Effects of Preimplantation and Prenatal Exposure of Endocrine Disrupters on Next Generation

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Let me begin by thanking everybody concerned in this symposium and everybody at the Ministry of the Environment, Government of Japan, for providing me with the opportunity to deliver a special lecture at the fifth International Symposium on Environmental Endocrine Disrupters today.

I am an obstetrician-gynecologist. Our duty is to examine mother and fetus from the early stages of pregnancy, and after the birth of a healthy infant, see the mother and the baby released from the hospital together. Being able to experience the joy of a new birth and hope that the baby grows up to be happy, I think it is a wonderful profession.

Recently however there has become a problem whereby the intrauterine environment during the fetal stage is connected with postnatal health and various illnesses as well as growth of the fetus. Thinking back on it, about fifty years ago, DES administered to expectant mothers as a miscarriage preventative had an effect on the fetuses, resulting in vaginal cancer in females, a historically bitter experience. This indicates that, when considering the issue of environmental endocrine disrupters, we must not forget that fetuses are highly sensitive to substances in the environment including chemical substances. The effect on fetuses may be expressed by the term “effect on next generation.”

Although late, the issue has been taken up from this perspective as a research theme in recent years. It is somewhat bold of me to stand up here in front of medical experts with many years of research experience including those from abroad, but I would like to consider this issue together with all of you here today.

We have become a “chemical civilization” whereby our lifestyles are supported by chemicals we make, which weren’t very much of a problem a hundred years ago. As was mentioned prior to this lecture, endocrine disrupters, also known as “environmental hormones,” have contaminated our environment, and the effect of endocrine disrupters are described as causing reproductive disorders in all sorts of wild animals, as shown here, such as birds, fish, shellfish, mammals and feminization of alligators as Dr. Guillette of University of Florida pointed out.

Endocrine disrupters, or environmental hormones, are new chemical substances created by human beings that affect the action of hormones, primarily estrogen. The chemicals are characterized by being hard to metabolize and hard to discharge. They therefore build up in the human body. If the accumulation of chemicals exceeds a certain limit, they may have an effect on the next generation or could cause a problem that could affect the future of mankind.

You have probably heard a lot about substances to be possible endocrine disrupters. Representative examples include dioxins, DDT and PCBs. Because these substances are toxic, we stopped producing them twenty or thirty years ago. Once the environment is contaminated, however, the chemicals can still be detected in our bodies and even in the bodies of infants.

200,000 to 300,000 tons of chemicals such as bisphenol A, phthalic acid and nonylphenol are consumed in Japan annually. Bisphenol A, for example, is used in the coating of canned drinks such as coffee that touches the lips of the consumer. We should also take note that phthalic acid of the microgram order is found in cooked rice that has been touched with PVC gloves. The Ministry of the Environment has also pointed out the danger of nonylphenol, which is considered to be a possible endocrine disrupter. A considerable amount of nonylphenol comes out in food wrapped in plastic food wrap when heated in a

microwave oven. There are all sorts of endocrine disrupters in our environment, a considerable amount of which gets inside of our bodies.

With that as one point of view, in the case of effect on the next generation, the life cycle of the next generation whereby life which begins with fertilization and then develops into an embryo, grows in the oviduct and uterus, develops through the medium of the placenta in the uterus, and then grows into a new child of next generation, is a mechanism controlled by various hormones. The hormone that plays most importantly is estrogen. Environmental endocrine disrupters disrupt the effect of estrogen. When this happens, we need to see to what degree the environment is currently contaminated and to what degree endocrine disrupters affect our health and the health of the next generation.

Today I would like to talk mostly about dioxins and bisphenol A. We obstetrician-gynecologists are using a technique called "*in vitro* fertilization." With this technique, we ascertain the condition of the ovum in the ovaries using ultrasonography, and vacuum out the contents of each ovum at a certain stage. Here we have follicular fluid and here we have seminal fluid. *In vitro* fertilization is accomplished by manipulating the sperm, fertilizing the ovum with the sperm and then returning the fertilized ovum to the uterus. About hundred thousand *in vitro* fertilizations are conducted in Japan per year. About ten thousand children have been born using this technique.

Follicular fluid is the fluid that nourishes the ovum. If you analyze this fluid, you will find it is contaminated by dioxins at a certain concentration and there is no one in the world who is not contaminated. If you check for endometriosis, you will find that both those with endometriosis and those without are contaminated with dioxins. Dioxins can also be detected in sperm at considerable concentrations. What surprised me was that the sperm of normal people contains a considerable concentration of dioxins. We also found that the seminal fluid of people requiring treatment for "oligospermia" whereby the sperm count is abnormally low contained relatively little dioxin. So then we want to know about dioxin in the blood. When we measured dioxin content in the blood, we oppositely found the concentration to be lower in normal people and higher in patients with low sperm counts. What we were ultimately able to confirm that individual sperm cells themselves were contaminated. People with a high sperm count therefore have a relatively higher concentration of dioxin in the seminal fluid. This suggests that people are contaminated with a certain amount of dioxin from the moment the ovum is fertilized by the sperm, the very beginning of their existence.

The next thing is, because the baby gets its nourishment from the placenta, we obtained informed consent of expectant mothers to take samples of blood from the umbilical cord at birth, the mother's blood and amniotic fluid at the same time. I'd like to show you the results. The mother's blood contained approximately 20 pg of dioxin at the time of birth. The blood samples taken from the umbilical cords at the same time contained 10 pg, about half that amount. This means that dioxins migrate to the fetus via the placenta. There is somewhat of a concentration gradient with a positive proportion whereby; the concentration in the mother is higher, the concentration in the baby will also be higher.

Another thing that must be noted is the fact that the amniotic fluid contains a significantly high concentration. Once dioxin migrates to the fetus through the placenta, it circulates in the fetus and contaminates the amniotic fluid, which serves as the environment for the fetus. If you analyze this according to the number of times the mother gives birth, the concentration of dioxin in the mother's blood gradually drops each time she gives birth. This means that the mother is passing the accumulation of dioxins in her body on to the fetus when she gives birth and through the mother's milk, causing the concentration in the mother's blood to drop. The blood in the umbilical cord also indicates the concentration in the firstborn child is significantly higher than the second and third.

This means the contamination in the mother's body is passed on to the fetus, and by doing so presents us with the problem of how this affects the next generation. An examination of the dynamics here

shows that the concentration is somewhat less at the late stages of pregnancy and delivery than at the early stages for the first pregnancy. This is accounted for by the portion that migrates to the fetus via the umbilical cord and placenta. Contamination is halved for the second birth. This is possible due to the portion that migrates to the fetus via the placenta plus through the mother's milk. Contamination tends to increase proportionally with age. One problem with this is the fact that, in Japan, children that are highly contaminated with dioxin are being born because the birth rate is low (1.3 children per couple) and the average age of the primiparity is rising.

We also studied contamination of follicular fluid and seminal fluid with bisphenol A in the same manner as was previously described. When we measured bisphenol A in 30 women, a concentration of 2 ng/ml was detected in all 30. Approximately the same amount was detected in follicular fluid and seminal fluid, so we think that, just as with dioxin, bisphenol A contaminates the reproductive organs, and it implies that the contamination also extends to the ovum and sperm. If the concentration in the follicular fluid is high it doesn't mean that the results of *in vitro* fertilization will be bad. I don't think the contamination is at such a level that it will inhibit the ability to fertilize.

If you compare the level of concentration of bisphenol A between mother and child, concentration in the mother is lower in the early and late stages of pregnancy than when not pregnant. Higher levels of concentration are oppositely detected in umbilical cords and the babies' blood than in the mothers' bodies. The reason for this is bisphenol A contaminates the fetus through the placenta. Although bisphenol A is easier to metabolize than dioxin, it contaminates the fetus through the placenta. Another problem is the fact that the concentration in the fetus is rather higher than in the mother with a positive proportion. The amniotic fluid is the part surrounding the fetus in the uterus and called the fetus' "inner space." The fetus' urine and secretions are mixed in with the amniotic fluid, and the fetus swallows and absorbs the amniotic fluid. This fact makes it even more fetus' inner space. A little while ago I have showed you the data obtained during childbirth including cesarean sections. We also examined amniotic fluid in the early stages of 15 and 18 weeks as part of our prenatal examinations.

According to the latest data we have obtained, a comparison of mothers' blood at the early and late stages of pregnancy with amniotic fluid of 10 months such as at maturity or cesarean section, shows the amniotic fluid to be contaminated at about the same level as the blood. The concentration of bisphenol A detected in the amniotic fluid in the early stages of pregnancy from 15 to 18 weeks is about 5 times greater than that in the later stages. A study of this shows free bisphenol A that has not been metabolized accumulates. The results of glucuronidation by the liver affect the mother's body. As birth approaches liver function becomes mature even in the fetal stage and is able to cope with it. Premature fetuses however are unable to metabolize bisphenol A, and it oppositely becomes concentrated. If you oppositely keep in mind that the fetus is highly susceptible in the early stages of development and the concentration is relatively high during the period in question, I think it may be necessary to come up with various countermeasures and safety limits.

When we studied concentration of bisphenol A taken on an empty stomach, we got some unexpected data. When measured on an empty stomach, concentration of bisphenol A was statistically significantly higher in males than in females.

More than the fact that males have more opportunity to ingest bisphenol A from canned drinks such as coffee, we believe that there is a variation or difference in metabolism according to sex. Obstetrics and gynecology includes a disease called polycystic ovary syndrome, or PCO. This disease inhibits ovulation and causes an increase in male hormones, hairiness and infertility. The cause of the disease is not clearly understood, but in all cases, we know that the concentration of androgen, a male hormone, is high. When we measured the concentration of bisphenol A in such patients, we found the concentration to be higher than that of females at large. This is because there is a metabolic function of bisphenol A related to

androgen, which is not as strong in males as in females. Bisphenol A is therefore surmised to be higher for the pathology of PCO.

If we take a look at the relationship of male hormones and bisphenol A, we can actually see there is a correlation. If you take the fact that metabolism of organisms varies according to bisphenol A, a substance unknown to mankind, it will provide a clue when we make a study on PCO, a disease that is not well understood. We indicated 1 to 2 ng for bisphenol A, 10 ng in some cases, and a high concentration when concentrated in the amniotic fluid. How should we go about studying this? If we convert this to molar concentration, 5 or 10 nmol. This means that bisphenol A exists in the environment on the nanomol level. Environmental contamination on such a level – sometimes 1 nmol – in sewage and rivers means the same concentration exists in our blood, follicular fluid, seminal fluid, as well as in the blood of our fetuses.

How should we evaluate contamination in such cases? Is it something that can ultimately be tied in with people's health and effect on the next generation? Animal experiments are a way to see whether such substances are still at a level that we don't have to be alarmed about. I think animal experiments are good for studying the cycle that creates the next generation.

As a famous experiment, there is one report by Dr. vom Saal and his group concerning bisphenol A. The report involves administering extremely small doses of bisphenol A to pregnant mice and then looking at various indices for the babies born. The babies were born normally without miscarriage or stillbirth compared with controls. The body weight at birth was also normal. The babies' weight increase after birth was however significantly higher for those whose mothers were administered small doses of bisphenol A. It was also reported that female babies born to mothers injected with minute quantities of bisphenol A reached sexual maturity faster than those born to mother who were not. In the case of human beings, this would be the equivalent of reaching the age of the first menstruation period. An analogy may be drawn from these findings to the fact that in developed countries, the first menstruation period, menarche, has advanced from 16 to 12 years of age.

One of the reasons for this Dr. vom Saal thinks that chemical substances act as a toxin at strong concentrations of the milligram or gram order. Of course substances such as dioxins, PCBs and DDT are poisonous at high concentrations. At extremely minute quantities such as a thousandth, a millionth, or hundred millionth of that which actually exist in the environment, we get the low-dose effect, which acts differently from large doses of toxic substances. This suggests that if exposed to bisphenol A at low doses in the fetal stage, changes could occur after birth in weight and in time it takes to reach sexual maturity.

One of the techniques we are using for *in vitro* fertilization involves cultivating an ovum – a fertilized ovum consisting of 2 cells – to an 8-cell embryo. We cultivate this another 24 hours to form a blastocyst, which is the part that actually becomes the body and the placenta, and allow them to grow in units of time. When the blastocyst is cultivated, it is hatched and implanted in a dish where the portion that will become the placenta grows. By measuring the dimensions, we can see how much it has grown. In such an experiment, by the way, we can obtain ES cells which have become the topic of much discussion recently. This experiment involves observing what happens in terms of indices of dimensions, differentiation and growth when specimens are administered bisphenol A.

If you look at the percentage that will become the 8 cells, at 100 μmol, there is quite a high concentration, but the cells are not affected. If you look at the percentage that will become the blastocyst, however, it is significantly suppressed at 100 μmol. At a concentration of 100 μmol, bisphenol A suppresses growth and differentiation of the embryo. At 1 nmol or 10 nmol, the level that actually exists in our bodies, it oppositely speeds up development of the embryo rather than suppresses its growth. It there works in the opposite manner. At the next step where we cultivate these blastocysts and evaluate the dimensions, growth is spurred at 1 nmol or 10 nmol and suppressed at a relatively high concentration of

100 μmol. When suppressed, even by successive monitoring, it becomes large, but statistically small. At the environment / human body contamination level of 1 nmol, it is promoted.

We compared various effects and concentrations. At 1 nmol, promotion was positive and at 100 μmol, it was negative. The way it works differs according to concentration. We think a low-dose effect is observed at the lower concentrations.

In order to verify this theory, we must consider why this phenomenon occurs. We already know that bisphenol A acts on estrogen receptors. Estrogen receptors include the α type and the β type. Ova originally possessed estrogen receptors. The expression of receptors temporarily decreases when the ova are fertilized and disappears when it reaches 8-cell stage. Receptors are expressed in blastocyst stage and mediate an effect when bisphenol A works even at minute quantities. They are sometimes compared to a key and a keyhole. This can be interpreted as the keyhole has no function until bisphenol A functions as the key. This means that the receptors have an intermediary function.

You might ask what we have done to corroborate this. Tamoxifen is a chemical that antagonizes the effect of estrogen at a concentration that has a promoting effect. It antagonizes on the receptor level. It is used for breast cancer medicine which affects the keyhole. Tamoxifen inhibits accelerator effect and also rescues the suppressing effect. The conclusion is that there is an effect that works through the receptors.

Of course we cannot say that all effects work through the receptors, but at least some such as bisphenol A do. As the ovum grows, lives in the oviduct and grows in the uterus, there is some effect via the receptors at the level in our blood, the level at which we are actually exposed. The following concerns implantation.

In vitro fertilization and embryo transplantation is a technique whereby an embryo is returned to the uterus and a child is born. This applies to human beings as well. What we have done is to expose embryos to bisphenol A during preimplantation period and then return them to the uterus of a recipient mother that has not been exposed by bisphenol A. This means you can tell the effect of bisphenol A on the ovum in the uterus such as causing miscarriage, implantation not to take, stillbirth or deformation by looking at the baby after birth.

Out of 7 embryos, you had obtained 4.3 babies under ordinary circumstances. If administered 1 nmol of bisphenol A when still ova, you should get 4.1, which is not different from ordinary circumstances. Those for which growth was somewhat suppressed by 100 μmol produced 5.3 after implanted. This indicates that implantation rate and miscarriage were not affected at all. Weight at birth was also unchanged.

What surprised us was, whereas weight at weaning during the third week was normally 9.7, the specimens administered bisphenol A when still in the ovum stage weighed slightly in excess of 13.0. This shows that they developed more than 30% faster. It has become apparent that with exposure when still in the ovum stage, although the environment of the mother's body doesn't change at all, the substance has some effect on postnatal growth, thereby having an effect on the next generation.

This agrees almost perfectly with the results of Dr. vom Saal's group administering the substance to the mothers body in the fetal stage. Ova exposed to bisphenol A prior to implantation and fetuses exposed while still developing resulted in exactly the same change. We can therefore say that exposure to bisphenol A during the fetal stage or when the ovum is fertilized has some sort of effect on the next generation.

Because of this difference in growth rates, it is necessary to view this comprehensively.

I think the DNA microarray method of analysis is an important research method. Although it is not limited to bisphenol A, if you look at the expression level of genes while the embryo is at the initial stage of development when exposed to various chemical substances or substances said to be endocrine disrupters, the genes for which expression increased as a result of administration of bisphenol A, or the

opposite, are grasped even when the embryo is at the initial stage. When we studied various organs of specimens after birth, we found that expression of genes in testicles of animals for example that have been exposed to bisphenol A in the fetal stages is strange. Even if they possess the same genes, the fact that the genetic expression pattern of the next generation caused by exposure during the fetal stages is a problem that must be clearly stated.

We still do not know if we can really tie contamination by such endocrine disrupters into the changes that are now occurring in mankind. I think we are at the stage where we can investigate the connection between endocrine disrupters and the problems that are now surfacing. What I would like to discuss today however is whether they are actually affecting reproductive function. Since we have also become able to rank the problem of sex ratio – the proportion of males to females born – the same as the problem of environmental endocrine disrupters, I would like to think about this a little. And then endometriosis is a big problem for obstetrician-gynecologists.

Endocrine disrupters may have also given rise to the problem of attention deficit hyperactive disorder, in other words hotspurred children. This is not a problem for which we can draw a conclusion, but I would like to talk about the current state of this problem.

Concerning reproductive function, there was originally the concept of infertility. One in ten couples was infertile; this has been a problem for some time now. Recently this has grown to 1 in 7 couples. The number of people seeking the aid of *in vitro* fertilization has grown to 100,000 *in vitro* fertilizations per year, and 10,000 people have been born through this method in Japan. Of approximately a million people born in Japan, about 0.8 to 1% were born through *in vitro* fertilization. It is very important for obstetrician-gynecologists to raise success rate of *in vitro* fertilizations, but we are currently approaching this from the problem of environmental endocrine disrupters.

Concerning in what cases *in vitro* fertilization is necessary, it is primarily appropriate when the oviduct is blocked because of endometriosis, or it is difficult for fertilization to take place in the oviduct because there is not enough sperm. The fact that such cases are increasing is one of the reasons why there is said to be an effect on the next generation based on animal experiments.

According to Professor Skakkebaek's report, sperm count was once 100 something million. The count has gradually decreased to little more than 50 million during the 1990s. If it continues to decrease at this rate, I have been told that human sperm may disappear altogether. This has been the subject of much discussion, and a conclusion has yet to be drawn.

Concerning problems if sperm count is decreasing, here is the result of experiments. If more dioxin is administered to the mother's body, these experiments have proven that males born to that female will have lower sperm counts. If sperm count is actually decreasing, therefore, there is still no clear conclusion at the level where influence of dioxin must be considered. Many of you attending this symposium are expected to give a lecture that provides an answer to this problem.

Here is the data that Professor Mocarelli provided to "Lancet." Sex ratio is calculated by dividing the ratio of males by that of females. An explosion at the Seveso chemical plant exposed area residents to a massive quantity of dioxins. Immunological studies have been conducted by Professor Mocarelli of Italy and others for a long period of time now and there have been various reports. One of the most well known reports is a report concerning sex ratio whereby, in the case of males being exposed to large quantities (15 ppt or more), there are extremely few males; almost all are females. This does not have much to do with exposure of females. In other words, there is data that suggests that if males are exposed, there could be fewer males in the next generation.

There are still various opinions concerning this, but characteristic of countries the world over is the fact that the ratio of males is decreasing. This has been observed in common in all European and American countries as well as Japan at about the same time.

This shows the sex ratio in Japan since the 1940s. The ratio gradually rose up to about 1970. When we were students in school, boys stopped dying due to sanitation and obstetrics and gynecology. Since olden days, boys counted for a lot of the perinatal mortalities, and even after birth had a higher mortality rate than girls. This is said to be due to improvements in tocology management and diffusion of the concept of sanitation among the public. The trend was however reversed around 1970, and the reason for this is unknown. If we consider and extrapolate the data of Seveso, we cannot currently deny the possibility of dioxin contamination.

This shows sex ratio among miscarriages and stillborn infants. The fact that the percentage of male infant mortalities is recently on the rise is a huge problem for tocology management. Whether environmental endocrine disrupters have anything to do with this is still a research theme in the field.

Another significant disease is endometriosis. Endometriosis is a rare disease that occurs when the endometrium develops in the ovaries, oviduct or peritoneum in addition to the uterus where it belongs. In this way, we learned about this disease when we were still studying in medical school. If you look at the number of patients examined in obstetrics and gynecology these days, while the number of women contracting diseases such as hypertension, cancer and diabetes subsequent to menopause is increasing in other medical fields. Currently the typical female patient in the so-called “reproductive age” receiving hospital examinations has endometriosis.

We checked hospital charts 10, 20. 30 and 40 years ago, the percentage of women suffering from endometriosis, and although there of course were cases 40 years ago, they were limited to only 1% of the total number of female patients. This grew to 5% 30 years ago, 10% 20 years ago and 15% 10 years ago. The number of cases of endometriosis is increasing with the passage of time. This percentage has recently risen to 30%, so the number of women suffering from endometriosis is increasing when we examine our patients.

It is possible that we had not been so vigilant for endometriosis up to now, and may be a problem shared by civilized countries in general. This tendency however did coincide with an increase in exposure to dioxins, but that does not necessary mean there is a connection.

In the research of Rier et al, they gave rhesus monkeys extremely low dosages of dioxin (100 pg), which apparently did not affect their health condition. Years later after the experiments were conducted, they found that 71% had developed endometriosis. Although the percentage for the control group was 33%, when the dose is increased, the percentage increases. Light cases of phase 1 existed among the control group, but advanced progressively as the does was increased to 600 pg from 100 pg.

This report came out 10 years ago and suggested the connection between dioxin and increasing incidence of endometriosis – plus, at 100 pg, the dose was minute – for example the daily intake from mother’s milk in Japan is 100 to 200 pg. The fact that endometriosis is being induced and accelerated at normal concentrations rather than extremely high concentrations was a big shock to us.

Various experiments have been conducted, and research involving human exposure, animal experiments and molecular genetics research have been conducted. To provide a brief description, let me take for example dioxin receptors for dioxin. In terms of molecular genetics, comparing with cells of the ovaries and ovarian cancer, there have been reports genes are highly sensitive to dioxins in the case of endometriosis. This is our data. When we examined surgically removed fat tissue, we found the incidence of advanced stages such as stage 3 or 4 to be prevalent over lighter cases such as stage 1 or 2.

Concern has developed over the connection of it with dioxins. The Rier team says they think endometriosis could possible be caused at the 100 pg level. If this is true, if human beings ingest dioxins on the 100 pg level from mother’s milk, if this is what happens to monkeys at 100 pg, babies ingesting 100 pg would probably develop endometriosis.

In Japan, people have kept a mother and child notebook containing the type of nursing. If you refer to the notebook, you can see what sort of nursing you had. Thousands of people cooperated in this study. 51% of the endometriosis patients had been breast-fed. 68% of people not suffering from endometriosis have been breast-fed. It is undoubtedly a fact that more dioxin is ingested by breast-feeding. Just from looking at this data, you would think there would be a much higher incidence of endometriosis, but there doesn't appear to be that much more. Oppositely I thought there might be some new benefit in mother's milk for decreasing incidence of endometriosis. When I told Ms. Rier about this, I was told that exposure in the fetal stage was more important than breast milk, and that the data was accurate, but she believe that exposure of the fetus to dioxin is important, so we were unable to draw a conclusion.

We just got a report on the correlation between dioxin contamination and endometriosis in the people of Seveso the other day. Here we divided the degree of dioxin contamination into less than 20, 20 to 100 and over 100 and then checked out incidence of endometriosis for the residents of Seveso. We had hoped that we could draw a conclusion from the results, and if you look at incidence, there was 1.7%, 2.7% and 4.3%, and relative risk was 1.6 and 2.8 to 1, and seems to become higher as concentration rises. But the fact that it was not significant, in other words, we can conclude that we can't declare there is a correlation, we can say there is an inconclusive correlation between dioxin and endometriosis. It is a fact however that incidence of endometriosis is increasing, but we currently do not know the cause of it. By tackling the problem of dioxin with animal experiments and molecular genetics as a new opening, I think we can expect a new interpretation and a new breakthrough.

Attention deficit hyperactive disorder (ADHD) has become a big problem recently. This disorder occurs most often in boys. It is characterized by inability to concentrate, impulsive behavior and hyperactivity. The network of the brain is the cause of the disorder.

This is an experiment with rats conducted jointly with Professor Kato's group of the Department of Psychiatry at the University of Tokyo just recently. This experiment quantitatively evaluates the movement of rats in the open field. Compared with females, the males represented here in white in the control moved very little; the males moved around less than the females. The motion gradually decreased the first, second, third and fourth day as they became increasingly familiar with their surroundings. The females moved around more than the males, and gradually moved around less and less. It has become an accepted theory that males born to mothers administered 0.1 μg of bisphenol A move around a lot, about the same level as females. Compared to the control, the movement hardly decreases at all, and does not change that much at 50 μg. Thus, behavior is altered at a minute dosage.

The Morris water maze is used to evaluate the effort that drowning rats make to rescue themselves. Compared with the control, the males exposed to 0.1 took longer to learn. One interpretation/hypothesis is that endocrine disrupters pass through the brain barrier in the fetal stage. Estrogen does not pass through, but bisphenol A does. It also impedes neuron apoptosis and affects secretion of dopamine. Thus there are various hypotheses such as formation of an excessive network making possible syndromes such as ADHD. This has been made clear by such animal experiments. A phenomenon similar to that of animal experiments has been observed, but any conclusions will be left up to future research. This poses a problem.

Finally, as a physician, I would also like to consider a project for taking dioxin out of the body from the perspective of preventative medicine and treatment. I think this would consist of many things such as accidental exposure or eliminating the anxiety about mother's milk and placenta from mothers containing relatively high concentrations. One hint is an extremely high concentration in the bile. We can evaluate about 1 ng circulating in the enteric canal, and our aim is to pull this out. We have tried several methods of cutting off circulation in the enteric canal, but do not have anything that can be shown as data today, but it

is circulating simultaneously with cholesterol. The fact that a drug to reduce cholesterol or to suppress re-absorption of cholesterol provides a chance is what I can tell you about today.

Finally there is no direct relationship between animals and endocrine disrupters, but if you cross a horse with an ass, the offspring will have half the genes of a horse and half the genes of an ass. There will be no difference in the appearance of the offspring regardless of whether the father was a horse or an ass, but the disposition will differ depending on whether the father was a horse or an ass. An ass can withstand extremely rough food and perform hard work, but a hinny (father is a horse) eats a lot, and will not listen to human trainers or work. Even though they have the same genes, the expression is subtly different and they have different dispositions and different personalities.

From all sorts of research data, I showed a little while ago that the effect of endocrine disrupters on the next generation works in the fetal stage. I think various types of research have been conducted, in which it becomes clear that endocrine disrupters may affect gene expression. In such a case, it could change the way a person's heart should be.

More than a thousand years ago, a poet named Okura Yamanoueno wrote a poem singing "There is no treasure more precious than a child." Even a thousand years later our hearts have not changed and we can still understand that feeling. There is no guarantee that people one hundred or a thousand years from now will thrive with the same spirit. If we do anything at all, I think we had at least better overcome the problem of endocrine disrupters.

Although there are many problems, not only myself but also many scientists are working together to overcome the problem of environmental endocrine disrupters. Having been provided the opportunity to deliver this special lecture today, I hope more and more people will work together on this problem from different angles. This brings my lecture to a close. Thank you very much.