

**Endocrine Disruptors**  
**14-15 February 2012**  
**Holiday Inn, Munich**



**Day One: Tuesday 14 February 2012**

**08.30 COFFEE AND REGISTRATION**

**09.20 Welcome and Introduction to Endocrine Disruptors 2012**

**Session 1: Regulatory Aspects**

**09.30 EU's approach to the regulation of endocrine disruptors**

Endocrine disruptors: definitions, criteria and risks

Analysis of EU's initial strategy for endocrine disruptors

- Monitoring programmes
- Establishment of a priority list of substances
- Impact on future regulatory developments

EU legislative action

- REACH (registration, identification of substances of very high concern "SVHCs", Annex XIV, Annex XV dossier etc)
- The new EU Plant Protection Regulation
- Proposed Biocidal Products Regulation
- Cosmetics Regulation
- Food contact materials legislation
- Water Framework Directive

Approaches in individual EU Member States

Does existing or proposed legislation adequately address the issue?

Action points for industry

*Giles Chappell, McKenna Long & Aldridge LLP, Belgium*

## **10.00 OECD activities on endocrine disruptors testing and assessment**

*Nathalie Delrue, OECD, France*

## **10.30 COFFEE AND NETWORKING**

### **Session 2: Test Strategies and Methods**

#### **11.00 The evaluation of control performance in the amphibian metamorphosis assay (OPPTS 890.1100) and in the fish short-term reproduction assay (OPPTS 890.1350) with emphasis on weight of the evidence evaluation of the endpoints for potential interaction with the androgen, estrogen and thyroid hormone systems**

The United States Environmental Protection Agency (USEPA) has begun to execute a new paradigm with the Endocrine Disruptor Screening Program (EDSP). The goal is to screen chemicals for potential endocrine or thyroid activity. Screening is being conducted using 11 Tier 1 assays. Two of those assays are the Amphibian Metamorphosis Assay (AMA) and the Fish Short-Term Reproduction Assay (FSTRA). Although the OPPTS 890 assay guidelines are very specific, it is not a trivial matter to meet the acceptance and performance criteria. This presentation will evaluate control performance data from at least 12 assays conducted over the past 10 months and compare this performance against recommended acceptance and performance criteria presented in their respective OPPTS 890 and OECD guidelines. In addition, this presentation will propose weight of evidence (WoE) designation of critical endpoints like developmental stage and thyroid histopathology from the AMA and fecundity, fertility, gonad histopathology, gonadal somatic index, blood plasma vitellogenin and male tubercle score from the FSTRA. The WoE designations will also take into consideration endpoint results from the other 9 Tier 1 EDPS assays.

*Ronald C Biever & Mark A Cafarella, Smithers Viscient, USA*

#### **11.30 Guidance to clients on their testing strategies: difficulties and constraints**

One of the difficulties with changing regulations is knowing how to be ahead in the game? Companies discovering and developing new Crop Protection Compounds, Biocidal products or Specialty Chemicals are looking at the Global market opportunities for their inventions. The time scale to develop such compounds from discovery to market may be several years and the costs extremely high. In addition, the cost for re-registration and maintenance of such compounds are becoming critical to some companies.

There is the need to ensure that any studies conducted for safety and regulatory purpose are conducted according to the best scientific knowledge and latest guidelines of the day. It is important that the studies are conducted only once using harmonized global guidelines. This ensures that the costs for development are kept low; the study will be accepted in all key countries and that no unnecessary animal experiments will be conducted.

The presentation will focus on the Changing European Regulations covering Crop Protection Compounds, Biocides and Reach and the difficulties for companies in these uncertain times.

*Mike Neale, Matthew Kane & Thorsten Behsen, LKC Switzerland Ltd, Switzerland*

### **12.00 Evaluation and lessons learned in the in vitro tier 1 endocrine disruption screening assays**

The Endocrine Disruption Screening Program (EDSP) was initiated by the Environmental Protection Agency (EPA) in order to develop a screening program to evaluate whether or not certain chemicals could elicit hormonal effects in humans. As a result Tier 1 assays, in vitro and in vivo were selected to evaluate endocrine, androgen, and thyroid effects of commercial chemicals and environmental contaminants. CeeTox has established these assays and run over 25 test materials through each of these 5 in vitro assays: Steroidogenesis, Estrogen receptor (ER) binding, Androgen receptor (AR) binding, Aromatase and ER Transactivation.

There is a need to know the strengths and weaknesses of the Tier 1 assays and whether additional endpoints should be added to make them more robust. One example of this is the in vitro Steroidogenesis assay where only 2 endpoints are evaluated: Testosterone and Estradiol. Effects on the steroidogenesis pathway can occur at multiple steps. By adding more endpoints in the steroidogenesis pathway (for example DHEA, Corticosterone, 17OH progesterone, progesterone, androstendione etc), a more comprehensive evaluation of test chemicals impact on steroidogenesis can be assessed. This type of evaluation for each of the Tier 1 in vitro assays would significantly strengthen the predictability and could provide a more comprehensive screening program.

*Dr Colleen Toole, DP Blakeman, S McColley, C Haines, B. Wallace, H Wagner, J Willoughby P Wilga & JM McKim Jr, CeeTox, USA*

### **12.30 Q & A SESSION**

### **12.45 LUNCH AND NETWORKING**

### **14.00 Lack of androgenicity and estrogenicity of the three monomers used in Eastman's novel polyester plastic Tritan™**

Eastman Tritan™ copolyester, a novel plastic from Eastman is manufactured utilizing three monomers, di-methylterephthalate (DMT), 1,4-cyclohexanediemthanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD) in various ratios. As with most any polymer, the monomers along with the high molecular weight oligomers, whose toxicity is

most commonly represented by the monomers, make up the predominate amount of free chemicals available for leaching into the environment and/or foods. In light of the high level of public concern about the presence of endocrine (primarily estrogenic) activity ascribed to certain plastics and chemicals in the environment, Tritan's™ monomers were evaluated using QSAR for binding to the androgen receptor and estrogen receptors (alpha and beta) as well as a battery of in vitro and in vivo techniques determine their potential androgenicity or estrogenicity. The findings were universally negative. When these data are coupled with other in vivo data developed to assess systemic toxicity and developmental and reproductive toxicity, the weight of evidence clearly indicates that these monomers do not pose an androgenic or estrogenic risk to humans. Additional data presented also support such a conclusion for terephthalic acid (TPA). TPA is also a common polyester monomer and is the main mammalian metabolite formed from DMT.

*Dr James Deyo, Eastman Chemical Company, USA*

### **14.30 Endocrine disruption in aquatic environment from mechanistic to predictive approach**

Several field studies have demonstrated that aquatic population and especially fish, are impacted by environmental releases of certain endocrine disruptor compounds (EDCs). Obvious disruptions include induction of the egg yolk protein in males and juveniles, feminization of the reproductive tract of males, steroid hormones disruption, etc. These effects have been scientifically described as the consequence of the ability of these molecules to act as an internal hormone or interact with hormonal regulation pathways. Basic pathways, such as hormone-receptor interaction have been described to explain recurrent observation after fish exposure. However, other pathways have been pointed out due to growing knowledge on fundamental research on hormonal function, in particular by the availability on the market of -omics tools which allow to focus on higher functional levels in a given biological system. By today, the major scientific challenge is to define methods that meet criteria such as efficiency, reproducibility and representativeness to predict environmental fate and ecotoxicity of molecules within an environment. Through this presentation, we aim to investigate the ability of laboratory methods to determine the potency of EDC on an organism *versus* the limitation of available methods to predict the environmental impact.

*Dr Stéphanie Nadzialek, Albemarle Europe Sprl; Prof Patrick Kestemont & Sophie Depiereux, FUNDP Namur, Belgium*

## **15.00 COFFEE AND NETWORKING**

### **15.30 A highly sensitive in vivo assay for the detection of estrogenic activity using tg(cyp19a1b-GFP) transgenic zebrafish embryos**

Aromatase is the only enzyme converting androgens into estrogens, which are key actors in reproductive biology. Teleost fishes have two copies of the cyp19a1 gene that encode two isoforms of aromatase: cyp19a1a encodes ovarian aromatase, while the cyp19a1b gene encodes brain aromatase. Our recent work showed that, in zebrafish, aromatase B is

strongly expressed in a unique brain cell type, the radial glial cells (RGC). In mammals, such cells act as stem cells during embryonic development before disappearing in adults. On the contrary, in fishes, RGCs persist in adult where they act as neuronal progenitors allowing the brain to constantly keep growing. We have also shown that, intriguingly, the *cyp19a1b* gene is very sensitive to estrogens, through a mechanism that involves a well conserved ERE. This feature makes this gene an outstanding biomarker of xeno-estrogen exposure, and we have developed an *in vivo* assay allowing detection of estrogenic activity with a very high sensitivity. This assay is based on a transgenic zebrafish *tg(cyp19a1b-GFP)* line that expresses GFP in RGCs. By quantifying GFP expression in live fish, we show that short-term exposure of *tg(cyp19a1b-GFP)* embryos from 0 to 120 hpf to a variety of well established estrogenic compounds (estradiol, estriol, estrone, ethinylestradiol,, zearalenone and its metabolites, nonyl, octyl and tert-pentylphenol, BPA, benzophenones derivatives, etc) turns on GFP expression in a concentration-dependent manner. Overall, we demonstrate the remarkable usefulness of the *tg(cyp19a1b-GFP)* embryos as a reliable, sensitive and rapid *in vivo* estrogenic screening assay. As we have also evidenced an effect of estrogens on the neurogenic activity of zebrafish, abnormal exposure of fish embryos to estrogenic endocrine disruptors is likely to affect the neurogenic process. *Supported by the ANR NEED and the NEMO programme*

*François Brion, University of Rennes, France*

### **16.00 Pharmacophore models for predicting endocrine disrupting effects of xenobiotics: concept and case studies on steroid modulating enzymes**

Pharmacophore models are well established tools for the discovery of bioactive compounds. They consist of a 3D arrangement of chemical features, which are responsible for triggering or blocking a biological response *via* a specific pharmacological target. While widely used by the pharmaceutical industry, pharmacophore-based *in silico* screening has hardly been applied outside the drug discovery-focused research environment.

As pilot projects in the field of endocrine disruptors, two models for steroid-modulating enzymes were developed and employed to virtually screen a 3D structural database of putative endocrine disruptors (ED database). The first model was developed for 17 $\beta$ -hydroxysteroid dehydrogenase 3 (17 $\beta$ -HSD3), which catalyzes the transformation of androstene-3,17-dione into testosterone. Among the virtual hits, the class of UV-filters was dominant. Biological testing of virtual hits and other UV-filters revealed that several benzophenone-type UV-filters inhibit 17 $\beta$ -HSD3 with IC<sub>50</sub> values in the low micromolar range. Among the benzophenones, a clear structure-activity relationship was observed, which could be used for the development of safer UV-filters. The second model was devoted to the search for 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) inhibitors. 11 $\beta$ -HSD 1 and 2 convert cortisone to cortisol and vice versa, which is essential for a balanced glucocorticoid concentration in many tissues. Virtual hits from screening the ED database suggested, amongst others, Unisilan 41 as 11 $\beta$ -HSD inhibitor. *In vitro* assessment confirmed that this silane coupling agent inhibited 11 $\beta$ -HSD2 with an IC<sub>50</sub> of 6 $\mu$ M. These two case studies led to the identification of bioactive chemicals amongst a low number of

tested hits. The *in silico* filter was thereby a powerful tool to pre-filter potentially active compounds out of thousands of chemicals.

In conclusion, integrating pharmacophore models in the field of environmental and industrial chemical safety assessment is a promising approach to speed up the identification of potentially harmful chemicals and to develop safer chemicals for the future.

*Dr Daniela Schuster, Institute of Pharmacy/Pharmaceutical Chemistry, Innsbruck, Austria*

**16.30 Q & A SESSION**

**16.45 END OF DAY ONE**

**18.00 EVENING SOCIAL EVENT**

**Day Two: Wednesday 15 February 2012**

**08.15 COFFEE AND NETWORKING**

**Session 3: Mode of Action/New Effects**

**09.00 Mechanisms of endocrine disruption in humans**

There is growing scientific evidence to support the hypothesis that chemicals present in the environment may interact with hormonal systems leading to adverse health effects in human and wildlife populations. Most studies of the health effects of endocrine disrupting chemicals have focused on well-documented hormone receptor-mediated activities, including reproductive and thyroid hormones, but additional modes of action, including damage to the liver damage, disrupted glucose and lipid metabolism and other health effects have increasingly been reported for certain widely encountered chemicals. The extensive potential for low-dose effects has added to the controversy about possible hazards and whether currently recommended exposure thresholds require revision. To date, the majority of scientific evidence to support these hypotheses has come from animal and laboratory studies and debate about the possible health effects of endocrine disrupting chemicals in humans has been hindered by the lack of human bio-monitoring data with sufficient statistical power to detect plausible low-dose effects.

This talk will provide an overview of our most recent results in exploring the human health effects of endocrine disrupting chemicals of high public health concern, including the environmental phenol bisphenol A and perfluorochemicals including perfluorooctanoic acid (PFOA), in human populations. We have previously reported on associations between exposures to these compounds and human health effects including diagnoses of cardiovascular disease, diabetes and thyroid disease. We have subsequently been exploring the changes in gene expression that accompany exposure to these chemicals in human

subjects. We have been conducting quantitative expression analysis *in vivo* in circulating white blood cells using Taqman low density arrays, exploring correlations between gene expression changes with metabolite concentrations measured in blood and urine samples and with detailed biometric data, and validating these associations in isolated tissues and cells. Our most recent results will be presented and their significance in terms of public health protection discussed in more detail.

*Prof Tamara Galloway, David Melzer & Lorna Harries, University of Exeter, UK*

### **09.30 Role of endocrine disruptor chemicals on the tissue levels of AhR and sex steroid receptors in premenopausal breast cancer patients : comparison with benign female tumours**

Environmental endocrine disruptors are the prototypic ligands of the aryl hydrocarbon receptor (AhR) which promotes tumour formation in some reproductive model systems. Breast cancer affects Iranian women at least one decade younger than their counterparts in other countries and the incidence of breast fibroadenoma, uterine fibroadenoma and ovarian cysts are growing in the last two decades in Tehran. This study aimed to compare the AhR levels in premenopausal breast cancer and mentioned reproductive disorders with appropriate normal groups. Possible associations of AhR with dietary, lifestyle and reproductive risk factors and other fundamental genes of breast cancer and reproductive disorders were the other major goals of present study. To conduct the comparisons all possible reproductive, environmental and lifestyle risk factors of mentioned diseases were recorded in 100 breast cancer, 100 breast fibroadenoma, 137 uterine fibroadenoma and 137 young women with ovarian cysts and compared with 400 women in normal group from 2009 to 2011. AhR overexpression in epithelial cells of premenopausal patients emphasized the susceptibility of these cells to environmental induced reproductive disorders. The AhR overexpression was contributed to ER-/PgR- immunophenotype and GdA overexpression in malignant tissues. Weight gain (after 18 and after pregnancy), long term (>5yrs) OCP consumption, smoking, severe stress, history of ovarian cysts, hormonal deregulations, living near PAHs producing sources, nutritional factors especially increased intake of animal fats were correlated with increased risk of breast cancer and reproductive disorders and were correlated with elevated tissue levels of AhR. It seems that increased risk of breast cancer and other reproductive tumours in Tehran may be the result of exposure to environmental endocrine disruptors. Long term exposure to environmental estrogens can increase the tissue levels of AhR and deregulate the expression pattern of sex steroid receptors and other genes in target tissues.

*Dr Sepideh Arbabi Bidgoli, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran-Iran*

### **10.00 PPAR-mediated metabolic disruption by phthalates**

Metabolic homeostasis requires a controlled balance between energy storage and consumption; several NRs and their coregulators are instrumental in these processes. Among these, the PPARs act as lipid sensors that cooperate in different organs to adapt

gene expression to a given metabolic status. PPARs are sensor receptors with a rather large ligand binding domain, which can accommodate a variety of ligands, primarily lipid derivatives. Plasticizers, surfactants, pesticides, and dioxins can modulate PPAR activity, although fairly little is known about the molecular mechanisms and the physiological outputs involved. The phthalates are another group of well-characterized peroxisome proliferators. *In vitro* transactivation assays and intact cellular systems were used to reveal that phthalates and their metabolites bind and activate the three PPARs, among others NRs. MEHP is pro-adipogenic in a cell culture model, suggesting that it may act as a metabolic disruptor and promote obesity *in vivo*. Paradoxically, *in vivo* experiments partially contradicted these results since adult mice treated with high or low doses of DEHP were protected from weight gain, in a mechanism that involves PPAR $\alpha$ . However, this paradoxical phenotype was also completely abolished in PPAR $\alpha$ -humanized mice (mice in which the mouse PPAR $\alpha$  alleles were replaced by the human PPAR $\alpha$  gene). Together with further molecular studies, our observations highlight the species-specific EDC activity of some compounds, at least in part due to evolutionary differences in the receptors interacting with them.

*Béatrice Desvergne, University of Lausanne, Switzerland*

### **10.30 COFFEE AND NETWORKING**

#### **11.00 Epigenetic effects of endocrine-disrupting compounds**

Imprinting is an epigenetic form of gene regulation that mediates a parent-of-origin-dependent expression of the alleles of a number of genes. It consists in a methylation of CpGs in specific sites within or surrounding the gene, called differentially methylated domains (DMDs). The appropriate transmission of genomic imprints is essential for the control of embryonic development and fetal growth. A number of endocrine-disrupting compounds (EDCs) affect male reproductive tract development and spermatogenesis. It was postulated that the genetic effects of EDCs might be induced by alterations in gene imprinting. We tested 3 different categories of EDCs: methoxychlor, vinclozolin and dioxin. Their administration during gestation induced in the offspring a decrease in sperm count and significant modifications in the methylation pattern of a selection of paternally- and maternally-expressed canonical imprinted genes. The observation that imprinting was largely untouched in somatic cells suggests that EDCs exert their damaging effects via the process of reprogramming that is unique to gamete development. Interestingly, the effects were transgenerational, disappearing gradually from F1 to F3. A systematic analysis showed a heterogeneity in the CpG sensitivity to EDCs. We propose that the deleterious effects of EDCs on the male reproductive system are mediated by imprinting defects in the sperm. The reported effects of EDCs on human male spermatogenesis might be mediated by analogous imprinting alterations.

*Dr Ariane Paoloni-Giacobino, Swiss Center for Applied Human Toxicology, Switzerland*

## **Session 4: Case Studies**

### **11.30 Epidemiological studies of Bisphenol A in adults**

Bisphenol A has been extensively studied in laboratory models, but human evidence is scarce. This is puzzling, as the metabolism and excretion of BPA is very different in humans compared to rodents.

The first major epidemiological analyses of adult health effects associated with exposure to BPA involved a study of 1455 adults with measured urinary BPA (uBPA) from the US National Health and Nutrition Survey (NHANES) 2003-2004 (Lang et al., 2008). Higher BPA concentrations were associated with cardiovascular diagnoses, and this association was again evident in a separate sample in NHANES 2005/06 (n=1493). Initial associations with diabetes and some liver enzyme changes did not reach significance in the 2005-2006 data, but remained significant in pooled data. ([Melzer et al., 2010](#)). These cross-sectional studies were recently augmented by a longitudinal study. Associations between urinary BPA concentrations and carotid atherosclerosis have also been reported. The mechanism by which exposure to BPA affects CAD incidence is unknown, but seems to be independent of classical CAD risk factors.

To establish definite causation, randomized trials would be needed in humans, but these would face severe ethical and practical barriers. The implications of this 'evidence impasse' for regulation will be discussed.

*Prof David Melzer & Prof Tamara Galloway, University of Exeter, UK*

## **12.00 Q & A SESSION**

### **12.15 LUNCH AND NETWORKING**

### **13.30 Effects of perinatal exposure to Bisphenol A on the mammary gland**

There is concern that exposure to low doses of BPA, defined as  $\leq 5$  mg/kg-bw/day, may have developmental effects on various hormone-responsive organs including the mammary gland. Here, we asked whether perinatal exposure to a range of low doses of BPA is sufficient to alter mammary gland hormone response later on in life, with a possible impact on breast cancer risk. To mimic human exposure, we added BPA to the drinking water of C57/Bl6 breeding pairs. Analysis of the mammary glands of their daughters at puberty showed that estrogen-dependent transcriptional events were perturbed and the number of estrogen-induced proliferative structures was altered in a dose-dependent fashion. Importantly, adult females showed an increase in mammary epithelial cell numbers comparable to that seen in females exposed to diethylbestrol (DES), a compound exposure to which was previously linked to increased breast cancer risk. Molecularly, the

mRNAs encoding Wnt-4 and RANKL, two key mediators of hormone function implicated in control of mammary stem cell proliferation and carcinogenesis, showed increased induction by progesterone in the mammary tissue of exposed mice. Thus, perinatal exposure to environmentally-relevant doses of BPA alters long-term hormone response that may increase the propensity to develop breast cancer.

*Prof Cathrin Brisken, National Center of Competence for Molecular Oncology, Switzerland*

#### **14.00 Polycystic Ovary Syndrome (PCOS) and Bisphenol A (BPA): experimental and clinical data**

Bisphenol A (BPA) is an estrogenic contaminant used primarily in the manufacture of polycarbonate plastic and epoxy resins. With a broad spectrum of applications including use in certain food and drink packaging, in many plastic consumer products as well as dental materials, it is now considered one of the most ubiquitous industrial compounds. This has placed BPA at the center of a debate over its adverse effects on human health that has attained great scientific and public interest during the last decade.

Though numerous experimental studies have documented its adverse reproductive effects, the clinical consequences of BPA exposure in the human population are relatively unknown. Biomonitoring studies have reported that BPA can be measured in human tissues and fluids and has been quantified at higher levels in the follicular fluid of women with polycystic ovarian syndrome (PCOS) compared to healthy fertile controls.

Polycystic ovarian syndrome is a complex and heterogeneous disorder that represents the most common endocrinopathy among women of reproductive age. Well documented reproductive features of the syndrome are anovulation, hyperandrogenism and polycystic ovaries. These abnormalities are associated with hyperinsulinemia and high prevalence of significant metabolic aberrations with long-term health hazards including increased risk for cardiovascular disease. The etiology of the syndrome remains enigmatic and the potential influence of environmental factors is under intensive investigation.

A possible role of BPA in the pathophysiological mechanisms of PCOS is likely since serum BPA levels are higher in women with PCOS when compared to normal peers and neonatal rat exposure to the chemical induces a PCOS resembling phenotype in adult life. The underlying mechanisms are not yet fully determined though an interplay between androgens and BPA is implied.

When considering the potential association between PCOS and BPA exposure two questions are reasonable to be posed: 1. Is there any biological plausibility for BPA to interfere with the reproductive and metabolic features that characterize the syndrome and what are the potential pathogenetic pathways mediating this interaction? 2. If so, what are the available data to support this interaction?

*Dr Evanthia Diamanti-Kandarakis, Medical School University of Athens, Greece*

## **Session 5: Evaluation of Data including Risk Assessment**

### **14.30 Tiered approach for exposure assessment within an integrated risk assessment framework of endocrine disruptors in the Uruguay River**

A tiered approach for exposure assessment composed of in vivo and in vitro bioassays and chemical analysis of endocrine disrupting compounds (EDCs) of increasing specificity was designed within an integrated risk assessment framework. Its application was demonstrated at a watershed of the Low Uruguay River where industrial (Kraft bleached pulp mill), domestic (city discharges) and agricultural potential sources (soy crops) are present.

The first tier evaluated the general river health by applying the acute bioassays with *Daphnia magna* and *Pimephales promelas* and water quality indicators. The second tier pondered reproductive, developmental and sub-lethal effects through the application of the *Ceriodaphnia dubia* reproduction test, *Pimephales promelas* embryo test, and *Hyaella curvispina* growth test, and quantified chemical markers of endocrine disruptors from point-source discharges and non-point pollution sources.

The third tier targeted EDCs concentrations and evaluated endocrine disruptive effects using a combination of in vivo and in vitro assays, at gene, molecular, tissue, and organism levels. The receptor binding techniques employed to assess estrogenicity were: the yeast estrogen screen and the ER-CALUX<sup>®</sup> assay, and to evaluate androgenicity, the AR-CALUX assay. The 21 days *Pimephales promelas* fish exposure test was developed, exposing fish to both municipal wastewater and pulp mill effluent. In these tests, several end-points were measured: vtg gene transcription, VTG, fecundity, nuptial tubercles and histopathology. In the case of pulp mill effluents, the effects were not present at tissue level, but a decrease in fecundity was observed and effects at gene level. In the case of municipal wastewater, fecundity and VTG were affected. Tissue residues of exposed fish suggested probable responsible EDCs.

Field studies carried out with the sentinel wild fish *Astyanax fasciatus* aided in demonstrating effects at geographical and population levels and linking the results to laboratory experiments. Some of the most prevalent EDCs in fish tissue were nonylphenol, but also a complex mixture of endosulfan, chlorinated organic chemicals, phytoestrogens and wood extractive substances.

*Diana Míguez, Technological Laboratory of Uruguay, Uruguay; Elise Cartmell, Ana Soares & Simon Pollard, Cranfield University, United Kingdom*

### **15.00 National study on sperm quality in Switzerland**

Introduction:

For decades, numerous studies have reported a decline in sperm quality among industrialised countries. Explanations for this decline point toward environmental factors acting on animals and humans before and during their reproductive life. More recently, the detrimental role of endocrine disruptors during foetal development has also been hypothesised. In order to test whether these effects are operating in Switzerland, a survey study among Swiss young men was initiated in 2005 and will cover within the next two years the entire country. At the same time a human biomonitoring is underway. A large panel of substances is analyzed such as phthalates, PCBs, BPA and PCPs.

#### Material and Methods:

One month before recruitment, all conscripts are informed about the study. When interested, the volunteers send a consent form and two questionnaires (personal and parental) to the Swiss army physician in charge of supervising the study. Four recruiting centres (Lausanne, Rütli, Windisch, Monte Ceneri) are actively involved in the sample collection phase. At the end of their recruiting camp, volunteers, aged between 18-20, are invited in a nearby laboratory for biological investigations. Sperm samples are analysed according to WHO recommendations (2010) and the following parameters are recorded: sperm concentration, motility and morphology using a computerised system (CASA, SCA Microptic, Spain). Identical procedures are used in all four laboratories. Medical, anamnestic and biological data are stored in a centralised database (FileMaker Pro), from which data can be extracted for further statistical analysis (SPSS, Somers NY, USA). All samples of sperm, blood and urine are frozen at -80°C and stored in Lausanne for further analysis.

#### Results:

Results are grouped according to a geographic stratification of Switzerland and depending on place of living of the conscript: 1) Plateau west and center, 2) Jura, 3) Alps, 4) Plateau north and east. The median, p25 and p75 values of the sperm parameters were computed for the entire studied population (N=1768). Results indicate that the median values are above the WHO reference for all parameters. The p25 values are close to these references for all parameters indicating that about 25% of the samples are below the WHO thresholds (2010). The median value of sperm parameters were calculated for the 4 described regions. A significant lower sperm concentration, motility and morphology were detected in regions 3 and 4. Different levels of phthalate, specially DEHP, are observed in the four regions.

#### Conclusion:

This finding is currently further investigated by enlarging the cohort and identifying the causes of such differences. The results of the human biomonitoring are now analysed by the statistician and will allow to give conclusions in the next months.

*J Vargas, R Parapanov, A Senn & M Germond, FABER Foundation, Lausanne, Switzerland;  
J Mendiola, University of Murcia, Spain; E Stettler, Swiss Army Medical Services, Switzerland*

### **15.30 Q & A SESSION**

**15.45 COFFEE**

**16.00 CLOSE OF PROCEEDINGS**



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