Discussion
"Frogs・Mammals / Thyroid Hormone"

Chairpersons: **Katsutoshi Yoshizato** (Hiroshima University, Japan)
Taisen Iguchi (Okazaki National Research Institutes, Japan)
Panelists: **Yun-Bo Shi** (NICHD, National Institute of Health, USA)
Barbara Demeneix (Muséum National d'Histoire Naturelle, France)
Robert J. Denver (University of Michigan, USA)
Ming-Jer Tsai (Baylor College of Medicine, USA)
Jacques Samarut (Ecole Normale Supérieure de Lyon, France)
Sheue-yann Cheng (National Cancer Institute, USA)

Yoshizato: Welcome to the last session of this afternoon meeting. We have heard 2 previous sessions, one for amphibian presentations and the second is mammals. The third one will be, as I said, the joint meeting of amphibian and mammalian groups.

Just keep your attention on this trivial slide. Originally as you can read in the published abstract, this session entitled "Discussion with Frogs and Thyroid Hormones." But I would like to change the title to "Discussion of Frogs and Mammals." Maybe you can understand (why I am making) this change in light of the previous 2 sessions.

Although there are some minor differences between mammals and amphibians for the response of thyroid hormone, we believe that most of the processes affected by thyroid hormone are very common between these 2 species. So, I'd like to change the discussion title to "Frogs and Mammals" instead of "Frogs and Thyroid Hormone."

As a start, I would like to pre-introduce this session using some slides. We have prepared a plan for this session, but we are very welcome to have any interruptions and free talking during this session.

At the start of this session, I will talk about the effect of bisphenol A on the process of amphibian metamorphosis, and Dr. Shi will discuss the following 2 issues: how to make observation in amphibians relevant to mammals, especially humans, and the second issue, how significant is thyroid hormone metamorphosis in endocrine disruption.

As the third presenter, Dr. Samarut, will give some comments on the discussion made by Dr. Shi. Dr. Demeneix will discuss on the OECD proposal for testing endocrine disrupting activity and whether we can come to some consensus on possible tests to recommend. Some comments will be given by European experts for the discussion by Dr. Demeneix. Finally, we will have concluding remarks by Dr. Iguchi.

In the first session of this afternoon meeting, I have said I would present some recent data on the detection of chemicals that affect the thyroid axis utilizing transgenic *Xenopus laevis*. You are now familiar with these TR β A1 promoter sequences.

From this slide, you can see some similarity between T_3 and bisphenol A, BPA. BPA is a chemical from which we can prepare or we can produce various types of plastics. This is a very common monomer chemical to make artificial plastics. As compared to this basic structure of T_3, this compounds lacks this part, but you see bisphenol is common, BPA does not have iodo atoms; instead methyl. So there are some differences in the structure.

We found that this bisphenol A inhibits T_3-induced tadpole tail regression, one of the representative phenomena during the amphibian metamorphosis. We first treated these *Rana* tadpoles with this concentration of T_3. So this is a control as you see experiment. Shrinkage or tail resorption you can see by the yellow line here. So nice tail regression took place in response to T_3.

However, if we treat tadpoles not only by T_3

and with BPA at this concentration, there is no significant difference for the inhibition, the concentration is very high and continues at this level, but there is no significant difference in concentration. But anyway, BPA significantly inhibits the T_3 action on tadpole tail regression.

Now we made a transgenic tadpole as I said utilizing the method as I said in the first session using this vector containing promoter region of *Xenopus* thyroid hormone receptor βA1 genes and EGFP as a reporter gene, and made germinal transgenesis with *Xenopus*.

Also, we have thyroid hormone analogue T_3, this is active thyroid hormone, and this is latent thyroid hormone T_4 and TRIAC and TETRAC. These chemicals are very potent to induce thyroid hormone-like response on the animals. These chemicals are very common in thyroid hormone research. You can see the difference between these groups and these groups.

First, this is a kind of positive control experiment. We exposed transgenic tadpoles with T_3 and T_4 at this concentration starting from 0.1 nM to 1.0 nM. Please note the difference of the concentration utilized; this is very high. Maybe you can see some EGFP response at this concentration for T_4 at 10 nM and maybe here you can see some response for T_3, but at a much lower concentration as compared to T_4.

The same experiment was done by exposing tadpoles with these concentrations of TRIAC and this concentration of TETRAC. Notice that the transgenic tadpoles responded very well for TRIAC and TETRAC by expressing reporter genes. And the dose response was determining the extent of the intensity of EGFP fluorescence induced by these thyroid hormone related chemicals.

You see this response for 1.0 nM T_3 and a little bit less response for T_4; this is very explainable and expected, and also as expected from other experiments, TRIAC and TETRAC had a much stronger effects on tadpoles as compared to the native forms of thyroid hormone.

In contrast, bisphenol A does not affect EGFP fluorescence in transgenic tadpoles. We tested these various concentrations of BPA, and you see this is control and response induced by T_3, but there is no response for EGFP fluorescence expression with this concentration of BPA. This is quite different from the response by TRIAC and TETRAC.

However, it is very interesting to see this graph, because BPA, bisphenol A inhibits EGFP fluorescence induced by T_3. This is T_3 alone: you see this is after 20 hours some response on the hind legs. This response is dose dependently inhibited by bisphenol A at 1.0 nM, and this is equimolar concentration of BPA. A 1000 times higher concentration of BPA completely erases the response of the expression of EGFP.

This is just a quantitative result from the previous photograph. This is the positive response to T_3 alone, and if we treat together with BPA dose dependently the response by T_3 decreased.

So, this is our current idea about the mechanism of inhibition of T_3 action by BPA, bisphenol A. As you know now, with hollow receptors this receptor activates the downstream genes. In the presence of BPA, maybe BPA plays some role as a competitor for T_3, or maybe the presence of BPA interferes the process of the activation reactions by T_3. I would like to introduce the collaborators for today's talk. Thank you.

All the panelists, could you come up to the stage? As we have planned, the first, Dr. Shi will discuss the 2 issues. Could you start your discussion?

Shi: Actually I do not think I will discuss anything, instead I would just like to raise the issue so maybe everybody can comment. Because being someone who works on amphibians exclusively, in fact, *Xenopus laevis* exclusively, basically I have 2 questions with regard to endocrine disruption.

Number 1 is we know from the talks today that thyroid hormone-dependent metamorphosis can be influenced by many non-endocrine disrupters. But the question is, if we ask the reverse question, there are many abnormalities of amphibian development, or defects in nature; how many of those are caused by potential endocrine disrupters, and are those actually relevant in policy making in

terms of affecting human health?

That is, how significant in terms of metamorphosis as a model as a detection or even as a discovery tool in the discovery of potential endocrine disrupters. That is one question that I would like some of you in the audience or some other panelists can answer.

The second question I have is, being that I work on amphibians exclusively, what I want to find out is actually what we need to do as amphibian biologists to convince the rest of the world that whatever we find in terms of endocrine disruption is relevant to mammals and humans.

That is, if we find something, what evidence would you need to see to convince you that whatever our finding is, or how it would be, relevant to policy making, let us say, to allow the drug or potential chemical to be continued to be used or should be banned. What evidence do you need from amphibian studies that will convince you of that? That is just my question, and I do not have an answer for that.

Yoshizato: OK, maybe Dr. Samarut has some comment on this?

Samarut: I might make a comment on the second part of what Dr. Yun-Bo Shi just said. My feeling is that we should not screen for these putative endocrine disrupters only on amphibians, but also it has to be performed on some mammalian models, and I think for 2 reasons.

The first one, and it was illustrated in some presentations, we have some different binding affinities, for example of PCBs to some carrying proteins, which are different between frogs and mammals. Another reason is that maybe some of these compounds might be metabolized differently in mammals and amphibians.

And there is a third reason, which also might be quite important, which is that the diversity of receptors in mammals in terms of isoforms is greater than in amphibians. For example, I am not sure that there are $\beta 2$ and $\beta 3$, at least $\beta 3$… maybe $\beta 2$? Is there a $\beta 2$ in amphibians?

Shi: It is not quite the same.

Samarut: Also, for the α isoforms, clearly there is no $\alpha 2$ in amphibians, because $\alpha 2$ is very specific to mammals. Also, the truncated isoforms, so-called delta α, also are very specific to mammals, and it was proposed quite recently at the ATA meeting that these short isoforms might bind specifically T_4 and reverse T_3 but not T_3.

Clearly there are some major differences in terms of proteins encoded by all these genes and I think this has to be kept in mind for testing putative endocrine disrupters.

Yoshizato: About the diversity of receptors, I was quite impressed by your presentation that double knockout α / β mice can survive. So maybe some unknown mechanism, you have an idea in your mind.

Samarut: Clearly there is no TR γ. We screened the human genome and there is nothing, which looks like third nuclear receptor for thyroid hormone. Bjorn Vennstrom at Karolinska has performed some binding of T_3 in double knockout mutants and he could not evidence any specific binding. So clearly there is no third receptor which would have the same affinity as TR α and TR β.

What I want to say is that the double knockout mutants are alive, but in a very carefully controlled laboratory environment, and we know that these animals have a strong defect in B cell production for example. I think these animals would not resist aggression from the natural environment. So, we have to be careful. I am sure these animals are not normal, anyway.

Yoshizato: From the floor, we welcome any comments or questions about the diversity of thyroid hormone receptor. Are there any comments? Yes, No?

Question: Could I make a comment? Actually, it is a question for Dr. Samarut. The question goes to the possible non-nuclear actions of thyroid hormones. As you know, there is growing evidence for steroid

hormones acting through possibly membrane receptors. How important is that for thyroid hormone action and development?

Samarut: There has been a long debate for many years for some non-genomic actions of thyroid hormone. So far, I have no answer for that. But from the double knockout animals we are now in a position where we could address this question. We have not yet done anything in that field.

But I am coming back to some recent data which were presented at the ATA meeting. These truncated isoforms that I mentioned which do not bind DNA are located only in the cytoplasm and it was shown by this American group that they could bind T_4 or reverse T_3, and that with open binding these proteins could modulate actin network reorganization in the cell. So there is not yet a clear demonstration of that, but maybe these isoforms might be mediators of this non-genomic action of thyroid hormone.

Cheng: I also would like to add to the non-genomic action. In addition to the proteins that Dr. Samarut just talked about which are possibly present in the cytosol, there are some very rapid actions presumably go through some membrane action. So, that is another aspect we should consider as the non-genomic action of thyroid hormones.

Tsai: I would also like to add some precaution raised by Dr. Samarut. The drug metabolism in mice versus humans are quite different, and I am quite sure from the frog to the mammal will be more different, too. Since the xenobiotic receptor CAR specificity of the mice and humans are so different, probably due to their diets, and therefore, they have completely different requirements.

So I am quite sure that the metabolism of this disrupter will be quite different as well. However, I think we cannot, because all of these reasons, discard the use of frog for testing environmental disrupters; the frogs are much easier and much cheaper to test for carcinogens and other disrupters. People using the Ames test in the bacteria for initial testing, and that does not mean the results will completely apply to human beings, but at least this should be used as an initial testing. Later one eventually has to come back to test in humans.

Shi: I just want to add maybe one point. I think that Dr. Tsai has mentioned that amphibians should be used. But in fact, my limited knowledge about the endocrine disrupters says to me that, very often endocrine disrupters are first discovered or very often first found because they affect amphibians in the wild.

In fact one example recently, and maybe this is disputable, but it has definitely attracted a lot of attention, is atrazine. It is found to affect amphibians, but the question is how soon will that knowledge be translated or be accepted or even be proven, validated in other species and mammals because until one shows or accepts that this chemical is affecting mammals, policy-making will not be there.

So, the question is; how do we do to speed things up? Because from the findings in amphibians to actual policy-change could be years, and there may be ways that nowadays we can do it by making use of the knowledge that exists or the tools that are available to shorten this time span, starting with amphibians.

Demeneix: Well, I do not know if Dr. Yoshizato agrees, but this maybe the point to bring in the idea about the OECD testing proposals?

Yoshizato: Yes.

Demeneix: The documents were recently made available that suggest that first of all amphibians should be used, not exclusively, but they should be used, and I think most of us here would agree on that. But they would be used as a first level of testing and obviously tests would have to be applied to mammals as well.

So if we are using amphibians the question then becomes what sort of tests we should be using. A great deal of effort has gone into a 28-day metamorphosis assay, which is a very interesting assay, but it does have the drawback that it is a

28-day assay and it involves following the animals for 28 days and then doing histology on them.

I think the question has to be raised to whether the big advances in genomics and genetic germinal transgenesis and somatic transgenesis could not be exploited to have shorter tests that could as well be combined with a metamorphosis assay test, and how quickly we can formulate the types of assays that should be used and which species we should be applying them to and how quickly they can be tested across the community.

These are questions that are really quite burning questions if we are going to have good models for assessing the great number of molecules that need to be tested for their endocrine disrupting potential.

Yoshizato: Barbara, could you briefly outline the recent activity of the OECD? Maybe most of the audience are not so familiar with the activity of the OECD itself.

Demeneix: Well, a number of national experts were asked to review quite an extensive document in the last few months which addressed these problems of what sort of tests, what sort of species, if it was to be a laboratory species whether it was to be *tropicalis* or *laevis*, if a wild type species should also be included in the test, and for instance, if another test was to be used besides the 28-day metamorphosis assay whether we should be bringing in perhaps DNA arrays and whether the tests would be ready in an appropriate span of time.

I imagine that a lot of people here saw this draft document that was prepared by the United States Environmental Protection Agency, I believe. Certainly an American body prepared it.

Yoshizato: Later we can have some comment from the floor, but before this, the OECD is now considering the possibility to utilize frogs, amphibians, for detecting the environmental disrupters.

Demeneix: Yes definitely, an amphibian committee.

Yoshizato: In this case what species of amphibian should be the standard? This is a very important issue.

Demeneix: Yes, I think this is a very important point. This is why I raised it briefly in my talk because if we are using a laboratory animal, there should be a decision as to whether it is *laevis* or whether it is *tropicalis* if it is *Xenopus*, which I think most people would agree that it should be. Obviously we need to come to some consensus as to which animal should be used and which sort of tests can be reasonably and most effectively applied to these animals.

Iguchi: I would like to invite 2 scientists in the audience, Dr. Werner Kloas from Germany and Dr. Joseph Tietge from the USA, could you come to the microphone? We had a satellite meeting before this international one, and they have very nice data. Could you summarize what you are doing and the OECD purpose?

Tietge: Is this one working? Yes, OK. I am with the United States Environmental Protection Agency, and we have been working on an abbreviated assay, which lasts for 14 days. It utilizes *Xenopus laevis* and we are using the premetamorphosis phase.

Anyhow, we are finding very good sensitivity in that 14-day protocol including clear, this is with the classic synthesis inhibitors: methimazole, perchlorate, 6-PTU, and within 8 days we get substantial histological changes, which are important to us because it is our opinion that developmental delay has to be distinguished or diagnosed from a thyroid specific effect, and at least the thyroid histology data indicates that we have a thyroid specific effect going on.

The assay can be expanded, and we also have some molecular approaches that we are using. I will not go into it now, but we have a gene array that is specific for the thyroid pathway in *Xenopus* which is used to publish sequences of relevant genes or genes that we thought were relevant and some additional biochemical measurements. But the basic assay is a 14-day assay that uses the prometamorphosis phase.

Before I give the floor back to Werner here, I would just like to make a few comments. We started to focus on the receptor a couple of years ago and then realized as Dr. Denver pointed out that most of the action in terms of thyroid activity is not mediated through the receptor; it is mostly involved with metabolism uptake and synthesis of thyroid hormone. So once in a while I think we should remind ourselves that there is not very much evidence that there is receptor activity.

Dr. Shi, in response to your original question, I think it is incumbent upon the amphibian researchers to develop a rigorous comparison of conserved processes between amphibians and mammals and those that are divergent, so that you understand that whichever protocol is adopted, you understand where it has strengths and where the effects can be extrapolated to other species.

Kloas: We had a ring test and unfortunately it will be more or less ready 14 days after I will be back in Germany. As Barbara already mentioned it is based on a 28-day test system, and we started also with premetamorphic tadpoles at stage 48-50.

From a theoretical point of view, as you before already mentioned, there could be also some positive thyroid hormone mimicking substances and if you start out with premetamorphic stages, you will have a much better sensitivity to detect, also if you have an endpoint developmental staging, then you have much better sensitivity to detect positive effects.

However, we agreed that our data and our resources are really in agreement with each other. So, to be on the safe side, I would prefer, this is a rough assumption on such a test system, it is a very simple test if you look at developmental staging. And if you do it for 4 weeks, for 28 days, you will be on the safe side, including everything, which is changing during the metamorphosis, for instance, also binding proteins or maybe enzyme induction of excretion to enzymes.

So, we start out with 48-50, so the normal average end in a normal control might be stage 59-60. I think this may probably cover all. In addition we did also some stuff on gene expression for biomarkers. If you look for TRβ expression you can expose animals only for 1 day and you will get the significant 8- to 10-fold increase of mRNA expression for TRβ at very early stages. So if you use stage 50 that is a very good reason to do something like this to get faster results, but that is limited then to positive effects.

I think all taken altogether, therefore making a very simple and for practical reasons also a test which could be done in every lab all over the world interested in doing some ecotoxicological stuff, and I think a morphological endpoint might be very good. Of course, I agree completely if we could substantiate this by adding some other methods, this would be great. Thank you.

Yoshizato: Are there any comments on the talks made by these 2 European scientists?

Tsai: Since you mentioned about to make the frog more like a human. You cannot do it completely, but I think humanizing the frogs can be a way to do it.

In order to get humanized mice, I believe 2 things need to change: one is TR, and the other is CAR which is responsible for xenobiotic metabolism. I think you can replace the frog's CAR with human CAR and the frog's TR with human TR. If you do that and I think this frog will be at least some degree similar to humans and they can be used for screening for environmental disrupters.

Yoshizato: Good proposal.

Cheng: I was just wondering whether there is such an assay that there is a very specific metabolite, which is secreted into the surroundings under some defined conditions that can be colorimetric determined and this metabolite is thyroid hormone specific. I thought if there was such a metabolite, this could simplify the assay.

I was just wondering, Yun-Bo, is there such a metabolite, which is secreted by amphibians at a defined stage of development that could be used, some sort of metabolite from some enzyme's action? This is just a question. I though I could simplify the assay.

For example, in humans sometimes people determine metabolites in the urine. I was just wondering if there is some sort of metabolite secreted by the tadpoles or frogs during metamorphosis at a certain stage, and maybe that metabolite could be used if that can be easily and conveniently determined by some sort of colorimetric or fluorescence method. It is just a question, a possibility to consider.

Comment: May I make a comment please? Thank you. Just a follow-up, because the question came up what about mammals. To follow up a reminder that the OECD program there also includes development of screens in rodent mammals to screen for thyroid specific activities as well. So those activities are moving along in parallel with the development and the frog metamorphosis types of assays.

Indeed, I think that there is great promise, for great discussion in June at the OECD-EDTA meeting that Dr. Kuder presented about the need for these multi-modal types of screening assays in mammals early in the tiered evaluation process. We are moving ahead, I think with those types of assays in terms of developing, standardizing, and validating those. Thank you.

Yoshizato: Thank you for this comment.

Samarut: OK, for this mammal and mouse screening, I do not know what kind of tests you were thinking about, so maybe you could give more information, but in my mind, what we would have to look at is gene expression using DNA chips which are relevant to expression of thyroid responsive genes in different tissues.

I think this could be the most sensitive assay, because if we look or if we expect histological examination alteration I am not sure we could see them unless we are using a tremendous amount of compound, which makes no sense.

Denver: Yes. I think these screening assays are based on whole animal models. So you are focusing on hormone level and also on histopathology; they are primarily the endpoints. Indeed there is the limitation on the amount of compound that you need to have, and of course, you have a question about sensitivity and specificity.

But indeed in the OECD validation effort those are the types of questions that are meant to be answered: relevance, reliability, sensitivity and specificity. Maybe at the end of the day one can have a series of compounds for which you evaluate in a mammalian system the same types of compounds in the frog metamorphosis assays, or the other types of assays using amphibian.

One can then compare and decide which perhaps is the best type of assay for a specific class of chemicals, or perhaps one for which you need more information than you can get, you might trigger perhaps additional studies that are more molecular in nature. We are still in early stages on both, but they are under way in parallel.

Samarut: I just want to mention one point. We have constructed a recombinant mouse, which is a reporter mouse for thyroid hormone. So using this mouse with a label reporter we can see where thyroid hormone is located in the body.

This mouse could be used, for example, to screen for compounds which would abrogate binding of thyroid hormone or which would mimic thyroid hormone. Also, this mouse could be used to isolate specific tissues and to establish cell lines which would contain this reporter system and which would be cell lines isolated from very specific tissues.

Yoshizato: We now all understand we have 2 nice animals, which respond well to thyroid hormone, mammals and amphibians. To my opinion, we have many, well maybe not so many in amphibian, biologists and scientists who are interested in the mechanism of thyroid hormone action in mammals and amphibians.

But to my opinion, these 2 groups, 2 big and small groups, I mean the mammal group is very big and the amphibian group is not so big. These 2 groups are maybe doing same studies as far as the action mechanism of thyroid hormone is concerned. But these 2 groups are completely isolated, there is

no information exchange. I think these 2 groups should contact each other much more frequently and exchange ideas and problems.

So for to detect endocrine disrupters we need a comprehensive understanding of the action of thyroid hormone on mammals and amphibians. Barbara, the OECD headquarters is located in Paris, so you know very well about the recent activity of the OECD. Is there some trend or activity to join these 2 groups together?

Demeneix: Unfortunately, just because I am located in Paris does not mean to say I have got any particular access. I think I am just the same as if I were in Hiroshima.

But I think that there will be in some time some meetings to see between the 2 committees I think it is absolutely logical and I think as experts we should certainly propose this idea before the 2 committees get too far in their work, that there should be some combined meetings to thrash out some of these problems together.

Yoshizato: Yes, to my knowledge this is the really first chance for us to have this type of joint meeting for mammals and amphibians. So this is a memorable meeting, I think.

Demeneix: Certainly, absolutely. Yes. That is a very important point.

Samarut: I think this could be a good opportunity to have some kind of international action in that field which might be supported by several countries simultaneously.

Denver: Can I say one last thing? Thus far, we have been talking about how to use amphibians as a model for mammals and for humans. But we should not lose sight of the fact that it is very important to consider the conservation of biological diversity, perhaps even more important than focusing exclusively on how these compounds affect humans directly.

Because amphibians have been, or are considered in many ways as sentinel species, they can detect deterioration of habitats and loss of amphibians is significant for the human population, not so much as they are indicators of what is going to happen to us as we are exposed to a compound, but rather loss of biodiversity is a significant problem that we have to keep our eye on, also.

Yoshizato: Back to the xenobiotic aspect of thyroid hormone actions. First, Dr. Denver you presented nice data on the effect of PCBs on the development or metamorphosis of anurans. Can you compare the effect of PCBs between mammals and amphibians?

Denver: The short answer is there are some similarities in terms of competition for binding proteins and alterations of thyroid hormone metabolism. I think there are a lot of similarities, but I think there are differences also as I was pointing out in terms of specificity of binding proteins.

But it is much more complex than that. Amphibians have very different life histories from mammals. They live in very different environments. So, the way that they take up potential endocrine disrupters, their exposure to them is going to be different.

I think it is important, and this was mentioned, the uptake of these compounds, their metabolism, is probably just as important, if not, more important than their potential for interacting with the nuclear receptors. But ultimately if the availability of thyroid hormone is altered, then receptor activation or repressor functions of the receptors are going to be affected.

Demeneix: I would just like to insist on that point. Because as much as I agree that these 14-day and 28-day assays are important, because they will take into account the whole animal physiology, one cannot have an effect on development of thyroid histology unless there is at some point an effect on a receptor. Even if we do take into account these putative non-genomic effects, there is 99% probability that any effect that we do see on histology or development, in our current state of knowledge, must involve an effect at the level of a nuclear receptor.

Denver: Yes, but also the real question is: the disruption of thyroid function and then the phenotype that one sees, is that a result of the disruption of thyroid function? Because PCBs, for example, can have effects that go well beyond effects on thyroid function or effects on hormones, they can have direct neurotoxic effects. So, evaluating whether the phenotype that one sees is in fact an effect of thyroid disruption is, I think, very complicated.

Actually, I have a question for Dr. Yoshizato with the bisphenol A, and it goes to the question of uptake and how these compounds are administered. This is an important question in developing any assay system; normally compounds are administered to amphibian tadpoles by placing the compound in the water. I assume that that is the way that you administer the bisphenol A?

Yoshizato: Yes.

Denver: Is it possible that the bisphenol A was not necessarily altering thyroid hormone receptor function, but maybe altering the uptake of T_3? That is something that we all need to consider in any experiments or assays that we are designing is the route of administration because tadpoles take things up from their environment and that is the basis for the acceleration of metamorphosis by adding T_3 to the water. That uptake could perhaps be altered by these industrially derived compounds.

Yoshizato: But you know there is some structural similarity between bisphenol A and thyroid hormones.

Denver: Yes, but the structural similarity is based on modeling of PCBs and other compounds that would interact with thyroid hormone receptors. I am not sure that that would be an argument for it actually interacting with the receptor. It could be that it gets into some transporter molecule, perhaps a membrane transporter, and just plugs up the system.

Demeneix: Regarding bisphenol A, there was a paper that came out very recently showing that it does displace T_3 from the receptor.

Yoshizato: Dr. Oofusa, do you have some comment on the effect of bisphenol A?

Comment: One of our subjects before was looking at the sexual differentiation effects of bisphenol A and sexual differentiation during the development. I think also the same data presented in the action between T_3 and bisphenol A; I think you did also some positive control experiments with estradiol.

So, there is some interaction, and although I think you would not mention your question concerning estradiol and doing a similar treatment and you will also reduce the tail regression at the same time. I think there were also some data presented at the poster session 2 days before. Something like that. I do not think it is a question of uptake of T_3. I think also I would support more this idea that says interaction….

Denver: That is for the bisphenol A, but it may not extend to other compounds.

Comment: Yes.

Yoshizato: Is Dr. Oofusa here? Do you have some comment on the bisphenol A?

Ofusa: We plan to perform the experiment using receptor ligand binding assay, asking does bisphenol A interfere with the interaction between the thyroid hormone and its receptor or not.

Yoshizato: That is enough for your question, no?

Demeneix: I was making the point that there was a paper that came out in *Endocrinology* about 4 weeks ago that did show this, that there was displacement by bisphenol A of T_3 from its receptor. Yes, that was my point.

Yoshizato: Thank you.

As I showed to you in the first session, thyroid hormone has a great effect on the growth

and differentiation of tadpole epidermis. My question to Dr. Samarut: you made double knockout transgenic mice with respect to TR β and α. Did you not notice any affect on the development of skin?

Samarut: We did not see some overt effect on the development of skin, but what we have observed, and we have not yet looked in depth at that, but this is something, which is interesting, we have observed some wound-healing defect with these animals.

Yoshizato: OK, thank you. Do you have any other suggestions or comments on this session, also from the audience? OK, now we would like to have concluding remarks from Dr. Iguchi.

Iguchi: I have not thought anything about the concluding remarks. At the beginning of thinking of this symposium we had a meeting. In Hiroshima we have a very nice amphibian institute. Prof. Yoshizato is in it. This symposium we would like to discuss about the thyroid hormone.

We had 2 sessions, and how do we manage this? So I just proposed to put it together. That is why I am here. You should be in the chair. I do not need anything to do.

I think this is a very good discussion. I am representing the validation management of VMG-eco for OECD, and of course we are working on the frog, fish and birds, that is the Japanese quail. Only the frog side is thinking frog, but the bias comes when this frog system has to be used for humans. But we need to think about the frog itself, also.

The frog is not only for metamorphosis, sex differentiation, and other various things. So I think this is a very good start. I think the thyroid system we can work on the mammalian side independently. And I think if both sides can work independently and then get together sometimes and discuss the progress or the differences or consistencies.

I think this is a good start to think about this key word of thyroid hormone or thyroid hormone receptor. But we need to think about the environment of all species not only mammals, not only frogs or amphibians but other species.

Thank you very much for a good discussion and for participating in this discussion time. I want to close this session. Thank you very much.