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Abstracts

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Session 1 Some Considerations on Epidemiological Studies

Difficulties in Conducting an Epidemiological Study for Endocrine Disrupting Chemicals

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There have been a limited number of epidemiological studies conducted to examine the effects of endocrine disrupting chemicals (EDC) on health outcomes. These are even rarer in Japan. In western countries, however, driven by strong interests in the effects of PCB and agricultural pesticide use on humans, several important studies have been conducted, in particular, for breast cancer. These have been cohort studies, using stored serum samples, and large scale case-control studies, with biomarker measurements. These are two possible designs which can provide reliable evidence. These kinds of studies should also be conducted in Asian countries since there exist differences among countries and ethnic groups in: EDC exposure level, incidence of the disease of interest, endogenous hormone level, hormonal drug use, intake of phytoestrogen (such as soy isoflavone), and genetic variability. The purpose of this presentation is to describe the difficulties of conducting epidemiologic studies for EDC, particularly in Japan, from our experiences in conducting a large scale cohort study (more than 140,000 participants) to examine the effects of life style on the disease (JPHC Study), a nation-wide population based case-control study to examine the effects of lifestyle on childhood leukemia and brain tumors, and other studies.

Because randomized controlled trials to examine the effects of EDC on humans are unethical, the most reliable evidence for humans comes from prospective cohort studies involving stored biological samples, such as serum or urine. In these studies, the biggest difficulty is how to ascertain individual health outcomes. Without a legally supported disease registry, it is almost impossible to ascertain individual health outcomes for thousands of people, during long terms of follow up. In Japan, we have no legally supported disease registry for cancer. Our study showed self-reports of cancer and other diseases are not reliable.

An alternative to the prospective study, which is not quite as reliable, is the case-control study, where cases and controls are compared in terms of EDC measurement levels, with adjustment for other, possibly confounding, factors. The biggest difficulty in case-control studies is how to sample representative controls. Ideal controls should be selected randomly from the same population from which cases arise. Unfortunately, however, our experience is that the participation rate by mail requests is about 30%, and this is the common experience in Japan. No analysis can protect against selection bias with this low rate of participation. As a substitute, hospital controls are often selected from the same hospital as cases, but this leads to an underestimate of the risk if the reason for the hospital visit of controls may be associated with the exposure of interest. Our experience indicates that using controls from a different population from cases is often misleading, since exposure distribution in controls varies largely between populations. As for exposure, it is well known that the validity of the estimates is unclear since they are made after the case's diagnosis and may be affected by disease status.

Any of these study designs can provide different results between studies, due to confounding or chance, even if the studies are conducted very well. It is therefore important to validate the results by multiple epidemiologic and biological studies. That is the only way to get reliable evidence.

Despite the difficulties of epidemiological studies for EDC, several studies are being conducted now in Japan. In addition, it is important to establish monitoring systems of exposure and diseases, and also to establish multipurpose cohort studies.

Evaluation of Endocrine Disrupters in Women and Children: Lessons from the CHAMACOS and Seveso Women's Health Studies

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There is a growing concern about whether endocrine disrupting chemicals pose a risk for the reproductive health of men and women, and for the health and development of children. Although endocrine disupting chemicals, including PCBs, certain pesticides, and dioxins, have been well-studied in animals, the effects of these chemicals have been poorly studied in human populations. Study of the impact of environmental chemicals on human health is complex. The purpose of this presentation is to present the methodology used to study two populations exposed to endocrine disrupting chemicals, and the key results. We use, as examples, a study, which focused on the reproductive health of women residing in Seveso, Italy and exposed to 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (TCDD), and another study, which focused on neurodevelopmental functioning in Mexican-American farmworker children living in California and exposed to pesticides which are potentially endocrine disrupting.

In 1976, a chemical plant explosion in Seveso, Italy exposing the nearby residents to the highest exposure to TCDD known in humans. Twenty years later (1996-1998), we initiated the Seveso Women's Health Study (SWHS), a retrospective cohort study, to determine whether exposure increased risk for reproductive disease. We enrolled almost 1000 women who lived near the chemical plant and who had blood stored since 1976. As part of this study, we measured TCDD in the stored specimens, and women received an interview, gynecologic exam with pelvic ultrasound, completed a menstrual diary, and had a blood draw. The median serum TCDD was 55.8 ppt, (range: 2.5 - 56,000), with higher levels in those that were young. We have examined the relationship of TCDD in blood and spontaneous abortion, infantbirth weight, menstrual cycle characteristics, age at menarche and at menopause, endometriosis, fibroids, and breast cancer. We will present a summary of the findings of the SWHS to date.

The second example will be from the CHAMACOS project, a longitudinal birth cohort study that enrolled 600 primarily low-income Mexican immigrant farmworker pregnant women living in the agricultural Salinas Valley, California. Over 500 children were born and most have been followed until 5 years of age. Mothers were interviewed twice during pregnancy, at delivery, and when the children were 6 months, 1, 2, and 3.5 years of age. Urine, blood and breastmilk samples were collected. Neurodevelopmental assessments of the children and home inspections were conducted at each age. Currently, we are conducting visits with the children at 5 years of age. The aims of the Center include 1) assessment of pesticide exposure in pregnant women and young children; 2) the potential health effects of pesticides on childhood growth, neurodevelopment and respiratory disease; 3) the mechanisms of pesticide neuro- and immuno-toxicity; and 4) community-based outreach and interventions to reduce take-home pesticide exposure to children of farmworkers. We will present a summary of the findings of the CHAMACOS study to date.

Interpreting the Significance of Small Effect Sizes in Epidemiological Studies

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The sizes of the putative effects observed are often small in studies that rely on functional endpoints such as neurodevelopment as the critical health effects. Because the mean deficits implied are more modest in magnitude than are those that correspond to the clinical criteria used to diagnose "disease," some observers dismiss them as inconsequential. The mean deficits take on greater import when viewed as effects on a population rather than on individual members of the population. Several considerations germane to an effort to reconcile these perspectives will be discussed: (1) the relative sensitivity of clinical diagnoses and continuously distributed scores on neurobehavioral tests as indices of adverse effect, (2) the syndromal nature of many diagnoses in behavioral neurology and the implications of shifting nosology, (3) the role of neurobehavioral test score scores as markers of or as prodromes for clinically significant deficits, (4) the implications of the distinction between individual risk and population risk, and (5) the tendency of the distributions of a risk factor in population to move up and down as a whole. The clinical and population perspectives on neurobehavioral toxicity are complementary rather than incompatible.

Session 2 Risk Communication: Present Status and Future Directions

A Systematic Guide for Risk Communication with Special Focus on Risk Characterization, Risk Amplification, and Precaution

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The paper deals with four major challenges faced by risk communicators: (1) how to provide a transparent, consistent, and reasonable risk characterization, (2) how to communicate uncertainties, (3) how to deal with risk amplification, and (4) how to justify appropriate precautionary actions?

Risk communication is a fundamental component of the risk analysis paradigm. Not only is risk communication necessary among experts of various disciplines in order to assess the risks, but also is an essential tool for bi-directional sharing of information, values, and preferences between experts, decision-makers, stakeholders and the public. In this light, it is sensible and - indeed - crucial for the risk communication efforts to integrate a sound risk characterisation. I propose an "evidence framework", that allows for a transparent summarization of the experts' line of reasoning. This framework helps to describe the most crucial arguments for as well as against a risk suspicion, the conclusions of the experts, and the remaining uncertainties.

However, social science research on risk demonstrates that basing risk communication solely on a scientific risk characterization falls short of the mark. The current social perception and potential for emotional and social risk amplification, as well as their dynamics, must also be considered.

Furthermore, a successful risk communication program should also focus on the risk management strategies. Special attention deserves the question "How will people react to the implementation of the precautionary measures?". The key is the impact of precautionary measures on risk perceptions.

My proposals for risk communication that address these critical issues will be based on our recent empirical research into risk perception and risk communication.

The Current Status of Risk Communication in Japan

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The author reports the current status of risk communication in Japan. Although the concepts of risk and risk communication, and even the concept of communication, were not well recognized in the Japanese society, risk communication has been becoming one of the major concerns these days.

As to the topic of endocrine disrupters, in the early stage of risk communication on the endocrine disrupting chemicals, they are introduced as, to some extent, 'unknown' risks. The communication seemed to fail in the sense it caused a nationwide scare at that time. The scare was mainly attributed to the 'sensational' journalism and was in part attributed to the so-called 'emotional' laypersons. However, the author knows of no empirical data supporting such a contention. More plausible explanation could be that the ambiguous nature of risks of endocrine disrupters would lead people to act in a rather conservative way to refuse technology.

In addition, the author and the colleagues did a national survey to grasp the current attitudes toward endocrine disrupters among the Japanese. The main findings were as follows: (1) While approximately 2/3 of the respondents had never heard of the technical term "endocrine disrupting chemicals", approximately 90% of them knew of the term "environmental hormone" instead, which is not scientifically correct. (2) Whereas the need for information was potentially high, scientifically incorrect knowledge (what is called "laypersons' knowledge") was very common. (3) More than 70% of the respondents did not know the fact that the relation between endocrine disrupters and human health damage has not yet been scientifically proven.

The interpretation of the above-mentioned results could be that the interest in endocrine disrupters among Japanese has decreased recently and the knowledge of endocrine disrupters has not yet reached to the satisfactory level.

As is usual the case with other risks containing ambiguity, the conflict between risk experts and laypersons exists in the endocrine disrupters. There could be a sharp difference between risk experts and laypersons over the preference in precautional measures in particular and/or the evaluation of risk assessment in general.

In conclusion, a new way of risk communication is necessary to establish mutual understanding and to encourage all the stakeholders including laypersons to actively involve in risk communication of the endocrine disrupters.

Session 3 Community and Ecosystem Level Assessment of Human Impacts

Impacts of Endocrine Disruption on Roach, *Rutilus Rutilus*, Populations Living in UK Rivers

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Endocrine disruption is now a well-established phenomenon in both freshwater and marine fish. One of the most common effects reported in fish is the feminisation of males and some of the most comprehensive studies in this regard have been conducted on wild roach, *Rutilus rutilus*, living in UK Rivers. Over the past 10 years we have established that intersex (the simultaneous presence of males and female features in the same gonad) occurs commonly in wild roach in rivers contaminated with wastewater treatment works (WwTWs) effluents and this condition can be induced experimentally on exposure to oestrogenic effluents and to some individual oestrogenic chemicals. We have also shown that some of the oestrogenic chemicals of concern bioconcentrate in roach. Importantly, roach with altered gonadal development have an altered timing to maturation and/or produce gametes of poorer quality with a reduced capacity for fertilisation. Thus the ability of feminised male roach to reproduce is compromised and, depending on the severity of the condition, individuals can be rendered infertile. Given that feminisation of male roach in UK Rivers is extensive - a recent survey identified intersex roach at 44 out of 51 sites studied throughout England and at some of these sites all males were feminised, a population level impact is a very real possibility. Roach comprise up to 50% of the lowland UK freshwater fishery, thus endocrine disruption could also fundamentally affect the riverine freshwater ecosystem.

In the second part of my presentation I will focus on the challenges we now face in trying to understand the population level consequences of endocrine disruption in wild roach. In the first instance we need to develop a more complete understanding of the basic breeding biology of the roach, of which we know very little, because the parentage dynamics could have a major bearing on how the population is impacted by endocrine disruption. For example, if in natural populations only a small percentage of the males contribute their gametes to the gene pool, then a certain level of intersex can likely be accommodated without harm to the population. If on the other hand gametes from most reproductively active males contribute, then endocrine disruption might serve to reduce the normal genetic heterogeneity of the population. For other fish species, we have evidence to show that reproductively compromised males compete in the spawning act with other males and can alter the breeding capabilities of other 'fit' males. The importance of age in breeding roach populations is also not known and given the fact the severity of the intersex condition in fish in effluent contaminated rivers increases with age, this could also have a major bearing on the reproductive success of the population. We are developing DNA microsatellite markers in roach and propose to experimentally manipulate breeding colonies to address some of these questions. Other questions we need to address in developing our understanding of the population level effects of exposure to endocrine disrupting chemicals (EDCs) include how fish might acclimate and adapt to EDCs in the aquatic environment. Until we have this information our ability to predict roach population and thus ecosystem level impacts of EDCs, even with modelling approaches, is limited.

40 Years of Change on Iriomote Island

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An island is conceptually easy to grasp as an ecosystem. It is an isolated, stable ecosystem without external influences, but is also fragile if external influences penetrate.

Evidence suggests that there was human settlement on Iriomote Island from at least around the 8th century and human activity has been continuous since then. Although there has been a long history of the construction of new villages by immigrants and the demise of other villages due to malaria, it is true to say that the coexistence between the environment and humans has been maintained with little adverse environmental impact.

Ten years after the end of the Second World War and after malaria was eradicated, immigration restarted and Iriomote Island began to change.

I have been observing the natural environment of Iriomote Island since 1965, when the Iriomote cat was first discovered. In those days there was no road linking the east and west coasts of the island. About 3000 people lived on the island but the environment was preserved, a variety of shellfish could be found in the sea and the fishing catch was plentiful. In 1972 Okinawa returned to Japan. After that, the population of Iriomote decreased and the population remained at about 1200 into the 1980s.

About 1800 people live on the island now. The harbor and road have been upgraded and the number of cars has increased, power and water are supplied throughout the island and a fairly comfortable standing of living is guaranteed. On the other hand, exotic plants have increased, including Spanish needles (Bidens), and the native vegetation of Iriomote is disappearing along the road. The presence of exotic animals such as the giant toad (Bufo) etc. has been confirmed. The fishing catch has reduced.

In my estimation, the Iriomote cat population has remained at about 80-100 animals since it was first discovered, but the causes of death have greatly changed. Dog attacks and trapping used to be the main factors, but now most Iriomote cat deaths are caused by traffic. This is one example of the adverse effect of humans on the environment and this effect is increasing. Such impact was inconceivable twenty years ago. These effects are easy to understand because there is clear physical evidence in front of us. However, the effects on the ecosystem of chemical substances and environmental hormones are difficult to see, so often countermeasures are taken too late. However, we should be aware that they are advancing quietly and deeply even though we can't see them.

There are three important steps to ensuring coexistence between the natural environment and humans, although of course it is not easy for people who know convenience to return to the old ways. The first is to take effective protective measures for protected areas and to develop designated development areas efficiently, the second is to put limits on the amount of road construction and development, and the third is to control the introduction of pets, foliage plants and other exotic organisms.

Biological Interactions: Important Factors in Structuring Organic Communities and Controlling the Pesticide Impact on Ecosystems

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Biological interactions, such as predator-prey and competition, are very important in structuring the lake zooplankton communities. For example, fish induce the development of zooplankton community dominated by small-sized species such as small cladocerans and rotifers by selective predation on large-sized species. On the other hand, large-sized species dominate the zooplankton communities in lakes with less abundant fish, because the large species out compete the small ones by competition for food.

The large zooplankton species tend to be more sensitive to toxic chemicals than small ones. It would be expected, therefore, that the contamination with pesticides in lakes reduce the large species but increase the small species, which are released from competitive pressure by the large species. The increase of the small species is a result of the indirect effect of pesticides through biological interactions. The indirect effect should be understood in assessing the pesticide impact on ecosystems.

To analyze the indirect effect, we performed a mesocosm experiment using experimental tanks, where a zooplankton community was established in the tanks with different densities of the predator cyclopoid copepoda, and was exposed to the insecticide carbaryl. Rotifer populations showed different responses to the carbaryl application between the tanks of different predator densities. They decreased largely in the tanks with abundant predators due to mortality caused by the pesticide toxicity and predation. In contrast, they increased in the tanks with low density of predators, because the rotifers were released from the competitive pressure by cladocerans, which were damaged more intensively by the pesticide than rotifers.

Impacts of biological interactions (natural factors) and of toxic man-made chemicals (anthropogenic factors) on ecosystems are discussed.

Session 4 New Approaches to Mechanistic Understanding of Endocrine Disrupters

Effects of Persistent Organic Pollutants on mRNA Expression in Avian Neuronal and Hepatic Cells Using FRAP-PCR and SAGE

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In recent years there has been considerable interest in using new methods for assessing the effects of endocrine-disrupting chemicals (EDCs) on gene expression in wildlife species in much the same way that the methods are being developed and applied for human toxicology. With wildlife species there are many important scientific and technical challenges for adopting such approaches. The DNA sequences for critical genes that are known to be impacted by EDCs are not available for most wildlife species, thus limiting the application of large-scale studies with cDNA microarrays in most laboratories. In our laboratory, we are exploring the possibilities of using 'open' methods of mRNA expression for determining the effects of selected persistent organic pollutants such as polybrominated diphenyl ether (PBDE) flame retardants and perflurorinated organics in wild birds. Our studies involve both examining the effects of EDCs *in* ovo and in primary cultures of neuronal cells (from the cerebral cortex) or hepatic cells. The molecular methods that we are using include a new Fluorescent Arbitrarily Primed PCR (FRAP-PCR) technique and Serial Analysis of Gene Expression (SAGE). In this presentation, we will describe the methods, and will present some of our recent results that have been used to discover novel effects of PBDEs and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mRNA expression in avian neuronal and hepatic cells.

Germ Cell Apoptosis in Rat Testis is Induced by Oxidative Stress via Oral Administration of di (2-ethylhexyl) phthalate, and is Significantly Prevented by Treatment of Antioxidant Vitamins or Special Six-Carbon Monosaccharides

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Phthalate esters have been used extensively as plasticizers of synthetic polymers. Recent studies have revealed that these esters induce atrophy of the testis, although its pathogenesis remains unknown. The present study describes the possible involvement of oxidative stress in the pathogenesis of atrophy of the rat testis induced by di (2-ethylhexyl) phthalate (DEHP).

Liver enlargement occurred in rats fed either a 1 or 2 % DEHP-containing diet in two weeks. However, testicular atrophy accompanied by aspermatogenesis was induced by feeding with the 2% but not with the 1% DEHP-containing diet. This suggests that the critical DEHP dose for gonadotoxicity is higher than that for hepatotoxicity.

Biochemical and immunohistochemical analysis revealed that oral administration of DEHP increased the generation of reactive oxygen species, with concomitant decrease in the concentration of glutathione and ascorbic acid in the testis, and selectively induced apoptosis of spermatocytes, thereby causing atrophy of this organ. Oxidative stress was selectively induced in germ cells, but not in Sertoli cells, treated with mono (2-ethylhexyl) phthalate (MEHP), a hydrolysed metabolite of DEHP. Furthermore, MEHP selectively induced the release of cytochrome c from mitochondria of the testis. These results indicate that oxidative stress elicited by MEHP principally injured mitochondrial function and induced the release of cytochrome c, thereby inducing apoptosis of spermatocytes and causing atrophy of the testis.

Using the 2% DEHP-dose, the effect of simultaneous administration of antioxidant vitamins (= vitamins C and E) was next examined. It was found that the vitamin supplementation significantly prevented the testicular injury. The results suggest that antioxidant vitamins can protect the testes from DEHP-toxicity.

Our recent work has elucidated that some six-carbon monosaccharides which are rarely present in nature (i.e. D-psicose and D-allose) are also effective in prevention of the testicular injury. Microarray and proteomic analyses have been applied to elucidate the genes and proteins which are involved in the DEHP-toxicity and the protection mechanism.

Effects of Estrogenic Chemicals Estimated by Gene Expression Profile

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Recently, many chemicals are suspected to affect reproduction and they are called endocrine disrupters. They are considered to mimic or disturb the action of the endocrine systems. Among the endocrine disrupters, many of chemicals have been reported to have estrogenic activities. For example, nonylphenol and bisphenol A are ones of the chemicals that have been shown to have estrogenic activities. Although it is known that the chemicals can weakly bind to the estrogen receptor, it is unclear whether all reported effects of chemicals are attributable to their estrogen receptor binding activity because chemical evaluation methods had been rather limited. In order to examine if these chemicals have similar effects to the natural hormone, estradiol, we used a mouse model to examine the effects of chemicals on gene expression.

Mice (C57/BL6/J) were ovariectomized at eight weeks of age and the ovariectomized mice were injected intraperitoneally with a chemical. Total uterine and liver RNAs were extracted at 6 h after chemical administration and gene expression profiles were analyzed by mouse genome U74A (Affymetrix) Gene Chip. In addition to physiological and non-physiological estrogens, other chemicals suspected to be endocrine disrupters such as phthalates were examined. Dose dependent uterine gene expression patterns by physiological and non-physiological estrogens showed similar but distinct profiles, suggesting difference of gene activation mechanism among estrogens. In contrast to the similar effects of physiological and non-physiological estrogens observed in the uterus, in liver, gene expression was more markedly affected by non-physiological estrogens. In addition, it was found that non-physiological estrogens could activate another set of genes that is distinct from estrogen-responsive genes. These results indicate that non-physiological estrogens have similar effects to estradiol on gene expression in uterine but not in liver tissue, indicating that tissue-specific effects should be considered in order to elucidate the effects of endocrine disrupters.

Session 5 Development of Testing Methods for Endocrine Disruption

The Validation Management Group for Non-Animal Testing (VMG-NA) of the OECD Task Force for Endocrine Disrupting Testing and Assessment (EDTA)

Patric Amcoff

Organization for Economic Cooperation and Development (OECD)

At the EDTA6 meeting in Tokyo, Japan, in 2002 it was considered appropriate to establish a 3rd validation management group for development of relatively cheep and quick *in vitro* tests and screens for the level 2 of the EDTA Conceptual Framework. The group was named the "Validation Group for Non-Animal Testing (VMG-NA)" and was established in early 2003. The first meeting was held in Paris in March 2003 where a number of tests were discussed and further work outlined. At the 1st meeting it was evident that there were no tests available that had been validated and could readily be turned into official OECD Test Guidelines. However, a number of assays were considered as promising and further optimisation and pre-validation work was considered appropriate for several different tests. Four "Detailed Review Papers (DRP's)" have been developed within the areas of Steroidogenesis, Aromatase, Metabolism and Thyroid disruption (also a DRP on in vitro tests for Vitellogenin analyses has been suggested), respectively, that have provided further guidance in the selection of suitable level 2 tests. A 2nd meeting of the VMG-NA was held in October 2004 and a 3rd meeting will be held at OECD in Paris in December 2005. It is anticipated that this meeting will consider the development of a number of assays at different stages of validation, including assays for steroidogenesis, aromatase, receptor binding and transcriptional activation. Their validation status and possible inclusion in the work plan as suitable Test Guideline prospects will be discussed. Also QSAR's for ED potential are covered by the ED QSAR Task Group of the VMG-NA, which is coordinated by the VMG-NA, but monitored by the QSAR ad hoc group.

Development of OECD Test Guidelines for Endocrine Active Substances in Environmental Species

Anne Gourmelon

Organisation for Economic Cooperation and Development (OECD)

In 1997 the OECD member countries started a special activity for Endocrine Disrupters Testing and Assessment, under the umbrella of the Test Guidelines Programme. This initiative stemed from concerns that: *i)* observations from wildlife showed feminisation of fish downstream certain industries and effluent treatment plants, and *ii)* the existing OECD Test Guidelines for the testing of chemicals did not allow for the detection of substances active on the hormone systems of mammalians and wildlife species. Promising test methods were identified both for the protection of human health and the environment. Most of the methods, especially for the environment, were relatively new and brought from the science; validation was necessary to evaluate their performance and to enable regulatory acceptance.

OECD Test Guidelines are covered by the principle of the Mutual Acceptance of Data. The 1981 OECD Council Decision concerning the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals is built on the OECD Test Guidelines and Good Laboratory Practice Principles (GLP). The Council Act requires OECD governments to accept chemical test data developed for regulatory purposes in another country if these data were developed in accordance with the OECD Test Guidelines and GLP Principles. This means new data for notifications or registrations of a chemical only have to be developed once, and are then used across OECD countries.

Since 2001, member countries and stakeholders from industry and environmental organisations have worked together to validate assays aimed at detecting endocrine active substances in fish, amphibians, birds and invertebrates. A Validation Management Group for ecotoxicity testing was established to oversee progress and to advise on validation issues and needs. A substantial amount of information is now available on assays using fish and amphibians, which has sometimes confirmed initial hypotheses and other times demonstrated the limits of the methods developed. Efforts to generate the data have been significant both in terms of financial resources committed and of time dedicated by all those involved: member countries and industry. With the data on the table, member countries now have to go a step further: agree on Test Guidelines and formally adopt them. This phase also requires time because OECD Test Guidelines are adopted by consensus.

A screening assay using fish has been under the validation programme to evaluate its performance: it reliably detects estrogen, aromatase inhibitors and androgens; but there are issues for the detection of anti-androgen, as demonstrated in the validation studies. The limitations of an assay are as important as its capacities in this context. The endpoints under discussion are vitellogenin, secondary sex characteristics, gonad histopathology and fecundity. An Amphibian Metamorphosis Assay is also under validation to evaluate substances active on the thyroid system; multi-chemical and multi-laboratory testing is underway. An enhanced Daphnia reproduction test, with offspring sex ratio as an indicator of endocrine activity, is considered for ring-testing. Other life-cycle or multi-generational tests including development and reproduction are evaluated and may also become part of the overall framework for testing and assessment.

The limitations of an assay are as important as its capacities in this context. To facilitate regulatory acceptance of future Test Guidelines, it will be necessary to define their scope and purpose, based on the data available. Sometimes guidance material may be added for using the data produced in the assay.

Environmental Risk Assessment of Endocrine Disrupters: Status and Needs of Biomarkers

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In order to maximise the scientific benefits of using biomarkers, they have to be biologically relevant and reproducible. This presentation will argue that biomarkers are increasingly important tools in environmental risk assessment and are best used as 'mechanistic signposts' to guide longer-term studies of adverse effects in laboratory and field populations (Hutchinson et al., 2006). Over the past decade, biomarkers have proven to be invaluable tools for tracking spatial and temporal trends in fish exposed to endocrine disrupting chemicals (EDCs). Moreover, through the use of mechanistic biomarkers such as vitellogenin induction, implications of EDC exposures for other wildlife populations (eg amphibians and birds) can be addressed in a more efficient manner. In ecotoxicology, this has the potential to provide a parallelogram approach to endocrine disrupter effects assessment, adapted from the model previously developed for mammalian genetic toxicology. For biomarkers to fulfill their potential they should be mechanistically relevant and reproducible (as measured by inter-laboratory comparisons of the same protocol). VTG is a good example of such a biomarker in that it provides an insight to the mode of action (oestrogenicity) that is vital to fish reproductive health. Inter-laboratory reproducibility data for VTG are also encouraging; recent comparisons (using the same immunoassay protocol) have provided coefficients of variation (CVs) of 38 - 55% (comparable to published CVs of 19 - 58% for fish survival and growth endpoints used in regulatory test guidelines). Biomarkers also provide linkage between field and laboratory data, playing an important role in directing the need for, and design of, fish chronic tests for EDCs. It is the adverse effect endpoints (e.g. altered development, growth and/or reproduction) from such tests that are most valuable for calculating ^{adverse}NOEC (No-Observed Effect Concentration) or ^{adverse}EC10 (concentration giving a 10% effect) and subsequently deriving Predicted No-Effect Concentrations (PNECs). With current uncertainties, ^{biomarker}NOEC or ^{biomarker}EC10 data should not be used in isolation to derive PNECs. In the future, however, there may be scope to increasingly use biomarker data in environmental decision-making, if plausible linkages can be made across levels of organization such that adverse outcomes might be envisaged relative to biomarker responses.

Background references: Handy et al (2003) Ecotoxicology 12: 331-343; Hutchinson et al., (2006) Environ. Hlth. Perspect (special issue); Sumpter & Jobling (1995) Environ. Hlth. Perspect., 103 (suppl 7):173-178; van der Oost et al (2003) Environ. Toxicol. Pharmacol., 13: 57-149.

Session 6 Current Initiatives on Risk Assessment of Chemicals

Roadmap for Implementing the USEPA Endocrine Disrupter Screening Program

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Under the Food Quality Protection Act (FQPA) of 1996, the United States Environmental Protection Agency (US EPA) is required to develop a chemical screening program for pesticides and environmental contaminants to determine if these substances may have an effect in humans and wildlife that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect as the Administrator may designate. With the input of a federal advisory process, the Agency developed a two-tiered approach for an Endocrine Disrupter Screening Program (EDSP) to carry out Congress' directives. There are over 1,000 pesticide active ingredients, and over 2,700 pesticide inert ingredients that are eligible for screening under FQPA. One of the greatest challenges the Agency faces is the development of tools to identify available, relevant information to prioritize data needs for testing and risk assessments. EPA is implementing the EDSP in three major steps: 1) Develop and validate the screening level Tier 1 assays and Tier 2 tests. (Tier 1 assays are intended to identify the potential of chemicals to interact with hormone systems, while Tier 2 tests are designed to determine if the pesticide chemical causes endocrine effects that may be quantitated in a dose-response relationship.) 2) Develop the procedures the Agency will use to require screening. 3) Finalize the priority setting for selecting the initial list of chemicals to undergo screening. The Office of Research and Development within the US EPA is conducting ongoing research and development for *in vitro* and *in silico* techniques to aid in pre-screening chemicals. This research is being conducted in concert with the OECD QSAR Task Group.

This abstract has been subjected to review by EPA's Office of Pesticide Programs and was approved for submission. Approval does not signify the contents reflect the views of EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Developing Approaches to Chemicals Management - A UK View

Michael John Roberts

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The development of national and international policies on the management of chemicals continues to be a dynamic area, with several initiatives in progress.

Within the European Union, work is underway to establish a more effective system to assess the short and long-term risks posed by industrial chemicals and to take action where necessary - referred to as the Registration, Evaluation and Authorisation of Chemicals (REACH).

In addition, the EU has launched an "Environment & Health Strategy", known as SCALE (Science, Children, Awareness, Legal Instruments, Evaluation), which aims to establish a good understanding of the link between environmental factors (including chemicals) and:

* Childhood respiratory diseases, asthma and allergies;
* Neurodevelopmental disorders;
* Childhood cancer;
* Endocrine disrupting effects.

Similarly, regional priority goal 4 of the World Health Organisation (Europe) led initiative on Environment & health - the Children's Environment & Health Action Plan for Europe (CEHAPE) - requires action on hazardous chemicals.

There are also currently a number of international agreements on chemicals management - for example: *the Stockholm Convention on Persistent Organic Pollutants (POPs) and the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade.*

At the same time as working with European partners to develop more effective EU legislation, the UK Government is working with stakeholders to develop solutions that can be applied to reduce the risk of chemicals damaging the environment, at national level.

Japan's Approach for the Prevention of Environmental Pollution Caused by Chemicals

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Law concerning the Evaluation and Regulation of Industrial Chemicals

Due to increasing concerns about environmental pollution caused by industrial chemicals such as PCBs, the Law concerning the Evaluation of Chemical Substances and Regulation of their Manufacture, etc. was enacted in Japan in October 1973. The aim of the Law was to prevent environmental pollution by chemicals that had PBT properties (i.e. persistency, bioaccumulation and toxicity) in order to protect human health. Since then, the manufacture and import of the designated PBT chemicals as "Class I Specified Chemicals" have strictly been prohibited. This regulation has contributed a lot to avoid environmental pollution of POPs.

In 1991, the Law was amended aiming to improve international harmonization of chemical regulation as well as to address the pollution caused by non-PBT chemicals, such as trichloroethylene and tetrachloroethylene. These chemicals are not highly bioaccumulative, however their persistency and toxicity have still potential for posing environmental problems. At that time, the control of these chemicals was ensured by introducing a risk-based approach into the Law, and based on the results of the assessments it was legally enabled to put upper limits on the annual production/import volume for designated chemicals as "Class II Specified Chemicals".

More recently, in 2003, the Law was further amended by incorporating the protection of the ecosystem to its purpose, which came into effect in April 2004. At that time, the regulations of the Law were also streamlined for improving its efficiency by putting more attentions to the likelihood of emissions of chemicals to the environment.

Environmental Monitoring and PRTR

The environmental survey and monitoring have systematically been carried out in Japan, and their outcome has been used as major input to environmental exposure assessments of chemicals.

Japan's Ministry of the Environment (JMoE) has initiated an environmental monitoring program in 1973 in cooperation with local governments. This program covers multi-environmental media (i.e. water, sediment, biota and air) of all over Japan, and has successfully provided useful data. In some cases, they got triggers of taking action under the above Law.

Utilizing its experience, JMoE advocated a creation of the POPs environmental monitoring program at the East Aisin region in 2002. After that, with strong supports from the countries in the region, JMoE have held a series of workshops on environmental monitoring of POPs.

Japan's PRTR has started in 1999. The PRTR data have been used in carrying out exposure assessment of chemicals.

Environmental Basic Plan

In Japan, the work for developing the 3rd Environmental Basic Plan has been progressing. The Environmental Basic Plan is a governmental one where comprehensive measures for protecting the environment are set out. The 3rd the Plan will set targets for the next two decades. It will be released around the end of 2005, and a strategic plan for the reduction of risks caused by chemicals will be a part of the Plan in order to tackle outstanding issues that still remain in that area, such as acceleration of examination of industrial existing chemicals.