Discussion
Children's Health / Risk Assessment

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Panelists:	**John A. Mclachlan (Tulane University, USA)**
	Jorma Toppari (University of Turku, Finland)
	Fumiki Hirahara (Yokohama City University, Japan)
	Masatoshi Morita (National Institute for Environmental Studies, Japan)
	Frederick S. vom Saal (University of Missouri – Columbia, USA)
Commentators:	**John P. Myers (United Nations Foundation)**
	Richard A. Becker (American Chemistry Council, USA)

Mori: First I will show about four slides that briefly explain the target of our discussion and then I would like to ask each panelist to make remarks. As everybody knows, risk assessment includes exposure assessment in addition to hazard identification and dose response assessment. As for risk assessment, it was proposed by the National Research Council in 1983, and now various risk assessments are being conducted for each chemical individually.

There has been much scientific advancement in recent years, and many have expressed the opinion that the existing risk assessment has some shortcomings. One of today's themes is combined effect and we will deal with the effect of combinations of chemicals rather than single chemicals. Then there is susceptibility. There is concern that fetuses and children may be more susceptible to chemicals than adults. Third is long-term effect and delayed long-term effect, whereby changes appear after an extended period of time rather than the end point after exposure currently used for assessment.

In addition there is low-dose effect. We are beginning to hear opinions that a new end point must be added to current risk assessment.
Recently, we have compiled a book concerning the fact that we are beginning to change from current awareness to countermeasures and measure for coping with the issue of chemical substances up to now based on human fetuses and children in particular.

Along with considering the impact on human beings, we must now consider the combined effect of multiple contamination and exposure. We must also consider endogenous hormones and various chemical substances that enter the body from the outside. As one of the keywords, we must consider the fact that children and fetuses do not exist in the same condition as adults do.

This is not just being said by Japanese researchers or a certain researcher, but rather we are hearing from sources all over the world about the need to think of the environmental background of childhood and adult diseases with fetus as an international criterion.

In the discussion session of this symposium, our commentators and panelists have been provided with six keywords: Children's health, Risk assessment based on the children and fetus, Susceptibility, Combined effect, Delayed long-term effect and Low-dose effect. I would like to hear an exchange of your opinions about these from various standpoints. Although it is difficult to draw a conclusion, let's first listen to every one's opinions. Hopefully we would slightly have an idea that it is required to take international collaboration and cooperation in the future to realize such risk assessment. This is the purpose of this discussion. First we shall hear from Dr. Myers. Please Dr. Myers.

Myers: Thank you, Dr. Mori. It is an honor to be with this group again. This is my 4th year at the

International Scientific Symposium on Endocrine Disruption in Japan. I am very pleased to see how much progress we are seeing made with every year, with every addition of new scientific results.

What I would like to do in my 10 minutes is to ask you to rise above the very tight focus we have had on specific details, to, instead of looking at the trees, look at the forest of the issues that we have been discussing. What I would argue is that as you think about endocrine disruption and children's health, we are in scientific heaven but regulatory hell. What do I mean by that?

We are living midstream in a scientific revolution that is radically changing our understanding of the links between contamination and health. The papers presented at this meeting are carrying us forward in that revolution; just in the last presentation we learned new things about hypospadias and bisphenol A. It is truly astounding.

If you go to an academic library today, and carry out a search in one of the computer databases on how many publications have there been within the last year on one substance, on bisphenol A, for example, it is astounding from how many different laboratories, on how many different endpoints, how many different methods people are now using around the world to look at these links between contamination and health.

You will find publications on aggression and behavior, findings from Japan on concentrations in human biological fluids, and some really remarkable results looking at the interference of bisphenol A with the standard treatment for prostate cancer.

Another fascinating study from Ehime University examines possible involvement of bisphenol A in increasing the conversion rate of pre-adipocytes to adipocytes, raising the possibility that bisphenol A is involved in the epidemic of obesity that the world is now experiencing.

New results like these are being published virtually every week, making it difficult even for specialists to keep up with this torrent of science. It is truly extraordinary to see what is happening.

But it leads us to regulatory hell. I can imagine the regulators who are trying to keep track of just this one compound, bisphenol A. It must be like the Dutch boy and the dam, where there is one leak in the dam over here, and there is another leak over here, a 3rd one over here, a 5th one over here, but all of the sudden in a completely unexpected direction the dam breaks because we have completely new results coming from a completely unexpected direction.

We have heard a lot today and over the last couple of days about some deep issues related to this regulatory hell. One of them was an issue that Fred vom Saal touched on earlier, which is the inadvertent contamination of experiments through endocrine disruptors in food that is not yet understood or endocrine disruptors in some of the lab equipment that are giving us false controls and leading us to a literature that is probably now polluted with many false negatives.

More deeply, we are also seeing data from multiple studies of endocrine disrupting compounds that are repeatedly falsifying the operating assumptions at the heart of risk assessment process as it is currently practiced. I am going to summarize what some of those core operating assumptions are for you.

First, the dose makes the poison. The simplistic application of this assumption is now clearly falsified by a whole host of data showing low dose effects with non-monotonic dose response curves. In fact, at the Yokohama conference we had a panel of scientists including industry spokesperson Dr. Jim Lamb agree that this is no longer a valid assumption. So that is out.

We have seen some elegant work looking at thresholds of effects. Dr. Daniel Sheehan, David Crews, and others have shown that for systems in which hormonal systems are already activated, there is no practical threshold for effect in that system. So that one is out.

We heard an elegant paper by Andreas Kortenkamp this morning rejecting the notion

that you can derive useful regulatory guidelines by basing your work only on single chemicals at a time. This is just no longer adequate in this modern era. So that is out.

We also have an assumption, increasingly prevalent, that you have to demonstrate adverse effects in order to start regulating. But when you look at what we really understand between changes in biological systems and the ultimate developmental processes, you can see that we would be incredibly arrogant to assume that we can predict adverse effects based on our current understanding of what those changes actually mean. So that is out.

Finally, if you listened to Dr. Elizabeth Guillette earlier this afternoon you heard an absolutely spectacular discussion demonstrating that the animal studies that prevail throughout regulatory science, as important as they are, are probably not sufficiently sensitive to get to the endpoints that parents really care about.

There has been some work trying to employ operant conditioning techniques and behavioral studies with animals to get at more subtle behavioural effects, but they do not play a very large role in the regulatory process and they do not get us to the sorts of endpoints that Dr. Guillette was talking about in her work.

So we have got to be looking for other sources of guidance. Is epidemiology a good source? Epidemiology has some real problems in dealing with endocrine disrupting compounds, also.

First, nonmonotonic dose response curves are deeply challenging to human epidemiology as it is currently practiced. So is the fact that as development unfolds in the womb, the same compound at different times in development can lead to completely different disruptive patterns. How does epidemiology currently cope with that? Not at all, at least in *in utero* studies.

The converse is also true: disruption of the same developmental process in that same developmental window by different compounds can lead to the same effect, dramatically increasing the statistical noise in epidemiological work, unless at the same time you are able to monitor all the relevant compounds that might be interfering with that developmental process.

Finally, as Dr. Mori mentioned, long latencies can intercede between cause and effect, decades sometimes between exposure and impact.

So what is the regulatory answer here? First of all, we have to acknowledge that the current process is fatally flawed by its dependence upon falsified assumptions and polluted literature. We have got to start over.

We have got to build a new science-based approach, not one that is based on false assumptions, but is based on real data, that begins with models that are incorporating some of the factors that I mentioned: incorporating the low-dose nonmonotonic response curves, incorporating the fact that there can be no threshold, incorporating a focus on mixtures at the core, and demonstration that if we find an effect in regulatory research, we find an effect in a developing organism, we should then reverse the burden of proof so it is not incumbent upon us to demonstrate an adverse effect, it is instead incumbent upon those who would wish to use the product to demonstrate that an effect is not adverse. This will require a compete reversal of the burden of proof.

Dr. Mori, with that I will stop. Thank you very much.

Mori: Thank you. Next, Dr. Becker please.

Becker: It is my pleasure to be here as well and address this group. First, let me congratulate Dr. Myers on a very impassioned speech. This is a scientific symposium, so let us focus on the science, I think.

I would like to begin with, as we have heard some very provocative statements, let me be a bit provocative here as well, and I offer this quote from Dr. Koop. As you recall Dr. Koop is the former Surgeon General and a distinguished pediatrician in his own right.

Dr. Koop, of course, says at the top that

children as we all recognize are our most precious resource, and we as parents and guardians must do all we can to protect them. But he goes on to say something that I found to be very stimulating, and I would like to offer that for the group's consideration here.

He says, "I have been concerned in recent years that in the understandable quest to protect children, our society's priorities have been inverted. This inversion has caused us to attempt to eliminate purely hypothetical risks to children, while the real risks to children prevail, almost unattended."

So I thought I would start out with this and perhaps use that as a way to investigate a little further what we are talking about here with children's health.

This slide shows life expectancy in the United States from 1901-2000. You can see that life expectancy in the United States has risen dramatically over this period of time. A child born in 1901 had a life expectancy of about 45-46 years. A child born today has a life expectancy in the United States of close to 80 years, quite an improvement over time. So clearly the health of children has been improved.

What about infant mortality? This slide shows infant mortality from 1950 to 1999, again in the United States. Infant mortality rates, neonatal mortality rates, post-neonatal mortality rates. All have declined substantially since 1950, dropping by some 75%. Again then, we have made tremendous progress in improving the lives of our children over our lifespan.

However, much more remains to be done. I apologize this is out of focus. This shows under-5 mortality rates from 1995-2000 in industrialized countries here, and then in different regions across the world. This is Latin America, the Middle East, and Sub-Saharan Africa. You can see that there is quite a disparity; 10-, 20-, even 30-fold higher rates of neonatal mortality in the developing world. Clearly much more needs to be done.

This slide is from UNEP showing global causes of mortality in children, the top killers of children under-5 in 1999 on a worldwide basis. Look at this — this is remarkable. Clearly more than 50% of the children that die worldwide, even today in 2002, die from malnutrition and diarrheal diseases. The vast majority of causes of mortality in children are infectious diseases that are transmitted through unclean water, vaccine preventable diseases, malaria and respiratory infection.

Let us contrast it to the leading causes of death in children in the US in 1999. This slide illustrates that unintentional injuries, congenital anomalies, malignant neoplasms are the leading causes of mortality in US for children. None of the top 5 causes of mortality in children in the United States' are related to any of these infectious agents.

On a global basis, the leading causes of death of children are preventable with the knowledge that we have today, through the products of chemistry to disinfect drinking water, through the products of chemistry to prevent malaria, through the products of chemistry and technology to improve nutrition.

Now, again I would like to emphasize that if we are looking at the developing world and the developed world, we need to focus on different things. The leading causes of death in the United States in children less than one year of age are: congenital anomalies, short gestation, problems with gestation and SIDS, sudden infant death syndrome. There is a very different pattern of causes of death for children in the US from ages 1 to 4 and 5 to 9. In these age groups the leading cause of mortality, accounting for more than 50% of deaths, is unintentional injuries.

Innovative chemistry, can improve the lives of children across the world. From chemicals to disinfect water, from pharmaceuticals to treat disease, from innovations in chemistry to improve agriculture and nutritional products – chemistry contributes to public health enhancement. We must not turn our back on our knowledge that we have gained in the science and technology of chemistry, physics, and engineering. In fact, I want to offer

you a figure.

Let me just pause for a minute and offer you a figure. The United Nations says that 11 million children die each year due to preventable causes. That is 900,000 children each month, 30,000 children a day that can be saved if we apply our knowledge now to provide water treatment systems, to prevent malaria, to prevent infectious diseases.

What about environmental factors? Again, despite the common perception that environmental factors are synonymous with environmental synthetic chemicals, there is limited empirical evidence for an etiological role for such chemicals in childhood diseases.

Susceptibility: susceptibility of children depends on the substance and the exposure. Again, I will not go through this entire slide. The important point to note is that it is incorrect to presume that children are always more sensitive than adults. In terms of metabolism the newborns capacity increases rapidly. By 6 months of age children are usually not more sensitive to chemical toxicity, and in many cases they may be less sensitive than adults.

Low dose: we have gone through this before, but I will go through it again one more time quickly, as shown on this slide. There are serious shortcomings with the low dose hypothesis. The effects have not been reproducible for the same agent across different labs; the effects are not consistent with different agents operating via the same mode of action, and biological significance of the reported effects are unknown.

This is important to note: in March of this year EPA looked at all the collected information from the NTP low dose expert panel report and made a determination. They did not apply any additional precaution. What they said, and this is taken from their statement, is that "it is premature to require routine testing of substance for low dose effects. Additional research is needed to better understand the hypothesis."

Chemical testing provides data that is relevant to assessing hazards to children. This is not a complete slide of all tests; I have just illustrated some of the tests that are run. This slide shows the screening information data set, the OECD SIDS set, or the HPV challenge set.

I just want to point out that even in this screening set, information relevant to children's health is gathered, from developmental toxicity studies to reproductive toxicity studies to genetic toxicity studies, and neurotoxicology is evaluated in every in vivo study.

Mixtures: obviously prediction is difficult. We have heard about additivity, antagonism or synergy. There are many shortcomings and it is difficult to do the research, and I think there was some agreement even today that evaluation of mixtures and risks needs to consider naturally occurring hormonally active agents such as phytoestrogens.

But I want to point out that we have not ignored mixtures issues. At least in the EPA in the US, for more than 10 years, effect additivity has been the common approach employed in communities' risk assessments.

As a final slide, to provide additional perspective for this discussion session, I would like to present the death rates for leading causes of death among persons in the US 1-24 years of age; this slide covers the period 1950-1999. Unintentional injuries cause from 6- to 10-fold greater mortality in children even today than cancer and heart disease. Indeed mortality from cancer is declining, as is mortality from heart disease in young people. Today a child born has about half the risk of dying from cancer or heart disease as someone born in my generation. Thank you.

Mori: Thank you. Next is Dr. John Mclachlan.

Mclachlan: Thank you, Professor Mori, and thanks to the organizers for this meeting, not only for inviting me and my beautiful partner in life but also for organizing this discussion, which I think we will have some good debate after we have our presentations.

This is my grandson Lyle; so I am speaking

today as a father, as a grandfather, as a scientist who has worked on mechanisms of developmental toxicology for 30 years. I am also speaking as someone who has worked with obstetricians, gynecologists and pediatricians to insure that data and ideas that come up in basic science can be applied most effectively to clinical science.

What I will talk about is prenatal determinants of postnatal health. I do want to point out that I am also a member of the board of the Children's Environmental Health Network, which also has many distinguished pediatricians, most of whom are in practice, and who review all of the literature that relates to potential causes of adverse health in children, both from prenatal exposure and from exposure as kids.

There are some things that I think Dr. Becker mentioned that I would be eager to debate when we get to the debate part, but I think one comment I have to make before I proceed is that we certainly are interested today not in whether or not a child dies, but what the long-term quality of life of that child is, and whether or not the environment, however, we defined it, affects that. Here are some of the prenatal conditions that I think are accepted by almost everyone that affect postnatal health.

One of them is teratology, or birth defects: fetal malformations which are shown following fetal exposure to radiation that was done first, drugs, chemicals and viruses.

There are some cancers of childhood that occur so early in a young person's life that almost everyone thinks there must be some known prenatal influence. I do not mean to imply it is environmental; it could be genetic, it could also be some disposition *in utero* that we just do not know.

We also know that cancers in adulthood following prenatal exposure to the synthetic estrogen diethylstilbestrol, or DES, are really established as the first example of transplacental carcinogenesis in humans. The animal models and animal studies for these, I think, have been very similar to what has been seen in humans.

Let us review a little bit what are some of the milestones in teratology and why now we are thinking mostly of long-term effects or subtle defects that have effects later in life.

In 1956, as everyone in this room knows, in Minamata, Japan, mercury, which is known to be toxic to the mother, was also shown to be toxic to the fetus. There were long-term damages to the mother and her offspring. This I think helped us first see that there was no real barrier or protection for the fetus to environmental toxicants exposed to the mother, and if they were toxic to the mother, they could and would be toxic to the fetus. We still had the idea, though, that there had to be some toxic effect of the chemical in order for it to affect the fetus.

In 1961 that idea was wiped out with the findings that a drug, thalidomide, a sedative given to women that had no noticeable side effects to the mother was toxic only to the fetus and caused limb reduction defects in a high percentage.

In case we did not learn that lesson in 1969, in the 1980s, another drug, Accutane, which was a form of what was considered a natural chemical, retinoic acid, a hormone, was shown when women put this on their face for acne, to cause craniofacial abnormalities in their offspring at birth. The mechanism for how Accutane works is now well known, the mechanism for thalidomide less so.

In 1971, we had one other apparent assumption taken away from us, and that is that diethylstilbestrol had no apparent toxicity to the mother or to the fetus, there were no abnormalities or malformations at birth. We only saw the toxicity many years later, and these were functional defects in the offspring, and in some cases, very rarely, including cancer.

Where we are today, and where I think meetings like this are most useful, is to try to bring together recent evidence from scientific research and apply it to the real life problems we may or may not be developing when working with.

This is a slide taken from a paper in

Developmental Biology, by Li-Yan Ma, in which this is one of the first times a study was done linking defects in specific genes to defects in reproductive tract development and the defects based on knocking out or disrupting one of these members of the Hox gene family were almost identical to what was seen with diethylstilbestrol.

So the premise that many people have had over the years that chemicals at low doses and a critical periods of development can alter genes in irreversible ways, I think this paper is a seminal paper to lead us along that direction.

What I think we need to pay attention to, and one of our charges was to look at how we have irreversible effects after prenatal exposures, and that is if we go beyond teratology and birth defects and think more in terms of transplacental toxicology which is a term that Jim Lamb and Ken Koenig and I made up in 1979, I think, where you go beyond malformation and look at what could be called molecular teratology: actually functional persistent defects. If it is persistent, it must be in the genes.

We do not know why hormones could work through genes, since they are not mutagens. There is a whole body of literature that we have not paid attention to in frogs and birds that date back to Bert O'Malley in the 1970s showing an epigenetic memory in genes after they see estrogen in these 2 species.

We now know that genes can be imprinted by estrogens through epigenetic changes in their DNA which include DNA methylation and chromatin acetylation, building in whatever change is there for generations in that cell.

Estrogenic chemicals, natural and synthetic may exhibit this same property. Clearly, it is a matter of when in the cycle of cell differentiation these chemicals are there, it matters how much of the chemical is there, and it matters the form. This, I think, is a common feature we are finding in more and more compounds.

Finally, I want to say that the collaboration among Japanese laboratories and American laboratories and European laboratories has been one of the most effective I have ever seen in my 30 years of research. I invite all of you to visit our website which is on the bottom. We are sending a signal around the world as a group.

I will put my comments in a much better form on our website and they will be available to anyone who would like to get a more clear presentation and keep the slides, including our interpreter, to whom I did not do such a great job in explaining to him ahead of time what I was going to say. Thank you very much.

Mori: Thank you, and also thanks to all of the commentators for punctual presentations. Now time is exactly on schedule, and next we move to today's morning session. Professor Morita, could you please take over the morning session?

Morita: We heard about risk assessment in the morning session. I would like to add one more, exposure assessment, to today's discussion.

Whether or not children are more or less susceptible to substances, it probably varies depending on the types of substance. This is the target of much interesting and important research. Even if it does not vary, there exists a type of exposure particular to children. I would like to tell you that it is extremely important to be concerned about this problem to protect children's health.

As Prof. Mclachlan mentioned a little while ago, Minamata disease is caused by methylmercury. Exposure to methylmercury in the fetal period would result in a classic case of fetal Minamata disease. Mother's milk contains substances such as PCBs, DDT, dioxins, and the baby is most exposed to substances contained in the milk during infancy. In the case of dioxin for example, children often ingest about twenty times the allowable daily adult dose in this period.

Children tend to have the highest concentration of heavy metals such as lead between the ages of two and four. This happens as a result of children eating for example the dust

and dirt in the house. We must therefore pay strong attention to the fact that children have opportunity to be exposed to such substances more than do adults.

If we arrange this somewhat, we get the following condition: transplacental delivery is important for exposure at the fetal stage; a lot pollutants are known concerning this stage and I think several animal experiments contribute to these findings. Infants have the opportunity for exposure to such substances through milk and baby goods. They are also often exposed to estrogenic substances through cosmetics as we heard today. Because children's metabolism is extremely fast, respiratory volume and food consumption per body weight unit is very high, so air is very important.

An example is provided below. Mass arsenic poisoning once occurred in Japan. The source of the poisoning was powdered milk contaminated with arsenic. The poisoning killed 180 and over 10,000 people complained of minor symptoms of poisoning. It was later discovered that some of those people had developed intelligence impairment.

PCBs, DDT and dioxins have not been clearly identified as having such an effect, but there have been cases of concentrations in excess of the permissible value obtained through the mother's milk and there have also been cases of such exposure through cow's milk as well.

When children grow a little more, particularly when they pass a year, they begin crawling around the house and putting objects in their mouths. Food is an important route of contamination, but there are also toys, objects in the room, dirt, air and water which children may put in their mouths. This is particular to children. Adults for example do not put dirt into their mouths, but a child will eat dirt, so contaminants in the dirt find their way into the child's body. The Japanese Ministry of the Environment has recently begun establishing an environmental standard for soil. In particular, the ministry is about to establish the most stringent standard in the world for lead to tighten up the regulations with the prospect that children eat dirt.

Children may also accidentally come in contact with pesticides; there is a risk of them coming into various substances when they are too young to be taught. In this sense, besides taking note that children may be more susceptible to chemicals than their parents, we must be aware that children have more opportunity to be exposed to such substances, and we must work to reduce the opportunities if they are exposed much more than adults.

Mori: Thank you very much. Next to Dr. vom Saal.

vom Saal: I want to address a couple of issues discussed in the talk I gave this morning.

First of all, it is important to realize that at various life stages there are very different background levels of endogenous hormones. For instance, every physician knows that the effect of drugs and chemicals after menopause in women (when endogenous estradiol goes down to very low levels) are very different than effects prior to menopause in young adulthood.

Interestingly, during aging in men endogenous estradiol levels relative to testosterone go up, whereas in women at menopause they drop off. There are also many other changes in the endocrine system at different life stages. As a result, studies in adults are not going to have any relevance at all to the physiological state of pregnancy and the types of hormones that are present in a pregnant woman and the hormones to which the fetus is exposed.

Clearly, the background level of hormones has to be considered as an important issue with regard to where the presumed no effect level is for the exogenous chemical that is acting in a similar manner to this endogenous hormone.
For instance, Dr. Becker is still referring to the fact that the studies that the chemical industry sponsored did not replicate my findings. Now that we have done the follow up research to understand how that could happen, we have found is in the chemical industry study conducted by Cagen, the endogenous levels of estradiol in

fetuses were dramatically elevated through the use of a different type of food that has an endocrine disrupting effect associated with it. As a result, Cagen increased the endogenous level of estradiol to a point where it actually was producing a maximum response in the male reproductive organs. This is important because in the developing prostate, we know that there is an inverted-U dose response curve for the effect of estradiol on prostate size. Once the prostate is maximally stimulated by estrogen, it cannot respond to more estrogen by becoming larger.

After you have reached that maximum stimulation point, you cannot see low dose effects of bisphenol A anymore. This is what Dr. Myers was referring to in terms of being worried about experiments where false negative conclusion of safety of bisphenol A are being drawn because those conducting the research do not understand all of the background hormonal changes that occur when experiments (such as the Cagen study) are not proper replications.

One of the issues here is what is normal, what is adverse, what is acceptable? Clearly, for instance there are occasionally animals in our lab, a very small number, maybe 1 or 2%, that have high levels of estradiol. This is a different graph than before; this time the estradiol dose is across the bottom, and the number of animals responding is on the y-axis, the frequency.

In response to very high levels of endogenous estradiol, we see animals with enlarged prostate and damage to the neck of the bladder where the urethra enters the bladder and the sphincters control urination. When you are exposed to bisphenol A, we will be publishing a paper showing that this region of the urethra is malformed, it is damaged and estrogens of any kind damage this system.

Does the fact, that this malformation occurs naturally, mean that it should not be labeled as an adverse effect of exposure to bisphenol A? If bisphenol A results in 100% of the population now shows what maybe could occur naturally as a malformation in 1-2% of the population, is it reasonable to declare that the bisphenol A effect is not adverse? Birth defects do occur in about 3% of babies born of unknown causes.

Here is a situation in which with chemical exposure the entire population now has malformations. So I think just because something can occur due to a natural cause (such as elevated endogenous estradiol), if a chemical that mimics estradiol causes everyone to have that abnormal characteristic, it would seem to me that would fit the definition of an adverse effect. Thank you.

Mori: Thank you very much. Next, we move to the afternoon section. Dr. Toppari, please.

Toppari: Yes, I would like to make a comment as a pediatrician that we all appreciate the improved health of our children, and as a Finn I am very interested in perinatal mortality, where Finland has long been competing with Japan for the lowest figures, and Japan has passed Finland as the best in the world in that section.

Still, I think there is no contradiction here. We all want the good health in developed countries and in developing countries. But at the same time, we do not want to pay any cost for that, that we would do it by any means.

We appreciate that malaria can be prevented by the use of DDT. Still, we would like to have some other chemicals to fight against malaria and not to use DDT, because we know all the bad things about DDT.

So when we appreciate the chemicals and technology, at the same time we want to be sure that the technology and the chemicals that we use are safe for ourselves and to our children, and that is why we are here. I think that we all agree on that. I would not like to raise any contradiction here but rather work all together. I think just to save time I will limit my comments to that.

Mori: Thank you. Finally we have Professor Hirahara.

Hirahara: We don’t have much time so I’ll keep it simple. I view this issue from the perspective

of congenital birth defects. As Professor Mclachlan mentioned a little while ago, the number one factor in the infant mortality rate in advanced countries is congenital birth defects. The system for monitoring congenital birth defects originated from the thalidomide tragedy occurred in the latter half of the 1950s and has since become able to identify what drugs are teratogenic that cause congenital birth defects .

As was previously mentioned, materials that can be identified by such one-to-one correspondence as the toxicity of vitamin A derivatives have been found by making a thorough investigation. Concerning problems such as chronic low-dose exposure and compound exposure, which are today's themes, with these as an opening, how to approach monitoring of congenital birth defects is a huge problem.

Work that was originally the responsibility of the Ministry of Health, Labor and Welfare has been turned over to the Ministry of the Environment, and has been provided to us as a research theme. You could never lightly say there is something wrong with the data that was discussed today. However, if we don't continue to conduct solid research, it will be like we just took a slice out of a mountain. I recognize the problem this time that we should become aware of a need for solid research .

Mori: Thank you very much. Now let us start the discussion with each panelist and commentator. Anybody want to go first? Dr. Myers?

Meyers: Thank you. I embrace with Dr. Becker the success that we have made over the last century in improving childhood mortality. There is no question, but we have benefited from a great deal of progress. But why stop there? We have signals in the environment in our public health data that there are new problems emerging.

These data are from the state of California in the United States, showing the rate of autism in children. The number of autistic cases diagnosed in California by a function of year of birth showing a fairly strong increase post-1980. There are some debates about what percentage of that is due to changes in diagnosis; a recent study has basically concluded that there may be some diagnosis effect, but there is a huge increase in autism rates in California.

So why do we have to stop with improving infant mortality? Do we have to admit defeat?

Becker: I think we are in violent agreement, and disagreement on some other matters. Certainly, we do not need to stop, but I want to provide a perspective. Again we need to think about how to improve the lives of the children throughout the world. Now just to comment on this slide, because it is incorrect. This represents utilization of services, not diagnosis.

Myers: No, actually.

Becker: The original report represents utilization of services, and some things happened in the health care program in California that shifted utilization from private sector to public sector over some of that time period. So it remains to be determined – the actual rate of autism. In fact, CDC has said that that clearly in the United States there are no solid data on rates or trends of autism that are available today.

I would like to just continue and comment. Another point, there was a comment on exposure and certainly we all recognize that again, the issue of exposure and developmental sensitivity or windows of sensitivity. I think we are well beyond the case of discussing whether or not it is just the dose that makes the poison.

Of course, there are susceptibility issues. That has been well recognized for many, many years now. So we have to take into account the different sensitivity of the developing organism. But I just want to point out that in standard toxicology studies that are conducted for products and for materials, developmental toxicity studies are included, and they have been included for a great number of years since the body of science

discovered the issue of thalidomide and became aware of concerns related to perinatal toxicology.

Reproductive studies as well have been included to try and get at these endpoints. Certainly, does improvement need to be made? I think we all agree improvement needs to be made. We are working globally through the OECD to develop and standardize and validate additional models. But we need to get beyond this issue of some of the rhetoric and focus on the science.

Now one more point about exposure if I could, please. Exposure does not equal to risk, and we recognize that. So we need to do in the context of risk assessment to consider exposure in the context of what the effects are or potentially could be observed to calculate risk.

Mori: Dr. vom Saal, please.

vom Saal: One of the initial statements by Dr. Becker was that a 6 month old baby might not really be at more risk than an adult, and even in fact at less risk. Apparently Dr. Becker believes that there have been in place developmental toxicological studies that would be capable of predicting they types of damage to fetuses that we are discussing here.

Dr. Mclachlan presented data about the adverse effects of DES on development. Traditional developmental toxicology studies require gross malformations to detect anything, so our prototype endocrine disrupting chemical, DES, completely fails as a problem chemical in the traditional developmental toxicological test. DES would be predicted to be completely safe, and we know that conclusion is absolutely not true.

In the 1993 National Academy report on pesticides in the diet of children, a statement was made by this esteemed panel of physicians and scientists that I think everybody here would agree with: children are not little adults. That is the working premise of every pediatrician and of every developmental biologist with regard to chemical or drug exposure.

I think that the idea of heightened developmental sensitivity to chemicals is now accepted. It is also accepted that the prior lack of appropriate studies with regard to the traditional toxicological approach in which only severe toxicity and malformations were detected allowed harmful endocrine disrupting chemicals to be declared safe. Also, the long latency outcomes following developmental exposure is another new issue that we need to focus on. We thus have to recognize that the chemicals in commerce today have not been subjected to the new tests that hopefully will be soon be approved that detect the types of adverse effects that we now know are caused by endocrine disrupting chemicals.

Mori: Thank you. Also, how about Dr. Morita from the risk assessment?

Morita: What we face when assessing risk and in the process of switching to risk management that determines standards for risk assessment is always the lack of scientific information. What we can often do just as a certain action is needed is to take the approach of minimizing risk by eliminating those at relatively high risk although there may not be absolute risk. Thus, because children have a much higher risk of exposure, it is necessary to create standards for them or develop products to minimize exposure of them. I therefore sense that this has to do with the wisdom of our lifestyles.

Mori: Thank you very much. Is there any comment?

Mclachlan: A general comment that I think is important in dealing with all of the science we already know is that environmental chemicals, if adults are exposed to them, may or may not have adverse effect. There are ways to look for that.

In children and in fetuses, there is a big difference, and that is what we could call a change of state in which we are not just looking at something that comes in and goes out as an adverse effect. I am actually thinking of some work that was done, I am looking at Jim Lamb sitting out there who collaborated with me many

years ago.

What we found, or what Jim found, and I was a coauthor with on several papers, is, if a mouse is treated with an estrogen early in its life as a fetus, you irreversibly change the pattern of localization of cells in the uterus, cervix and vagina, and that pattern is changed and stays changed.

If an adult mouse gets estrogen and it is withdrawn, the pattern goes back to where it was before. I think that is something that in all of these discussions we have to keep embedded in our thinking, whether it is lead affecting a developing nervous system or whether it is a hormone, there is a change of state that occurs during development that persists throughout the lifetime of that individual.

They may not show up as a direct damage at the time, but that is embedded in the system and it stays often for life. That is one of the things I think we have to definitely emphasize.

Toppari: Could I show a couple of pictures I brought with me just to remind us of some basic facts of development of reproductive organs that we know, but I think it has not been shown today, so I will just show them again.

First there is a big difference between the male and female fetus in terms of endocrine activities. The male fetuses are very active in producing testosterone, and that activity is in 2 phases, which here we see only the postnatal peak of hormones that we see in the boys, whereas the ovary is quiet all the way to puberty.

Boys have also very active testosterone production during fetal life, so any endocrine disruptor that could affect this hormone production, either during the fetal period or perinatally, might have an effect.

In the girls, if there are exogenous hormones they might have effects in their system as clearly demonstrated by diethylstilbestrol, which did affect the reproductive system of girls quite drastically.

Next shows the basic facts of the testicular differentiation, which we heard about yesterday night. Again in the boys the sexual differentiation in humans is critically dependent on hormones, whereas in the girls it is largely hormone independent. There are genes like Wnt4 that is needed for development of the ovary and other genes, but it is largely hormone independent.

This has, of course, big consequences when we think of endocrine disruption. Then, anything that disturbs the hormones that regulate the male sexual differentiation will cause undermasculinization or feminization, whereas in the girls any additional hormones, both estrogens and androgens, might cause harm. We know about androgens, they virilize the girls, if they are exposed during these weeks to any androgenic compounds or if their endogenous androgen levels, for some reason, are increased.

I think this has to do with what Fred vom Saal has tried to tell us many times now, that the background hormone levels and the endogenous hormone levels are very important and it is very important what happens to our endogenous hormone levels by the exogenous compounds.

Phytoestrogens seem to decease the endogenous hormone levels or endogenous estrogen levels when there are measurable levels, and then they function as agonists when there is no endogenous hormone production. The same can be true for many other compounds. Thank you.

Mori: Do you have any comments?

von Saal: I had one follow comment to that, and that is that we know, for instance, that female fetuses do not have the kind of endocrine feedback systems that an adult would have. An important element of endocrine disruption in the fetus is that a lot of toxicology is based on the premise of repair systems being activated in response to toxic insult.

But endocrine disruption at low doses is not perceived as a toxic event in the fetus, and in fact the regulatory systems that would adjust hormone levels in the adult are different in the

fetus and totally unpredicted by anything that you see in an adult. So this is a very important element in endocrine disruption.

The fetus lacks the adult type of homeostatic systems and repair and regulatory systems that we are used to thinking about. This is why insult to the fetus by chemicals leads to such tremendous unpredictability of outcomes, because there is so much about what is going on in the fetus that we really do not understand in terms of how the control systems work. What we do know is that they do not work the way they work in the adult.

Mori: Dr. Becker, please.

Becker: A short comment here and just to reminder, I think, to all of us that many of these so-called environmental hormonally active agents in the environment, when you look at how they act, and classically you are talking about acting through receptor binding, their affinity for the receptors are hundreds, thousands, maybe even a million or more fold less potent than the endogenous substances, certainly less than DES.
I think we need to also keep in mind, as I think people would agree that it is a combination of the dose, the timing, and the susceptibility or the sensitivity of the window. But let us not forget the dose issue.

Mori: Thank you. Any more comments?

Mclachlan: Just to make another comment on what you just said, Dr. Becker. One of the things that we have not spent as much time on is looking at the dose to the cell and target cell itself.

A lot of these compounds are carried by serum binding proteins and others. DES is one of those compounds that actually does not bind serum proteins but binds the receptor. Estradiol, the natural hormone is sequestered by serum binding proteins, and there are similar effects with synthetic chemicals that do not bind serum proteins but bind the receptor, phytoestrogens, which binds serum proteins and less to the receptor.

So I think that one of our challenges and something that makes it not easy to just say their receptor binding capacity is orders of magnitude lower, therefore, they possibly cannot do anything, is to know how they move within the cell and I think it is not intuitively simple based just on receptor biding data alone.

vom Saal: Mr. Mori, do you mind if I make one additional comment? The way I got involved in bisphenol A research is our finding that essentially bisphenol A bypasses the plasma glycoprotein barrier to estradiol entering cells. As a result, the biologically active concentration of bisphenol A is much higher than the biologically active fraction of estradiol.

When you actually look at what gets into the blood and then what gets to the receptor, a lot more bisphenol A gets by the blood proteins into the cells relative to estradiol, and that needs to be fit into the mathematical equation of potency. The only reason I am studying this chemical is our initial finding about bisphenol A and plasma binding proteins which got us very interested in the true potency of this chemical.

Mori: Dr. Myers.

Meyers: I would simply remind all of us here about Dr. Kortenkamp's presentation today which provided some pretty strong evidence against the simplistic use of the sort of calculations you have just cited.

Becker: I was not implying that you could use those as calculations, but just as a reminder that these substances generally tend to be much less potent in those types of assays. Now how that gets applied *in vivo* becomes a different story. I do not think that even Dr. Kortenkamp indicated that his *in vitro* assays cannot be translated directly into *in vivo*, because it is a much more complicated system. Just that clarification.

One more point, I think it is important just to make regarding breast-feeding, and I think that

this is just a public health announcement. Everybody who has looked at this from a public health standpoint, WHO, and all of the other organizations, have said that breast-feeding is the best. Even with the levels of contamination that one is seeing, that should not scare women away from breast-feeding. So I want to be very careful about that, and I am not a pediatrician and I will turn to one of the pediatricians to make sure we get that message there.

Toppari: There we agree that the breast-feeding is the best and that is why we want to prevent contamination of breast milk to reduce the amount of toxic compounds through it, because it is good but we do not want to make any harm with that.

Becker: But we would not want a woman to abandon breast-feeding because of concern, because the benefits to the child are so tremendous.

Toppari: Yes, that is for sure, and that is why we also want to make sure that the breast milk is in every sense the best thing that kid can get.

Mori: I agree with you here. I want to ask to today's morning speaker, Dr. Kortenkamp, is there any trouble with the mixture especially the focus on the fetal or children's risk assessment. Could you please?

Kortenkamp: That, at the moment, on the basis of our data which I have presented this morning, is difficult to say. However, I would like to emphasize one point: we have used our assay as a simple model in order to probe a little the idea that endogenous hormones are so powerful that we can disregard the effects of less potent xenoestrogens, in this case.

Although I am very careful and cautious to extrapolate the findings of our in vitro assays into more complicated scenarios, I would like to stress and reiterate that really just by looking at the potency of hormones and taking this out of context and using this to argue that we can disregard the weaker active agents is absolutely untenable. This you cannot do in my opinion.

It will require a lot more work to repeat similar experiments in more expensive in vivo models. We are about to do this, I hope. We have to wait.

One can perhaps use the basic principles we have established in our simple assay to make predictions. One prediction I would like to make, I am sticking my head out here quite a lot, on the basis of fundamental pharmacological principles I would predict that we would see similar things in *in vivo* assays, although I admit it has to be proven.

Mori: Thank you. Is there any comment from Dr. Morita?

Morita: Along with assessing risk come several points of discussion. Although it is fact that we still do not have enough scientific knowledge, I would like to add another argument concerning mother's milk. The question of whether or not to breastfeed has come up three times in Japan.

The first time the question of breastfeeding came up was when mother's milk became highly contaminated with DDT around 1970.

The second time was about 1975 when the question of whether or not it was OK to breastfeed with milk contaminated with PCB.

The third time was around 1990 when there was a question of whether or not it was OK to breastfeed with milk contaminated with dioxin.

The conclusion of course was to advise people to use the conventional method of breastfeeding supplemented by powdered milk for those who were worried about contamination. There however was a question amidst all this that mother's milk should not be contaminated. Thus there is a problem with continuing to produce such substances until risk occurs. My first impression therefore is that we should be more proactive when it comes to children's health.

Secondly, concerning PCB contamination,

use of PCBs was first halted in Japan in 1971. Up to then, PCBs were considered to be the safest, most stable and easy to use and therefore have continued to be produced. When we first began using PCBs we used them recklessly by touching them with hand.

Toxicology has advanced as concern about PCBs has spread since 1971. We have since found that PCBs are toxic substances that affect reproduction as well as others. Recently we have also learned that they have a subtle negative effect on the thyroid function.

If we look at the history of toxicity assessment of such substances, we must eventually decide whether to quit or continue assessment, and I feel that there is a need to consider regulations to a certain degree to be on the safe side.

Mori: Thank you very much. Regarding mother's milk, I see Professor Tada on the floor. Do you have something to say concerning this?

Tada: My field is neonatology and pediatrics. As was mentioned by various speakers in the various discussions we heard today, there is a question as to what each and every endocrine disrupter may do to children. As a clinician, just as there are cases of hypospadias, I think it is extremely important to compare both to see what the effect on children is.

We heard today that the number of autistic children is increasing. It is a fact that children in Japan have begun behaving oddly. We sense this keenly in a clinical sense. Because there are various elements, however, it is extremely difficult to connect each fact with each element.

As Professor Morita just explained, we are monitoring dioxin in mother's milk. By regulating dioxin, we can gradually decrease the level of dioxin in the environment. We are looking at the effect of this, but that is not all. There are also various effects of substances other than dioxin. I hope that clinical research for children would be somehow tied in with toxicological research. I would also like to receive such information in the future.

Mori: Thank you for your comment. Are there any additional comments from the panelists or commentators? We are very close to the finishing time here. Dr. Becker.

Becker: I was looking through my notes here, and I think there is some degree of agreement between Professor vom Saal and myself on this issue of variability and trying to define what is normal. Because I think that is critical to pushing forward our understanding of the scientific status.

Perhaps some additional experimentation with different diets, with different strains of animals, looking very carefully at normal distributions, hormone levels, and of development, might really provide a key set of data and data bases from which we can then can evaluate subtle changes, whether or not they are adverse, or effects of environmental chemicals or just alterations in normal physiological data.

Mori: Thank you. Now the time is just 5:00; we have to finish and close this discussion. Thank you for all of the participants and all the audience; we had a fruitful discussion. Thank you. Thank you for you attention. This brings our discussion to a close.