Session 3 Wildlife

Current Status of Contamination by Organotin Compounds and Imposex in Gastropods from Japan and Possible Physiological/Biochemical Mechanism of Organotin-Induced Imposex in Gastropods

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Imposex (a superimposition of male reproductive tracts, such as penis and vas deferens, on female prosobranch gastropods) is known to be typically caused by organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), which have been used worldwide in antifouling paints for vessels and fishing nets since the mid-1960s. Approximately 150 species of prosobranch gastropods (meso- and neogastropods) have been observed to be affected by imposex. Severe stages of imposex lead to reproductive failure due to oviduct blockage by vas deferens formation and spermatogenesis in ovary, resulting in population decline and/or mass extinction. Here, we present the current status of organotin contamination and imposex as well as population declines resulting from imposex-effected reproductive failure in prosobranch gastropods of Japan, such as the rock shell (*Thais clavigera*) and the ivory shell (*Babylonia japonica*). Endocrine disruption has also been observed in abalone from Japan, including disturbed reproductive cycles between females and males and ovarian spermatogenesis. Results from field studies and *in situ* exposure experiments concerning endocrine disruption in Japanese abalone stocks will be submitted. Laboratory experiments have confirmed that TBT and TPT exposure causes ovarian spermatogenesis in adult female abalone. Therefore, masculinization of female abalone seems to resemble the imposex in meso- and neogastropods, although no penis formation is observed in abalone which belongs to the order Archaeogastropoda. To clarify the mode of action of TBT- and/or TPT-induced imposex in prosobranch gastropods, we have also studied the basic biology of gastropods, with respect to their steroid hormones and respective receptors, the role of cytochrome $P450_{arom}$ (CYP19A1, aromatase), and neuropeptides. Results to date from this study are shown, and possible mode of action of TBT and/or TPT on the development of imposex in gastropods will be discussed. Finally, a new hypothesis about the imposex induction mechanism will be presented.

Endocrine Disruption in Wildlife of the Great Lakes of North America, Past and Present

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The Great Lakes-St. Lawrence River ecosystem is the single largest freshwater ecosystem on Earth, representing almost 20 percent of the world's available fresh surface water. The quality and quantity of these waters play a major role in determining the health and welfare of the people, and the diversity of the wildlife living in and around their shores. These waters contribute to transportation, agriculture, natural resource extraction, fisheries, energy, and industrial capacity fundamental to the growth and prosperity of the region, and of the United States and Canada as a whole.

However, these economic and life-sustaining values are frequently forgotten by ever expanding human populations and industries. In the 1960s, ranch mink fed fish from both Lakes Michigan and Erie and Lake Trout, once abundant in Lake Ontario, suffered reproductive failure. We now know that this was due to PCBs and dioxin-like activity, respectively. Wildlife biologists documented reproductive failure of Bald Eagles, Double-crested Cormorants, and Herring Gulls nesting on islands and shorelines of some of the lakes. Intensive studies of Herring Gulls revealed that reproductive failure was due to both embryo toxicity and abnormal parental behaviour. Congenital malformations were found in chicks of nine species of fish-eating birds. Biologists observed hyperplastic thyroids in introduced salmon and Herring Gulls. Some Herring Gull embryos were feminized, and the sex ratio of some of their populations as well as those of some Lake Michigan fish were markedly skewed towards females.

Public and scientific concern about pollution of the lakes culminated in the governments of the United States and Canada signing the Great Lakes Water Quality Agreement in 1972. The agreement focussed on pollution and particularly on those forms that contribute to eutrophication. It committed the governments to regulation, research and surveillance, and has been amended to cover a wide array of chemicals and their sources, and now calls for the virtual elimination of persistent toxic substances.

In keeping with the agreement, governments have supported research and surveillance which has resulted in a greater understanding of the variety, incidence, and severity of contaminant-associated effects in fish and wildlife, and the application of more sensitive methods of detection. Recently, considerable effort has gone into the comparison of relatively "clean" and "contaminated" sites. This has led to the discovery of decreased hatching success, congenital malformations, and feminization in Snapping Turtles, and the presence of the egg protein vitellogenin in the plasma of male Snapping Turtles and Herring Gulls. Use of functional tests has revealed a delayed and decreased increase in corticosterone in response to injected ATCH in prefledgling Herring Gulls and adult Mudpuppies (*Necturus* sp.), and suppression of T-cell-mediated immunity in Herring Gulls, Caspian Terns, and Black-crowned Night Herons, and altered B-cell response in Herring Gulls and Caspian Terns.

In particular, I have been focussing on effects on thyroid function. The effects differ between adults, prefedgling, and pipping Herring Gulls, and although plasma thyroxin levels may be normal, the thyroid glands are often greatly enlarged and contain a decreased concentration of thyroxin. Plasma free thyroxin concentrations are significantly correlated with plasma hydroxyl-PCB concentrations. We have also found thyroid effects in hatchling and adult Snapping Turtles. Currently, the most common thyroid disease in humans in the state of Michigan is endemic goiter. Across the Detroit River, in Windsor, Ontario, the rate of hospitalizations for females under 25 with thyroid gland disorders was a startling 208 percent higher than for the province as a whole, between 1986 and 1992. Thyroid hormone substitutes are currently the most frequently prescribed drug in Canada.

Aquatic Species in Ecosystems at Risk: Assessing Normal and Abnormal Endocrine Responses in Wildlife

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Over the last decade, we have described abnormalities in the reproductive, endocrine and immune systems of alligators living in contaminated lakes from central Florida, USA. To better understand the extent of these problems, we have performed studies on other wildlife species, such as the Mosquito fish, and Florida gar, living in these lakes. We have examining three lakes, Lake Woodruff, a reference population with low contamination by pesticides and nutrients, Lake Apopka which is heavily contaminated with pesticides and nutrients and Orange Lake, with low pesticide levels but elevated nutrients. We have found that Mosquito fish living in Lake Apopka have reduced gonapodial size, sperm counts, tissue testosterone concentrations and male behavior. Exposure of reference males to water from Lake Apopka for one month alters their reproductive behavior and sperm counts. Male Florida gar from Lake Apopka have altered plasma testosterone concentrations, as seen in alligators from the same lake and display an altered stress response. Additional studies with frogs suggest that nutrients in two of the lakes can also act as endocrine disruptors as female frogs exposed to environmentally relevant concentrations of nitrates exhibit altered steroidogenesis, altered plasma IGF-1 concentrations and altered gene expression for thyroid receptor alpha. Major questions currently being tested include studies of natural and contaminant altered sex differentiation and reproductive function at the gene to organism level in fishes, amphibians and reptiles.

Testing Strategy for Addressing the Effects of Endocrine Disrupters on Invertebrates

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The effects of endocrine disrupters on invertebrates are well documented. However, due to the complexity of endocrine mediated responses, and the diversification of physiological characteristics within the large number of Phyla and Classes included as invertebrates, designing testing strategies for this group is particularly difficult.

Endocrine systems in invertebrates are quite different from those observed in vertebrates and therefore, when addressing endocrine disrupting chemicals two main issues should be considered, the effects on invertebrates of chemicals interacting on vertebrate hormonal systems and the effects of chemicals mimicking invertebrate hormones.

The knowledge on invertebrate endocrine systems is limited to a few taxa, and physiological differences in reproductive patterns and strategies, even among closely related groups, create large difficulties for extrapolating the ecological relevance of expected effects.

An alternative testing strategy, including new assessment tools, is proposed. The strategy includes an initial assessment on the likelihood of ED effects for the chemical on the basis of available information on other groups, and the development of two alternative approaches, when a potential for ED effects is suspected.

The first alternative can be applied when the mechanism of action of the substance is known, and the available information allows the selection of invertebrate species and test endpoints appropriate for covering the interactions associated to this particular endpoint. The knowledge on the sensitivity of different invertebrate groups and endpoints to chemicals acting through vertebrate endocrine activities, particularly estrogens, is experimenting an exponential increase. In addition, new testing possibilities and tools are emerging. Both facts are enhancing our capacity for selecting adequate assays. The information regarding effects of industrial chemicals acting on invertebrate endocrine mechanisms is scarce and mostly limited to molting (ecdysones) and juvenile (JH) hormones. However, a large amount of information has been generated on insect systems due to the development of insecticides acting as insect growth regulators. The IRAC (Insecticide Resistance Action Committee) classification on mode of action includes pesticides acting as juvenile hormone mimics, mite growth inhibitors, chitin biosynthesis inhibitors, and ecdysone agonist/disruptors. The available information on interspecies sensitivity for these groups will be used for helping on the testing strategy for chemicals showing activities on related mechanisms.

However, in most cases, the available information may suggest a potential role of ED activities, but will not give enough evidence for confirming the mechanisms of action. A second alternative covers this possibility, using a combination of toxicity tests on a battery of invertebrate groups with different reproductive strategies. An invertebrate multispecies toxicity test has been developed by INIA for covering simultaneously three taxonomic groups and three reproductive strategies; guarantying comparable exposure levels among organisms. The assay covers in 46 days a population study on the cladoceran *Daphnia magna*, a multi-generation study on the chironomid *Chironomus praxinus*, and a reproduction study on the snail *Limnaea peregra* (Sanchez and Tarazona, 2002). In this way, ecologically relevant effects on crustaceans, insects and molluscs, and parthenogenetic, sexual reproduction with dimorphism and sexual reproduction with hermaphroditism, can be addressed simultaneously with a single assay.

Reference

Sanchez P and Tarazona J.V. (2002) Development of a multispecies system for testing reproductive effects on aquatic invertebrates. Experience with *Daphnia magna, Chironomus prasinus* and *Lymnaea peregra*. Aquatic Toxicol. 60: 249-256.

Effluent Induced Disruption of Sexual Function in Roach (*Rutilus rutilus*) in Rivers in the United Kingdom - A Case History and Future Perspectives

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Almost 15 years ago in the UK, we found that exposure of male fish to effluents from sewage treatment works (STWs) induced the synthesis of vitellogenin, an oestrogen-dependent protein normally found only in the blood of maturing female fish. This oestrogenic activity was shown to persist in rivers for considerable distances (many kms) downstream of effluent discharges and it was recognised that it could potentially impact on riverine wildlife. We chose a common cyprinid fish, the roach (*Rutilus rutilus*) as our sentinel species to investigate the possible effects of this hormone activity on wild riverine fish populations. Extensive field studies established widespread, and in some cases severe, gonadal disruption in roach and this was correlated with the amount of effluent in those rivers. Controlled exposures of various life stages and for periods of up to a year have proven that some of the feminising effects seen in wild roach arise as a consequence of effluent exposure. Furthermore, roach with altered gonadal development have an altered timing to maturation and/or produce gametes of poorer quality with a reduced capacity for fertilisation. Steroid oestrogens (natural and synthetic) and alkylphenolic chemicals have been identified as the potential feminising agents in effluents from STWs and our lab-based exposure studies have provided substantial evidence to support this. In the laboratory we have shown that long term exposure to the pharmaceutical ethinyloestradiol, a synthetic oestrogen used in the contraceptive pill, causes reproductive failure in fish populations at aqueous concentrations of 1 part in billion. Effluent discharges, however, contain more than 60 000 chemicals, excluding their products of degradation, and we have been conscious to keep an open mind on the chemical causation of sexual disruption. Indeed, our mixtures experiments with oestrogens have shown that they can have additive effects. Adding a further dimension (and complication) to this work we now know that effluents in the UK have anti-androgenic activity (which could similarly result in feminising effects). Our present studies are exposing roach to effluents and identifying the mixture of oestrogenic and anti-androgenic chemical that get into the fish (in bile) to get a more complete picture of the feminising insults faced by wild roach. Two major questions that have yet to be answered in the story of sexual disruption in roach in UK Rivers are how does this disruption occur? (the mechanisms) and, what are the population level consequences (in the contexts of abundance and genetic integrity)? We are applying molecular techniques to address both of these questions. Mechanisms of disruption are being investigated by cloning specific genes known to play central roles in reproduction and determining how known endocrine disrupting chemicals (EDCs) and effluents with hormone activity affect their expression. We know, however, that some chemicals can have multiple target sites in the body to disrupt physiological function. Thus, in parallel we are undertaking similar experiments using, transcriptomics - employing gene micro arrays. Collaborating with Liverpool University and colleagues in Japan we are working to establish a 10 000 gonad gene cDNA array for this purpose. To start to get an understanding of possible population-level consequences of sexual disruption, we are undertaking studies with the zebrafish as our laboratory model, a species that has similar breeding biology to the roach. We are using DNA microsatellite markers to determine the paternity and maternity in populations of zebrafish and we will then investigate the impacts of life long exposure to environmentally relevant concentrations of EDCs on the breeding dynamics and genetic integrity of the laboratory maintained populations.