked at the permeation of short peptides and [inaudible], and they do get through the skin. They don't last long because they are hydrolyzed by peptidases in the skin. But there is a compound secreted by the skin of one of the Amazonian frogs, I believe it is called [inaudible], which has D amino acids instead of L amino acids, so it's not as readily hydrolyzed. We have applied that to the skin, and it gets through and it survives. So, molecules with weight of 501 and above certainly do permeate skin.

McCarthy: I think for the typical models that we do, 500 to 1,000 do get in, they just diminish the amount that gets in. Gerry, do you want to say typically 1,000 and above is considered de minimis? 500 to 1,000.

Kasting: I think what you have to factor in is getting to small amounts, you've got small amounts of people with compromised skin, too, and in any exposure scenario. So, compromised skin is going to be much less size selective than intact skin. So, I think it becomes not important to try to set an absolute level of molecular weight because it's going to be exceeded by people that have skin that's not in good condition. So, I think you have to factor in the fact that these molecules are less well absorbed, but there are going to be scenarios where they, some is absorbed.

McCarthy: What we have done internally, is we have used paper [inaudible], which is very rough assumptions based on molecular weight and optimal water partition coefficient. And simplistically, it's 0% for the large polymers. 10, 40, 80, 100% dermal penetration. [Inaudible] actually came back and said really nothing happens at 100%. Even worst case is 50%, but even so, I'm going to stick with the [inaudible]. And then what we did from our, the research I said with compromised skin, if the product indication is clearly for compromised skin, again, the diaper rash, the eczema, we will put in a, we will take whatever the perceived dermal penetration in intact skin, and then out a safety factor up to 100%, so again, it's not [inaudible] 5X, because if the base is assumption of 40% dermal penetration, we're not going to assume 200% dermal penetration. It maxes out at 100. But we will do that for product lines based on the data

we showed, we'll just put a 5X in just to cover that lessened barrier function. But again, the basic premise is optimal water partition coefficient and molecular weight are major drivers, yes, vehicle is a driver as well.

[Inaudible]

Yourick: I think we had some concluding slides. Are they up? Go ahead. The recorded session and the slides will be available on the SOT FDA Colloquium site. And you can see, these are the topics that are going to be coming up for this year for the addition colloquia for this calendar year, so please consider attending those, too. And here is the recording slide and captioning text that is available at no charge online. There is going to be a survey that will be sent out to all of the participants to comment back, so please make comments. It helps improve the process for all of the following colloquia, too. Thank you.