PNRPE

PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

International Workshop 2016

Abstract Book

ENDOCRINE DISRUPTORS EFFETS ON WILDLIFE AND HUMAN HEALTH

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RÉPUBLIQUE FRANÇAISE

Ministère de l'Écologie, du Développement durable et de l'Énergie

anses
agence nationale de sécurité sanitaire alimentation, environnement, travail
Connaître, évaluer, protéger

EDITORIAL

De nombreuses substances chimiques, les perturbateurs endocriniens, modifient la régulation hormonale des êtres vivants, avec de possibles effets sur la reproduction, la croissance, le développement ou encore le comportement, aussi bien chez l'homme que pour la faune. On soupçonne ces effets d'être en lien avec des troubles de la reproduction, des cancers, des maladies métaboliques, des troubles de la croissance, etc. Certaines populations sont particulièrement vulnérables comme les femmes enceintes et les jeunes enfants. Les perturbateurs endocriniens sont présents dans de nombreux produits ou objets, comme des détergents, des cosmétiques, des matériaux variés présents dans des produits d'usage quotidien.

Le ministère de l'Écologie, du Développement durable et de l'Énergie (MEDDE) et l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses) organisent en ce début 2016 un colloque sur les perturbateurs endocriniens.

Ce nouveau rendez-vous fait suite à celui organisé à Paris, fin 2012 qui avait réuni plus de 300 personnes. Ce premier événement avait permis de dresser un large panorama des recherches réalisées en France ainsi qu'à l'étranger. Il avait en particulier fourni l'occasion d'exposer les projets soutenus par le MEDDE et l'Agence de l'environnement et de la maîtrise de l'énergie (Ademe) dans le cadre du programme national de recherche sur les perturbateurs endocriniens (PNRPE) et par l'Anses dans celui du programme national de recherche environnement santé travail (PNREST). Les travaux conduits dans le cadre du PNRPE avaient contribué à des avancées majeures remettant en cause certains points considérés comme acquis, tels que, pour certains perturbateurs endocriniens testés, la relation dose-effet «monotone». Par ailleurs, il a été confirmé qu'un mélange de substances pouvait produire des effets inattendus, potentiellement plus délétères, par rapport à la somme des effets individuels de chaque substance. Des travaux sur des méthodes de détection de perturbateurs endocriniens ont offert de nouvelles perspectives pour l'élaboration de tests réglementaires.

Une nouvelle rencontre était nécessaire pour faire un point sur les travaux les plus récents. Un appel international à communication a été lancé en 2015. Il a suscité beaucoup d'intérêt, puisque près de soixante-quinze propositions ont été soumises. Vingt-cinq interventions orales ont été sélectionnées. Pour introduire les travaux exposés, plusieurs conférenciers de niveau international ont été invités : Jean-Pierre Bourguignon de l'université de Liège, Germaine Buck-Louis du National Institute of Child Health & Human Development (Etats-Unis), Patrick Fénichel du Centre hospitalier universitaire de Nice, René Habert du Commissariat à l'énergie atomique et aux énergies alternatives,

Christy Morrisey de l'université du Saskatchewan, Leonardo Trasande de l'université de New-York et Andreas Kortenkamp de l'Institut de l'environnement de l'université de Brunel. Enfin, ce colloque se veut aussi un lieu d'échanges entre chercheurs, notamment autour de cinquante posters qui seront affichés lors de cet événement.

Les présentations s'organisent autour de sept sessions : les effets de perturbateurs endocriniens sur les espèces naturelles, les troubles du métabolisme et les diabètes en lien avec les perturbateurs endocriniens, l'épigénétique et les effets transgénérationnels des perturbateurs endocriniens, les effets des perturbateurs endocriniens sur le système reproductif, les effets des mélanges, les effets du bisphénol A et de ses substituts, l'expertise, l'évaluation du risque et le coût économique.

Cet événement s'inscrit dans la priorité accordée par les pouvoirs publics aux perturbateurs endocriniens. Le gouvernement a en effet adopté le 29 avril 2014 la stratégie nationale sur les perturbateurs endocriniens, qui a conforté l'interdiction du bisphénol A dans les biberons et dans les matériaux au contact des denrées alimentaires. D'autre part, la France accroît ses actions d'évaluation de risques sur des substances susceptibles d'interférer avec la régulation hormonale ; l'évaluation de cinq nouvelles substances est confiée chaque année par le gouvernement à l'Anses. L'objectif est de mieux protéger la santé et de faire de la France un pays moteur de l'adoption par l'Europe de règles plus protectrices.

De manière générale, la qualité de l'environnement est un important déterminant de santé. En santé-environnement les pouvoirs publics sont confrontés à deux impératifs : d'une part, agir parfois en situation d'incertitude, notamment sur la qualité de l'environnement, et d'autre part, continuer à accumuler des connaissances. En ce qui concerne les perturbateurs endocriniens, c'est bien dans cette double perspective que s'inscrit le 3ème plan national santé environnement (PNSE). Il vise en effet d'une part à traiter des enjeux de santé tels que les pathologies en lien avec l'environnement et, d'autre part, il affiche la perturbation endocrinienne comme l'une des grandes questions de recherche en environnement-santé. En matière de gestion de risques, la réduction de l'exposition des populations aux perturbateurs endocriniens est une des dix actions immédiates du 3ème PNSE. Il s'agit de limiter l'exposition des populations, en particulier celle des femmes enceintes et des jeunes enfants.

La communauté de recherche partage ces préoccupations. Les trois alliances nationales de recherche, Allenvi pour l'environnement, Aviesan pour les sciences de la vie et de la santé et Athena pour les sciences humaines et sociales ont publié l'initiative française pour la recherche en environnement santé (IFRES), qui décrit un plan d'action dans les domaines de la toxicologie, de l'écotoxicologie, de l'épidémiologie et des sciences sociales.

Plusieurs objectifs prioritaires y sont inscrits en termes de nouvelles méthodes à développer, de disciplines à mobiliser ou d'approches à favoriser. La question des perturbateurs endocriniens y est fortement prise en compte et celle-ci est l'objet d'un des neuf programmes de recherche ciblés identifiés dans ce plan d'action comme nécessaires au côté de programmes de financement généralistes pour animer des communautés scientifiques sur des sujets majeurs de santé publique ou de risque environnemental.

Trois ans après le colloque de 2012, ce nouveau rendez-vous scientifique porté par le ministère et l'Anses fait le point en ce début 2016. Nous souhaitons que vous puissiez trouver durant ces deux jours des réponses à vos questions ou des pistes de nouvelles perspectives pour mieux connaître et mieux gérer les perturbateurs endocriniens.

Décembre 2015

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Commissariat général au développement durable
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SUMMARY

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PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

Session 1. Effects of endocrine disruptors on wildlife

Plenary Conference

Endocrine disrupting chemical effects on avian migration: exploring links to global migratory species declines

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Although migration is a phenomenon that spans the animal kingdom, many long-distance migrant birds are currently in steeper decline relative to non-migrant species. Environmental contamination by various pollutants has been identified as an important potential factor contributing to these declines. The physiological processes involved in initiation of migration are under direct hormonal control and are finely synchronized so as not to interfere with other life cycle events such as reproduction and moult. Birds must perceive environmental cues to initiate the onset of migration, undergo periods of intense feeding bouts (hyperphagia) to produce and store significant amounts of fat, increase activity levels, directionally orient and navigate the route, and optimize energy expenditure during flight. Long distance migrants are often exposed to endocrine active pollutants (e.g. PCBs, PBDEs, PAHs, dioxins) but the potential of these EDCs to affect migratory behaviour, morphology or physiology have yet to be established. This prompted new questions about latent effects from exposure to EDCs during development or during migration to understand how this might impact patterns of migration behaviour and physiology in birds. Using shorebirds and passerine model species, we have taken a holistic approach to link the small scale mechanistic effects of industrial contaminants on cells to organisms to understand the broader implications for migratory bird populations using contaminated sites during key life stages of early development or on migration. This includes 1) determining key locations of exposures for long distance migrants, 2) understanding latent effects on neuro-endocrinological controls of migration and 3) developing methods to test cellular responses from dioxin exposures in vitro. To date, we have identified the exposure and hotspots of marine pollutants (PAHs and other dioxin-like compounds) to long distance migratory shorebirds using bioassays (H4llE) of sediment collected from shorebird staging areas across the Americas in partnership with numerous international collaborators. Also, we found that a model migrant passerine (European starlings) exposed as nestlings to the PCB mixture Aroclor 1254, a ubiquitous industrial contaminant, alters their migration orientation and moult. We further tested these same birds in a set of learning and memory trials and found that the high dose males failed to learn a spatial task suggesting PCB-induced changes in brain development. We have also been developing in vitro models (primary avian hepatocytes, fibroblast cultures and fibroblast stable cell lines) derived from multiple bird species (starlings, sanderlings, etc) to study the mechanisms of contaminant interference with adipocyte differentiation and

triglyceride production and utilization involved in fuelling and fattening during migration. Given more than 40% of the migratory bird species are declining worldwide, a lack of understanding of chemical effects limits our potential to employ conservation measures to protect migratory species. This work is critically important to identify the key life stages and consequences of pollutant exposure on migration success.

Integrated modeling of endocrine disruption in zebrafish at different biological levels (MOZAIC)

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Aim of the work

The translation of subtle functional deficits within individuals into population-level effects is identified as a main challenge for the hazard and risk assessment of endocrine disruptor compounds (EDCs) in fish. Among model species for ecotoxicological investigations, zebrafish (*Danio reiro*) is a vertebrate organism extensively used for scientific purposes and an increasing amount of toxicological data of EDCs toward zebrafish have been gathered during the past years. Thus, the aim of MOZAIC project was to propose an integrated modelling framework for zebrafish to assess how EDCs modify the level of hormones in individuals and how such disruption will impact individual fitness and population dynamics.

Methods

In order to achieve this goal, we developed a physiologically-based pharmacokinetic (PBPK) model for zebrafish for a panel of EDCs. To develop a PBPK model as generic as possible, toxicological parameters were computed using QSAR/QSPR models. A hypothalamic-pituitary-gonadal (HPG) axis model for male and female zebrafish was also developed and was coupled with the PBPK model to predict the dynamics of the circulating vitellogenin and steroid. To perform predictions for populations stressed by EDCs, a population dynamics model for zebrafish was built, coupling a model of individual bioenergetics (DEB model) with an individual-based model (IBM) taking into account the main ecological factors. This last model included relevant dose-response relationships relating hormone levels to effects on individual performances in zebrafish.

Key Results

The first phase of the project focused on the generation of new experimental data to fulfill the lack of knowledge concerning the zebrafish endocrinology and physiology. For example, to support the development of the zebrafish PBPK model, new experimental data on the physiology was produced at INERIS (e.g., volumes, lipids and water contents of the studied organs).

These original datasets were used to develop a PBPK for the zebrafish using validated and/or new QSAR/QSPR models to predict some toxicokinetic parameters. Model predictions were compared to available toxicokinetic data for zebrafish for many compounds including endocrine disruptors. Our model predicted accurately the different datasets, which proved the robustness of our calibration of the physiological parameters and of the QSAR/QSPR models developed to predict partition coefficients and gill permeability.

Finally, the predictions of the DEB-IBM were compared to existing observations on natural zebrafish populations (unexposed) and the predicted population dynamics agree with empirical observations. Then, the impacts of the ethinylestradiol predicted by the DEB-IBM at the population level were compared to the observation of Kidd et al., (2007). Taking into account the specificity of the eco-physiology of zebrafish, the predictions provided by the model were conformed to experimental observations.

Conclusions

During this project, we thus developed a first battery of models covering many biological levels. Models which remain to be developed are those relating these different biological levels, in particular those linking hormonal disturbances and individual performances. In the future, molecules with many new toxicological data could be used to find robust statistical and mechanistic relationships for this linking.

Thyroid active pesticides identified using the Xenopus Embryonic Thyroid Signalling Assay

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Aim of the work

The Xenopus Embryonic Thyroid signalling Assay (XETA) was designed as a screening assay to provide information on the potential of a test substance to alter the normal functions of the thyroid system. The XETA provides a rapid (<72h) way to measure the response of embryonic stage tadpoles to potential thyroid disrupting chemicals, allowing an efficient method for screening thyroid disruptors. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the in vivo Amphibian Metamorphosis Assay (AMA - OECD TG231). The AMA test is based on the study of the metamorphosis of tadpoles after three weeks of exposure to a given chemical, and includes histological examination of the thyroid gland. XETA could provide an alternative test that can be performed quickly, providing information that would be useful for screening large number of molecules or testing environmental samples that couldn't be stored or sampled in large quantities. OECD has started a validation study to publish a test guideline for the XETA. The objective of the validation study is to establish the relevance of the assay by assessing its sensitivity to detect disruption of the thyroid system by compounds active at different points within the thyroid system. The validation is intended to determine the performance and transferability of the assay across a range of both experienced and naïve laboratories. This process is divided into several phases, different molecules are tested in each phases and the protocol is modified in accordance to lesson-learned between each phase.

Methods

XETA utilizes free-living X. laevis embryonic-stage animals (stage 45 up to stage 47) in a multi-well format to detect modulation of thyroid receptor signaling by potential thyroid active chemicals. The assay is transcriptional-based, and uses a transgenic tadpole line containing the THbZIP genetic construct to detect the activity of Thyroid active molecules that work through various mechanisms. This assay could be applied for the detection of thyroid disrupting activities in various samples including surface water, wastewater, cosmetics, pharmaceuticals and food.

Key Results

The phase I ring test experiments gave the expected results for the chemicals chosen. A statistical approach was determined and a great consistency of the results was observed between laboratories. The XETA Phase I results demonstrate that the assay provides reasonable sensitivity with the chemicals tested and is reproducible, with a few exceptions, across replicates and labs.

The XETA phase II has started mid 2015 and active chemicals with modes of action that were not covered in phase I are tested to challenge the assay and the statistical analysis procedure. As pesticides are a major concern regarding thyroid disruption, two pesticides, Atrazine and Linuron have been included in the phase II. In parallel of the OECD validation, experiments were performed on different pesticides showing the ability of the XETA to detect the thyroid disrupting activities of compounds such as Diniconazole or Diazinon.

Conclusions

The data acquired by WatchFrog and by the different participating laboratories during the OECD ring tests shows the XETA to be efficient to detect thyroid active molecules and transferable across laboratories. This test offers a reliable quick screen for the detection of thyroid active chemicals, including pesticides, in various matrixes.

Identification of Embryonic Periods Sensitive to Disruption of Sex Determination in a Model Fish

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Aim of the work

Numerous laboratory and field studies have conclusively proven that sex determination and maintenance in fish can be disrupted by exogenous hormones or endocrine disrupting chemicals. It is also widely accepted that certain, critical developmental stages are more sensitive than others to this type of dysregulation. To our knowledge, no cartography of developmental sensitivity to hormonally-induced disruption of sex determination has been published. Using the medaka (Oryzias latipes) as a model we set out to systematically chart developmental sensitivity to reference androgen and oestrogen axis agonists and antagonists.

Methods

We developed a transgenic medaka line harbouring a genetic construction comprising of the promoter of the 42sp50 gene driving expression of GFP. This line expresses GFP in developing oocytes, starting at around 3 days post fertilisation (dpf).

Eggs of the 42sp50 line were collected immediately after fertilisation and split into three groups (solvent control and two pharmacological treatment groups). The treatment was carried out as a 24 h pulse at a range of developmental stages. Following treatment, embryos were washed, returned to clean media and raised to 16 dpf. Pharmacological treatments were carried out at high concentrations to induce phenotypic changes in a 24 h window of exposure.

At 16 dpf the presence/ absence of GFP fluorescence in the developing gonads was recorded. Fry were then genotyped for dmy, indicating the presence of a Y chromosome.

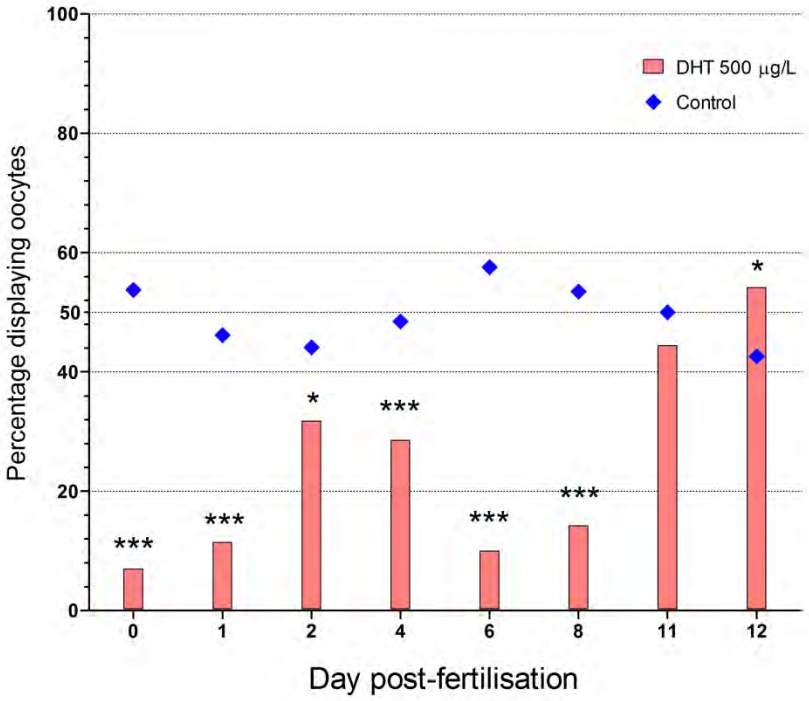
Key Results

The presence of oocytes was easily observable by fluorescence and was a reliable indication of the phenotypic sex of the fry.

Following a 24 h pulse of 500 µg/L 17β-oestradiol (E2), an increased percentage of phenotypic females was observed when the treatment occurred between dpf 0 and dpf 11.

The same concentration of dihydrotestosterone (DHT) reduced the percentage of phenotypic females when the embryos were exposed between dpf 0 and 8. However, dpf 2-4 appeared to be less sensitive than the earlier and later exposures. As expected a lower concentration of DHT (2.5 µg/L) induced less dramatic effects on phenotypic sex. Treatment with the androgen receptor (AR) antagonist flutamide was ineffective. This appeared to be due to its inability to penetrate the chorion. Genetic sex determination confirmed an increased percentage of XX males and XY females, compared to controls, in the E2 and DHT groups respectively.

Figure 1: Percentage of fry exhibiting gonadal fluorescence at DPF16. Following a 24 h pulse of DHT (500 µg/L) at different developmental time points. The percentage of fry displaying GFP+ cells in the solvent control group are shown in blue.



Conclusions

Sex determination of medaka embryos is sensitive to a 24 h pulse of either an oestrogen receptor (ER) or AR agonist at pre-hatch stages. When the ER was stimulated, sensitivity peaked from dpf 1-5. When the AR was stimulated, peaks in sensitivity were observed at dpf 0 and dpf 6. This intriguing result suggests that the relatively insensitive period (dpf 2-4) may be due to a lack of continuity between maternal and zygotic expression of AR. Around dpf 11, sex determination of the newly-hatched fry appears to be insensitive to hormonal treatment. It remains to be determined whether sensitivity returns at later developmental stages. These data will allow a better understanding of hormonal control of sex determination as well as the impact of endocrine disrupting chemicals.

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PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

Session 2. Metabolic disorders, Diabetes related to Endocrine Disruptors

Plenary Conference

Environmental endocrine disruptors: new diabetogens?

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The prevalence of metabolic syndrome, obesity and type 2 diabetes (DT2) has dramatically increased worldwide during the last few decades and exceeds World Health Organisation's predictions. It is not possible anymore to explain this real pandemic only by genetic predisposition and/or by classical lifestyle changes such as sedentary lifestyle or energy-dense diet. Three fundamental concepts have emerged these last few years, trying to explain how different environmental factors could influence obesity, insulin-resistance and insulin secretion impairment, all contributing to DT2, and able to partly support this epidemiological transition. Developmental origin of chronic disease (DoHAD) was first proposed by D. Barker in the eighties showing epidemiologically that fetal exposure to a deleterious nutritional, toxic or metabolic environment, may contribute to influence the occurrence of adult metabolic and/or cardiovascular diseases.

More recently, it has been reported that external manipulations of the gut microbiota in rodents could increase obesity and DT2, when submitted to high fat diet, even in genetically not susceptible strains. Finally, participating likely in the two first concepts, there is increasing experimental and epidemiological evidence suggesting that exposure to environmental endocrine disrupting chemicals (EDCs) may also contribute to the incidence of DT2.

EDCs are natural or synthetic chemical compounds, present in the everyday domestic environment, interfering with hormonal regulation systems critical for energy homeostasis. Many are present in the food chain and after absorption are sequestred in adipose tissue. They may represent after low-doses exposure during sensitive windows or via chronic exposure to cumulative doses, one aspect of the genetic / environment interface, involved in the pathophysiology of DT2.

In rodents, exposure to bisphenol A is responsible for modifications of insulin synthesis and secretion in pancreatic beta cells but also for modifications of insulin signaling in liver, skeletal muscle and adipose tissue, which both lead to insulin-resistance. In humans, some epidemiologic reports suggested a strong link between exposure to some persistent EDCs (pesticides, polychorinated biphenyls, bisphenol A, phthalates, dioxins, polycyclic aromatic hydrocarbons) and DT2, especially after acute and accidental releases of EDCs (Seveso plant explosion, Vietnam war veterans).

Other cross-sectional studies around the world reported suggestive to strong association between diabetes and obesity and EDCs exposure, especially for persistent organic pollutants, which should be considered as insulin-resistance risk factors. In vivo and in vitro experimental studies have shown that EDCs act through nuclear receptors (ERs, AhR, PPARγ, ERRγ) involved in metabolic control and are able to induce in specific windows of exposure (fetal, perinatal, pre-pubertal periods) epigenetic changes (DNA methylation, histone modifications, miRNA dysregulation) programming later obesity, insulin resistance, and/or β cell failure. It is still necessary to better understand the involved molecular mechanisms, to develop additional prospective, longitudinal case/control epidemiological studies, and to identify biomarkers of exposure, in order to minimize the difficulties linked to chronic exposure to mixture of EDs and to assess the real implication of such factors in high risk patients.

Association between urinary bisphenol A-glucuronide and the incidence of type 2 diabetes in the French prospective cohort study D.E.S.I.R.

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Aim of the work

There are accumulating animal and human data linking exposure to endocrine disrupting compounds such as bisphenol A (BPA) with type 2 diabetes (T2D). However, all but one of the human studies supporting this association are cross-sectional, limiting inference about causality. We investigated the relation between exposure to BPA and the risk of developing T2D in the French prospective cohort study D.E.S.I.R. (Data from the Epidemiological Study on the Insulin Resistance Syndrome).

Methods

Using a case-cohort design, we analyzed data from 755 participants, aged 30-65 years at baseline (1994-1996) and followed-up for 9 years, including 201 incident cases of T2D and a random sample of the healthy cohort members (‘subcohort’).

Urinary BPA-glucuronide (BPA-G), a major metabolite of BPA, was assessed as a proxy of BPA exposure in spot urine samples collected at baseline. BPA-G was assayed by liquid chromatography-mass spectrometry after dansyl chloride derivatization. Prentice-weighted Cox regression models were used to estimate adjusted hazard ratios (HRs) of T2D per standard deviation increase in ln-transformed BPA-G concentration. Analyses with BPA-G coded in tertiles were also conducted.

Statistical models were adjusted for baseline covariates selected using a directed acyclic graph: age, sex, urinary creatinine level, level of education, employment, smoking status, physical activity, caloric intake, hypertension, use of lipid-lowering medication, fasting

glucose, liver enzymes, body mass index (BMI), waist circumference (WC), and family history of diabetes. Effect measure modification by sex and BMI were investigated.

Key Results

The median (25th percentile-75th percentile) BPA-G concentration was 1.5 (0.5-3.0) ng/mL in the subcohort. Overall, BPA-G concentrations were not significantly associated with incident T2D, with a HR (95% confidence interval, CI) of 1.09 (0.93-1.28). HRs (95% CI) were 0.97 (0.78-1.20) in men and 1.14 (0.80-1.61) in women (p for interaction=0.12). There was evidence of effect modification by overweight status at baseline (BMI < or ≥ 25 kg/m^2), with HRs (95% CI) of 1.25 (1.01-1.54) for overweight participants, and 0.86 (0.65-1.15) for normal weight participants (p for interaction=0.05). Results from analyses in tertiles are reported in the figure. Among overweight participants, those in the highest tertile of BPA-G had a significantly higher risk for T2D (adjusted HR=2.18, 95% CI: 1.32-3.58) than those in the lowest tertile, whereas no significant association was seen among normal weight participants (p for interaction=0.01).

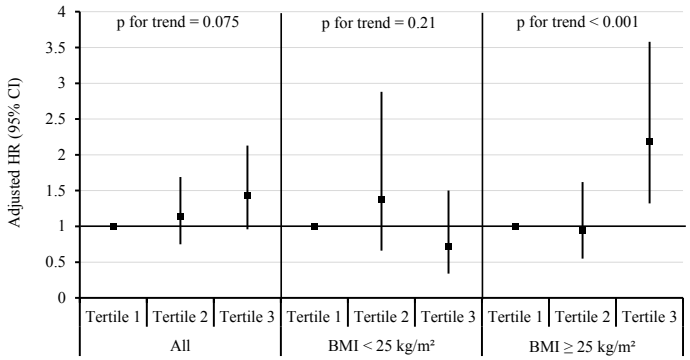

Figure: Adjusted associations between uBPA-G concentrations at baseline in tertiles and incident type 2 diabetes in the whole study population and according to overweight status

Conclusions

This is one of the first prospective studies on BPA and T2D. We found that higher BPA-G concentrations were significantly associated with future T2D incidence in the overweight participants, independently of traditional risk factors. This finding is consistent with previous epidemiological studies and might be explained by differences in BPA metabolism or by a synergistic effect of fat mass and BPA on T2D. Caution in interpretation is needed due to the limited ability to predict long-term BPA exposure from spot urine samples. More longitudinal studies are required to better understand the relation between BPA exposure and the development of chronic metabolic disease such as T2D.

Dysregulations of mucosal and systemic immune responses at adulthood after perinatal exposure to bisphenol A (BPA): possible involvement in food adverse reactions and inflammatory diseases

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Aim of the work

The effect of perinatal exposure to BPA on immune system is a relatively new issue. Particular concerns have been raised from animal studies regarding the risk of developing food intolerance, increasing susceptibility to infection, and dysregulated inflammation into the gut (Braniste et al. 2010; Menard et al, 2014). In human, the estimated BPA dietary intake was highest in infants and toddlers (up to 0.875 µg/kg bw per day). Recently, European Food Safety Authority established a temporary Tolerable Daily Intake (t-TDI) of 4 µg/kg bw per day. In rodents, we suggested the ability of perinatal BPA to interfere with the maturing gut immune system at a dose (5 µg/kg bw/d) close to the current t-TDI. We aimed to investigate whether developmental immunotoxicity during perinatal exposure to this low dose of BPA resulted from long term alterations in immune responses in adult offspring.

Methods

Dams were treated with BPA [5 or 50 µg/kg BW/d] or vehicle (corn oil) from day 15 of gestation until weaning (postnatal day (PND) 21). Fecal lysozyme activity and lysozyme fluorescence intensity in ileal Paneth cells were quantified in adult female offspring (PND45). Total and commensal *E.coli*-specific IgG responses in plasma sampled from adult offspring were measured. Immune cells from spleen, mesenteric lymph node (MLN) or jejunal lamina propria (LP) were also harvested. Phenotypic analysis of dendritic cell (DC), T regulatory (Treg) and innate lymphoid cells (ILC3) was performed by flow cytometry using specific cell markers. Retinoic acid expression was measured in separated intestinal cells from jejunum by flow cytometry. Th1/Th17 cytokine profile was assessed in supernatant of splenocytes after CD3/CD28 restimulation.

Key Results

We report that adult mice perinatally exposed to 5µg/kgBW/day of BPA showed reduced retinoic acid (RA) production by intestinal epithelial cells (IEC). A decrease in lysozyme activity linking to a default of lysozyme expression in Paneth cells was observed in perinatally exposed offspring compared to control.

Total IgA and IgG production in feces was also reduced. These alterations were associated with a defect in DC maturation from LP and spleen. Frequency of these DC subsets increased in gut mucosa while it decreased in spleen, suggesting a domiciliation defect after perinatal BPA treatment. Concomitantly, a decrease of regulatory and activated T cells in LP and MLN was observed in BPA-treated mice, while these subpopulations were increased in spleen. Interestingly, an alteration in the frequency of innate lymphoid cells ILC3 producing IL-17 and IL-22 occurred in LP, associated with a decrease of aryl hydrocarbon receptor (AhR) expression, known to drive the development of gut ILC22. Perinatal exposure to BPA promotes in female mice offspring inflammatory response in the spleen, with a sharp increase of IFN-gamma and IL-17 production by T lymphocytes.

Conclusions

Our results demonstrated that perinatal exposure to BPA weakens protective and regulatory immune functions of IEC associated with an alteration of DC and T cells populations and ILC3 development. These disturbances in the progeny might impair mucosal tolerogenic responses, and favoured inflammatory systemic immune responses, thus increasing susceptibility to food adverse reactions.

Seasonal Variation in Urinary UV filters in Danish Children Aged 3-5 Years

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Aim of the work

Chemicals with UV blocking properties (known as UV filters) can act as endocrine disruptors1. UV filters are used in sunscreens and other cosmetic- and personal care products, as well as in food packaging, clothing and furniture textiles. Daily sunscreen topical applications are a normal procedure in Danish kindergartens from early spring and during the whole summer. We have investigated difference in summer (s) and winter (w) urinary excretion of UV filters from kindergarten children 3-5 years of age and evaluated it according to the given information about sunscreen application.

Methods

Spot- and first morning urine were collected in two Copenhagen kindergartens. In 2013 at one summer- and one winter day, spot urine (su) (1-7 samples per child) were collected in kindergarten from lunch time and during the afternoon followed by first-morning urine (mu) collection at home the day after. In total 265 urine samples were collected from 55 children. Urine were analyzed for content of benzophenone (BP), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), 5-cholro-2-hydroxybenzophenone (BP-7), 4-methyl-benzophenone (4-MBP), 4-hydroxybenzophenone (4-HBP), 3-(4-methylbenzylidene)-camphor (4-MBC), and 3-benzylidene camphor (3-BC) by LC-MS/MS. Winter and summer urine samples were examined and further more evaluated in accordance to given information about sunscreen application. Habits of sun protection were examined by questionnaire.

Results

BP-1 and BP-3 were present in the highest concentrations; median levels (ng/mL) were 0.85 (BP-1) and 1.17 (BP-3) in winter and 2.01 (BP-1) and 3.98 (BP-3) in summer spot urine and were detected in a majority of all samples both in summer and winter urine (limit of detection (LOD) (ssu) > 97%; LOD (wsu)>75%). BP and 4-HBP were detected in respectively one third and two thirds of samples with median concentrations below LOD for BP and 0.26 ng/ml and 0.71 ng/ml for 4-HBP in respectively winter and summer samples. Higher amounts of UV filters were measured in first morning urine compared to spot urine.

As expected, significantly higher levels were excreted in summer spot- and morning urine compared to winter spot- and morning urine (BP: psu=0.029, pmu=0.005; BP-1: psu=0.011, pmu=0.049; BP-3: psu=0.002, pmu=0.056; 4-HBP: psu=0.025, pmu=0.001). Thirty-one percent of the children were applied sunscreens at home before collection of summer spot urine in kindergarten. Interestingly, were concentrations of BP-1 and BP-3 higher among children who didn't received application of sunscreen before urine samples collection compared to children who did (BP-1: psu=0.043, pmu=0.012; BP-3: psu=0.009; pmu=0.006). No statistically significant differences were observed between children with- and without sunscreen application for BP and HBP-4. Urine concentrations of BP-1 vs. BP-3 and BP vs. 4-HBP was significantly correlated (R2 =0.823 and R2=0.687). Information collected in the questionnaire shows that sunscreens are the preferred method of protection against the sun among 59% of children's parents while 31% ranks shadow as their last choice of protection against the sun among hat, cloths, sunscreens and shadow.

Conclusions

- Kindergarten children were exposed to UV filters both summer and winter
- Summer exposures were significantly higher than winter exposures
- BP-1 and BP-3 concentrations were highest in children without sunscreen application
- Sunscreens are the preferred method of protection against the sun

Reference

Krause et al. Sunscreens - are they beneficial for health? Int J Androl. 2012, 35, 424-436

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PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

Session 3. Epigenetic and gene expression

Plenary Conference

A mechanistic insight into neurodevelopmental endocrine disruption

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It has been shown recently that neurodevelopmental disorders including intellectual disability, autism and attention deficit-hyperactivity disorder account for 132 out of 157 billions Euros of annual cost of Endocrine Disrupting Chemical (EDC) effects. Not included in those disorders, disturbed neuroendocrine control of homeostasis of energy balance and reproduction in the hypothalamus are also part of neuroendocrine disruption. Alterations of behaviour, cognitive abilities and hypothalamic control of homeostasis by early exposure to EDCs involve mechanisms that are still to be elucidated. Using the rodent model, our research has focused on two EDCs, Aroclor 1254 (ARO), a commercial mixture of polychlorinated biphenyls (PCBs) and Bisphenol A (BPA). Three brain regions were studied: cortex, hippocampus and hypothalamus.

PCBs are persisting environmental contaminants possibly causing cognitive deficits and learning disabilities. We tested the hypothesis that prenatal exposure to ARO can concomitantly alter thyroid hormone (TH) levels and TH-regulated events during cerebral cortex and hippocampus development. Pregnant rats exposed to ARO showed reduced total and free serum thyroxin levels by the end of gestation. Gestational and lactational exposure to ARO resulted in reduced serum total thyroxin levels that normalized after weaning. Proliferation of progenitor cells was not affected neither in the fetal cortex nor in the dentate gyrus early postnatally. In the fetal cortex, increased cell cycle exit of the neuronal progenitors and delayed radial neuronal migration were observed. Proliferation was modestly reduced in the dentate gyrus of adult animals. Subtle alterations in development of synaptic function were observed.

Bisphenol A (BPA) is a ubiquitous EDC present in polycarbonate plastics, epoxy resins, dental cements and thermal paper. The European Food Safety Authority recently set the daily tolerable intake of BPA at 4 µg/kg.day. In France, the daily BPA exposure in children, adolescents and pregnant women is 50-60 ng/kg.day and twice as much in infants. Female rats were exposed to vehicle or BPA 25 ng/kg.day or 5 mg/kg.day from PND 1 to 15. After exposure to the low dose, vaginal opening was delayed following a delay in developmental acceleration of pulsatile gonadotropin-releasing hormone (GnRH) secretion. Inversely, exposure to BPA 5 mg/kg/d resulted in early vaginal opening following a premature acceleration of GnRH secretion.

On PND 20, the mRNA expression of hypothalamic genes involved in GABAA neurotransmission showed opposing changes depending on the dose of BPA. This finding was confirmed by studies on GnRH secretion in presence of GABAA receptor agonist/antagonist. Among 10 genes showing opposing changes in expression depending on BPA dose, some could represent new targets of neuroendocrine disruption.

Taken together, these studies confirm that developmental exposure to EDCs result in complex structural and functional alterations in different brain areas. Though thyroxin levels are reduced at some points during and after PCB exposure, the mechanistic involvement in the observed alterations remains to be established.

Estrogen receptor beta regulates locus-specific DNA methylation
A possible mechanism for epigenetic effects of endocrine disrupters

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Aim of the work

Estrogen receptors, ERalpha and ERbeta, are targets of endocrine disrupters (EDCs) such as bisphenol A (BPA). Exposure to EDCs induces locus-specific epigenetic alterations, in particular DNA methylation changes, which in turn are associated with increased susceptibility to a number of diseases. However, the molecular mechanisms underlying these changes are unclear. Our aim is to investigate if and how ERs, particularly ERbeta, is directly involved in regulating epigenetic processes, and if this function could underlie the effects of EDCs on epigenetic patterning.

Methods

We compared genome-wide DNA methylation in mouse embryonic fibroblasts (MEFs) derived from wildtype (wt) and ERbeta (berko) mice using reduced representation bisulfite sequencing (RRBS). Methylation patterns were validated by bisulfite-pyrosequencing and expression of differentially methylated genes was analysed by qPCR. To investigate if ERbeta interacts with regulators of DNA methylation, we investigated the interaction between ERbeta and thymine DNA glycosylase (TDG), a protein that is involved in active DNA demethylation, using yeast-2-hybrid assays and chromatin immunoprecipitation. Finally, to analyse the effect of BPA on DNA methylation and the involvement of ERbeta therein, we analysed Fkbp5 methylation and expression in differentiating mouse hippocampal cells in the absence and presence of ERbeta using bisulfite-pyrosequencing and qPCR, respectively. BPA's effects on Fkbp5 expression and DNA methylation were also tested in male Wistar rats exposed to 40 microgram BPA/kg/day during pregnancy and lactation.

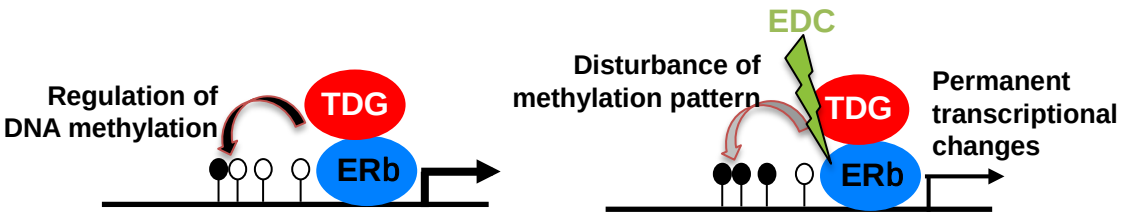
Key Results

Using RRBS, we identified around 8000 differentially methylated regions (DMRs). Validation and further analysis of DMRs showed a clear correlation between methylation status and expression levels of the closest gene. Additionally, re-introduction of ERbeta into the knock-out cells could reverse hypermethylation and reactivate expression of some of the genes. Furthermore, we found that ERbeta interacts with TDG, and that TDG is ERbeta-dependently recruited to identified DMRs.

One gene found differentially methylated was *Fkbp5*. *Fkbp5's* gene product FKBP51 is an important regulator of the stress response, and its genetic and epigenetic dysregulation is associated with several psychiatric conditions. In differentiating mouse hippocampal cells, we found that *Fkbp5* expression and methylation is affected when ERbeta is knocked-down. Similar effects were seen when the cells were treated with BPA during the differentiation process. Finally, we found sexual dimorphic differences in *Fkbp5* expression and methylation in rats exposed to low doses of BPA in *utero*, thus confirming *Fkbp5* as epigenetic BPA target *in vivo*.

Conclusions

Our findings suggest a model in which ERbeta can recruit TDG to specific sites in the genome, thus targeting regulation of DNA methylation to these sites. This implies a novel function for ERbeta that could underlie epigenetic effects of EDCs such as BPA.



Exposure to phthalates and male infertility: role of the genetic background in the epigenetic response

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Aim of the work

Among environmental factors, endocrine disruptors (EDs) have been suggested as highly detrimental to male and female reproductive organs development and function.

We previously reported the effects of two ED pesticides - vinclozolin and methoxychlor – after administration in pregnant mice, on the fertility of the male progeny, and on the methylation pattern of imprinted genes in sperm and somatic cell DNAs. We observed transmission of these dysmethylation marks over generations, in the FVB/N mouse genetic background, with a trend to attenuation over the generations.

Di-(2-ethylhexyl)phtalate (DEHP) is a plasticizer with ED properties, found ubiquitously in the environment. Studies in rodents and in humans revealed that it is an aggressive reproductive system toxicant. We recently investigated the impact of prenatal exposure to DEHP on spermatogenesis and DNA sperm methylation in two distinct, well characterized, and sequenced mice strains. Our hypothesis was that the genetic background can play a role in the epigenetic modifications potentially induced by specific EDs and mediating spermatogenesis defects.

Methods

FVB/N and C57BL/6J mice strains were exposed during gestation to DEHP (oral administration to pregnant females).

Key Results

Interestingly, a significantly decreased spermatogenesis was observed in the C57BL/6J, and not in the FVB/N background F1 mice. In the sperm of DEHP exposed F1 mice of the two strains and unexposed mice. MBD-sequencing analysis was performed on sperm DNA. This cutting-edge technique allows measurement of DNA methylation on a genome-wide scale. The DEHP exposure revealed statistically significant DNA methylation changes in several genes and genomic regions, in a strain-distinct manner. Transcription level of a subset of targets was tested by RT-qPCR on sperm RNA.

Conclusions

Our work confirms our previous studies on the detrimental role of specific EDs on male fertility. It also shows that prenatal exposure to DEHP can decrease spermatogenesis in a strain-dependent manner and affect sperm DNA methylation in promoters of large sets of genes, among which genes potentially linked to spermatogenesis. The key message is that the genetic background, may play a role in the epigenetic modifications induced by EDs, and that individuals may therefore be unequal with regards to the risks of diseases linked to environmental exposures.

Pregnancy Exposure to Select Phenols and Phthalates and Pulmonary Function in Five Year-Old Male Offspring

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Aim of the work

Endocrine disruptors, in particular from the phenols and phthalates families, are suspected of adverse effects on respiratory health. Such an effect is supported by their immunomodulatory and pro-inflammatory properties. In humans, studies on the effects of early-life exposure to phenols and phthalates on objective pulmonary function measurements are lacking, except for bisphenol A.

Our aim was to evaluate associations between maternal pregnancy exposure to select phenols and phthalates and pulmonary function measurements in male offspring at 5 years of age.

Methods

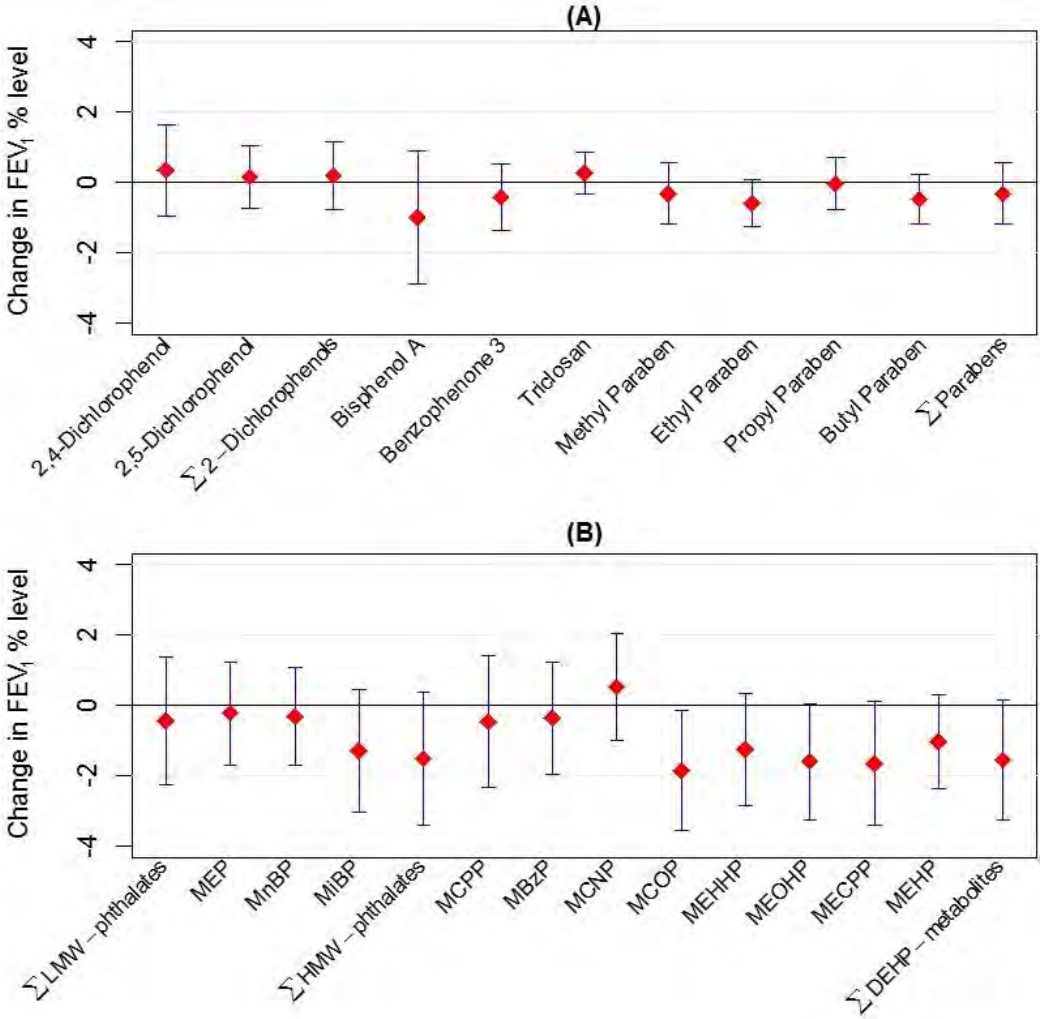
Among 228 boys participating in a mother-child cohort, nine phenols and eleven phthalates metabolites were quantified in spot maternal urine samples collected between 23 and 29 gestational weeks. Boys were followed until age 5, when Forced Expiratory Volume in 1 second (FEV1) was measured by spirometry. Associations of each urinary metabolite concentration with FEV1 in percent predicted (FEV1%) were characterized by linear regression adjusted for potential confounders.

Key Results

Monocarboxy-isooctyl phthalate (MCOP), a di-isononyl phthalate (DINP) metabolite, exhibited evidence of adverse association with FEV1% (β for 1-ln unit increase, -1.86; 95% CI, -3.58, -0.15, Figure 1).

Boys in the highest tertiles of two di(2-ethylhexyl) phthalate (DEHP) metabolites had FEV1% decreased by 4.15% (mono(2-ethyl-5-oxohexyl), or MEOHP, 95% CI, -7.74, -0.55) and 3.80 % (mono(2-ethyl-5-carboxypentyl), or MECPP, 95% CI, -7.38, -0.22), compared to the lowest tertiles. Trends in favor of reduced FEV1% with increases in ethyl-paraben concentration were also suggested (Figure 1). No association was observed for the other phthalates and phenols.

Figure 1



Conclusions

This study is one of the first to suggest that prenatal exposures to ethyl-paraben, DINP and DEHP may decrease pulmonary function in early childhood.

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PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

Session 4. Effects of Endocrine Disruptors on the reproductive systems

Plenary Conference

Endocrine Disruptors and Couple Fecundity

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Endocrine disrupting chemicals (EDCs) comprise a diverse array of persistent and non-persistent chemicals that have entered the environment and ecosystems serving as a route for human exposure. In light of EDCs ability to interfere with endocrine function among other organ systems, concern has arisen about their potential impact on human reproduction and development. We designed the Longitudinal Investigation of Fertility and the Environment (LIFE) Study to assess EDCs and a spectrum of reproductive and developmental outcomes beginning with couple fecundity, defined as the biologic capacity of couples for reproduction.

The LIFE Study recruited 501 couples who were discontinuing contraception for purposes of becoming pregnant. Couples were recruited prior to conception and followed daily until pregnant or up to 12 months of trying at which time they were censored for analysis. Following the enrollment interview and completion of a standardized anthropometric assessment, each partner of the couple provided blood and urine samples for the analysis of persistent (metal, persistent organic pollutants) and non-persistent (BPA, benzophenones, phthalates) chemicals using high-resolution mass spectrometry protocols. Women used urinary home fertility monitors to help time intercourse relative to ovulation and sensitive digital home pregnancy test kits.

We assessed each class of environmental chemicals in relation to couple fecundity, defined as time-to-pregnancy (TTP) or the number of observed menstrual cycles required for a couple to become pregnant, which is a proxy of human fecundity. Specifically, we used Cox analysis for discrete survival time to estimate fecundability odds ratios (FORs) and 95% confidence intervals (CIs) for each chemical and TTP after adjusting for potential confounders depending upon the analytes under consideration, but typically including partners' ages, body mass indices, serum cotinine concentrations, and research site while accounting for time off contraception (left truncation). FORs <1 are suggestive of diminished fecundity or a longer TTP.

TTP tended to be significantly longer for increasing concentrations of male partners' blood lead concentrations (FOR=0.83; 95% CI 0.70, 0.98), specific polychlorinated biphenyl congeners (FORs ranging from 0.74 to 0.83), specific phthalates (FORs ranging from 0.80 to 0.81; 95% CI 0.6, 0.9), and for 2,2',4,4'-tetrahydroxybenzophenone (FOR=0.69; 95% CI 0.49, 0.97). When considering female partners' concentrations, TTPs were longer for specific polychlorinated biphenyl congeners (FORs ranging from 0.79 to 0.82); however, shorter TTPs were observed for specific phthalates (mono-octyl phthalate, FOR=1.18 and

mono 3-carboxypropyl phthalate, FOR=1.22). The findings underscore the importance of assessing both partners' exposures to minimize erroneous conclusions based upon a single partner, and suggest more consistent evidence of diminished couple fecundity for the male partners' exposures.

Steroid production and gonad formation are regulated by the most used anti-diabetic drug, metformin

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Aim of the work

Metformin is the most prescribed oral antidiabetic for the treatment of type 2 diabetes. Because metformin is widely used, and not metabolized by the organism, several studies have detected metformin in wastewater (Trautwein & Kûmmerer 2011) and in river. More recently, a potential impact of metformin exposure is suspected in the establishment of fertility in fish species. Thus, fish exposed to metformin at environmental concentrations present alterations in the development of intersex gonads in males fish and a reduced fecundity suggesting that metformin could be an agent as endocrine disruptors (Nieumuth et al 2015) and raises questions about the human health risk.

The need to evaluate consequences on differents species is a real challenge (Khan & Nicell 2015). To increase knowledge about the metformin effect on reproductive function, we have investigated consequences of metformin exposure at puberty, and adult stage to evaluate fertility, hormonal regulation in two species (mouse, chicken).

Methods

The first approach is based on a metformin administration in drinking water (150mg/kg/day) to chicken at pre pubertal stage 6 to 9 week old male chicken. A second approach analysed the consequence of a mouse embryo exposure to metformin. Metformin was administrated through the drink water (300mg/kg/day) during all pregnancy and an investigation of the male offspring was realized. Controls are only water without addition of metformin.

Key Results

After metformin exposure during pre-pubertal stage, chicken displays a significant reduction in testis weight (206±17 mg vs control 339±42, $p<0.05$) and in seminiferous tubule diameter (191±6 µm vs control 224±9, $p<0.05$). Testosterone level in serum is two fold lower than in control (0.8±0.3 ng/ml vs control 1.8±0.3, $p<0.05$) and the testicular expression of protamine, a marker of mature germ cells is reduced (5.8±0.4 ng/µg vs control 8±0.4, $p<0.05$). These results suggest an effect on the start of puberty if metformin was absorbed at this period.

In mice, a metformin exposure *in utero* did not induce consequences on the ano-genital distance of pups, or the age of puberty. The first estrus appeared at the same age than control and the duration of the estrus was not altered (2.25±0.5 days vs control 1.5±0.5). In male, testis weight was not clearly affected, despite the seminiferous tubule diameter was decreased (130±1 vs control 140±1, $p<0.05$). However, males exposed in utero present more abnormal sperm than control mice, with abnormal head (20±2 vs control 13±1, $p>0.05$). The pituitary LH concentration was also lower after metformin exposure (1.4±0.2 ng/µg protein vs control 2.8±0.4 ng/µg protein). To complete this study, we have analysed the litter size and we have noted that litter size was negatively regulated by metformin (5.6±0.9 pups/litter vs control 8.1±0.3). Hence, it appears than a metformin exposure exerts a negative effect on male offspring fertility.

Conclusions

Taken together these results complete those of Tartarin et al 2012, which has described a negative effect on the mouse and human fetal testicular functions (i.e., testosterone secretion, cell proliferation). However, both results on chicken and mice are not able to demonstrate the strong phenotype suggesting an endocrine disruptors activity as described in fish by Niemuth et al. We have to determine in a near future the molecular mechanism of metformin action.

A new front of endocrine disruption: analgesics from pharmaceutical and non-pharmaceutical sources and reproductive health

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Aim of the work

Our aim was to investigate the endocrine disrupting properties of analgesics.

Methods

We review the evidence from epidemiology, animal experiments, and human ex vivo model-systems.

Key Results

Mild analgesics comprise non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol. They are classically used against pain, fever, malaise, inflammatory diseases and chronic musculoskeletal pain. They are among the most used and environmentally released pharmaceutical drugs. Recent biomonitoring data evidence the omnipresence of paracetamol and salicylic acid in European populations, even in people who did not take paracetamol or aspirin (acetyl salicylic acid), indicating that secondary sources of exposure (non-pharmaceuticals) do exist. Metabolic conversion from ubiquitous industrial compounds such as aniline, 4-aminophenol, methyl salicylate or intake from natural products (salicylic acid: fruits …) is most likely to occur. Recent studies from the two groups presenting this abstract highlight the endocrine disruptive properties of several analgesics (paracetamol, aspirin, ibuprofen…) and of aniline, with a number of negative consequences in terms of reproductive development, in rodents (in utero, ex vivo) and humans (epidemiology, ex vivo) in both sexes.

Conclusions

Investigation of these newly characterized endocrine disruptors leads to the merging of the two classical branches of toxicology, namely pharmacotoxicology and environmental toxicology.

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Vulnerability of the neural circuitry involved in the expression of male sexual behavior to adult exposure to low doses of endocrine disruptors

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Aim of the work

Endocrine disruptors are natural or man-made pollutants able to interfere with endogenous hormones or their signaling pathways. In this context, we have recently shown that adult, and not perinatal, exposure to bisphenol A reduces sexual motivation and performance at the Tolerable Daily Intake (TDI) dose in male mice. No effects were observed at the No-Observed-Adverse-Effect-Level (NOAEL) dose. Furthermore, the use of a genetic mouse model lacking the neural androgen receptor suggested that BPA could act as an anti-androgenic compound in the neural circuitry underlying sexual behavior. The present study aims to determine whether the vulnerability to adult exposure to bisphenol A extends to other endocrine disrupters. Nonylphenol (NP) and di (2-ethylhexyl) phthalate (DEHP) are two emergent compounds widely used in the industry and day life. This results in a large environmental animal and human contamination. DEHP is described as an anti-androgenic molecule, while NP seems to act as estrogenic and antiandrogenic molecule. In the male nervous system, testosterone controls the expression of reproductive behaviors and signals directly through androgen receptors or indirectly through estrogen receptors after neural aromatization into estradiol.

Methods

We therefore exposed adult males for 1 month to oral DEHP or NP at the TDI dose (50 µg/kg body-weight/day) and to lower doses close to the environmental human contamination (0.5 and 5 µg/kg body weight/day). Behavioral analyses included sexual behavior, attractiveness, olfactory preference, ultrasonic vocalizations and other behaviors modulated by sex steroids and able to interfere with mating when altered such as locomotor activity and anxiety-related behavior. Neuroendocrine, neuroanatomical and molecular studies were conducted in order to determine how exposure to DEHP or NP affects the neural circuitry underlying sexual behavior.

Key Results

The obtained results show effects of adult exposure to DEHP and NP at low doses on the expression of sexual behavior, thereby confirming that the vulnerabimity of this circuitry extends to several endocrine disrupters. However, the two molecules acted differently, suggesting that they may affect different signaling pathways. Analyses are in progress in order to determine the targets of these molecules in the neural circuitry underlying sexual behavior.

Conclusions

The obtained data could help to understand how low doses of environmental contamination could interfere with neural functions during adulthood, an under-estimated period in the field of endocrine disruption.

Monitoring TDS indicators in France: updated results

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Aim of the work

During the last decades, worldwide studies reported limited but worrying trends in reproductive health outcomes and debates arose about a possible global reproductive weakening due to environmental causes. Sperm quality impairment, testis cancer and congenital malformations of the male urinary tract (cryptorchidism, hypospadias) compose the testicular dysgenesis syndrome (TDS), which is suspected to be causally linked with early/prenatal exposure to endocrine disruptor chemicals (EDCs). In the context of the growing ubiquitous exposure to EDCs in France since the 50's, we aimed to analyse temporal and spatial trends of TDS indicators in the French general population, for public health purposes and in order to see whether they are congruous with the TDS hypothesis.

Methods

The national ART register (FIVNAT) provided data to study semen characteristics in France between 1989 and 2005. We analyzed three semen quality indicators from a sample of men partners of infertile women (bilateral fully blocked/absent tubes), therefore close to the general population. The French National Hospital Discharge Data Base (PMSI) provided data to study indicators of testis cancer, cryptorchidism and hypospadias from 2002 to 2014, using diagnosis codes based on international classification of diseases, 10th revision. For each set of data, we selected the best suited statistical model, based on Akaike or Deviance Information criteria, to describe temporal, spatial and spatiotemporal trends. Trend for each analysis was assessed by a spline function to capture potential non-monotonic relationship with TDS indicators, when spatial correlations were captured by a Besag, York and Mollie model.

Key Results

Temporal trends show a nationwide continuous impairment of male reproductive health: in 1989-2005 for sperm concentration and sperm morphology; and, in 2002-2014, for testis cancer and cryptorchidism. The trend for hypospadias in 2002-2014 is quite stable. Geographical differences and spatiotemporal trends are under analysis and preliminary results will be presented.

Conclusions

Our updated results confirm the male reproductive impairment monitored through TDS, except for hypospadias. For urogenital malformations, limits remain due to possible variations in medical practices. To analyse whether spatial trends are congruous with the TDS hypothesis, an improvement would be accessing localization at birth instead of localization at ART attempt.

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Session 5. Mixtures

Effect of mixtures of endocrine disruptors in zebrafish: the MIXEZ project

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Aim of the work

Until now, most *in vitro* and *in vivo* assays that have been employed to address mixture effects focused on estrogen receptor alpha (ERα) and liver vitellogenin expression leaving unexplored mixture effects of estrogens on other ER subtypes and ER-regulated genes. In this context, the aim of our project was to acquire knowledge on the effects of binary mixtures of endocrine disruptors with direct and indirect estrogenic activities, especially in fish, by using zebrafish specific *in vitro* and *in vivo* bioassays allowing assessment of mixture effects on the three different zebrafish ER subtypes and on the brain specific ER-regulated *cyp19a1b* gene.

Methods

For that purpose, recently established zebrafish-derived tools were used: (1) *in vitro* transient reporter gene assay (ZfCyp19a1b-luciferase/ zfER-α or -β2) in a human glial cell line (U251-MG) and (2) *in vivo cyp19a1b*-GFP transgenic zebrafish embryo assay (EASZY assay).

Concentration-response relationships for all single chemicals were measured, modeled, and used to design the binary mixture experiments following a ray design. The results from mixture experiments were analyzed to predict joint effects according to concentration addition (CA) and independent action (IA) models. Two different statistical approaches (Jonker's dose-response surface models and Hewlett and Streibig's isobole-based models implemented by Sorensen) were used to assess deviations from the CA model and to characterize interactions between the components of the mixtures (synergism/antagonism).

Key Results

Our work shows that binary mixtures of natural and synthetic estrogens (strong ER agonists) generally have additive effects on the expression of a known estrogen-regulated gene, the *cyp19a1b* gene, in the central nervous system of the developing zebrafish.

As regards binary mixtures of (xeno-)estrogens, our study highlights an antagonism between estradiol and genistein, or THB and 4BP in mixtures. In contrast, binary mixtures of estrogens with pro-estrogenic compounds, i.e. estradiol and testosterone, or ethynilestradiol and levonorgestrel, exert synergistic and additive effects respectively.

To our knowledge, this is the first study reporting an antagonistic, synergistic or additive effect between these compounds in binary mixtures on the expression of an estrogen-regulated gene (*cyp19a1b*) in a neuro-glial cell context whatever their concentration and their ratio in mixtures. Even if the *in vitro* bioassay partially accounts for the effects observed *in vivo*, only the *in vivo* bioassay seems to highlight some of the synergistic effects of estrogenic compounds on the *cyp19a1b* gene expression. Such differences between *in vitro* and *in vivo* responses to mixture effects may rely on the bioavailability and the pharmacodynamic of test compounds in the embryo assay, allowing a better characterization of the mixture effects of estrogens and pro-estrogens.

Conclusions

This study provides original data on the effect of mixture of (xeno-) and (pro-)estrogens in fish in a glial cell context, and shows the usefulness of these bioassays to address the issues raised by mixture effects of EDCs. The *in vivo* bioassay on *cyp19a1b*-GFP transgenic zebrafish embryos appears as relevant to further study these questions.

Acknowledgment: the MIXEZ project was funded by the PNRPE program from the French Ministry of Environment.

A chronic exposure to EAS mixtures including bisphenol A, vinclozolin and genistein affects the reproductive axis and testicular transcriptome of the unexposed progeny of exposed fathers

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Aim of the work

A previous study in male Wistar rats exposed by gavage, from conception to adulthood, to environmental levels of EAS mixtures has shown the deleterious impact of these exposures on various endpoints of the reproductive axis as well significant modifications of the expression of numerous testicular genes, in the neonatal period, at puberty and in the adult. An unexposed progeny (F2) sired from exposed fathers (F1) has been generated for further studies. Various reproductive endpoints and the testicular transcriptome at different developmental stages in the unexposed F2 were investigate and compared it with the effects in the exposed F1.

Methods

The offspring (unexposed F2) of male rats orally exposed lifelong from conception (F1) to bisphenol A (B, 5µg/kg/day, <<NOAEL, a dose similar to the 2015 EFSA t-TDI) alone and in mixture with the phytoestrogen genistein (G, 1mg/kg/day: compatible with an Asian diet) and/or the antiandrogenic dietary contaminant vinclozolin (V, 10µg/kg/day <<NOAEL and below the 2014 EPA TDI, 25µg/kg/day) were studied, as their fathers, neonatally (PND3), prepubertally (PND25), postpubertally (PND50) and as young adults (PND100) using conventional reproductive endpoints and testicular mRNA expression profiles (Affymetrix, GeneChip Rat Gene 2.0 ST) followed by the integrative search of the functions modified using the Ingenuity software.

Key Results

A reduced relative anogenital distance in all groups, a delayed puberty onset and a significantly decreased sperm production for most groups were the main phenotype changes in the F2 sired from exposed fathers in comparison to controls. In addition, relative testis and prostate weights were found to be significantly decreased for some exposures at various developmental periods, including neonatal, pre and post puberty.

Hundreds to thousands of testicular genes according to the developmental period in the progeny and exposure modality in the fathers were found to have an expression significantly modified. Unsupervised analyses according to Ingenuity systems revealed significant alterations of several major functions in the adult testis, for example, "Survival and cell death" and "Endocrine system disorders". Especially, the expression of testicular key-genes such as Caspase3, CatSper4, Star and Cyp17A1 was found significantly modified and consistently with the phenotypic changes.

Conclusions

These first results suggest that exposures to EAS alone or in mixtures, at relevant environmental doses, may disrupt various reproductive endpoints including testicular gene expression of the unexposed progeny of exposed fathers. These original results may have implications for the understanding of the so-called “low-dose effects”, their impact in the progeny and the human risk assessment.

Mixtures of chemicals found in amniotic fluid disrupt thyroid signalling and brain development

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Aim of the work

Early embryonic development, particularly neurogenesis, is a critically vulnerable window for exposure to endocrine disrupting chemicals (EDCs). This is particularly the case for thyroid disrupting chemicals (TDCs) as thyroid hormone is essential for normal fetal brain development. Humans are exposed to a spectrum of industrially produced chemicals, many of which are found in amniotic fluid raising the question of effects on early neurogenesis. Mnay of the chemicals found in amniotic fluid have been studied individually for their effects on thyroid hormonal signaling. However, no experiments have addressed their combined effects nor the potential consequences of exposure on brain development.

Methods

1: Use of the Xenopus embryo to analyse the effects of thyroid disrupting chemicals on early neurogenesis. Given the experimental and ethical difficulties of working with early mammalian embryos we exploited the high conservation of thyroid hormone signaling across vertebrates with the numerous advantages of the Xenopus embryo (Fini et al 2007, 2012) to address effects of TDCs on early neurogenesis.

2: Thyroid disrupting effect of chemical mixtures found in amniotic fluid:

We examined the separate and combined effects of a set of 15 chemicals documented as present in urine or serum of pregnant women (Woodruff et al., 2011). Effects of the chemicals, individually or as a mixture at concentrations reported in amniotic fluids, were assessed on thyroid hormone signaling using a line of transgenic thyroid hormone reporter Xenopus embryos (Fini et al, 2007).

2. Effect on tadpole mobility

The short-term effects of different concentrations of this mixture (0.1x, 1x, 10x) were assessed on mobility of tadpoles exposed at the same period of development. Using DanioVision (Noldus), we measured total distance covered by the tadpoles with or without a light stimulus.

3. Endogenous gene expression

Other batches of tadpoles were exposed from NF45 for 3 days to quantify endogenous gene expression. Brain tissue was dissected and placed in lysis solution. RNA was later extracted using RNA pure Ambion kit.

Quality was assessed using Agilent Bioanalyzer and all RNA with RIN >8were kept. Then RNA were retrotranscribed and gene expression was quantified using qPCR 384 well plate with *odc* and *ef1a* as normalising genes.

4. Immunohistochemistry

Tadpoles NF45 exposed for 3 days to mixture (0.1x, 1x, 10x) were fixed in PFA 4%. Antibodies against PLP and beta3 tubulin were used to quantify oligodendrocyte and neuronal populations respectively.

Key Results

Nine chemicals out of 15 showed thyroid-disrupting effects. Further more the mixture tested at 0.1x, 1x, 10x gave a dosed dependent increase in thyroid signaling readout.

Exposure to the mixture modified mobility and total distance covered by tadpoles. Notably exposure to the highest mixture concentration (10x) lead to an almost total loss of spontaneous mobility.

Genes encoding deiodinases and thyroid hormone transporters expression were significantly modified by exposure to the mixture, as were levels of *tubulin* and *mbp*. This latter result suggests brain maturation, like mobility, was affected by exposure to mixtures.

Conclusions

Taken together these results show that tadpoles exposed to TDCs during early brain development, at concentrations equivalent to those measured in amniotic fluid, display a number of defects in brain maturation with consequences of mobility.

Low-dose effects: experimental challenges for endocrine disruption

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Aim of the work

The issue of endocrine disruption has generated a large stream of mechanistic research as well as controversies about principles of toxicology and hazard and risk assessment. Classical hazard and risk assessment is largely based on adverse health effects findings in experimental animals according to globally agreed test guidelines. Nowadays, parameters of physiological function, such as reproductive and thyroid hormone levels, provide additional data that need interpretation in terms of adaptive changes versus adverse effects.

The question arises whether and to what extent compound-induced changes in hormone levels that are within normal homeostatic ranges should be considered adverse in the absence of concomitant toxicity.

Furthermore, a host of *in vitro* models have been designed and applied for detecting endocrine effects of compounds. Findings indicate that even at very low concentrations effects can be observed in such models. Moreover, data have been reported suggesting non-monotonic concentration-responses. Conflicting studies have been published, feeding the controversy about the reality and significance of such findings. *In vitro* derived data in reductionist model systems need to be interpreted in the context of possible adversity in the intact organism. This discussion occurs in an era of innovative changes in toxicological hazard assessment, which drives towards mechanistic approaches, increasing information about molecular mechanisms of compound effects, and requiring toxicologists to interpret a host of novel molecular parameters in view of toxicity. This presentation will address these issues, and discuss principles of toxicology and hazard and risk assessment in the context of the endocrine disruption.

Synergistic Activation of Human Xenobiotic Receptor by Binary Cocktails of Pharmaceutical and Environmental Compounds

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Aim of the work

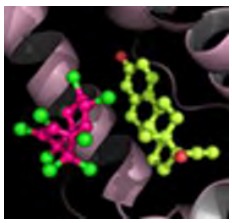
Humans are chronically exposed to multiple exogenous substances simultaneously, including environmental pollutants, drugs and dietary components. Many of these compounds are suspected to impact human health, and their combination in complex mixtures could exacerbate their harmful effects. To explore the outcome of combined exposure to chemicals and establish a detailed mechanistic understanding of this emerging paradigm, we focused our attention on the xenoreceptor PXR (pregnane X receptor; NR1I2) which has been identified by the US Environmental Protection Agency (EPA) ToxCast's program as a major front-line target of chemicals.

Methods

In addition to compound screening, we have used a battery of cell-based, biochemical, biophysical, and structural approaches to characterize the interaction between PXR and binary mixtures of exogenous substances.

Key Results

Here [1] we demonstrate that a pharmaceutical estrogen, the active component of contraceptive pills 17α-ethinylestradiol (EE2), and the persistent organochlorine pesticide trans-nonachlor (TNC), both exhibiting low efficacy when studied separately, cooperatively bind to the pregnane X receptor, leading to synergistic activation. Biophysical analysis shows that each ligand enhances the binding affinity of the other so the binary mixture induces a substantial biological response at doses at which each chemical individually is inactive. High-resolution crystal structures reveal the structural basis for the observed cooperativity.



We therefore propose the concept of a *supramolecular ligand* that defines a molecular assembly consisting of two or more compounds that interact with each other inside the ligand-binding pocket of a receptor, resulting in the creation of a new entity with improved functional characteristics in regard to those of its individual components.

Conclusions

Our results suggest that the formation of such *supramolecular ligands* contributes to the synergistic toxic effect of chemical mixtures, which may have broad implications for the fields of endocrine disruption, toxicology and chemical risk assessment.

[1] Delfosse *et al.*, Nature Communications, 2015 (in press)

An assessment of the effects of chemical mixtures found in meat on human PXR activation: application of the concentration addition model

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Aim of the work

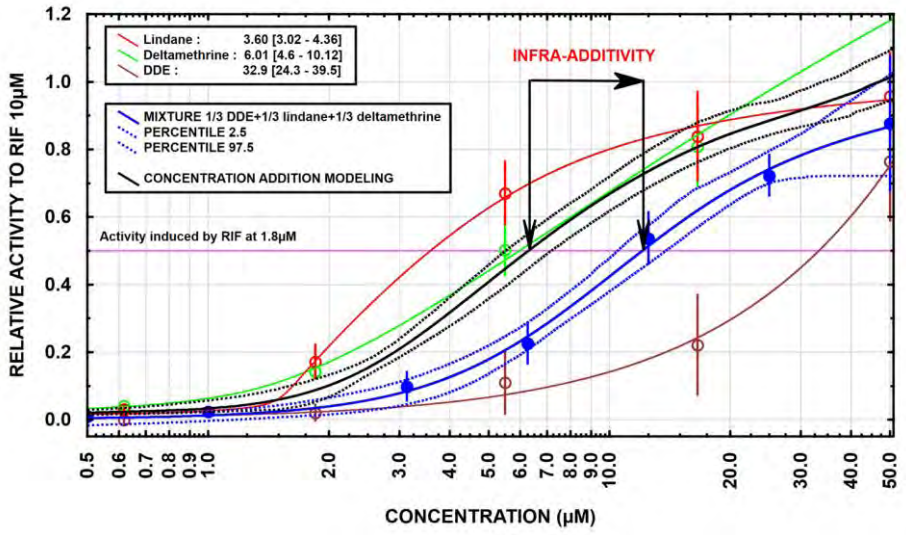
Although most of the chemical residues found in meat are present in relatively small quantities, this causes humans to be exposed to many compounds at low dose. There is now evidence that these chemicals can work together to produce additive, supra-additive or infra-additive effect hat can impact human health. The mixtures effect cannot so be ruled out, even if all the components of the mixture are present at their individual NOAELs or NOECs, as the risk assessment considers only single substances. Therefore, the exposure to multiple chemicals that exhibit similar modes of action on a given nuclear receptor can act like a single molecule in the ligand-binding domain. Among those receptors, we focused on the human PXR *(hPXR)*, which notably regulates the transcription of genes coding xenobiotic detoxication systems.

Methods

We tested the hPXR transactivation potency of 8 meat's chemical residues, belonging to various classes (PCBs, pesticides, antibiotics, mycotoxins), using a stable hepatoma cell line expressing luciferase under hPXR control (*HepG2-hPXR*). Different mixtures *(chemicals in equimolar proportion)* were also tested at 7 concentrations. hPXR activation was modeled *(5 parameters curves)* using the bootstrap method. We applied the concentration addition model to the whole data set generated after bootstrapping to obtain the uncertainties of the predicted dose-response curves.

Key Results

After modeling of the dose-response curves by bootstrap techniques, it appears that three pesticides, namely DDE, lindane and delthamethrine, as well as HBCD and PCBs activate, significantly the hPXR. The effect of mycotoxins and Cd was very low or even had no effect. A ranking could be established by reference to rifampicine (the agonist model, set to 1): lindane = 0.52, deltamethrine and HBCD = 0.30, DDE and PCBs = 0.084. The very weak hPXR transactivation potency of the other compounds *(cadmium, zearalenone, fumosinin B1)* did not allow their classification. As for the "mixtures", all significantly activate hPXR in a dose-dependent manner. As an example, the modeling applied to a mixture only composed of 3 pesticides showed an infra-additive effect when compared to the experimental data *(see below)*.



Conclusions

This work was performed in the frame of the SOMEAT project *(Safety of Organic MEAT)*, which aims to provide scientific data to contribute to the sanitary quality of organic meat in regard to their possible chemical contaminant contents and the potential resulting toxicity potential for consumers. As our diet is contaminated by a large number of chemicals, there is undeniably an increasing need to update the pesticide legislation which currently only considers active substances taken individually. The present study demonstrates that mixtures of chemicals, found in meat, can act as hPXR agonists which may represent a risk factor contributing to endocrine system disorders in humans. The mechanistic cause for the deviation from CA observed with some hPXR agonist mixtures is unknown, but do not exceed a 1.5 fold. Since any such deviation was only minor, the CA model is recommended for risk assessment, as it is typically more conservative than the alternative, independent action model.

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Session 6. Effets of BPA and BPA substitutes

Plenary Conference

Are Bisphenol A substitutes safe?

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Low doses of Bisphenol A (BPA) has been incriminated in the etiology of an increasing number of human diseases, such as diabetes, obesity, cardiovascular, chronic respiratory and kidney diseases, breast cancer, behavioral troubles, tooth developmental defects and reproductive disorders in both sexes. Thus, during the last years, BPA regulation has been tightened, particularly to protect against exposure during fetal and neonatal life. For instance, BPA was banned in baby bottles in Canada in 2008, in France in 2010 and in the European Union in 2011. Recently, the French National Agency for Safety in Food, Environment and Work (ANSES) have recommend to lower BPA TDI to 0.1 µg/kg/day and BPA has been banned in any food or beverage packaging from January 2015 in France. Manufacturers are more and more using alternatives to BPA. However, many alternatives to BPA are other bisphenols. Their structures are closer to that of BPA and we must fear that their effects will be similar to those of BPA.

We'll review toxicological data obtained with some BPA substitutes using biochemical, structural, *in vitro*, *ex vivo* and *in vivo* approaches. Taken together, these data show that the relative effect of substitutes depend on their nature and, more surprisingly, on the experimental approach. Furthermore, because the susceptibility to Endocrine Disruptors varies from one species to another [1] a special attention will be paid to human data. Specifically, using a culture system that we developed (the Fetal Testis Assay, FeTA), we previously showed that BPA concentrations relevant to environmental internal exposure (0.01 µM) reduced basal testosterone secreted by testicular explants from human fetuses [2]. Using the same experimental system, we demonstrated that BPS and BPF have similar anti-androgenic effects in both human and mouse fetal testes [3].

In conclusion, the toxicological data dealing with BPA substitutes are presently limited. BPA cannot be replaced by a single substitute in its various uses and numerous potential substitutes will be necessary. Their individual risk assessment will be a huge work site especially since the mechanism of action of BPA is not yet clearly established.

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Bisphenol S promotes obesity in male mice fed to high fat diet

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Aim of the work

Determine the impact of exposure to a substitute of bisphenol A, the bisphenol S (BPS), on energy metabolism in mice model.

Methods

C57Bl/6 pregnant mice were exposed to low dose of BPS 0.2; 1.5 and 50µg/kg bw/d BPS in drinking water. Treatment began at gestational day 0 and continued in offspring up to 23-weeks old. Four weeks after weaning, mice from treated-dams were fed with a standard diet or high fat diet (HFD; 60% kcal as saturated fatty acid). High fat diet induces obesity classically after 7-8 weeks. During their lifetime, body weight and fat body mass of female and male mice were monitored. Fat mass was measured using Echo-MRI® systems. At 17-weeks old, lipid load test was performed in male and female mice. Mice were force-fed with corn oil (0.5 ml) and the time-course in triglyceride level was analyzed. For each condition, about 10 female and 10 male mice were analyzed.

Key Results

Effects of BPS were dependent of gender, dose and diet. Only male mice fed to high fat diet and exposed to 1.5 and 50 µg/kg bw/d BPS exhibited a significant overweight (11 and 14%, respectively). Also, these data are in positive correlation with adipose tissue. In contrast, no difference was observed in food intake. Likewise, in male mice fed to HFD, the lipid load test indicated that plasma triglyceride levels were decreased in BPS-treated mice suggesting that BPS affects either lipid uptake or the intestinal lipoprotein process.

Conclusions

This study clearly demonstrates that BPS potentiates the obesity induced by a diet rich in saturated fatty acids. This effect is exhibited only in male mice after perinatal exposure at low doses. Combination of a low post-prandial hypertriglyceridemia and lipid accumulation in adipose tissue suggest that BPS improves the efficiency of peripheral lipid storage. Thus, BPS has the same obesogen potency that BPA. Then, BPA substitutes as BPS must be used with caution in term of energetic metabolism disruption.

Bisphenol A affects enamel quality and exacerbates dental fluorosis by modulating the expression of a restricted number of genes

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Aim of the work

Bisphenol A (BPA) is a widespread Endocrine Disruptor (ED) commonly used by the plastic industry and contributes to the development of several pathologies as shown by recent epidemiological and experimental studies. Anecdotally, molar incisor hypomineralization (MIH), a recently described enamel pathology affecting 15 to 18% of children, is increasing concurrently with ED related pathologies. Our previous data show that BPA impacts amelogenesis and generate similar enamel defects as those described for MIH.

The aim of the present study was to identify BPA target genes in the presence of fluoride, a widespread agent commonly used in dentistry to prevent caries.

Methods

Wistar rats were exposed daily to 5 µg/kg/day BPA from the conception to the sacrifice. After weaning, each dam was assigned randomly to one of the four groups. Three experimental groups (n=12 for each) were constituted and treated with 5 µg/kg/day BPA alone, with 5 mM NaF alone included in drinking water or with both BPA and NaF until they were killed on P65. The control group was treated with solvent alone.

RNAs from microdissected dental epithelia were submitted to microarray analysis.

Key Results

Rats exposed to both NaF and BPA presented a more severe phenotype than those exposed to NaF alone, characterized by the more pronounced discoloration of incisors.

Among the 19239 tested RNAs, only 41 were modulated (more than 1.5-fold) by BPA. Interestingly, among these genes, amelogenin and enamelin coding for specific enamel matrix proteins were ones of the highly up-regulated. Four other genes involved in mineralization process, SLC5A8, SLC26A4, SLC44A4 and Carbonic Anhydrase VB also appeared as BPA target genes which expression modulation may explain enamel hypomineralization.

Fluoride had essentially gene repressor effects, notably by reducing the expression of key enamel proteases, KLK4 and MMPs. Combined treatments with fluoride evidenced only nine common target genes, four of them being tightly involved in amelogenesis. Thus, three important groups of genes with major roles in amelogenesis (Enamel Matrix Proteins (EMPs), proteases and SLCs) were affected during exposure to both agents. Interestingly, despite some overlapping target genes (e.g. SLCs), BPA mainly promoted the expression of EMPs whereas fluoride impaired the expression of proteases. Both of these events lead to enamel hypomineralization.

Conclusions

BPA impacts enamel synthesis through expression modulations of a restricted number of enamel key genes. In consequence, exposure to BPA weakens enamel making it more susceptible to mineralization defects as MIH or dental fluorosis. Our study identifies hypomineralization genes that may enable the use of dental enamel as early biomarker of exposure to environmental toxicants.

Developmental exposure to related substitutes of bisphenol A alters murine and human female germ cells

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Aim of the work

The developing gonads are highly sensitive to numerous pollutants, especially to endocrine disrupters. These last decades, numerous studies have demonstrated the reprotoxicity of a widely used environmental endocrine disruptor: the bisphenol A (BPA). This has prompted the removal of BPA from consumer products and the replacement with related substitutes such as BPS or BPF. The aims of this study are the evaluation of the putative toxicity of some of bisphenols (BPS, BPF, BPAF, IRGANOX, BPM and BPAF) on human and murine fetal ovary.

Methods

The toxicity of these bisphenols was assayed using *in vitro* and *in vivo* approaches. Firstly, all substitutes were tested using an organotypic culture of fetal murine ovary. Then, the number of germ cells was measured. As BPAF and BADGE seems to have a significant effect on the germ cells, we have exposed pregnant mice to these molecules during second third of gestation. Additionally, we have developed a xenograft model of human ovary allowing a chronic exposure to low doses of these substitutes over several weeks mimicking “real life” exposure. The impacts of these substitutes on the survival and the differentiation of female murine and human germ cell were assayed.

Key Results

Using *in vitro* and *in vivo* models, we have demonstrated BPAF and BADGE have deleterious effects on murine female germ cells. Theses substitutes increase germ cells apoptosis and impair germ cell proliferation and differentiation. Interestingly, these substitutes have opposite effects that sign different pathways and mechanisms of action involved.

Conclusions

Identifying which substitutes can alter the reproductive function should help public deciders especially as numerous reproductive pathologies have been increasing over the last decades. For the first time, our study highlights the toxicity on the female fertility of two putative BPA substitutes that are already used in our environment: BPAF and BADGE.

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Session 7. Expertise, Risk assessment and Economic cost

Plenary Conference

Health and Economic Cost exposure to EDs in Europe

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Introduction

Rapidly increasing evidence has documented that endocrine disrupting chemicals (EDCs) contribute substantially to disease and disability.

Aim of the work

To quantify a range of health and economic costs that can be reasonably attributed to EDC exposures in the European Union.

Methods

A Steering Committee of scientists adapted the Intergovernmental Panel on Climate Change weight-of-evidence characterization for probability of causation based upon levels of available epidemiologic and toxicologic evidence for one or more chemicals contributing to disease by an endocrine disruptor mechanism. To evaluate the epidemiologic evidence, the Steering Committee adapted the WHO Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group criteria, while the steering committee adapted definitions recently promulgated by the Danish Environmental Protection Agency for evaluating laboratory and animal evidence of endocrine disruption. Expert panels used the Delphi method to make decisions on the strength of the data.

Results

Expert panels achieved consensus for probable (>20%) EDC causation for IQ loss and associated intellectual disability; autism; attention deficit hyperactivity disorder; childhood obesity; adult obesity; adult diabetes; cryptorchidism; testicular cancer; male infertility and mortality associated with reduced testosterone. Accounting for probability of causation, using the midpoint of each range for probability of causation Monte Carlo simulations produced a median cost of €157 billion annually across 1000 simulations. Notably, using the lowest end of the probability range for each relationship in the Monte Carlo simulations produced a median range of €119 billion that differed modestly from the base case probability inputs.

Conclusions

EDC exposures in the EU are likely to contribute substantially to a wide array of disease and dysfunction across the life course with costs in the hundreds of billions per year. These estimates represent only those EDCs with the highest probability of causation; a broader analysis would have produced greater estimates of burden of disease and costs.

Benefits for public health from exposure reduction to the endocrine disruptor pesticide chlordecone in Guadeloupe, the BAREPE project

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Aim of the work

Inhabitants of Guadeloupe are chronically exposed to low doses of chlordecone via local food. The magnitude of the health impacts is not known. This makes it difficult to design appropriate and cost-effective preventive actions. Despite uncertainties, public authorities have implemented an exposure control program since 2003. The aim of the work is to develop a quantitative health risk assessment method for non-genotoxic effects of chlordecone, and estimate the impacts related to exposures in Guadeloupe. Then we aimed at estimating the benefit as the difference in cost of impacts before and after 2003; finally we compared the benefits to the cost of the control program.

Methods

Relevant data are extracted from publications searched in Medline or Toxline. Knowledge about modes of action is used to select effects of chlordecone that could occur at low dose. A linear exposure response function (ERF) is derived for each possible effect of chlordecone at chronic low-dose. From epidemiological data, ERF is the delta relative risk (RR-1) divided by the corresponding delta exposure. From animal studies, ERF is the benchmark risk (10 %) divided by the best benchmark dose modeled with BMDS2.4.0. Distribution of exposure of the Guadeloupe population is extracted from several epidemiological studies that have used blood chlordecone concentration as internal exposure indicator. Risks are exposures multiplied by ERF, and impacts are risks multiplied by number of persons at risk. A no-effect threshold dose is included in the risk model to allow a comparison of results for different assumptions about the existence and level of the threshold. Impacts are monetized with DALY and VOLY (value of a life year) concepts.

Key Results

Four effects are possible at chronic low dose: cancer promotion, developmental impairment, neurotoxicity and hepatotoxicity. Only the latter is not driven by endocrine disruption. Two ERF are derived from epidemiological results: prostate cancer after 44y (0.0019 per $\mu g/l_{blood}$) and impaired neurodevelopment in boys (-0.32 QI_{point} per $\mu g/l_{cord\text{-}blood}$). Two are based on animal studies: liver cancer (2.69 per mg/kg/d) and renal dysfunction in women (0,0022 per mg/kg/d). Neurotoxicity in adulthood and other developmental effects lack quantitative data for ERF derivation. Assessed without threshold, annual impacts before the control program are: 5.4 liver cancers; 2.8 prostate cancers; 0.1 renal dysfunction and 1 173 IQ points lost.

The corresponding total cost is 32 M€/y [11-64] among which 62 % are from impaired neurodevelopment; 27 % liver cancers; 11 % prostate cancers; 0.6 % renal dysfunction. The decrease in exposure levels attributable to the control program implies a benefits of 11 M€/y. Direct costs of this program are around 3.5 M€/y. When threshold is included, the total damage cost is decreased to 23 M€/y [7-47], but the benefits remain about the same. This may be due to the fact that exposures in pregnant women have decreased less than in men during the same period of time.

Conclusions

This is the first study that quantified chronic non genotoxic effects of chlordecone exposures in Guadeloupe. The exposure control program is highly cost effective. Pregnant women should be a main target of prevention. Prevention efforts should be sustained as long as chlordecone remains in the soil. Toxicological and epidemiological research on health endpoints that could not be taken into account would also be required. The assumption of a no-effect threshold has a relatively small effect on the results.

Table 1: health impacts, annual costs and benefits from reduction of exposures to chlordecone in Guadeloupe, without threshold. The last column shows how the result would decrease with a threshold dose of 0,5 µg/kg/d

Health outcome	Main factor of variation	population at risk	Period of exposure measurement	n	Impact (number of annual cases)	CI low (Euros)	CI mean (Euros)	CI high (Euros)	Proportion (mean estimate)	Ratio with/without threshold
Liver cancer	Conversion from external exposure to internal exposure	All	<2003	436 968	5.4	5 738 000	8 540 000	17 480 000	27%	0.47
			≥2003	458 465	2.0	2 129 000	3 169 000	6 488 000	15%	0.30
			Annual benefit		**-3.4**	3 609 000	**5 371 000**	10 992 000	**51%**	0.58
Prostate cancer	$IC_{95\%}$ of RR	Men > 44 years	<2003	72 566	2.8	914 000	3 351 000	6 876 000	11%	0.64
			≥2003	76 049	1.0	319 000	1 169 000	2 398 000	5%	0.34
			Annual benefit		**-1.8**	595 000	**2 182 000**	4 478 000	**21%**	0.80
Renal dysfunction	$BMD_{10\text{-}HD}$	Women	<2003	227 417	0.10	17 000	200 000	500 000	0.63%	0.31
			≥2003	239 470	0.04	7 000	83 000	208 000	0.39%	0.25
			Annual benefit		**-0.06**	**10 000**	**117 000**	**292 000**	**1.1%**	0.35
Cognitive development	Equivalence between QI point and ASQ point	Male new born	<2003	3 747	1 173	3 949 000	19 745 000	39 489 000	62%	0.86
			≥2003	3 929	1 003	3 377 000	16 887 000	33 773 000	79%	0.65
			Annual benefit		**-168**	572 000	**2 858 000**	5 716 000	**27%**	2.08
Total			<2003			**10 618 000**	**31 836 000**	**64 345 000**	100%	0.47
			≥2003			**5 832 000**	**21 308 000**	**42 867 000**	100%	0.30
			Annual benefit			**4 786 000**	**10 528 000**	**21 478 000**	100%	0.58

« n » = Population from 2002 census data for the first period of time and 2006 census data for second period. Euros are expressed in 2006 value. Impact are expressed in number of death (mean estimate), except for cognitive development where the impacts are expressed in QI point lost. CI = Cost of Impacts (include only the value of disability adjusted life year lost).

Toolkit for uncertainty and knowledge quality analysis of endocrine disrupters' risk assessments: the case study of Bisphenol A (Dico-Risk)

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Aim of the work

The Dico-Risk project has been funded by the PNRPE Programme of the French Ministry of Ecology between 2011 and 2014. Its objective was to develop a toolbox for uncertainty analysis and knowledge quality assessment, to explore and understand the interdependencies between scientific and political dimensions of controversies surrounding the risks of BPA.

Methods

For building uncertainty analysis tools specific to this project, several methods have been associated:

- The method of expert elicitation, associated with the development of original typologies of uncertainty classes, to develop the tools of the family Qualichem

- Linguistic uncertainty analysis using Natural Language Processing (NLP), which has been used to associate sentences with a class of uncertainty. Three approaches were used: a rule-based approach, an approach based on supervised learning, and an approach based on an information retrieval system.

- The manual coding of risk assessment documents, to identify the expression of uncertainty. An original ontology comprising 28 uncertainty classes has been used to label the uncertainties identified. To build an automated coding of these documents, supervised learning techniques have been used.

Key Results

A family of tools called Qualichem has been developed and validated with BPA specialists:

Qualichem_in-vivo, for evaluating the quality of in vivo studies

Qualichem_epidemio, for assessing the quality of epidemiological studies

Qualichem_review, for literature reviews intended at identifying and characterizing the hazards of a chemical.

For the characterization of linguistic uncertainty, three approaches to automatic detection of uncertainty in the BPA risk assessment textual materials were tested: a rule-based approach, a supervised learning approach and an approach exploiting an information retrieval system.

The comparison between the three approaches showed that machine learning is the most effective, i.e. it handles the largest number of classes and sentences, and get the automated results the nearest to the manual coding results.

The analysis of the interdependencies between the linguistic expression of uncertainty in expert reports and political decision showed that there is more "precaution" expressed in the EU documents than in the US assessments. Also, uncertainty expressed by experts in the US decreases with time and with the accumulation of references, whereas in the EU it increases.

Conclusions

The results obtained with Qualichem indicate that there is considerable inter-individual heterogeneity among scientists assessing the quality of the same study, using the same criteria. Qualichem produces common results of several experts while allowing everyone to express themselves individually, even when their opinion is minoritary in a group. In addition, Qualichem assesses the overall level of uncertainty in a study, expressed as a confidence level.

The methods and tools using NLP were shown to be usable to perform automatic detection of uncertainty in chemical and food risk expertise documents. Tests conducted with two different datasets (chemical and dietary risk) show that the methods and tools are portable from one domain to others with reliable results.

Finally, the uncertainties expressed linguistically by experts seem to lead to risk management measures, instead of blocking the action. More so, the strongest decisions are made based on documents containing more uncertainty.

Endocrine disruptors: Challenges for Anses

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Anses has been involved for many years in providing support to research and conducting risk assessment of endocrine disrupters (EDs) and is recognized for its knowledge and know how in this field. It is currently fully engaged in the implementation of the French Strategy on Endocrine Disrupters (also called SNPE). In this context, with the contribution of its experts' panels, Anses provides scientific support and expertise for the regulation of these chemical compounds at European Union level involving sectorial legislations and the REACH, pesticides or biocides legislations

H How to perform risk assessment with such compounds is subject to intense scientific debate given the underlying public health, societal and economic implications. Should the classical risk assessment approach be applied or are there specificities linked to EDs that justify changing or adapting the current risk assessment methodology? Reflecting on the BPA risk assessment process and methodology developed by Anses in the light of new developments, studies and publications, is a very good exercise to identify all the issues related in particular to the hazard assessment of EDs: how to consider non GLP-OECD guideline studies in a weight of evidence approach? Is there sufficient evidence for non-monotonic dose-response relationship? What adverse apical outcome can be expected in later life from a modification in the development of certain organs during uterine life? …

This conference will use various examples of recent risk assessments performed by Anses and its expert panels to highlight some aspects related to risk assessment of EDs. Challenges faced by Anses and proposals as to how to move forward in this complex and controversial field will be presented.

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Posters

P1-Development of exposure assessment and preventive intervention of blood PCB level using Japanese birth cohort data

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Aim of the work

The health effects of fetal and children's environmental exposures to persistent organic pollutants (POPs) have been of concern. In Japan, it has been reported that food is the major source of exposure to POPs. Our previous studies revealed that colestimide (fiber; a medication prescribed for hyperlipidemia) and dietary fiber intake were effective to reduce PCBs/dioxins levels in human body. It is important to know how PCB contamination levels are affected by eating habits to reduce the contamination level effectively. The objective of this study was to find a preventive intervention method to reduce human exposure to PCBs/dioxins by analyzing data on the correlation between the results from Food Frequency Questionnaire (FFQ) and the PCB contamination level in the blood samples of mothers during pregnancy and fathers who participated in the Japan Environment and Children's Study (JECS). JECS is a long-term birth cohort study.

Methods

The blood PCB levels of 1,456 mothers and 47 fathers were analyzed and calculated by Packed Column Gas Chromatography Electron Capture Detector (GC/ECD). Subjects were recruited in Chiba Prefecture. The relationships between questioner (age, body mass index [BMI], number of delivery), serum lipid levels and FFQ (consumptions of foods and nutrition) from JECS participants and PCB concentration were analyzed by partial least squares regression (PLS) (number of predictor variables = 93).

Key Results

Prediction models were developed for mothers (n = 1,456) and fathers (n = 47). The expected and actual blood PCB levels in mothers, blood PCB level was positively correlated (R^2 = 0.302 and Q^2 = 0.292) with age, intake of fat-soluble vitamins, cholesterol levels, lipid levels, and was negatively correlated with intake of fiber and the number of delivery.

Among them, the mothers of first pregnancy were scrutinized (n=414), then blood PCB level was more strongly correlated (R^2 = 0.341 and Q^2 = 0.322) with age, consumption of fat-soluble vitamins, cholesterol levels, lipid levels, and was negatively correlated with intake of fiber.

The expected and actual blood PCB levels in fathers correlated positively (R^2 = 0.456 and Q^2 = 0.334) with age and consumption of fish, and was negatively associated with fruit/vegetable consumption.

Conclusions

It was indicated that food fiber intake can decrease the blood PCB level. The correlation was seen most strongly for fathers, next for mothers of first pregnancy, and last, for all mothers. This might be because that women with delivery (and probably breast feeding) experience decrease the PCB level dramatically. FFQ can be used to estimate the contamination level of PCBs/POPs, and in case high contamination is predicted, intake of food fibers can decrease the level.

This study was conducted as an adjunct study of JECS. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the government.

P2-Carbohydrate metabolism is disrupted by a combination of persistent organic pollutants in the human hepatic cell line HepaRG

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Aim of the work

Epidemiological studies have associated exposure to xenobiotics with an increased prevalence of metabolic diseases. Humans are exposed to mixtures of xenobiotics detoxified by the liver. The aim of the study was to measure the impact of two xenobiotics, both endocrine disruptors and persistent organic pollutants, on key genes of hepatic energy metabolism, in the human hepatic cell line HepaRG.

Methods

HepaRG cells were exposed to either 25 nM 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which binds to the transcription factor Aryl hydrocarbon Receptor, or 10µM α-endosulfan, an organochlorine pesticide, which acts via either the Pregnane X Receptor and/or the estrogen receptor or their combination. The expression of carbohydrate metabolism genes was measured following 30H of treatment by RT-qPCR. Radioactive glucose was used to measure its oxidation into CO_2 and the productions of lactate and glucose were measured with colorimetric assays after 72H.

Key Results

The expression of glucose transporter 2 (SLC2A2/GLUT2) and of glucose-6-phosphatase (G6Pc), were reduced 80-90% by the combination of TCDD and α-endosulfan, which was more than by either xenobiotic alone. The expression of phosphoenolpyruvate carboxykinase 1 and 2, liver pyruvate kinase, glycogen synthase, glycogen phosphorylase and pyruvate dehydrogenase kinase 2 also was decreased by the mixture. Under gluconeogenic culture conditions, a 40% decrease in the production of glucose was observed by the combined treatment. The oxidation of radiolabeled glucose into CO_2 decreased 30% after 72h exposure to the mixture under glycolytic conditions but lactate production was not modified. Treatment for 8 days with lower concentrations of TCDD (0.2 to 5 nM), α-endosulfan (3 µM) and their mixtures also resulted in a decrease in expression of both G6Pc and GLUT2.

Conclusions

These results demonstrate that mixtures of TCDD and α-endosulfan down-regulate several key genes of human hepatic carbohydrate metabolism, resulting in modifications of glucose metabolism. The results further suggest that a chronic exposure of individuals to low doses of persistent organic pollutants might alter hepatic carbohydrate metabolism and contribute to the development of the metabolic syndrome.

P3-Evaluation of Σ6 NDL-PCB-induced neurotoxicity in mice offspring: a multigenerational epigenetic study

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Aim of the work

Polychlorinated biphenyls (PCBs) are a group of organic pollutants produced from multiple industrial processes. Despite the ban of their production since the 80s, they persist in the environment and accumulate in the food chain because of their chemical stability and lipophilicity. Among PCB congeners, the non coplanar compounds called "non-dioxin-like" PCBs (NDL-PCBs) are the most widespread molecules in the environment and food matrices.

It has been shown that developmental exposure to PCBs at high levels through placental and breast milk transfer may induce changes in the neuroendocrine control of reproduction and cause long-lasting neurological damages in the offspring, besides, in our previous study, we demonstrated that a lactational exposure to the Σ6 NDL-PCBs at 10 ng/kg can cause developmental, behavioral and genomic alterations in mice of the offspring.

In this context, our present work aimed to evaluate the potential transmission of the neurobehavioral toxicity between the F1 and F2 generation and induced by a perinatal exposure to the Σ6 NDL-PCBs at environmental levels.

Methods

F0 pregnant dams were daily exposed from the 7th day of gestation until weaning [postnatal day (PND) 21] via free access to a food paste containing a mixture of the Σ6 NDL-PCBs at two doses: (1) 10 ng/kg considered low dose, used previously and that showed early and long-lasting neurological damages in the offspring F1 and (2) 1000 ng/kg equivalent to the TDI fixed by the ANSES (2007). The generation F2 has been engendered by mating F1 controls and exposed groups in order to identify the parental origin of the potential disorders. Cognitive outcomes were assessed in F1 and F2 generations using developmental (righting reflex, negative geotaxis and ultrasounds recording) and behavioral tests (rotarod and water escape pole climbing test) realized from PND 3 to PND 30.

Key Results

Results showed significant perturbations in the maturation of the vestibular function and the motor coordination measured between all exposed animals versus controls excepted at PND 30 (last and late stage).

Conclusions

Further studies such as functional measurements, including anxiety, general activity, depression, social behavior and memory assessments, will be performed at adult age to bring a global response on potential cognitive impairments and molecular studies including transcriptomics and epigenetics to identify and characterize the modifications of DNA and RNA supporting cognitive and motor coordination impairments.

Keywords: Σ6 NDL-PCBs, multigenerational mice model, perinatal exposure, epigenetic, neurotoxicity.

P4-Perinatal exposure to NDL- PCBs at environmental level induced changes in inflammatory status in brain mice

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Aim of the work

Polychlorinated biphenyls (PCBs) are a class of man-made organic compounds, hazardous for human health, biomagnified in the food chain despite a regulatory stopping of their production in the 80's. Among them, the toxicity of the six non-dioxin-like PCBs (6 NDL-PCBs; PCBs: 28, 52, 101, 138, 153 and 180), considered as indicators because of their significant occurrence in food and feed, are poorly studied. Since no data were available on their impact in a realistic context of exposure, this study enrolling in the NeuroDeveTox project (ANR-CESA-2011) focused on the effects of a naturally contaminated food matrix essentially fishery products which are the major source of human exposure to 6 NDL- PCBs. The postnatal period is a critical developmental window where inflammatory events may have significant and impacting effects on the brain development. As a consequence, neuroinflammation may induce alterations of emotional behavior and/or cognition and enhance susceptibility to chemically induced neurotoxicity, neurodegenerative diseases and multiple sclerosis in later life. In addition to that, these alterations are characterized by the activation of glial cells (microglia and astrocytes…) causing changes in their cellular morphology and the release of numerous inflammatory mediators.

In this context, this study aimed to assess neuroinflammation in brain of mice perinatally exposed to different regimes based on eel, source of the ∑6 NDL-PCBs.

Methods

Swiss albino female mice were daily subjected from gestational day 7 until postnatal day (PND) 21 to the ∑6 NDL-PCBs at 0, 85, 200 and 400 ng/kg/day via a food paste containing lyophilisate eel. At PND0, 14 and 21, protein levels changes of selected inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-4, TGF-β, IL-10, CCL2/MCP1 and IFN-γ) in offspring brain homogenates were assessed by ELISA as well as nitric oxide (NO) production.

Key Results

Results showed significant variations of selected pro-inflammatory cytokines TNF-α, IL-1β, CCL2/MCP1 and IL-6 as well as anti-inflammatory mediators IL-10 and TGF-β and NO production depending on the level of contamination, gender and age. Whereas, no significant variation of IL-4 and IFN-γ production was observed in exposed pups compared to controls.

Conclusions

This study demonstrated that the perinatal exposure to the ∑6 NDL-PCBs via a naturally contaminated food matrix is able to disrupt the inflammatory status in offspring brain with an enhanced susceptibility to produce pro-inflammatory mediators at early age. These findings should be correlated with the neurodevelopmental impacts highlighted by the functional and mechanistic analysis performed in this project in order to explain these defects.

P5-The Endocrine-Disrupter, p,p'DDT behaves as an Allosteric Modulator of the human Follitropin Receptor

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Aim of the work

Impact of endocrine disruptors (ED) on health is a growing concern as targets and effects on animals and humans are diverse, and the list of disruptors seems endless. Among ED, the insecticide p,p'DDT is an environmental persistent endocrine disruptor. Several studies have shown an association between p,p'DDT exposure and reproductive abnormalities. Previously, p,p'DDT has been shown to disturb the downstream signaling of the FSH receptor (FSHR). The FSHR is a plasma membrane receptor belonging to the G protein-coupled receptor (GPCR) superfamily. The FSH receptor plays a key role in the steroidogenesis and gametogenesis. A comparison of the structures of some of these allosteric modulators of FSHR with p,p'DDT show some rough structural homologies. Here, we investigate the assumption that p,p'DDT may interact with allosteric sites on the FSHR.

Methods and Key Results

We have investigated the impact of p,p'DDT on the FSHR activity and its interaction with the receptor, using CHO cells stably expressing the human FSHR. The receptor activity is assessed by measuring the intracellular cyclic AMP (cAMP) concentration by a technique of bioluminescence on living cells.

Key Results

After 5 minutes, p,p'DDT increased the maximal response of FSHR to follitropin by 32%, without changing or basal activity or sensitivity of the receptor. The potentiating effect of p,p'DDT was dependent on and specific to FSHR. Indeed, p,p'DDT reduced by 50% the activity of the LH receptor (a closely related receptor belonging to the same family as FSHR). Using pharmacological approach, we have shown that p,p'DDT does not act directly on adenylate cyclase or phosphodiesterase (enzymes involved in the metabolism of cAMP). In addition, p,p'DDT increases the sensitivity of FSHR to a low molecular weight agonist (16a), suggesting a role as an allosteric modulator for the ED. Using different mutants of FSHR, we show that p,p'DDT binds to the transmembrane domain of the receptor.

This is corroborated by *in silico* experiments, in which p,p'DDT and 16a agonist were docked into the FSHR simultaneously. p,p'DDT also increases the sensitivity of FSHR to human chorionic gonadotropin hormone (hCG). Finally, we show structural analogues of p,p'DDT (p,pDDE, o,p'DDT, Bisphenol A) differently modulate the activity of the FSHR.

Conclusions

p,p'DDT acts as a positive allosteric modulator of FSHR. The increased response to FSH in presence of p,p'DDT shown *in vitro*, and the gain of sensitivity to hCG, can therefore be deleterious effects *in vivo*. Finally, we conclued the effects on the FSHR signaling, and more largely on GPCRs should be considered when analyzing the mechanisms of the association between ED and diseases of the reproductive system. Indeed, GPCR, as exemplified here by the FSHR, are additional targets of p,p'DDT and other endocrine disruptors.

P6-The effects of DEHP on the Egyptian cotton leafworm, *Spodoptera littoralis*

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Aim of the work

Di-(2-ethylhexyl)-phthalate (DEHP)[1] is a plasticizer widely used in PVC to increase flexibility and known to have endocrine disrupting effects on vertebrate species. While insects have a great economical interest (pollinators, invasive species, vectors of diseases) only a few studies focus on the effects of DEHP on insect species.

In the frame of the ANR DISCO project (endocrine Disruption of Insect Sexual Communication), the current project focuses on the effects of DEHP on the Egyptian cotton leafworm, *Spodoptera littoralis*. First of all, since no data was available in our model, we investigated the effects of DEHP on the post-embryonic development (length and number of larval instars, larval weight and food consumption, titration of ecdysteroids) and the sex ratio. Then, we focused on the effect of DEHP on the female sex pheromone detection by *S. littoralis* males. Indeed, this crucial process for mating is under endocrine control in our species[2] and could be potentially disrupted by DEHP.

Methods

Larvae *Spodoptera littoralis* were fed, from the end of the 2nd instar to the last larval instar, with contaminated food at several concentrations (from 10pg to 40mg DEHP per gram of food).

The effect of DEHP on post-embryonic development was investigated using 50 larvae per condition and compared to larvae feeding on control food (without DEHP). Larvae and food were weighted at the beginning of each instar till the 6th larval instar, and every day from the beginning of the 6th larval instar. Timing of each larval instar was determined by a daily record of molt process as well as mortality. During pupal stage, we recorded mortality, duration of the stage and the sex ratio. To complete the post-embryonic study, we investigated the potential modifications in the ecdysteroid titration in hemolymph of the last larval instar using Enzyme Immuno Assay (EIA) as described in Porcheron et al., 2009[3].

We studied the sexual behaviour by recording for 15 min, in a behavioral arena under red light, a DEHP-contaminated male after introduction of a female. Mating success and several other courtship items were then assessed.

Key Results

Most of the experiments are still in progress. Preliminary results showed that DEHP is weakly toxic for *S. littoralis*. Mortality is increased only for the two highest concentrations. Some effect on larval growth rate was recorded and has to be confirmed in additional experiments.

Conclusions

Although we have to complete our work, preliminary results highlighted that DEHP can have some effect on *S. littoralis*.

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P7-Evidence of cross-talk between AhR and ER signaling pathways in fish and human reporter cell lines used for estrogenicity screening

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Aim of the work

In vitro cellular assays based on the stable expression of a reporter gene driven by estrogen receptor (ER) are recognized as valuable effect-based tools (EBT) to assess estrogenic activity of environmental mixtures. However, it is now established that ER-mediated cellular response can be influenced by different factors relative to the cell context studied, such as metabolism and cross-talk with other cellular signaling pathways. AhR ligands (i.e. dioxin-like compounds) are widespread environmental contaminants and generally occur concomitantly with estrogenic compounds. Interactions between aryl hydrocarbon receptor (AhR) and ER signaling pathways have been reported in numerous studies, both in fish and human. Characterizing such interferences in EBT used for estrogenicity screening appears crucial when interpreting ER cellular response to complex environmental mixtures.

Our work aims at characterizing three recently established zebrafish reporter cell lines stably transfected with zfERα (ZELHα), zfERß1 (ZELHß1) or zfERß2 (ZELHß2) subtypes (1) by comparing response profiles between human (MELN derived from MCF-7) and zebrafish (ZFL zebrafish liver cells) cellular contexts and (2) by assessing possible influence of zfER subtypes on the response, especially in case of zfERß2, a fish-specific ER subtype.

Methods

Cells were exposed to estradiol (E2) and 2,3,7,8-tetrachlorodibenzodioxin (TCDD), as model agonist ligands for ER and AhR respectively. MELN and zebrafish cells were exposed for 16h and 72h, respectively. Within a given assay, ER and AhR activation were monitored in living cells using luciferase activity and cytochrome P450-1A-related EROD activity, respectively. Concentrations of E2 and TCDD for co-exposure were chosen according to the sensitivity of each model. Results were confirmed in 3 independent experiments.

Key Results

Preliminary results on MELN, ZELHα and ZELHß2 cells show a reciprocal crosstalk between AhR and ER. In MELN, TCDD induced a slight estrogenic activity at the highest concentration of 10nM. In co-exposure with E2 10nM, TCDD enhanced ER transactivation by 30%. In contrast, TCDD alone had no estrogenic activity neither in ZELHα nor in ZELHß2 cells.

Co-exposure of TCDD 10 nM with estradiol lead to 50% and 25% decrease of E2-induced luciferase activity in ZELHα and ZELHß2 cell lines, respectively. Looking into reciprocal interaction, we showed that E2 inhibited TCDD 10nM-induced EROD response in all the models. The inhibition reached 43% in MELN, 20 % in ZELH-ERα and 22% in ZELH-ERß1. Surprisingly, only very low levels of EROD activity were detected in ZELH-ERß2 cells in both untreated and TCDD exposed cells.

Conclusions

Our results demonstrate that a reciprocal cross talk between AhR and ER do occur in our models and that zfER subtypes may influence the extent of the response. A negative crosstalk between ERα signaling pathway and AhR ligands has been previously reported in fish, suggesting ZELHα cell line to be representative of fish liver for that specific endpoint. However, less information is available regarding the implication of zfERß1 and zfERß2 subtypes in AhR/ER crosstalk. The unexpected low level of EROD activity in ZELHß2 would warrant further research. Taken all together, the opposite effects of TCDD on hERα and zfERα responses in human and fish reporter cell lines illustrate the need for understanding bioassay response when interpreting results, especially in case of environmental mixtures containing ER and AhR ligands.

P8-BPA exposure interferes with follicular stage progression in zebrafish

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Aim of the work

We studied the effects of Bisphenol-A (BPA) exposure on growth and progression of ovarian follicles. Zebrafish was used as animal experimental model and exposed to BPA (5µg/L) for 3 weeks. Unexposed animals were used as animal experimental control.

Methods

Ovarian sections from unexposed (control, CTRL) and BPA-exposed (BPA) fishes were processed for histological analysis using ematoxilin-eosin staining and observed under a light microscopy.

In both experimental groups, all the follicular stages, from primary to mature stage, were observed (Fig. 1A). Interestingly, ovarian sections from BPA-exposed animals appeared particularly enriched of mature follicles with morphological markers of atresia such as membrane disintegration, follicular cell proliferation, zona radiata breakdown, yolk resorption. The majority of these follicles appeared enlarged, extremely fragile to sectioning and frequently showed empty areas within the oocyte.

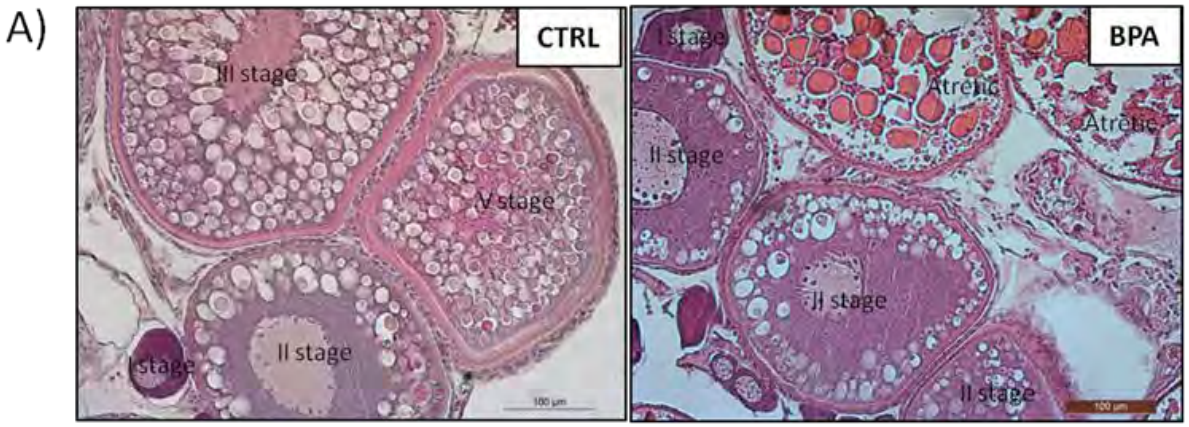
To validate the histological observations and decode the BPA-target follicular stages we analysed ovarian sections from both experimental groups and counted follicles in each developmental stage (Fig. 1B). Accordingly with Selman (1993), we classified five stages of follicular development. In particular, stage I (primary growth), stage II (cortical alveolar), stage III (early vitellogenic), stage IV (late vitellogenic and maturation competent) and stage V (mature) were classified and counted in both experimental groups. Using morphological markers of atresia, we also counted the mature follicles with atretic markers (henceforward referred as atretics).

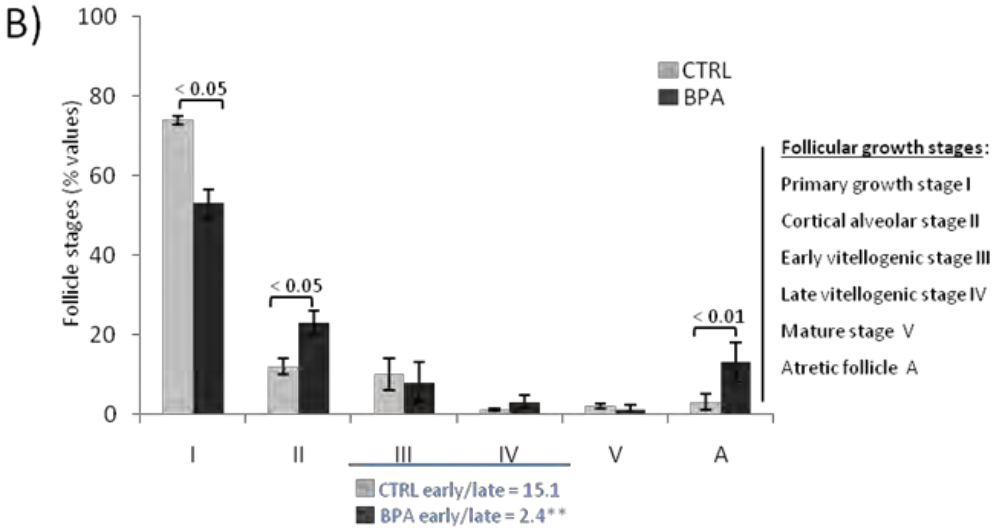
Key results

In BPA-exposed animals the number of stage I follicles significantly decreased as compared with the control group ($p<0.05$) while the number of stage II follicles was significantly higher ($p<0.05$). No effect was observed on the number of early (stage III) and late (stage IV) vitellogenic follicles although early/late ratio significantly decreased after BPA exposure ($p<0.01$). Interestingly, in BPA exposed animals no effect was observed on the number of mature follicles. Conversely, the atretic ones were numerically higher ($p<0.01$)

Conclusions

These observations suggest that BPA affects the previtellogenic and vitellogenic phases. In particular, BPA-exposure increases follicular recruitment by acting on primary stage I and forces follicular transition from stage III to stage IV. We suppose that BPA forces stage III follicles to acquire morphological futures of stage IV without to force the acquisition of maturational competence so that follicles undergo atresia.





P9-Effects of Biphenol A (BPA) on post-embryonnic development and sexual behavior of the cotton pest, Spodoptera littoralis

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Aim of the work

Bisphenol A (BPA) – found in many mass-produced products including medical devices, food packaging, perfumes, … - is considered as a major pollutant in several countries. BPA is mostly resistant to environmental degradation in water and soil (half-life from 1 day to 12 months). This EDC is found in virtually all regions of the world and can be widely dispersed in the environment through different ways, such as the sewerage systems, waste disposal and spraying. While little is known about his distribution and transport in the atmosphere, different ranges of BPA concentrations were measured in the atmospheric aerosols from urban, rural, marine and polar regions. Thus, the sources of aquatic or terrestrial organism's contamination are numerous: by ingestion (contaminated water or food) and by direct exposure (through external epithelia). Current researches on BPA's effects mainly focused on human and vertebrate health. Numerous studies on EDCs mechanisms of action have led to a better understanding of their disruption mechanisms on the steroid signaling pathways. Effects of EDCs, especially BPA, on invertebrates (95% of known animal species) are poorly studied whereas they are of great ecological importance in particular because they form the basis for most food-webs. Most of these studies had been realized on aquatic insects showing an impact of BPA on molting, growth and development. Only 10% of the studies on invertebrates were conducted on terrestrial models. Yet, numerous biological processes in arthropods are under endocrine control and could be disrupted by EDC. To date, only two studies were available on terrestrial insects**.** Considering the crucial importance in ecology, agronomy, medicine and economy of insects and particularly terrestrial ones, more studies on these species are required, especially for improving the strategies in environment's protection. In the frame of the ANR DISCO project (endocrine Disruption of Insect Sexual Communication), the current project focuses on the effects of BPA on the post-embryonic development and sexual behavior of the Egyptian cotton leafworm, *Spodoptera littoralis*.

Methods

We investigated the effects of BPA on the post-embryonic development by recording the length and number of larval instars, larval weight and food consumption, pupal weight, mortality of larvae, pupae and adults as well as duration of pupal stage and the sex ratio.

We completed this part of work by making titration of hemolymphatic ecdysteroids and studying expression level of several nuclear receptors (ECR, USP, E75, E78, BRC) involved in the ecdysteroid signaling pathway.

Adult and larval behavior was recorded using a behavioral arena under red light either after introduction of a female for BPA-contaminated male either after food introduction for larvae. Sexual or eating behavioral items were then analyzed using Ethovision software.

Key Results

Several doses have disrupted the duration of larval and pupal stage, the level of hemolymphatic ecdysteroid and the expression level of the nuclear receptors during the last larval instar as well as the sex ratio. Moreover, some behavioral items of contaminated males appeared to be affected at some doses of BPA.

Conclusions

As predicted in the DISCO project, BPA seems to disturb many biological processes in our model of terrestrial insect that are in normal condition under the control of the endocrine system.

P10-Perinatal BPA exposure contributes to obese phenotype in male mice at adulthood: an immunological point of view

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Aim of the work

Metabolic disorders (obesity and type 2 diabetes) are associated with inflammation and affect a quart of adults and over a million of children in United States. Its adverse effects on social, medical and economical aspects make it one of the biggest points of interest of research. During the last decade, a link between environmental endocrine disruptors (EDCs), in particular with the ubiquitous contaminant Bisphenol A (BPA), and expansion of obesity has been made (Review by B.S Rubin, 2011). A correlation has been reported between BPA plasma levels, inflammatory markers and visceral obesity in adult male human. White adipose tissue (WAT) expansion in obesity is characterized by increasing infiltration of proinflammatory immune cells responsible for chronic low-grade inflammation (Review by Sell et al, 2012). Based on these data, we aimed at investigating in aging mice whether perinatal BPA exposure at 50 µg/kg body weight (BW)/day alters gut immune system development, and promotes chronic inflammation at adulthood leading to metabolic disorders.

Methods

C3H/HeN mice were fed orally from day 15 of gestation until weaning of pups at postnatal day (PND) 21 with 50 µg BPA/kg BW/day or vehicle (corn oil) alone. Immune cells from spleen, mesenteric lymph node (MLN) or visceral WAT were harvested from young male adult offspring at PND45. Phenotypic analysis of macrophage subsets and regulatory T cells (Treg) was performed by flow cytometry using specific cell markers. IFN-γ and IL-17 secretions were assessed by ELISA in supernatant of spleen and MLN cell suspensions after *in vitro* anti-CD3/CD28 restimulation. Male offspring were weekly weighed, food intake was monitored, and oral glucose and intra-peritoneal insulin tolerance test performed at PND70 and 170, respectively.

Key Results

Perinatal exposure to BPA increased body and peri-gonadal WAT weight in male offspring compared to vehicle-treated mice. At PND45, higher production of inflammatory cytokines IFN-γ and IL-17 was observed in spleen and MLN in BPA-exposed mice compared to controls, without any modifications of glucose homeostasis. In aging animals, BPA-treated

mice exhibit impairment of glucose tolerance at PND70 and insulin resistance at D170, without increase of TNF-α levels in the pancreas. At PND170, BPA-treated mice exhibited an increased IL-17 production at the systemic (i.e. spleen) level, with significant reduction of Treg cells. Moreover, an increased frequency of proinflammatory macrophages subset M1 ($CD11c^{+}CD301^{-}$) occurred in WAT of BPA-treated mice, without variation of anti-inflammatory macrophages M2 ($CD11c^{-}$ $CD301^{+}$).

Conclusions

Perinatal exposure to BPA contributes to the development of metabolic disorders. For the first time, our results demonstrated that male offspring perinatally exposed to BPA showed a low-grade inflammation in the intestine preceding metabolic disorders occurrence, characterized by inflammatory M1 macrophage infiltration in WAT, and chronic systemic inflammation. These findings are in accordance with the appearance of an obese phenotype in these animals. These findings emphasized BPA exposure as a risk factor for metabolic syndrome.

P11-Prenatal Bisphenol A exposure induces sex-specific modifications in lipid profile of mouse liver

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Aim of the work

Bisphenol A (BPA) is a major concern to public health due to its properties as an endocrine disrupting chemical (EDC) and its important worldwide production. Extensively found in the environment, humans are exposed by many routes such as oral, inhalation and transdermal. BPA is metabolized in the liver to form BPA-glucuronide and excreted within urine. One main issue is the exposure during pregnancy. Indeed, the fetus can reactivate the inactive BPA-glucuronide and exacerbate the exposure to bioactive BPA [1]. It has been recently reviewed that estrogenic properties of EDC can be linked to the worldwide rise of metabolic diseases (obesity, insulinoresistance and Type 2 Diabetes) [2]. We assessed the effect of perinatal exposure to BPA on mouse liver metabolism by non-invasive *in vivo* ^{1}H Magnetic Resonance Spectroscopy (MRS).

Methods

Fertilised Swiss female mice (n=6) are injected intraperitoneally during gestation and lactation (from Embryonary day 1 until 3 weeks) with 20 µg/kg of body weight/day of BPA diluted in olive oil or vehicle only (olive oil). Male and female pups are separated in 4 experimental groups: Male Control (n=13); Female Control (n=16); Male BPA (n=19); Female BPA (n=16). At 6 weeks, Magnetic Resonance acquisition is performed on a 7T horizontal magnet (70/16 Bruker Pharmascan). PRESS (Point Resolved Spectroscopy) sequence (voxel 4x4x3 mm^3; TE=16ms; TR=3260 ms) is used to characterise the lipid profile in the right liver lobe. Concentrations are measured in mM by ERETIC method (Electronic Reference to access In vivo Concentration). The rTUFA (Total Unsaturated Fatty Acids relative to total amount of fatty acids: 5.4/1.3 ppm) and rPUFA (Polyunsatured Fatty Acid relative to total amount of fatty acids: 2.8/1.3 ppm) are calculated based on [3]. Welch-corrected t test is realised and $p<0.05$ is significant.

Key Results

A significant increase of lipid peaks have been measured in Female BPA (Cf. *Figure 1*): methyl (+71.4%), methylene (+78.8%), allylic (+68.9%), diallylic (+80.9%) and methine (+51.2%) *vs* Female Control (mean±SEM in mM: methyl=22.6±2.1; methylene=181.8±19.9; allylic=11.9±1.4; diallylic=15.7±1.7 and methine=19.7±2.9) ($p<0.05$).

There is no difference in lipid profile in Male Control *vs* Male BPA. No variation has been observed in rTUFA among groups but a decrease of 33.4% of rPUFA is measured in Female BPA group (*vs* Female Control: p=0.07).

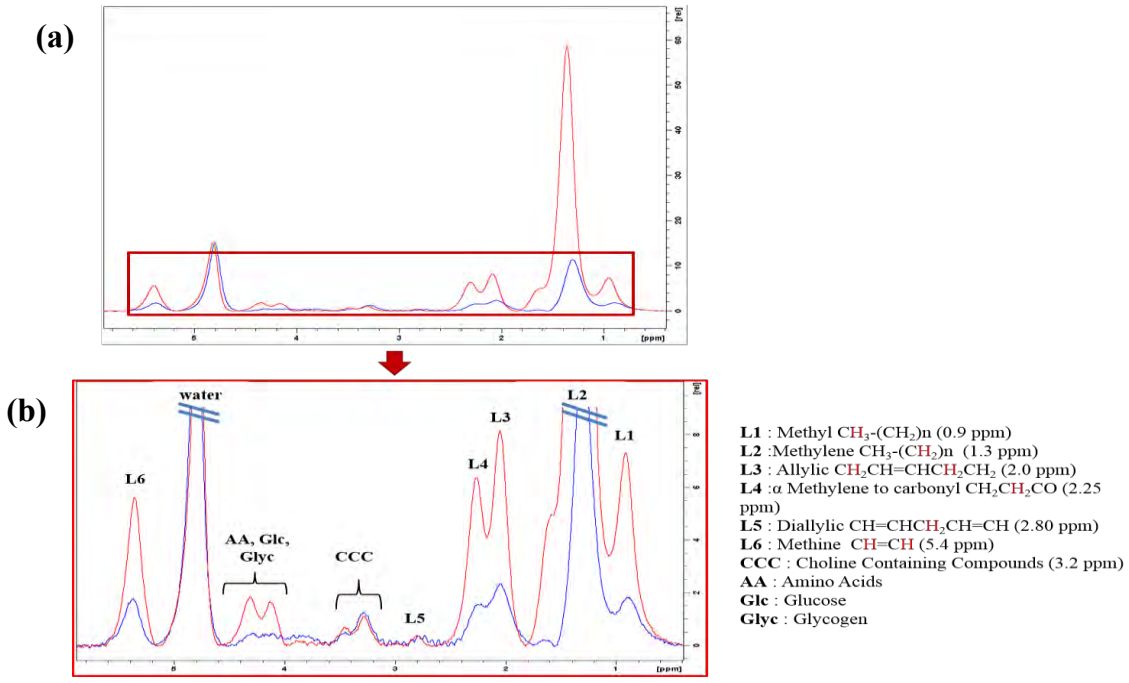

Figure 1 **(a)** *Superposition of female mice liver spectra and* **(b)** *shows insert in (a): female control (blue) and female BPA (red)*

Conclusions

We have shown that a perinatal exposure to BPA at a very low dose (20 µg/kg/day < Tolerable Daily Intake 50 µg/kg/day) induces a sex-specific alteration of the hepatic lipid composition in young female mice (6 weeks). It has been shown recently that the perinatal exposure to BPA (5 mg/kg/day in the diet) alters fetal liver biochemical maturation in female offspring only [4]. An increase in fatty acids (palmitic and oleic acids: major constituents of triglycerides) as well a decrease in the PUFA have been measured in mice directly treated with 50 µg/kg/day BPA [5]. A decrease in PUFA in mice (by its depletion in the diet) have been previously demonstrated to lead to hepatic steatosis [6]. BPA exposure during pregnancy and lactation alters fatty acids composition in female offspring at 6 weeks which might lead to hepatic steatosis later in adulthood.

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P12-Impaired expression of epigenetic machinery and possible epigenetic modulation of *kiss-1* in the testis of rats chronically exposed to low BPA dose

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Aim of the work

Bisphenol A (BPA) is an endocrine disruptor commonly used in the production of epoxy resins and polycarbonate plastics that interferes in the endocrine network of hypothalamic-pituitary-testis axis and compromises spermatogenesis and sperm quality. Beside the endocrine control of testis physiology, a finely controlled cascade of local modulators drives the progression of the spermatogenesis from the recruitment of proliferating spermatogonia toward meiosis, spermiogenesis and sperm maturation. In this respect epigenetic mechanisms (DNA methylation, histone modifications and chromatin remodeling) exert a direct step by step influence on the spermatogenesis modulating the expression of target genes.

The aim of the work was to investigate the effects of a low BPA concentration on testis physiology with a focus on the epigenetic machinery involved in the regulation of gene expression. Possible epigenetic modulation of *kiss-1,* a new central and local modulator of testis physiology involved in the control of Leydig cell activity and sperm functions- was also evaluated.

Methods

Six female (200-250g) Wistar rats were coupled and pregnant females were given BPA (0.1mg/l, 3 rats, group 1) o vehicle (0.1ml/l, 3 rats, group 2) in the drinking water in glass bottles starting from 6dpc. These treatments were maintained for all the time also for the newborns, assigning each newborn to the same treatment group of the mother. The newborns were finally sacrificed by anesthetic overdose at age 60d (9 male rats from group 1, 6 male rats from group 2), taking roughly an equal number of rats from each litter. Testes were quickly removed, stored at -80°C and used for the extraction of total RNA, quantitative PCR analysis (qPCR), genomic DNA (gDNA) extraction and its fractionating based on the degree of methylation status.

Key Results

BPA treatment differentially affected the expression rate of genes involved in the control of DNA methylation status and in chromatin remodeling. In particular, BPA treatment decreased the expression level of the maintaining DNA methyltransferase *Dnmt1,* of *de novo* DNA methyltransferase *Dnmt3a* and of histone deacetylases *Hdac1*, and increased the expression levels of *Hdac2.* Since qPCR revealed lower expression levels of *kiss-1* in the testis of BPA treated animals, possible epigenetic mechanisms were evaluated. Thus, gDNA from testes of control and BPA treated animals was fractionated in methylated and unmethylated portions and the presence of *kiss-1* gene was evaluated by PCR in both fractions. In the control group the *kiss-1* signal was higher in unmethylated DNA fraction than in methylated one; in BPA treated animals, higher *kiss-1* signal was observed in the fraction of methylated DNA.

Conclusions

A low BPA dose affects the epigenetic machinery in testis and may switch off the expression of *kiss-1* gene by changing the DNA methylation status. The present results bring attention to the risk of environmental BPA exposure for the male reproductive health.

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P13-Effect of continuous exposure to BPA alone or in combination with genistein and/or vinclozoline on taste preferences and submandibular of the offspring

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Aim of the work

Inducing changes in male taste preference and salivary gland development has been identified as neuroendocrine disrupting effects of maternal exposure to vinclozoline and genistein, alone or in combination (Kouidhi et al, 2012, 2013). In the aim to identify salivary gland biomarkers of taste disruption, this study aimed at comparing the effects occurred by chronic low dose exposure to bisphenol A (BPA) alone or combined with the estrogen-like genistein (G; 1mg/kg bw) and/or the anti-androgen fungicide vinclozolin (V; 10µg/kg bw) on taste preference and submandibular secretion in immature and adult Wistar rat males.

Methods

BPA(5µg/kg/d), alone or in combination to Genistein (1mg/kg/d) and/or Vinclozoline (10 µg/kg/d) was orally administered to F0 pregnant rats from the first day of gestation (G1) until the last day of lactation (LD21) and to F1 female offspring from weaning (PND21) up to the day before mating at adulthood (PND100). Sweet and salt preferences were evaluated at weaning (PND25) and at adulthood (PNDJ100) using the two-bottle choice method for 3 days. Animals were killed at PND110 and blood was removed for biochemical analysis; Salivary glands were removed to perform histological analysis of secreting structures and molecular analysis of exocrine enzymes (amylase, gustin) and endocrine (growth factors, sialorphin) secretions related to sweet preferences and/or feeding behavior.

Key Results

Exept a low increase in sweet intake in the male group receiving BPA/Vinclozolin mixture, Sweet and salt preferences were not significantly affected by a long-life exposure to endocrine disruptors either at weaning, neither at adulthood. At adulthood, histology of submandibular showed a strong deleterious effect on secretory organs (GCT) by BPA alone by comparing to the combination with genistein or vinclozolin, whereas the combination of the three compounds was safe.

mRNA expression of exocirine and endocrine markers were not affected excepted in BPA group (decrease of gustine mRNA expression and increase of EGF mRNA expression). By surprising, the mixture of the three compounds did not produce disrupting effect on taste preference and salivary gland disruption.

Conclusions

In previous works, we showed a synergic effect of perinatal co-exposure to genistein and –vinclozolin on sweet preference and salivary gland secretion. In this study, the effect occurred by a long life exposure were lowly pronounced and we observed a stronger effect of BPA by comparing to the exposure to the binary and ternary mixtures.

This study shown the difficulty to predict biological effects of mixtures and show that multi-exposition to chemicals having different endocrine properties could displays no effect.

This study has been supported by PNRPE, INRA, and Bourgogne –Franche Comté Regional Concyl.

P14-Temporal variability of urinary concentrations of phthalate metabolites, parabens and benzophenone-3 in a Belgian adult population

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Aim of the work

Phtalates, parabens and benzophenone-3 are three chemicals classes of non persistent endocrine-disrupting pollutants eliminated from the human body within a day. Since human exposure occurs daily, the question of the temporal representativeness of the urinary biomarker measurements is important for the interpretation of epidemiological studies.

Methods

In the present study, we investigated the temporal within-person variability of the exposure biomarker for phthalates, parabens and benzophenone-3 (BP3) in 32 Belgian adults, each providing 11 urine spots during 4 months. We calculated the intraclass coefficient correlation (ICC), the sensitivity and the specificity to assess the temporal reproducibility and to investigate the predictive ability of the spot measurements for these classes of chemicals. Additionally, we explored the temporal variability of the estimation of the cumulative risk of exposure to phthalates (hazard index; HI).

Key results

We observed fair ICC ranging from 0.55 to 0.68 for parabens, monoethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP) and BP3, but lower ICC, from 0.20 to 0.49, for monobenzyl phthalate (MBzP), mono-n-butyl phthalate (MnBP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxo-hexyl phthalate (5-oxo-MEHP) and mono-2-ethyl-5-hydroxy-hexyl phthalate (5-OH-MEHP). The ICC estimated for HI (0.49) reflected a moderate reproducibility.

Conclusions

The measurements in spot samples were moderate to good predictor of the 4-month level of exposure for parabens, MEP, MnBP, MiBP, BP3 and HI (sensitivity ranging from 0.67 to 0.77), but lower predictor for MEHP, 5-oxo-MEHP, 5-OH-MEHP and MBzP (sensitivity ranging from 0.58 to 0.63). The sensitivity could be increased when several spot urinary levels were averaged to predict the long-term level of exposure. Globally, our results indicate that a single spot measurement seems to correctly represent the long-term exposure for parabens, BP3, MEP, MiBP and HI. Additional spot samples seemed to be needed for the proper exposure assessment of the other target compounds.

P15-BPA or BPS perinatal exposure alter gene expression involved in lipid intestinal metabolism in mice

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Aim of the work

Determine the impact of exposure to bisphenol A (BPA) or bisphenol S (BPS) on lipid intestinal metabolism in mice.

Methods

C57Bl/6 pregnant mice were exposed to low dose of BPA or BPS (0.2; 1.5 and 50 µg/kg bw/d) in drinking water. Treatment began at gestational day 0 and continued in offspring up to 23-weeks old. Four weeks after weaning, mice from treated-dams were fed with a standard diet (SD) or a high fat diet (HFD; 60% kcal as saturated fatty acid. High fat diet induces an overweight classically after 7-8 weeks. At the sacrifice, jejunal mucosas were collected and mRNA expression of genes encoding for nuclear receptors (ERα/β, PPARα/β/γ, GR, ERRα/γ) and genes involved in intestinal lipid metabolism (CD36, LFABP, MTP, GPR120, PGC-1α, APOC2, APOC3) were assessed using real-time RT-QPCR.

Key Results

Transcriptional expression of genes encoding for nuclear receptors and proteins involved in lipid intestinal metabolism exhibited a pattern dependent on the doses and the diets. BPA and BPS induced an mRNA expression inhibition of key genes of lipid intestinal metabolism (CD36, LFABP and MTP) in standard diet condition. Interestingly, no difference was observed in HFD except for 0.2 µg/kg bw/d of BPA or BPS (similar to SD), the dose where no effect on the body weight was obtained. Concerning nuclear receptors, we observed principally an alteration of mRNA expression of glucocorticoid receptor (GR). BPA induced an overexpression of GR mRNA (in SD and HFD) whereas it was inhibited by BPS in SD with no effect in HFD. Except for ER alpha which was overexpressed, expression of others nuclear receptors exhibited changes in dose- and molecule-dependent manners.

Conclusions

Previously, we observed that BPA or BPS exposures induced an overweight in male mice fed to high fat diet at 1.5 and 50 µg/kg bw/d of BPA/BPS, and in correlation with an increased plasma clearance of triglyceride-rich lipoproteins secreted by intestine which suggests a potential impact on lipid intestinal trafficking.

However, molecular mechanisms involved need to be clarified. In this study, we demonstrate that BPA and BPS are able to modify mRNA expression of key genes implicated in lipid intestinal metabolism. Furthermore, we hypothesized that in SD, intestinal lipid metabolism is not affected by BPA/BPS-induced inhibition of gene expression. There are probably further mechanisms which counterbalance these disturbances. In contrast, in HFD, restoration of these gene expressions associated with compensation mechanisms (previously established) could result in a better lipid intestinal metabolism and notably chylomicron processing. As the composition of chylomicrons determines the plasma TG clearance, consequently it could favour lipid storage in peripheral tissues. BPA and BPS also appear to alter the expression of GR known to be involved in the intestinal maturation and homeostasis regulation. Furthermore, as we observed effect after perinatal exposure, it hypothesizes that disruption in development or potential epigenetic alterations. Indeed, BPA is already known to be involved in epigenetic mechanisms.

P16-New method for analysis of bisphenols in various matrix based on Molecular imprinted polymer

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Aim of the work

The aim of this work is to develop a powerful technique for analysis of Bisphenols in various complex matrix such as biological fluids and foods.

Methods

To analyze Bisphenols in various matrix, an original method is proposed for the clean-up and the pre-concentration for complex matrices using molecularly imprinted polymer (MIP) as selective sorbent for solid-phase extraction (SPE).

A MIP is a synthetic material with artificially generated three-dimensional network, able to specifically rebind a target molecule. Based on this technology, we have developed a powerful technique of selective solid-phase extraction and clean-up before analysis of this compound.

This product has been evaluated with a very broad range of Bisphenol analogues.

Key Results

The method was applied to the analysis of Bisphenols from a wide variety of matrices such as water, milk, infant formula, canned food, urine, …. Thanks to selectivity of MIP, perfect clean-up and high recoveries (>80%) were obtained even by LC-fluorescence.

Conclusions

A robust and powerful method based on molecularly imprinted polymers has been developed for various matrix.

P17-Urinary bisphenol S-glucuronide (BPS-G) and metabolic health in the French prospective cohort study D.E.S.I.R.

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Aim of the work

The growing concern over bisphenol A (BPA) exposure and its potential effects on human health, as well as its ban in several countries, have prompted the removal of BPA from consumer products and its replacement by other compounds. BPA can be replaced by structurally similar molecules such as other bisphenols (e.g., bisphenol S), for which experimental studies suggest similar endocrine disrupting effects. To date, no studies have examined the relationship between exposure to bisphenols other than BPA and metabolic health in humans. We studied BPS exposure and its association with markers of metabolic disease in the French prospective cohort study D.E.S.I.R. (Data from the Epidemiological Study on the Insulin Resistance Syndrome).

Methods

Using a case-cohort design, we examined data from 755 participants, aged 30-65 years at baseline (1994-1996) and followed-up for 9 years, including 201 incident cases of type 2 diabetes (T2D) and a random sample of the healthy cohort members (‘subcohort’). Urinary BPA-glucuronide (BPA-G) and BPS-glucuronide (BPS-G) were assessed as a proxy of BPA and BPS exposure in spot urine samples from baseline using chloride dansyl derivatization and liquid chromatography-mass spectrometry. The limits of quantification (LOQ) were 0.5 ng/mL. Due to low detection rate of BPS-G, we studied BPS exposure as a dichotomous variable, either exposed (BPS-G ≥ LOQ) or unexposed (BPS-G < LOQ). We performed cross-sectional analyses to study the associations of BPS exposure with individual characteristics and metabolic markers among participants of the subcohort.

Then, we examined the association between BPS exposure and incident T2D using Prentice-weighted Cox regression models adjusted for baseline covariates: age, sex, urinary creatinine level, level of education, employment, smoking status, physical activity, caloric intake, hypertension, use of lipid-lowering medication, fasting glucose, liver enzymes, body mass index (BMI), waist circumference, family history of diabetes, and BPA-G levels.

Key Results

Overall, 7% of the participants had detectable levels of BPS. In exposed participants from the subcohort, the median (25^{th}-75^{th} percentile) of BPS-G concentrations was 1.1 (0.7-2.8) ng/mL. BPA-G and BPS-G concentrations were not correlated (Spearman's Rho, -0.11, p=0.55). There was no cross-sectional association of BPS exposure with sex, age, education level, smoking status, physical activity, body mass index, waist circumference, caloric intake, fasting glucose, HbA_{1C} or insulin levels, blood lipids or blood pressure. In the prospective analysis, BPS exposure was significantly associated with an increased risk for T2D, with an adjusted hazard ratio of 2.49 (95% confidence interval, 1.37-4.55). There was no evidence of interaction of BPS exposure with sex (p=0.40) or BMI (p=0.66).

Conclusions

This is the first epidemiological study on BPS exposure and metabolic health. At baseline, there were no significant associations of BPS exposure with any of the standard metabolic markers. BPS quantification at baseline was significantly associated with the incidence of T2D, independently of traditional T2D risk factors and BPA-G concentrations. These preliminary results need further investigation. This study is limited by the low exposure prevalence. Samples were collected before strong concerns about the use of BPA existed, and it was not possible to characterize the dose response function of BPS potential effects on T2D.

P18-Bisphenol-A and other bisphenol-A congeners promote adipogenic differentiation of human adipose stromal/stem cells

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Aim of the work

The obesity epidemic and associated co-morbidities are at the centre of worldwide public health concerns. Besides classical lifestyle factors, mounting evidence is signaling exposure to endocrine disrupting chemicals (EDCs) as an additional risk factor for obesity and metabolic disorders. Major efforts are under way to understand the mechanisms underlying these environmental exposures. Reliable *in vitro* screening systems of adipogenesis are required for this purpose. Several investigations have been conducted using murine cell lines, but very few studies have explored this issue using human cell precursors.

The goal of this work is to assess the impact of bisphenol A (BPA) and its proposed substitutes bisphenol S (BPS) and bisphenol F (BPF) on adipogenic differentiation and lipid metabolism in adipose-derived human mesenchymal stem cells (ADSCs).

Methods

Effects of serial environmentally-relevant doses of bisphenols (10 nM-100 nM-1μM-10 μM) during the progressive maturation of ADSCs were tested at 14 and 21 days of culture in adipogenic differentiation media. The extent of adipogenic differentiation was assessed by staining intracellular lipid content with Oil Red O and quantifying the retained dye by optical density measurements. Gene expression of key lipid and adipogenic regulators such as PPAR-γ, C/EBPα, LPL, FABP-4, leptin, adiponectin and ERα, among others, was also assessed.

Key Results

Preliminary data indicate that adipogenesis in ADSC cells is significantly enhanced by low environmentally-relevant concentrations of BPA and its congeners. Hence, our results support the obesogenic potential of BPA described by other authors and raise questions about the use of BPS and BPF as candidate substitutes.

Conclusions

This study suggests the biological plausibility of a contribution by BPA and BPA congeners to the health challenge of obesity, raising concerns about the potential endocrine-disrupting obesogenic activity of putative BPA substitutes.

P19-Emerging endocrine disruptors: The fate of bisphenol S (BPS) in male and female Wistar Rats

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Aim of the work

Humans are daily exposed to a variety of man-made chemicals entering the food-web and/or present in the environment. Many are now known to behave as endocrine disrupters, and currently raise new challenges in the field of toxicology.

Bisphenol A (BPA) is one of the largest-selling chemicals worldwide. It is used as a monomer in the plastic industry in the synthesis of polycarbonate and epoxy resins, and the free monomer is also used in thermal papers. BPA is a model endocrine disruptor. Extensive literature documenting its low dose effects is now available. BPA effects span far beyond its classical reproductive system target, with adverse consequences reported on metabolism (obesity, metabolic syndrome) and cognition, among other. A great divide still exists regarding the effects of BPA on Human health, which are suggested not only by in vivo and in vitro studies, but also by increasing epidemiological evidences. All these data have leaded French Authorities to ban its use for food packaging purposes. Other countries have moved towards the limitation of BPA's use, and, consequently, "new" bisphenols are increasingly used, with bisphenol S (BPS) being one of the key candidates as a substitution solution for BPA. Several studies already suggest that BPS would also be an endocrine disruptor, since it was reported to be equally as estrogenic as BPA.

Methods

A single dose of radiolabeled Bisphenol S ([^{3}H]-BPS, 50 µg/kg bw) was administered to male (n=4) and female (n=4) adult Wistar rats by gavage. The 24 hr metabolic balance of BPS and the tissue and fluids distribution were explored. Metabolic profiling was performed on urine, bile and liver. Excreted metabolites were separated by high-performance liquid chromatography (HPLC), coupled to radioactivity detection for metabolite profiling. Metabolites were identified by high resolution mass spectrometry (HRMS).

Key Results

More than 90% of the administered radioactivity was recovered within 24 h following BPS administration. Urine was the main route of elimination (73% in males, 48% in females) whereas only 6% (males) and 4% (females) of the radioactivity was recovered in feces, and 13% (males) to 31% (females) was recovered in the digestive tract.

The amount of [^{3}H]-BPS residues measured in all tissues, including in reproductive organs, ranged from 0.01 to 0.28% of the administered radioactivity, with a higher amount of residues in liver, blood and skin.

In a separate 6-hr experiment in cannulated rats, 6% and 14 % of the administered [^{3}H]-BPS dose was recovered in males and females, respectively, indicating an extensive biliary excretion of BPS residues under our experimental conditions.

In urine, the parent molecule was still present after 24h, and 2 metabolites were identified: BPS-Glucuronide and BPS-Sulfate.

Conclusions

Following a 24-hr study, BPS was found to be excreted mainly in urine, with BPS residues being present in all tissues, including reproductive organs. BPS elimination was found to be greater in males than in females at 24h. Conversely, bile excretion was faster in females. These data strongly suggest an extensive enterohepatic cycling of BPS, with possible gender-related differences not previously observed for BPA.

P20-Post-weaning exposure to xeno-hormones affects plasmatic epidermal growth factor through submandibular salivary glands disruption in male but not in female Wistar rats

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Aim of the work

Salivary glands are mainly considered for their involvement in the control of oral homeostasis, taste perceptions and digestive process. Additionally, they promote other physiologic events such as gametogenesis and pregnancy, and their ablation cause infertility. These various functions are related to the endocrine secretion of specific peptides (EGF, NGF, TGFα, neuropeptides ...) whose synthesis is particularly controlled by estrogens and androgens. We therefore made of the hypothesis that the salivary glands can be a potential target for food xeno-hormones.

We previously reported that perinatal exposure to genistein, a phytoestrogen, and vinclozolin, an antiandrogen pesticide, differently affects submandibular salivary glands (SSG) development and endocrine secretions in both male and female Wistar rats when given alone or in mixture. This study aimed at identifying the effect of a later –in life chronic exposure to genistein and/or vinclozolin on submandibular gland of male and female rats.

Methods

Genistein, alone or mixed with vinclozolin, was administered orally at the dose 1 mg / kg / day to male and female rats from weaning (PND 21) until sacrifice in adulthood (PND100). The salivary glands were removed to determine the impacts on the submandibular secretory organs. The expression of sex hormone-dependent proteins, namely sex-hormone receptors, salivary enzymes (gustin, mucin..) and endocrine active peptides (EGF) were evaluated using RTq PCR. Blood was also collected to determine the circulating EGF levels.

Key Results

As a main result, female were not affected by the chemicals exposure. In males, Genistein and vinclozolin selectively affect in a similar manner the male SSG by increasing 1) the granular convoluted tubules (GCT) area, 2) the mRNA expression of the progesterone receptor and the EGF and 3) the EGF levels in plasma. In contrast, chronic exposure to the combination of Genistein and Vinclozolin had no effect neither on submandibular nor on the EGF blood level.

Conclusions

Our results revealed that the selective effects on male circulating EGF at adulthood through SSG endocrine activity disruption occurred by the post-weaning-exposure to estrogenic (genistein) or anti-androgenic (vinclozolin) chemicals could be lacking when a co-exposure. an Because an additive effect of the same mixture had been previously identified as a result of prenatal exposure, this surprising finding underline the difficulty to predict the effect of mixture on the basis of the singular properties of the chemical.

This study has been supported by PNRPE and Bourgogne –Franche Comté Regional Concyl.

P21- Do Artificial sweeteners act as endocrine-disruptor like? Sex specific effect of prenatal exposure to artificial sweeteners on female submandibular development

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Aim of the work

Intense sweeteners (EI) are increasingly used in diets as substitutes for sugars to decrease energy intake. They can represent a significant level of consumption for pregnant women and young children because they are strongly used to sweeten sodas and fruit juices, popular in the diets of these populations. Up to date, epidemiological studies cannot conclude on the risk-benefit effects of EI consumption during pregnancy or during lactation because the data are still fragmented and complex, due to exposure to cocktails whose the composition is often undefined. Nevertheless, on the basis of the INCA 2 expertise, EI intake by pregnant women and the population did not exceed the acceptable daily intake. This study aimed to identify the effects of a regular consumption of EI during gestation and lactation on the offspring's development of taste preferences and feeding behavior. For this purpose, we selected saccharin (tablets), acesulfame K and Rébaudioside A (food and drinking) because they could be used in a strong extent during pregnancy.

Methods

Female Wistar rats (6 / lots) were exposed per os to 1mg / kg / day (ie a lower dose than the ADI) saccharin, acesulfame K or rebaudioside A (stevia extract) from the first day of gestation until the weaning. Food consumption and body weight were recorded from birth until sacrifice. Sweet preference was evaluated at weaning (PND25), puberty (PND50) and adulthood (PND100) using the two-bottle choice method for 3 days. Sweet flavored solution contained either a single sweetener or a mixture of the three sweeteners at a concentration of 0.3% (w/w). Salivary glands were removed in adulthood to perform histological analysis of secreting structures and molecular analysis of exocrine (saliva enzymes) and endocrine (growth factors, sialorphin) secretions related to sweet preferences and/or feeding behaviour

Key Results

The observed effects on sweet preferences, feeding behavior and salivary gland vary according to the age, the sex and the EI chemical structure.

Saccharin had no effect while acesulfame K and stevia differently affected food intake in males and females in utero exposed. Histological analysis of the gland at adulthood clearly identifies the specific action of rebaudioside A in the development of secretory structures and/or profiles of salivary secretions of the submandibular glands of female but not males.

Conclusions

The sexual dimorphism of the salivary glands is very pronounced in rodents. In previous works, we showed that the targeted action of early exposure to estrogenic (genistein, bisphenol A) and anti-androgenic (vinclozolin) compounds on taste preferences was specially associated to changes in male salivary secretions. This study reveals a strong specific effect of EI on endocrine parameters of the female salivary glands that invite to consider EI as potential disruptive of neuroendocrine and metabolic systems.

This study has been supported by INRA (Metaprogramme Didit, SwitLipKik project, CS S Nicklauss) and by the Bourgogne –Franche Comté Regional Concyl

P22- Use of transgenic zebrafish models to study the endocrine effects of natural and synthetic progestins

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Aim of the work

Over the last decades, many studies have focused on the endocrine disruptive effects of estrogens and estrogen-like compounds on aquatic organisms. Comparatively, little research has been done on the biological effects of natural and synthetic ligands of the progesterone receptor (PR) on fish development and reproduction, resulting in a significant lack of data to assess the hazard and risk posed by these compounds on aquatic organisms. Yet, progesterone and its derivatives play key roles in development and reproduction in many vertebrates, including fish. Recent data showed that some progestins are present in the aquatic environment at concentrations in the ng/L range, potentially impairing fish reproduction and raising the need to characterize the effects of these compounds on the endocrine system of fish.

Methods

Our strategy combines mechanism-based zebrafish bioassays that use new transgenic models expressing Green Fluorescent Protein (GFP) under the control of steroidogenic genes, *cyp19a1b* and *cyp11c1*. The *cyp19a1b* gene is ER-regulated and encodes the enzyme Aromatase B, which is expressed in radial glial cells and is responsible for the biosynthesis of estradiol. The *cyp11c1* gene encodes the enzyme 11β-Hydroxylase, which is involved in the hypothalamus-pituitary-interrenal axis through cortisol biosynthesis.

At first, a selection of 21 PR ligands, derived from 19-nortestosterone, 19-norprogesterone, 17α-hydroxyprogesterone or spironolactone, was screened on the *cyp19a1b*-GFP zebrafish model to assess their potential estrogenic effect. These compounds were further tested on the zebrafish larval bioassay that uses the *cyp11c1*-GFP model to characterize their effect on corticosteroidogenesis.

Key Results

Among the tested compounds, we showed that neither progesterone nor progestins structurally related to progesterone (derived from 19-norprogesterone or 17α-hydroxyprogesterone) had an effect on GFP expression in the *cyp19a1b*-GFP zebrafish model.

However, all the testosterone-derived progestins tested so far such as Levonorgestrel (LNG), Norethindrone (NET) and Tibolone induced the *cyp19a1b* expression in a ER- and concentration-dependent manner. Furthermore, we showed that some of the pro-estrogenic progestins were also able to affect the expression of *cyp11c1* in the interrenal cells. Parallel experiments have shown that *cyp11c1* is up-regulated by Dexamethasone, a GR agonist ligand, and was accompanied by increased whole-body cortisol concentrations. Our data thus suggest that 1) some progestins are capable to affect the expression of a GR-regulated gene and 2) these compounds have multiple effects by interfering with the expression of hormone-regulated genes in radial glial cells and interrenal cells in developing embryo and larvae.

Conclusions

Overall, this study demonstrates the usefulness of combining different mechanism-based bioassays that use transgenic fish to characterize the endocrine disruptive potency of emerging aquatic contaminants by demonstrating their capacity to disrupt the tissue-specific expression of steroidogenic genes involved in estrogen and corticosteroid synthesis raising further questions regarding their developmental and reproductive effects. The effects of progestins will be further investigated using zebrafish transgenic models at adult stages.

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P-23 Ligand affinity of the *Lymnaea stagnalis* estrogen and retinoid-X receptors (LsER and LsRXR): implications for detecting endocrine disruptors

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Aim of the work

The freshwater gastropod *Lymnaea stagnalis* has been selected as a test species for the development of an OECD Guideline for reprotoxicity testing in molluscs. This *in vivo* test will appear as a Mollusc Partial Life-Cycle Assay at Level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors (EDs). It is meant at providing data on adverse effects on endocrine relevant endpoints related to reproduction. However, in *L. stagnalis* as in other molluscs, the existence, properties and ligand-affinity to ED-binding receptors are poorly documented and even controversial, so that the endocrine origin of adverse effects on reproduction cannot be ascertained. The present study was therefore designed to characterize the properties and ligand- affinity of receptors potentially able to bind EDs, namely the estrogen and retinoid-X receptors, in *L. stagnalis*.

Methods

Identification of LsER and LsRXR sequences

LsER cDNA sequence was inferred from RNAseq data (CNS and reproductive organs), using the RENILYS® strain. A contig was found, of which the longest ORF was 1380bp long and matched with 43% identity with Homo sapiens ERα. LsER sequence was checked using SANGER sequencing. A published full cDNA of LsRXRα was used.

Measurement of LsER and LsRXR transcriptional activity

LsER and LsRXR activities were monitored on (GAL4RE)5-Globin-luciferase reporter construct, pSG5- GAL4(DBD)-ER(LBD) and pSG5-GAL4(DBD)-RXR(LBD) expression plasmids. Transient transfection and luciferase assays were performed in U2OS cells using Jet-PEI. Luciferase assays were performed with the Promega dual-reporter kit. Renilla luciferase encoded by the normalization vector phRLTK (Promega) was used as the internal control for firefly luciferase normalization. Tests were performed in duplicate in 3 independent experiments.

3D-modelling of LsER and LsRXR ligand-binding pockets

LsER and LsRXR models were constructed using the modeling metaserver @TOME 2 and the crystal structures of the ligand-binding domains of molluscan ER and RXR as templates.

Key Results

Receptor transactivation test and structural profiling

Results showed that LsER activity is constitutive and not mediated by known ligands of HsER, including its natural ligand estradiol, the xenoestrogen bisphenol A, and the complete antiestrogen ICI. This is further supported by the analysis of the ligand-binding pocket 3D-model which showed that Tyrosine and Phenylalanine 1 residues prevent estradiol from binding to LsER. Phenylalanine 2 would be responsible for the constitutive activity of LsER. Similar results were previously obtained with CgER.

LsRXR activity is mediated by pharmaceutical (CD3254) and environmental (TBT) ligands, and the activity is ligand-dependent. Structural profiling showed that LsRXR is able to bind the natural RXR ligand 9-cis-retinoic acid. A similar result was obtained with BgRXR.

Conclusions

The present study confirmed the constitutive activity of ER in molluscs: LsER is insensitive to estradiol, confirming that its use as a positive control for testing endocrine disruption in molluscs is irrelevant. With respect to LsRXR, results are in line with recent findings obtained in *Nucella lapillus* RXR.

This *in vitro* approach will contribute to fill the knowledge gap on ED-binding receptors in molluscs and support interpretation of mechanisms underlying adverse reproductive effects of chemicals in *L. stagnalis* in the context of the forthcoming OECD Guideline.

P24- An Integrated Approach for the Characterization of the Interaction between Nuclear Receptors and Endocrine Disruptors

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Aim of the work

Humans are exposed daily to environmental contaminants, some of which acting as endocrine-disrupting chemicals (EDCs). EDCs are exogenous substances which inappropriately modulate the endocrine system and cause a wide range of diseases including hormone-related cancers or metabolic disorders. In particular, exposures during early embryonic stages can disrupt normal patterns of development and thus dramatically alter disease susceptibility during adulthood. Many EDCs are natural or man-made substances which have the capacity to mimic hormone action and interfere with nuclear receptor signalling. However, the chemical structures of these environmental pollutants are often distantly related to those of physiological ligands making their interaction with nuclear receptors hardly predictable. A better understanding of the coupling between chemical structures, binding mechanisms and compound activities will help in risk assessment and the design of safer chemicals.

Methods

We have developed a unique suite of multidisciplinary approaches using dedicated engineered cell-lines, biophysical and biochemical methods (mass spectrometry, fluorescence anisotropy, isothermal titration calorimetry,...) for ligand and coregulator interaction measurements, X-ray crystallography as well as bio-informatics studies to characterize the interaction between nuclear receptors and their environmental ligands from the atomic up to the cellular level.

Key Results

Our methodological approach will be presented through illustrative examples including the estrogen receptors (ERα and ERβ), the peroxisome proliferator activated receptor γ (PPARγ) and the xenobiotic receptor PXR. Our data reveal that structurally and chemically diverse compounds interact with nuclear receptors *via* varied and often unforeseen binding modes reflecting their differential activity, receptor specificity and binding affinity. A detailed analysis of the various binding/activation modes and their functional outcomes will be presented with a particular focus on bisphenol A and its halogenated analogs, organotins, phthalates, perfluoroalkyls and some pesticides. In addition, we have developed a bio-informatics tool for the *in silico* assessment of the interaction between nuclear receptors and environmental contaminants.

The web server which utilizes crystal structures to model any nuclear receptor – EDC complexes, estimate binding affinities and predict the hormonal activity of the tested compounds will also be presented.

Conclusions

Overall, this research program provides a wealth of tools and information which will aid in the development safer chemicals and, in turn, in decreasing the impact of endocrine disruption on human health. Indeed, a better understanding of the many ways by which environmental pollutants interfere with nuclear receptor signaling will help in predicting the residual hormonal activity of an existing industrial compound and rationalizing the synthesis of new analogues devoid of nuclear receptor-dependent activities.

P25- Chemical disruptors modify LXR transcriptional activity

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Aim of the work

Our team and others have described LXRs as putative pharmacological targets to prevent metabolic diseases, cancers, degenerative diseases, skin disorders. Even though synthetic molecules have been tested in human, secondary effects (hypertriglyceridemia, neurological disorders) remain so far too prominent and avoid their use for therapeutic approaches (Viennois et al. Expert Opinion Ther Targets 2011). Our previous studies have shown that disruption of pathways regulated by LXRs could participate to prostate disease development (Pommier et al. PLoS Genetics 2013; Viennois et al. Endocrinology 2012). Besides, emerging evidences have pointed out environmental disrupting chemicals (EDCs) in the increase of prostate cancer. To date, EDCs were primarily screened for their impact on the activity of steroids in prostate. Based on these evidences, we have started the screening of EDC effects on LXR transcriptional activity through a heterologous system and the development of new animal models.

Materials and Methods

The screening of putative LXR EDCs has been based on a molecular modelisation of the LXR-ligand binding domains to identify molecules able to fit in, followed by an *ex vivo* screening using HeLa cells transfected with a Gal4-UAS system. Two molecules known as EDCs and associated to the risk of prostate cancer development have been identified and tested for further analyses. These molecules have been tested in cell culture to check their effects on *bona fide* LXR-target genes.

Key Results

The two chemicals alter T0901317 induction of LXRα and LXRβ activity in a significant way on the Gal4-UAS system. They are also able to decrease accumulation of LXR-target genes. Their respective half maximal inhibitory concentration (IC_{50}) suggest that these molecules could affect *in vivo* the metabolic pathways regulated by both LXRs.

Conclusions

EDCs could alter LXR-activity. Deregulation of LXR-pathway by EDCs could thus be involved in the increase of prostate cancer incidence in westernized countries.

P26- Is 4-Nonylphenol capable to disrupt osmoregulation in the teleost euryhaline fish *D. labrax?*

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Aim of the work

In seawater the concentrations of 4-nonylphenol (4-NP), a surfactant degradation product, can reach maximum concentrations of several µg/L in polluted areas. Fish and other aquatic organisms often have the greatest exposures to such chemicals during critical periods in development or even entire life cycles. 4-NP is an endocrine disruptor chemical (EDC) that interact with the ligand binding domain of estrogen receptor subtypes. 4-NP can potentially modulate aspects of various estrogen-regulated functions including osmoregulation. The aim of this work was to investigate the effect of 4-NP on the osmoregulatory function in sexually unmature seabass *D. labrax*.

Methods

Juvenile seabass *D. labrax* were exposed during 2 weeks at two nominal concentrations of 4-nonylphenol (5 µg.L^{-1} and 25 µg.L^{-1}) or at the solvant as a control (0.0005 % methanol). The functionality of osmoregulatory mechanisms was evaluated at different levels: blood osmotic pressure, expression and activity of major proteins involved in ionic transport in gills (namely Na^+/K^+ ATPase NKA, chloride channel CFTR, cotransporter $Na^+/K^+/2Cl^-$ NKCC1), neuroendocrine control by the GH/IGF1 axis in pituitary gland. The expression of estrogen receptors ERβ1 and ERβ2 was also measured in gills.

Key Results

The osmotic homeostatis was significantly disrupted after 2 weeks exposure to 4-NP since the osmotic pressure in blood was significantly increased. In gills, the expression of the genes encoding the main ionic transporters involved in the regulation of ionic balance in seawater (NKAα, CFTR and NKCC1) was significantly decreased, as well as the activity of the Na^+/K^+ ATPase pump (Fig. 1). In the pituitary gland, the expression of GH was also significantly decreased, suggesting the disruption of the GH/IGF1 axis. Additionally, 4-NP exposure induced a significant modification of estrogen receptors expression ERβ1 and ERβ2 in gills.

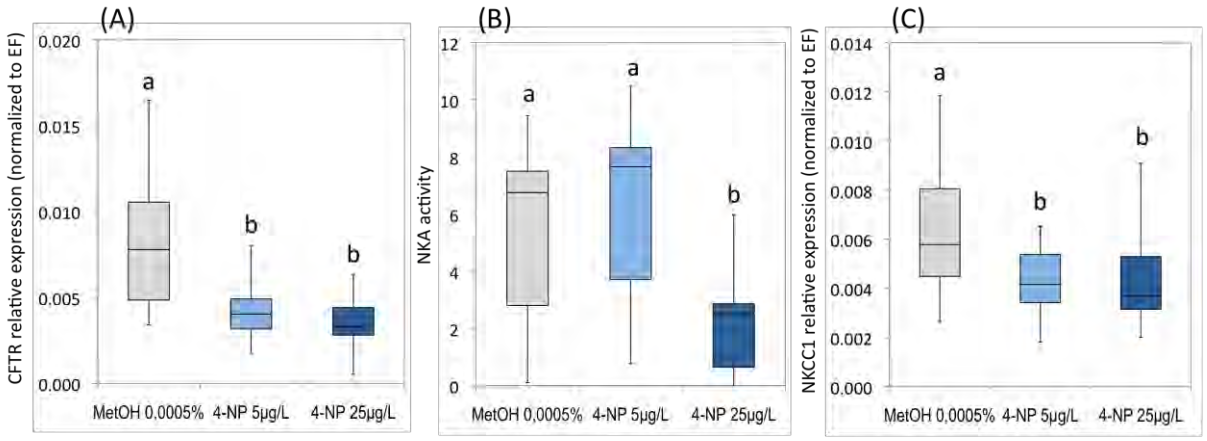

Figure 1: (A) Relative expression of the gene CFTR encoding the chloride channel, (B) enzymatic activity of the Na^+/K^+ ATPase pump, (C) relative expression of the gene NKCC1 encoding the cotransporter $Na^+/K^+/2Cl^-$ in gills of juvenile seabass *D. labrax* exposed during 2 weeks at 0.0005% methanol, 5 µg.L^{-1} or 25 µg.L^{-1} 4-NP (n=20, ANOVA, post-hoc Tukey, $p<0,05$).

Conclusions

These results highlight that 4-NP can disrupt osmoregulation in juvenile seabass at environmentally realistic concentrations. The pathways involved need to be further investigated. In particular, the mechanism of signal transduction by the estrogen receptors (ER) is complex and not fully understood.

P27 Exposure to a mixture of low-dose endocrine disruptors triggers insulin resistance in young adult male mice fed a diet-inducing obesity

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Aim of the work

Pollutants are suspected to contribute to the etiology of obesity and related metabolic disorders. However, while humans are life-long exposed to a large variety of chemicals at environmentally low dosage the current risk assessment does not take into account the cocktail effect. Recently, we demonstrated that a mixture of pollutants with endocrine disrupting activities, in the range of doses considered ineffective for humans could trigger sex-specific hepatic metabolic changes in the progeny of obese mice. Specifically in males, the expression levels of genes encoding proteins related to cholesterol metabolism were enhanced by week 12 [1]. Herein, we report the early signs of metabolic alterations in 7 week-old males.

Materials and Methods

The protocol [1, 2] consisted in feeding female C57Bl6/J mice for 5 weeks with a high-fat high-sucrose diet (HFSD) with/without a mixture of food pollutants made of 2,3,7,8-TCDD (dioxin), polychlorobiphenyl (PCB)153, DEHP (phthalate) and bisphenol A. Doses were adjusted resulting in mice exposure at the Tolerable Daily Intake dose range for each pollutant. Processing was continued at mating and throughout gestation and lactation. Offspring were fed the same diet than their dams after weaning until sacrifice. A control group was fed a standard diet (ST) throughout the experiment. Metabolic tests were carried out on 7-week old males and subsets of age- and treatment-matched males were euthanized. Other male mice were used for metabolic tests by week 11 and euthanized by week 12. Blood was recovered for biological assays. Liver and adipose tissues (AT) were rapidly dissected and frozen for RT-qPCR analyses. Data collected with 12-week old male mice have been published in [1].

Key Results

By week 7, HFSD-fed mice were heavier (24g) than ST-fed mice (20g) but they did not yet display all characteristics of obesity. For example, they had higher plasma leptin than ST-fed mice, intolerance to glucose and resistance to insulin but normal 6h-fasting glycaemia and plasma insulin. Exposure to pollutants did not affect body weight, lean/fat mass ratio, food intake, glycaemia, plasma levels of insulin and leptin in HFSD-fed males by 7 weeks.

However, metabolic tests indicated reduced insulin sensitivity ($p<0.05$ as compared to HFSD-fed mice) with no difference in glucose tolerance suggesting extra-hepatic insulin resistance. Consistently, we found enhanced expression levels of genes encoding inflammatory markers (IL1ß: +68%; TNFα: +67%) in the subcutaneous AT (not the visceral AT) as compared to non-exposed HFSD males. None of these significant effects were observed by week 12. Besides, the expression levels of the hepatic genes encoding proteins involved in cholesterol metabolism were not altered in the 7-week old pollutant-exposed HFSD males.

Conclusions

In summary, this work provides new observations on the metabolic effects of a lifelong exposure to a mixture of pollutants with endocrine disrupting activities in a context of obesity. Because 12 week-old mice fed-HFSD are clearly obese (33g), it is possible that the effects of pollutants seen by week 7 are eclipsed by the deleterious metabolic impact of HFSD by week 12. In addition with animal aging, more pollutants accumulate in liver which may lead to alteration of cholesterol metabolism as seen by week 12 [1].

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P28- Untargeted metabolomics approach to characterize environmental exposure of pregnant women to pesticides by UHPLC-HRMS

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Aim of the work

Pesticides are largely used to control pests and diseases in crops but current biomonitoring studies provide only a partial knowledge on the human exposure to these molecules. Biological measurement is a mean of assessing environmental exposures by an integrated approach accounting for multiple sources/exposure routes. In this context, characterization of pesticide exposure still represents a challenge since amounts of biological specimens available are low, and searching for possible compounds has to be as thorough as possible. In addition, from urine samples, pesticides are generally detected as metabolites whose chemical structures may be unknown. This project aims to develop a new strategy for the characterization of complex pesticide exposures of pregnant women.

Methods

The PELAGIE study was drawn to evaluate the consequences of the exposure to multiple contaminants on pregnancy and child health. This study enrolled a cohort of 3421 pregnant women living in a French area with high agricultural activities (Brittany). In our work, 333 women were selected according to the availability of a urinary sample collected at the early pregnancy, all stored in the same conditions. These samples were directly analyzed by UHPLC-HRMS (C18stationary phase, Electrospray ionization in both positive and negative modes, LTQ-Orbitrap mass spectrometer). Obtained data were processed with the MetWorks software (Thermo Scientific) to extract and integrate HRMS signals of 73 pesticides or adjuvants, and their known or theoretical metabolites. Moreover, a major advantage of this approach is the possible detection of compounds which are not present in the initial list. Right now, 507 masses were monitored by this way. Following their detection by UHPLC-HRMS, MSn experiments were performed to confirm or not the detected compounds as potential/probable metabolites. Some of them have also been confirmed by comparison with metabolites generated by animal experimentations.

Key Results

The study population corresponds to 333 women pregnant enrolled in 2004. Most of them live in the North and Center of Brittany, and are 25-34 years old (76%) with a median BMI at 22.8. 59.8% have a high educational level (post-secondary) and 71.3% didn't smoke while 12.7% stopped during the first trimester. Alcohol consumption was limited with only 15.8% of women reporting an occasional or regular consumption. Among the 507 signals, > 70 were detected and 28 pesticide metabolites were confirmed by MSn experiments. These metabolites correspond to 7 pesticides used in agricultural lands in 2004: 3 fungicides (azoxystrobin, fenpropimorph, procymidone), 3 herbicides (quizalofop-p-ethyl, chlorpropham and phenmediphame), and one insecticide (carbofuran). The 2 metabolites derived from carbofuran were only detected at trace levels.

Preliminary conclusions

The UHPLC-HRMS method developed in this work allowed to characterize many pesticide metabolites in an untargeted way in urine of pregnant women that are not routinely measured in environmental health, while the majority of studies has focused on several well-known pesticides. These results represent a major step to improve research on mixtures. The work goes further by a targeted quantification of pesticides and their metabolites by UHPLC-MS-MS. In addition, statistical analyses will be implemented in order to describe the impact of living near agricultural activities and pesticide exposure.

P29- Benzo(a)pyrene exposure during pregnancy: accumulation and adverse effects on human trophoblasts

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Aim of the work

The exposure to environmental contaminants is responsible for the development of human pathologies. Polycyclic aromatic hydrocarbons (PAHs) are notably found in diesel exhaust particles, foods and cigarette smoke. Bathing in the maternal blood, the syncytiotrophoblast is a main target of these environmental contaminants, which might affect its essential functions during pregnancy. The aim of this study was to evaluate 1) the placental uptake of Benzo(a)pyrene (BaP), the prototypical PAH, by using the *ex vivo* human perfused cotyledon model 2) its *in vitro* effects on the differentiation of human primary cytotrophoblasts in syncytiotrophoblast.

Methods

Placentas from uncomplicated full-term pregnancies were collected immediately after delivery and cotyledons were perfused with or without BaP. Placental uptake was calculated by determining endogenous accumulation during pregnancy or just after a perfusion experiment. BaP concentrations were achieved by mass spectrometry. The effect of BaP on trophoblast differentiation was characterized *in vitro* by the exposition of human cytotrophoblasts to this polluant. Cell fusion process and the formation of a functional multinucleated syncytiotrophoblast were studied. Finally, hormonal function was studied.

Key results

The mass of endogenous BaP in the cotyledon is about ng per g of tissue while after exposure, accumulation of BaP reached around □g per g of tissue. BaP is predominantly accumulated in trophoblast cells. Cell fusion assays indicated a decreased in syncytiotrophoblast formation by 37% while concomitantly an increase in hCG and hPL secretion was observed for cytotrophoblasts exposed to 1 µM BaP for 24 hours.

Conclusion

We have clearly demonstrated a placental uptake of BaP during pregnancy and we characterized the impact of BaP exposure on trophoblast differentiation and endocrine function.

P30- Endocrine disruptors and psychiatric disorders in children exposed *in utero*: evidence from a French cohort of 1002 prenatally exposed children and the example of diethylstilbestrol (DES) as a model for PE study

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Aim of the work

In-utero diethylstilbestrol (DES) exposure has been demonstrated to be associated with somatic abnormalities in adult men and women as well as shown for its trangenerationnal effect (1). Conversely, the data are contradictory regarding the association with psychological or psychiatric disorders during adolescence and adulthood (2). This work was designed to determine whether prenatal exposure to DES and/or Ethinyloestradiol (EE) affects brain development and whether it is associated with psychiatric disorders in male and female adolescents and young adults.

Methods

HHORAGES Association, a national patient support group, has assembled a cohort of 1280 women (spontaneous testimonies communicated after various informations) who took DES and/or EE during pregnancy. We obtained responses to detailed questionnaire (3) from 529 families, corresponding to 1182 children divided into three groups: Group 1 (n=180): firstborn children without DES treatment, Group 2 (n=740): exposed children, and Group 3 (n=262): children born after a previous pregnancy treated by DES and/or EE.

Key Results

No psychiatric disorders were reported in Group 1. In Group 2, the incidence of psychiatric disorders was drastically elevated (83.8%), and in Group 3, the incidence was still elevated (6.1%) compared with the general population (3). Total number of psychological/psychiatric disorders among the 982 DES-exposed adolescents (1002-20 stillborns) (Group 2) and post-DES adolescents (Group 3): Behavioral disorders, violence, aggressiveness, obsessive-compulsive disorders (n=110; 11.2%), Eating disorders (n=83; 8.4%); Schizophrenia (n=171; 17.4%), Depression, bipolar disorders, anxiety (n=257; 26.2%), Suicides (n=33; 3.4%), Suicide attempts (n=642; 65.4%). (Gynecol. Endocrinol. 2015, http://dx.doi.org/10.3109/09513590.20151063604)

Conclusions

This work demonstrates that prenatal exposure to DES and/or EE is associated with a high risk of psychiatric disorders in adolescence and adulthood. Molecular epigenetic (4) mechanisms subtending these toxic effects are in progress (5).

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P31- Prenatal exposure to organic solvents and child behavior at age 6: results from the PELAGIE mother-child cohort

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Aim of the work

Organic solvents (OS) are widely used in both occupational and domestic contexts. OS consist in various chemicals, including several endocrine disruptors. At least some OS are suspected to cross the placental and the blood-brain barriers (e.g. glycol ethers (GE)).The few available studies have suggested adverse effects of OS on the childhood neurodevelopment. In addition, based on the PELAGIE mother-child cohort, we previously observed higher level of attention deficit/hyperactivity and aggression among 2 years-old children of women who reported occupational exposures to organic solvents during the pregnancy.

This work aimed to explore the association between the prenatal exposure to organic solvents, using both self-reported maternal occupational exposures and maternal urinary concentrations of GE metabolites, and the child behavior at age 6.

Methods

Two hundred and three pregnant women from the PELAGIE cohort were asked to answer self-administered questionnaires at the beginning of pregnancy and at 6-year-old of their child. Specific items were dedicated to occupational exposures (missing values for 25 women). The Strengths and Difficulties Questionnaire (SDQ) was used to assess the internalizing (emotional symptoms and peer relationship problems), externalizing (conduct problems and hyperactivity), and prosocial disorders of the child at age 6. We measured six GE metabolites in 1st-trimester urine samples using gas chromatography coupled with mass spectrometry. Negative binomial and Poisson regressions were performed with adjustment for potential confounders.

Key Results

Overall, 88 (49%) women have reported occupational solvent exposures (38 occasionally, 50 regularly). We detected GE metabolites in 90.6 (ethoxyethoxyacetic acid) to 100% (phenoxyacetic acid) of prenatal urine samples. Score of the externalizing disorders (lower is better) was statistically significantly higher in children of women reporting occasional occupational exposures to solvents (beta= 0.34 [0.05 – 0.63]) but not significantly for regular exposures (beta=0.10 [-0.15 – 0.36]).

A statistically significant inverse association was found between the concentrations of ethoxyacetic acid (EAA) and the internalizing disorders score (lower is better), but only when comparing the 2nd versus the 1st tertile (beta 1st tertile = ref; beta 2nd tertile = -0.27 [-0.49, -0.05]; beta 3rd tertile = -0.09 [-0.33, 0.12]; continuous = -0.01 [-0.10, 0.09]). No other association was observed.

Conclusions

Association between self-reported occupational exposures to solvents during pregnancy and hyperactivity and aggression that was reported at age 2 of the children of the PELAGIE cohort, seems consistent with findings at age 6 in the present subcohort, but no dose-response relation exists. However, our study shows that prenatal urinary concentrations of GE metabolites were not associated with impaired child behavior at age 6. We consider that chance finding is likely to explain the inverse association between intermediate EAA level and internalizing disorders.

P32- Air to skin: An important exposure pathway for some Semi Volatile endocrine disruptors

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Aim of the work

Some environmental endocrine disruptors are semi volatile: e.g. phthalates (DEHP), PCBs, PBDEs, PAHs (benzo(a)pyrene), bisphenols (BPA) and pesticides (DDT, dieldrin, chlordane, heptachlor, lindane). At home, people are permanently exposed to these molecules through different pathways: cutaneous contact with the SVOC source, inhalation of indoor air (gaseous and particulate phases), ingestion of settled dust in addition to diet. If dust ingestion and air inhalation are two pathways often documented for residential exposure, this is not always the case for the dermal exposure. However, recent work suggests that it could have a significant contribution in the total exposure to SVOCs present in dwellings. The aim of this work was to identify residential SVOCs for which the dermal pathway has a significant contribution.

Methods

58 SVOCs detected in more than 10% of French dwellings were selected. A cutaneous permeability coefficient was estimated from physical-chemical parameters for each compound. It was multiplied by gas concentration in order to calculate dermal daily intakes by gas-to-skin pathway. Daily intakes by dust ingestion and air inhalation were calculated by multiplying SVOCs concentrations in dust and air (particle and gas phases) by ingestion and inhalation rates. Intakes were calculated for 6 age groups, from 0 to > 21 years old.

Key Results

Results are presented for one specific age group of [0-1 year]. For this group it was possible to assess total residential exposure for 43 SVOCs. For 4 of them (PCB-77, PCB-126, diazinon and fluoranthene) dermal exposure was contributing more than 50% for 29 between 5 and 50% and for 10 (DEHP, BPA, 6 PBDEs, PCB-180 and aldrine) less than 5%. It was not possible to assess total residential exposure for 6 SVOCs. For 5 of them (DMP, PCB-28, bisphenol ADGE, lindane and α-HCH) dermal intake was greater than inhalation, and for 1 (PCB-131) dermal intake was greater than ingestion. For the last 9 SVOCs dermal contribution was not possible to assess.

Main parameters that affect absorption through the skin are volatility and solubility of the studied compound. A sensitivity analysis revealed that key parameters for air-skin exposure assessment are gas concentration, octanol-water partition coefficient (Kow) and Henry's law constant (H).

Conclusions

These results support the importance to consider dermal absorption for assessing residential exposure to semi volatile endocrine disruptors.

P33- Caractérisation de l'exposition des agriculteurs aux produits chimiques cancérigènes au niveau de la région d'El Hajeb (Maroc)

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Objectif

L'utilisation des pesticides par les agriculteurs dans les communes rurales de El Hajeb est devenue systématique afin d'optimiser le rendement des cultures maraîchères et arboricoles. L'objectif de cette étude est d'évaluer les risques sur la santé humaine et sur l'environnement des pratiques phytosanitaires des agriculteurs ruraux de la région d'El Hajeb.

Méthode

L'étude s'est basée sur un questionnaire transversal traité auprès des 100 foyers d'agriculteurs dans 10 communes rurales.

Résultats

L'enquête a révélé que 74 préparations commerciales sont utilisées, dont 14 insecticides, 23 fongicides, 26 herbicides, 3 insecticide-acaricide et 1 nématicide. Les plus fréquemment utilisés sont les formulations de lambdacyhalothrine, de chlorothalonil, de mancozèbe et de deltaméthrine. La majorité des producteurs utilise des pyréthrinoîdes, des organophosphorés, des organochlorés et des carbamates. Parmi ceux-ci, peu de substances classées groupe 1, 2A ou 2B dans la monographie de CIRC. Parmi ces agriculteurs utilisant les produits cancérigènes 47.3% sont déjà exposés au tabac, et très peu de ces producteurs se confrontent aux règles d'hygiène pendant le traitement phytosanitaires, seuls 6.3 % portaient des gants imperméables et 2.5 % ont utilisé des lunettes de protection.

Tous les producteurs ont reconnu les dangers que peuvent poser les pesticides pour la santé humaine. La plupart ont rapporté qu'ils ont des irritations de la peau après l'application des pesticides, un rhume, des bouffées de chaleur ou des vertiges.

Conclusion

Les agriculteurs maraîchers et arboriculteurs utilisent des insecticides non appropriés. Ils ne bénéficient ni d'encadrement ni de formation continue. Ils se procurent sur le marché local des pesticides dont ils ne connaissent ni la toxicité ni le mode d'utilisation. Les modes d'utilisation, le manque d'équipements de protection adaptés constituent des facteurs de risques aggravants pour les agriculteurs.

Mots clés : *agriculture; pratiques phytosanitaires ; pesticide; El Hajeb, cancérigènes*

P34- Incidence and spatial trends of idiopathic central precocious puberty in France: a nationwide epidemiologic study

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Aim of the work

A large number of known or suspected endocrine disrupting chemicals (EDCs) are present ubiquitously in the environment and in consumer products, usually in trace amounts. Low doses exposures of the general population to certain EDCs are now proven in France. Early environmental exposure to EDCs could affect the development of reproductive functions: idiopathic central precocious puberty (ICPP) is a pathology increasingly studied for its possible causal link with EDCs exposure. The aim of this study was to estimate the incidence of ICPP and to describe spatial trends in France.

Methods

The incidence rates of ICPP were estimated using the French national health insurance information system (SNIIRAM) which provides nationwide exhaustive data on patient expenditure (hospitalization, drug treatment, long term disease, etc.). Incident cases of ICPP were identified by the first refund of GnRH agonists, for girls less than 9 years and boys less than 10 years, in metropolitan France, from 2011 to 2013 (studied period). Peripheral precocious puberty and central precocious puberty with central nervous system lesion were excluded by linking with hospital data from the French hospital discharge database (PMSI) and other medication reimbursement.

Key Results

A total of 3 519 girls were identified having a refund of GnRH agonist from 2011 to 2013, corresponding to about 1 173 new cases in girls per year in metropolitan France. The female annual incidence during the studied period is 2.68 per 10 000 girls younger than 9 years. During the same period a total of 352 boys were identified, corresponding to about 117 new cases per year in metropolitan France. The male annual incidence is 0.28 per 10 000 boys younger than 10 years. Spatial heterogeneity is observed across the country, with similar patterns for boys and girls.

Conclusions

The national incidences of ICPP among girls and boys were estimated for the first time in France. They are consistent with the results of the few available similar studies, especially the Danish nationwide study. Known and suspected risk factors (obesity, pesticides…), variations in medical practices or unknown risk factors of ICPP could be involved. Spatial heterogeneity must be further explored.

P35- Toward a multi-country monitoring system of reproductive health in the context of Endocrine Disrupting Chemical exposure

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Aim of the work

Worrying trends regarding human reproductive endpoints (e.g. semen quality, reproductive cancers) have been reported for decades. At the same time, evidence for the impact of EDCs on human reproductive health has grown, especially concerning the effects of developmental exposures. Other data suggest the potential for transgenerational transmission of effects. At the same time, the biological exposure of the general population to the most widely known EDCs such as dioxins, polychlorinated biphenyl (PCBs), bisphenol A, phthalates or selected pesticides (synthetic pyrethroïds, organochlorines, organophosphates), is observed in all places and countries where human biomonitoring studies have been performed. Therefore a growing collection of knowledge suggests a significant impairment of human reproductive health overflowing fertility outcomes, that could, at least in part, be caused by EDC exposure. Our aim is to answer this crucial question and, if so, apprehend to what extent EDCs might be causally involved and what other factors should be investigated. However, there is a striking lack of human data to fill the current knowledge gaps. Only epidemiologic surveillance at a wide scale will enable this question to be answered convincingly.

Methods

A multidisciplinary network named HURGENT (HUman Reproductive health and Global ENvironment Network) was created aiming at designing a European monitoring system for reproductive health indicators. Collaborative work allowed setting up the available knowledge to design such a system.

We also conducted an overview of 23 potential indicators, based upon a weight of evidence (WoE) approach according to their potential relation with EDC exposure It was based upon two international reports: the annex 1 of the recent report published by the European Commission for regulatory aims and the 2012 WHO-UNEP report on the state of the science on EDCs.

Key results

The framework and purposes of the surveillance system are settled as well as the approach to select suitable reproductive indicators. A set of core indicators were found with the highest scores according to the WoE approach: prostate and breast cancer incidence, sex ratio, endometriosis and uterine fibroid incidence, indicators related to the testicular dysgenesis syndrome (TDS), precocious puberty incidence and reproductive hormone levels.

Conclusions

Not only sentinel health endpoints, but also diseases with high burdens in public health are highlighted as prior indicators in the context of EDC exposure. Our work can serve as a basis to construct, as soon as possible, the first multi-country reproductive monitoring system. The next steps would consist of making positive choices and validation of indicators. Feasibility issues will be addressed including measurement and collecting methods, existing database exploitation, possible adaptations for existing databases, or de novo data collection. In depth knowledge of the existing databases in the participating countries and multidisciplinary collaborations will be required.

P36- Facilitating exposure assessment and improving health surveillance for gathering evidence of endocrine disrupting chemicals health impact

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Aim of the work

The WHO/United Nations Environment Programme (UNEP) publication, *State of Science of Endocrine Disrupting Chemicals - 2012*, emphasized that, despite substantial progress made in recent times, there were still gaps in the knowledge about, and understanding of, the health risks of endocrine disrupting chemicals (EDCs), and there is still a major lack of data from large parts of the world. To support and promote collection of information necessary for gathering evidence of EDCs health impact WHO organized an experts meeting in Bonn, Germany (July 2014).

Methods

At plenary and contact groups meetings international experts from all WHO Regions discussed the methodologies and tools used in assessing the risks of endocrine-disrupting chemicals (EDCs) to human health; experiences in the assessment of environmental exposure to EDCs, the human biomonitoring (HBM) of EDCs, the surveillance of endocrine-system disorders at the international and national levels; needs in relation to capacity-building and priorities for scientific research.

Key results

In many countries around the world there are monitoring programmes which could serve as a basis for planning the monitoring of EDCs exposure; however, some gaps have been identified, such as the lack of coverage of target chemicals, the lack of comparability of data and harmonization between programmes, and poorly addressed exposure sources, such as consumer products. HBM is also relevant for exposure assessment in the short and long terms, for persistent and short-life compounds, for assessment of early-life exposure and for raising awareness and driving policy decisions.

In addition to identification of EDCs in the environment, health outcomes linked with exposure to them need to be monitored. To the present there are no health-surveillance programmes that are fully applicable for this purpose. However, existing health statistics can serve for monitoring diseases relating to exposure to EDCs with some supplementary action, for example, to develop a consensus list of indicators, biomarkers and reporting protocols.

There is a huge gap among countries in the monitoring of exposure to EDCs and in the level of health surveillance development as a result of differences in capacity and human resources.

Building capacity in all the relevant professional groups, especially in developing countries is a priority, to facilitate activities related to EDCs at the national and international levels.

Conclusions

A number of recommendations resulted from the workshop discussion including on: integration of EDCs monitoring into existing national programs for monitoring environmental pollutants; step-by-step approaches to designing and planning monitoring, including human biomonitoring; and further development of health surveillance system to meet the needs of assessing impact of EDCs exposure. The leading role of the health sector in data collection and facilitating research, including epidemiological studies, was emphasized. Several areas of scientific research to promote collection of information necessary for policy development were agreed by the experts, in particular, development of exposure and effects biomarkers and methods for their investigation, improvement of toxicity screening frameworks and extrapolation of toxicological data from animals to humans, and strengthening understanding of the health endpoints impact of mixtures of EDCs.

P37- EDC-MixRisk: Integrating Epidemiology and Experimental Biology to Improve Risk Assessment of Exposure to Mixtures of Endocrine Disruptive Compounds

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Aim of the work

A large body of evidence supports associations between exposure to anthropogenic chemicals and endocrine disruptive effects, leading to disorders in humans and wildlife. Based on the scientific documentation it is beyond doubt that endocrine disrupting chemicals (EDCs) are of concern and need to be handled according to the risks they pose, as single chemicals or as mixtures. To develop chemical risk assessment to respond to these concerns, there is an urgent need to improve our understanding of the mechanisms and health effects of EDCs, in particular in mixtures. This will require selection, refinement and development of tools for assessment of EDC mixtures to bring current risk assessment procedures to a level where they can support risk management. This project is designed to promote the use of safe chemicals for the next generation. *EDC-MixRisk* aims to meet the societal need for improved decision-making regarding human exposure risks to mixtures of EDCs.

Methods

EDC-MixRisk will determine risks for multiple adverse health outcomes based on molecular mechanisms involved after early life exposure to EDC mixtures. The task is approached through interdisciplinary cooperation between experts in epidemiology, experimental toxicology and molecular biology, and risk assessment. This will be achieved by:

- Identification of EDC mixtures that are associated with multiple adverse health outcomes in three health domains (growth and metabolism, neurodevelopment and sexual development) in two epidemiological studies.

- Identification of molecular mechanisms and pathways underlying the associations between exposure and adverse health outcomes by the use and development of state-of-the-art experimental models and;

- Development of a transparent and systematic framework in risk assessment for integrating epidemiological and experimental research to facilitate the assessment of risk and societal impact, thus promoting better risk management for EDCs and their mixtures.

The workflow will start with biostatistical analyses of epidemiological material in order to identify mixtures associated with adverse health outcomes. The mixtures will then be prepared by two highly experienced chemical laboratories and tested in the different model systems, focusing on molecular initiating events and adverse outcome pathways.

Experimental results will subsequently serve as basis for assessment of risks posed by these mixtures, and of societal and ethical impacts of the findings. Additionally, they will be used to refine epidemiological and biostatistical analyses, generating novel information that, in turn, will be tested again in selected models.

Key Results

The project was launched on the 1st of May 2015. The project management and steering committee has been established and a website is in place: www.edcmixrisk.org.

The first critical mixtures associated with adverse health outcomes in the three health domains in focus have been identified using already collected data from the SELMA pregnancy cohort. The resulting mixtures are now prepared and will be distributed for exposure studies using standardized procedures. Meanwhile, a large number of health examinations and chemical analyses in the epidemiological studies have been initiated to refine the initial mixtures.

Conclusions

EDC-MixRisk has been designed to ultimately lead to a safer environment for our children.

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P38- Early life exposure to estradiol in neonate rats impairs mucosal and systemic immune response by modulating Th17/Treg cell balance

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Aim of the work

Recent studies emphasized the immune system highly sensitive to perinatal exposure to the ubiquitous contaminant bisphenol A (BPA), with long-term imprint on immune defenses and the establishment of oral tolerance in offspring. Whether these developmental effects are linked to estrogeno-mimetic properties of BPA remains to be explored. This requires preliminary studies on the influence of estradiol on maturation and responses of intestinal and systemic immune system in early life. The purpose of this study is to investigate the effects of subcutaneous and oral exposure to 17β-estradiol (E2) in rat newborns on immune cell subpopulations and immune responses in local and systemic compartment.

Methods

A dose-response study was first performed to determine median effective doses (ED_{50}) of E2 with physiological effects in the gut (i.e. total permeability to dextran) after subcutaneous and oral E2 treatment in rat neonates during the first week of life. On this basis, an ED_{50} of 10 ng/kg bw/d for subcutaneous injection (*sc* group) and 10 µg/kg bw/d for oral route (*oral* group) were daily given to male and female pups from postnatal (PND)5 to PND10. Immune cells from spleen or jejunal lamina propria (LP) were harvested at PND10. Phenotypic analysis of dendritic cells (DC), total ($CD4^+$) T cells, regulatory T cells (Treg), and Th1/Th17 cells was performed by flow cytometry. The frequency of immune cells expressing ERβ and ERα were analysed by flow cytometry. Th1/Th17 cytokine profile was assessed in supernatant of splenocytes after CD3/CD28 restimulation. Involvement of nuclear ERs on main observed effects was assessed using the ER antagonist ICI182.780 (2 mg/kg/d sc in corn oil).

Key Results

A sex-dependent effect of E2 occurred on DC subpopulations, whatever the route of exposure. Indeed, E2 increased DC frequency in the spleen, while in contrast, a decrease was observed in the LP, these effects being reported in female pups only. Regarding T cell subsets, in both sex, a decreased Treg frequency was observed in the spleen as well as in the LP in the *sc* group, while *oral* E2 exposure had no effect. In the *sc* group, a concomitant increase of Th17 cells was reported in the spleen and LP in female pups only.

This effect was associated to a marked increase in their ability to produce pro-inflammatory IL-17 cytokine, mainly in the spleen, when assessed *in vitro* after CD3/CD28 restimulation. The co-administration of the ER antagonist ICI182.780 completely abolished the enhanced IL-17 release by splenocytes, depicting the implication of nuclear ER pathway. In contrast, the same treatment had no effect on E2-induced Treg decrease, suggesting nuclear ER-independent mechanisms. In addition, E2 treatment in the *sc* group of pups led to a significant decrease of ERα expressing immune cells in the spleen, without change in ERβ, and this effect was abolished by ICI182.780.

Conclusions

Our results demonstrated that early life exposure to E2 directly affect immune cell population (both DC and T cells) and function in the spleen and the LP, two decisive locations for the development and the maintenance of immune homeostasis, including development of host defenses. These disturbances impair mucosal and/or systemic tolerogenic functions, depending on the sex, and promote Th17 inflammatory systemic responses in females. These findings may help for understanding the mechanisms underlying immunotoxic effects of BPA after perinatal exposure.

P39- Neo-Natal, Pre, Post-Pubertal and Adult Modifications of the Male Reproductive Axis and Testicular Gene Expression after a Continuous Dietary Exposure to Environmental Levels of Endocrine Active Substances Mixtures

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Aims of the work

The reproductive impact of a chronic exposure to mixtures of environmental endocrine active substances (EAS) in the male remains poorly known. The present study aimed at studying conventional reproductive endpoints and the testicular transcriptome of rats continuously exposed to environmental levels of estrogenic and antiandrogenic EAS mixtures at critical development steps.

Methods

Male rats orally exposed lifelong, from conception, to bisphenol A (B, 5µg/kg/day, a dose <<NOAEL and similar to the 2015 EFSA t-TDI) alone and in mixture with the phytoestrogen genistein (G, 1mg/kg/day, a dose compatible with Asian diet) and/or the antiandrogenic dietary contaminant vinclozolin (V, 10µg/kg/day, a dose <<NOAEL and lower than the 2014 EPA TDI, 25µg/kg/day) were studied neonatally, prepubertally, postpubertally and as young adults using conventional reproductive endpoints and testicular mRNA expression profiles (Affymetrix, GeneChip Rat Gene 2.0 ST) followed by the integrative search of the functions modified using the Ingenuity software.

Results

Conventional reproductive endpoints were weakly affected by the various mixtures. In contrast, the testicular transcriptome was significantly impacted by B and all EAS mixtures in the neonatal period with very significant changes in networks of genes involved in the development and functions of the reproductive and endocrine systems. The impact of GV exposure on these systems dwindled gradually from birth until adulthood despite the continued exposure.

Instead, BG, BV and BGV exposures from birth until adulthood increased significantly the modifications of expression of the numerous genes involved in the functions of the reproductive and endocrine systems. For example, significant changes in the expression of several crucial genes for the androgen biosynthesis (STAR, CYP11A1, CYP17A1, 17βHSD, CYP19A1 and SRD5A2) were found, in a different way, depending on the exposure modalities and the developmental periods.

Conclusion

Overall, pre and postnatal exposure to EAS mixtures at low environmental doses seriously affect testicular gene expression without marked changes of the reproductive phenotype. Thus, from the present study, we cannot conclude to an obvious deleterious impact of low-dose EAS exposures. More research is needed to determine the possible consequences of these exposures in the exposed males as well as in their progeny because the marked changes in the testicular transcriptome may indicate a disruption of the testis and sperm functions by mechanisms which are not considered through conventional endpoints. In aggregate, these results show the importance of not limiting studies to regulatory usual tests when it imports to improve our knowledge and the risk assessment of realistic exposures of low-dose and mixtures of EAS.

P40- Transcriptomic analysis and comparative effects of two estrogenic dietary compounds in a cell model of breast cancer

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Aim of the work

Numerous environmental compounds are able to bind estrogen receptor (ER) and to modulate its activity, it is the so-called xenoestrogens. In this study, we have analyzed estrogenic/antiestrogenic potency of two dietary xenoestrogens on an ER-positive breast cancer cell line. Apigenin, a flavone found in chamomile, parsley, grapefruit, is supposed to have preventive effects among which anti-proliferative and anti-inflammatory effects. Conversely, zearalenone, a mycotoxin produced by *Fusarium* species which is found in about 15 % of cereals consumed in Europe, is known to have adverse effects (genotoxicity, oxidative stress, etc.). These compounds are known to interact with ER but precise mechanisms involved to explain their different effects on human health, are yet elusive.

Methods

ER positive MCF-7 cells were treated with E2 at 10^{-9} M as positive control, with a dose effect up to 10^{-5} M of apigenin or with a dose effect up to 10^{-6} M of zearalenone. To decipher the activity of these compounds, cell proliferation assay and flow cytometry at 6 days of treatment, reporter luciferase assay, Proximity Ligation Assay to study the interaction between ER and non-genomic pathway, expression of estrogenic genes and transcriptomic analyses after 24 h of treatment were performed.

Key Results

Apigenin showed a partial and weak proliferative effect in the absence of E_2 and an antagonistic effect when it is used in the presence of E_2. On the contrary, zearalenone showed a full and powerful proliferative activity in the absence and presence of E2. Moreover, nongenomic effects of ER were assessed and shown that apigenin at 10-5 M reduce interaction between ER and Src whereas zearalenone at 10-8 M works as well as E2. Dose response effects showed that, apigenin requires high concentration (10-5 M) to induce the expression of E2-target genes, while zearalenone requires much lower concentration (10-9M). Our genome-wide microarray analysis of MCF-7 breast cancer cells identified 147 E2-regulated genes. Zearalenone treatment induced transcriptome changes very similar (98%) to that of E2. Conversely, apigenin treatment revealed 193 genes that overlap only at 53 % to E2-target genes. Among these genes, nucleolar genes represent an important cluster.

Conclusions

In conclusion, zearalenone behaves as a powerful estrogenic compound and could participate in breast cancer progression through estrogenic pathways. In contrast, apigenin behaves as an agonist/antagonist ER modulator and capable of inhibiting the growth of breast cancer cells in the presence of E2. Transcriptomic analysis showed that apigenin elicits the expression of a unique group of genes that were not regulated by E2 or zearalenone alone. The anti-estrogenic effect of apigenin may be explained by the modulation of several genes involved in protein synthesis and/or cell growth that are preferentially affected by apigenin and by the interference in the non-genomic pathway involving ER. This reinforces the possible use of apigenin in prevention or in therapy of breast cancer.

P41- Contribution of the environmental factors to the progression of the prostate cancer: involvement of ion channels

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Aim of the work

Recent data show that exposure to environmental factors called "endocrine disruptors (ED)" (pesticides, antibiotics, plasticizers such as phthalate or polycarbonate and nonylphenols), can have an impact on human health by influencing the endocrine system. These factors were shown important in the etiology of prostate cancer (PCa) and a link has been established between the abundance of environmental EDs and the incidence of hormone-dependent cancers, including prostate cancer. Moreover, recent studies suggest a potential role of these environmental factors in predisposition to the development of prostate cancer and the induction of proliferation of human prostate cancer cells. Therefore, it is important to determine the molecular basis of action of these environmental factors in human prostate cancer cells.

Our previous works have shown that alterations of calcium homeostasis and ion channels activity are involved in prostate carcinogenesis by modulating the proliferation, differentiation, migration and apoptosis of human prostate cancer cells. Environmental factors could intervene in the process by changing the calcium signaling *via* a modulation of the expression and/or the activity of the ion channels. In this context, we studied the effects of three environmental factors, Bisphenol A, and two widely used antibacterials in cosmetics and hygienic products (Triclosan (TCS) and Triclocarban (TCC)): 1) on the proliferation, differentiation, migration, apoptosis and secretion of epithelial and stromal cells, two cell populations of prostate cancer tumor microenvironment; 2) - on the expression and functionality of ion channels and on the calcium signaling in these cells and finally we studied 3) the involvement of ion channels and calcium in the effects of these environmental factors

Methods

Primary cell culture from human prostate cancer tissues, TUNEL and DNA laddering apoptosis tests, Immunofluorescence studies, cell migration assays (Transwell), western blot, RT-PCR, calcium imaging (Fura2 calcium probe), Electrophysiological techniques in whole cell configurations, ELISA tests for secretion of growth factors.

Key Results

We showed that BPA induces the migration of prostate epithelial cancer cells, through the modulation of protein expression of ion channels (Orai1). In these studies, we have also shown that the antibacterial TCS activates directly the calcium channels TRPA1 (in stromal cells) and RyR (in epithelial cells). The TRPA1 activation by TCS induces an important calcium entry in human stromal cells, known for their secretion of mitogen factors targeting epithelial cells and endothelial. The immunofluorescence studies have shown that the TRPA1 was preferentially expressed in human prostate cancer stromal cells. Furthermore, the TCS-induced increase of calcium was correlated with a secretion of HGF and VEGF, growth factors trageting epithelial and endothelial cells. Interestingly, in epithelial cancer cells, TCS induced a remodeling of ion channels expression correlated with a resistance to apoptosis.

Conclusions

This work allowed to determine the mechanism by which these environmental factors modified the physiopathology of prostate cancer epithelial and stromal cells by disturbing the calcium signaling in these cells. Taken together, these data suggest that by targeting the expression and/or the functionality of the ion channels, the environmental factors may promote prostate cancer progression. The present work suggests also that the ion channels could constitute markers of exposure to endocrine disrupting chemicals in human prostate cancer cells. Finally, these studies would propose preventive precautions to take in regard to the expositions to environmental factors to avoid the development and especially the progression of the human prostate cancer.

P42- Effets de mélanges de Perturbateurs endocriniens sur la plasticité mammaire et le cancer du sein

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Aim of the work

L'incidence de cancers hormono-dépendants ne cesse d'augmenter dans les pays occidentaux. Le cancer du sein est le cancer le plus fréquent chez la femme. Les facteurs de risque connus n'expliquent pas la totalité de ces cancers. L'hypothèse selon laquelle certains facteurs environnementaux, en particulier des composés perturbateurs endocriniens (PE), pourraient être impliqués dans cette pathologie constitue un enjeu considérable. De plus ces dernières années, un intérêt croissant est apparu pour les multi-expositions à des PE et leurs effets, avec une incertitude importante sur l'évaluation des risques liés aux mélanges, du fait d'une interaction possible entre ces composés. Pour mieux connaitre la nature et la toxicité des mélanges, il est nécessaire de développer des modèles biologiques pertinents et de nouveaux concepts. Plusieurs séries d'études réalisées par notre équipe, - des études experimentales sur les effets de mélanges de PE sur le développement et la carcinogénèse mammaire, et – une étude clinique (cancer du sein), seront présentées.

Methods

Dans une série de 3 études (intitulée 'effets de mélange de deux PE sur le développement et la carcinogénèse mammaire'), nous avons développé un modèle original d'exposition chez le rongeur, où un phyto-œstrogène de soja (génistéine, G) et un fongicide anti-androgénique retrouvé dans les légumes et les fruits (vinclozoline, V), sont administrés à 1 mg/kg/jour chez la rate gestante.

Résultats

Les deux premières études montrent que l'exposition *in utero* et pendant la lactation à ces composés (G ou V) altère le développement de la glande mammaire chez les animaux pubères. L'analyse transcriptomique montre que l'exposition au mélange GV altère également le profil d'expression génique de la glande mammaire différemment de l'exposition à chacune des substances. Dans une 3^{ième} étude réalisée avec un modèle de carcinogenèse induite (DMBA), nous montrons que si la carcinogenèse (incidence et nombre de tumeurs) diminue, après exposition *in utero* et pendant la lactation à chacune de ces substances (G ou V) par rapport au contrôle, de façon surprenante, ce n'est pas le cas après exposition au mélange (GV). Au contraire, les conséquences d'une exposition au mélange GV apparaissent plus sévères sur la croissance tumorale, comparées à celles observées avec les molécules isolées.

Conclusions

L'ensemble de ces études souligne pour la première fois le risque de la multi-exposition de PE appartenant à deux familles différentes sur le développement et la carcinogénèse mammaire. Le mécanisme d'action des PE le plus fréquent est la liaison aux récepteurs nucléaires hormonaux, facteurs de transcription capables de modifier l'expression des gènes. De nombreuses études épidémiologiques et expérimentales ont mis en avant l'association entre fonction anormale du récepteur AhR et cancer. Cependant, très peu de données sont disponibles en clinique humaine sur le cancer du sein. L'univers des ligands d'AhR s'est considérablement accru ces dernières années ; il inclut de nombreux composés industriels, tels la dioxine (TCDD, prototype environnemental et le plus puissant des ligands connus de AhR) et de nombreux PAHs (tels le benzopyrène présent dans la fumée de cigarette). Cet univers AhR inclut aussi des composés endogènes (FICZ et kynurenine), des phytoestrogènes (tels que flavonoïdes) et des composés pharmaceutiques (omeprazole, aminoflavone). Nous avons entrepris de déterminer si le récepteur AhR et ses partenaires sont des facteurs pronostics de ce cancer à partir d'une grande série de biopsies de cancer du sein. Les résultats nous ont conduits à analyser un certain nombre de gènes candidats et déterminer certaines voies de signalisation impliquées dans des sous-types de cancer du sein. Les mécanismes d'action impliqués dans l'activation de AhR et sa fonction sur la transcription génique seront explicités.

P43- Long chain alkylphenol mixture promotes breast cancer initiation and progression through an ERα36-mediated mechanism

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Aim of the work

Growing source of evidence suggests that exposure to estrogen mimicking agents is a risk factor for breast cancer onset and progression. Long chain alkylphenols are man-made compounds still present in household products, industrial and agricultural processes, leading to a global environmental and human contamination. These molecules are known to exert estrogen-like activities through binding to classical estrogen receptors. Recently, we have demonstrated that a realistic mixture of 4-tert-octylphenol and 4-nonylphenol can stimulate proliferation and modulate epigenetic status of testicular cancer germ cells through a rapid, Estrogen Receptor alpha 36 (ERα36)- dependent non genomic pathway (Ajj et al, 2013; doi: 10.1371/journal.pone.0061758). In a retrospective study of breast tumor samples, we also validated ERα36 expression as a reliable prognostic factor for cancer progression from an estrogen dependent proliferative tumor toward an estrogen dispensable metastatic disease (Chamard-Jovenin et al, 2015; doi: 10.1186/s12918-015-0178-7).

Since high ERα36 expression enhances expression of migration/invasion markers in breast tumors, we addressed the question of its involvement in response to alkylphenol exposure in vitro (MCF-10A mammary epithelial cell line and MCF-7 estrogen-sensitive cancer cells) and in vivo (C57/Bl6 mice).

Methods

In order to characterize the molecular events in alkylphenol exposed cells, ERα36 overexpression (knock in) or gene-silencing (knock down) strategies combined to microarray analyses of the mixture target genes were used in MCF-10A cells. Molecular and cellular biology experiments confirmed the predicted phenotypes. A customized database was designed to analyze comprehensive gene expression results, nonlinear correlation analyses, and mutual information computations helpful for the modeling of alkylphenol/ERα36-dependent pathways.

In vivo, alkylphenol mixture doses, representative of human exposure, were orally given to C57/Bl6 pregnant females and histological analyses were then performed on F1 mammary glands.

Key Results

Our results highlight a key role for ERα36 in alkylphenol non genomic src protein kinase /PI3-kinase/serine-threonine kinase Akt/ nuclear factor-*kappa* B signaling in non cancerous epithelial breast cells. Flow cytometry analyses, scratch-wound assays and caspase clivage measurements indicate that the alkylphenol mixture may promote a neoplastic like phenotype, *i. e.* proliferation, apoptosis escape and migration in MCF-10A epithelial cells through an ERα36 dependent pathway. These results are currently used to build a model of alkylphenol-directed breast cancer induction and progression.

In vivo, mammary gland hyperplasia is observed following alkylphenol exposure during embryonic life.

Conclusions

Hence, alkylphenol and/or ERα36-dependent control of the proliferation, adhesion and survival pathways opens the way to a better understanding of the link between endocrine disruptor exposure and the burden of hormone sensitive cancers.

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P44- Addressing the capacity of pesticides to act as thyroid hormone receptor antagonists

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Aim of the work

Pesticides are suspected to act at low dose as thyroid hormone disruptors, and, as a consequence, to compromise proper neurodevelopment. We addressed the possibility that they could act as antagonists of the nuclear receptors of thyroid hormone (TRs), without modifying the circulating level of thyroid hormone. If this hypothesis is confirmed, most toxicological studies would underestimate their neurotoxicity.

Methods

We used 2 reporter cell lines with complementary ability to detect weak antagonist activity. The first one is derived from human HEK293 cells and express luciferase gene under the control of a promoter which is regulated by a Gal4TR fusion. The second one derives from a mouse C17.2 neural cells in which thyroid hormone response was restored by stable TR expression. The cell also host a construct in which luciferase expression is driven by the promoter of Hairless, a gene which expression is highly inducible by thyroid hormone.

Key Results

Several pesticides act as TR antagonists is one or both assays. For some of them the activity is observed at low concentrations.

Conclusions

Genome wide analysis of gene expression in the C17.2 cells is currently performed to gain a broader view of pesticides toxicity in this cell line. This should clarify whether the observed effect reflect a global alteration of the thyroid hormone signaling pathway, and should guide later in vivo investigations.

P45- Facial dysplasia in wild chimpanzees: a preliminary study on the thyroid disruption axis hypothesis

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Aim of the work

Prenatal exposure to endocrine disruptors can disturb the development of endocrine system and organs that respond to endocrine signals. Effects of mother exposure to such chemicals present in the environment can produce profound and irreversible abnormalities in offsprings in humans but also in wildlife. Twelve out of the 60 identified and monitored wild chimpanzees in the northern area of Sebitoli, Kibale NP, Uganda suffered from anomalies including reduced nostrils, cleft lip, limb deformities, reproduction troubles, hypopigmentation that are likely from congenital origin. The chimpanzee territory is surrounded by industrial tea plantations and villager gardens. Chimpanzees used to visit crop and feed on maize stems and seeds. Among other hypotheses, we explore the hypothesis that chimpanzees may be exposed to teratogenic products that could produce such developmental impairments. Preliminary results showed that the mean levels of pesticides detected in maize stems and seeds, soils and sediments in the vicinity of the chimpanzee territory do not meet the standards, which may pose a health hazard to the consumers including wild animals. We aimed at analyzing biological samples from chimpanzees to diagnose potential effects of chemical inputs on their health including thyroid function.

Methods

In July and August 2015, 37 fresh fecal samples and 52 fresh urine samples of 16 chimpanzees (5 females and 11 males) including 5 with facial dysplasia were collected non-invasively. In the hour following emission, 0.5 g of feces were stored in 5 ml of 80% Ethanol. The same day, thyroid hormones were extracted by 1 minute manual centrifugation and then 45 minutes of sedimentation. Finally, 2 ml of the supernatant were sampled and stored at 2°C. Urine samples were kept at 2°C without extraction. ELISA analyses on T3 and T4 hormones will be carried out in september/october 2015.

Key results

Results of ongoing analyses will be presented.

Conclusions

This preliminary study brings new insights on the potential effects of chemicals present in the environment on the health of threatened large mammals. In order to confirm the potential effects of chemicals on facial dysplasia *via* a disruption of thyroid function, next steps will include T3/T4 analyses on more individuals and over a longer time but also biological sample analyses to detect presence of pesticides in the chimpanzees' organisms.

P46- Characterization of thyroid hormone transporter in Xenopus and their susceptibility to TDCs

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Aim of the work

Thyroid Hormones (TH) are crucial for brain development and maturation. Disruption of TH action, either due to genetic and/or environmental factors, has been implicated in neurological defects such as autism, attention deficit hyperactivity disorders and IQ loss. An example of genetic disruption of TH signaling is Allan-Herndon-Dudley syndrome where, in humans, the mutation of the brain specific TH transporter, monocarboxylate transporter 8 (MCT8), leads to severe intellectual disability. The disruptive effect of changes in MCT8 expression on neurodevelopment has also recently been documented in human fetuses suffering from IUGR (Intra uterine growth restriction), a condition associated with milder neurodevelopmental deficits usually arising from uteroplacental failure.

The actions of thyroid disrupting chemicals (TDCs) are suspected as environmental factors implicated in the current increase in neurodevelopmental disease. Disruption of TH signalling at various levels including the receptor, blood transporter and deiodinases by TDCs is well documented. The effect of TDCs on brain specific TH transporters including MCT8, however, is less explored.

Our hypothesises : Environmental pollutants affect TH signalling, especially expression and functioning of brain specific TH transporters, during early neuro-development and Effects of TDCs could be exacerbated under certain genetic backgrounds (hypothyroidism, hyperthyroidism) or in certain genetic disorders.

Methods

We are using *Xenopus laevis* and *X. tropicalis* as models due to the pre-existing TH literature, their fully sequenced genomes and transparency.

TH transporter expression profile: Different stages of *X. laevis* and *X. tropicalis* development were collected and temporal and spatial expression profiles generated using RT qPCR and whole mount *in situ* hybridisation (ISH)

TDC exposure: Stage NF45 Xenopus laevis tadpoles were exposed for 3 days to the TDCs cocktail mixture, with and without T_3. Brains were dissected on day 3 and subjected to RT-qPCR for different TH targets and neuronal markers.

Knock out: The CRISPR-Cas9 system is being used to generate a transgenic line of *mct8* knock out (on-going).

Key Results

Expression Profile: The expression of *mct8* begins from NF20 (neurulation) and remains relatively constant at least up until NF46, the time during which the general pattern of brain (fore, mid and hind brain) is established in Xenopus. ISH further showed *mct8* expression to be localized to specific brain areas such as the choroid plexus and the pituitary.

Effects of TDC exposure: Increased expression of *mct8,* and other TH transporters, was observed following exposure to low levels of the TDCs cocktail mixture. At higher concentrations, the expression of these TH transporters was significantly reduced.

Conclusions

Although preliminary, these results are the first to document that TDCs action during early neurogenesis modulated TH transporter gene expression. As such they highlight the need for a better understanding of the impact of TDCs, on TH transport into neuronal cells during brain development. The *mct8* KO will further allow us to test the hypothesis that TDCs can exacerbate genetic susceptibility in brain development.

We are currently applying an *in vitro* cell based transport assay, to analyze whether Xenopus *mct8* and other TH transporters are specific for the uptake of various TH and select TH orthologues. Further, we will test, *in vitro*, the disrupting action of various individual TDCs on Xenopus TH transporters.

P47- Mixtures of endocrine disrupting chemicals in the mouse embryonic stem cell test

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Aim of the work

Exposure to Endocrine Disrupting Chemicals (EDCs) is a global issue of concern due to the possible impacts on human health. Exposure to these chemicals, especially during pre-natal and post-natal stages might be linked to the development of endocrine diseases and disorders during adulthood. Current risk assessment does not fully assess for potential endocrine activity of a chemical, so information regarding to what degree humans are at risk is limited. Additionally, risk assessment nowadays focuses on the effects of single compounds, however humans are daily exposed to a mixture of compounds. The aim of this study is to investigate the effect of a mixture of EDCs in the mouse embryonic stem cell test (mEST).

Methods

The mEST is an *in-vitro* model designed to predict embryotoxicity. Three compounds have been tested in a dose-response, Flusilazol, Miconazol and Triadimefon. The endpoint of the test is the interference with mesoderm-derived cardiac muscle differentiation observed under the microscope as beating muscle foci. A dose–response curve for each compound was fitted using the log–logistic model, with PROAST software. The ID50 concentration, the concentration which corresponds with the concentration at which 50% of the embryo bodies were inhibited to differentiate towards contracting cardiomyocytes, was calculated. Based on the ID50 for each compound, the mixture dosages were calculated. For each mixture a dose response curve was fitted using PROAST software. The ID-50 for the mixture is calculated and a value of 1 means an additive effect; <1 Synergistic effect; >1 antagonic effect.

Key Results

All the tested mixtures showed an ID50 of 1. So, preliminary data show that a mixture of two EDCs with a comparable mode of action (MOA), have an additive effect on embryo toxicity in the mEST.

Conclusions

Future research will investigate mixtures of chemicals with a different MOA in the mEST. Additionally, mixtures will be tested in models measuring endpoints related to the endocrine system.

P48- Analysis of Estrogens compounds, a class of Endocrine Disrupting Chemicals Using Solid Phase Extraction based on Molecularly Imprinted Polymer

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Aim of the work

Endocrine disruptors are exogenous substances that act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones. Endocrine disrupting compounds encompass a broad class of molecules, including steroid hormones (17β-Estradiol, 17α-Ethynyl Estradiol….). Most studies have focused on the impact of environmental compounds with hormone-like action on human development and reproductive health. The aim of this work is to develop a powerful technique for analysis of Estrogenss in various complex matrix.

Methods

This poster shows a very powerful solid phase extraction (SPE) clean-up method based on molecularly imprinted polymers (MIP). The use of MIP is particularly suitable for the analysis of complex matrices such as food or biological fluids due to the selectivity of the material. Indeed, a MIP is a synthetic material with artificially generated three-dimensional network, able to specifically rebind a target molecule. For these analysis, a clean-up step is crucial to improve the sensitivity and the specificity before analysis.

Key Results

The method was applied to the analysis of Estrogens from a wide variety of matrices such as water, milk, infant formula, canned food, urine, ... Thanks to selectivity of MIP, perfect clean-up and high recoveries (>85%) were obtained.

Conclusions

A robust and powerful method based on molecularly imprinted polymers has been developed for various matrix. Thanks to selectivity of MIP, perfect clean-up and good recoveries were obtained.

P49- A rapid, quantifiable test to assess GPER-specific estrogen-like signaling

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Aim of the work

Endocrine disruptors exerting estrogen-like effects have been thought to act solely through the classical Estrogen Receptors (ERs), which are members of nuclear receptor superfamily and act as ligand-dependent transcriptional regulators. Recent data, suggesting that estrogen-like compounds may also act through the ERRgamma orphan nuclear receptor or the transmembrane GPER (G-Protein coupled Estrogen Receptor; aka GPR30), have challenged this view. However, cellular systems that allow the analysis of a specific alternative pathway are lacking.

Results

Our recent data show that human dermal fibroblasts in primary culture (which express GPER but not ERs) display a time- and dose-dependent morphological change when exposed to 17beta-estradiol (E2).

This remodeling, which involves a spatial re-organization of focal adhesions and of the actin network, is quantifiable, occurs within minutes, and is mediated by non-genomic mechanisms. Furthermore, this phenomenon is not sensitive to inhibitors of the classical ERs. Rather, inhibition of GPER by pharmacological (treatment with the G-15 inhibitor) or genetical (shRNA) means results in abrogation of E2-induced remodeling.

Conclusions

Our data thus allow to proposing a cell-based system to reliably analyze whether a given compound specifically acts through GPER.

P50- Development of a method for the use of toxicological data in the socio-economic analysis of chemical risks: the case of triclosan

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Aim of the work

Socioeconomic analysis (SEA) is currently used in the regulatory process in the European Regulation REACH, aiming at managing chemicals risks. A commonly used SEA method is cost-benefit analysis, comparing costs and benefits of each particular risk management option. For calculating the benefits (i.e., avoided health costs), the economic literature proposes the method of the population attributable fraction (PAF), using epidemiologic studies as input data. However, the quasi-totality of the data used in the REACH dossiers comes from toxicological studies. It becomes therefore important to develop a method able to use toxicological data for SEA.

We developed such a method, using in vivo studies for the evaluation of the dose-response relationship and its extrapolation to humans. The validated method of the PAF provides us a term of comparison for the results obtained with the new method.

Methods

We used triclosan (TCS) as a case study for comparing the two methods for the calculation of the share of the population showing an adverse effect. Using the dose-response modelling, this share was calculated for three health endpoints obtained from toxicological studies on TCS, i.e. decrease in the weight of vas deferens, decrease of thyroid hormone, T_3, and earlier pubertal development.

The hybrid approach was used to convert the continuous data into quantal data. The converted data were analysed with the software PROAST to obtain the dose-response function. Using available biomonitoring data, a modelling approach using the software R was employed for calculating the share of the population with an adverse effect. This share was applied on the size of the target population to obtain the final number of cases.

The PAF method was used for three endpoints addressed in epidemiological studies on TCS, i.e., increase of T_3, increase of BMI points (obesity), and earlier pubertal development.

Key results

The comparison of the results obtained with the two methods was possible only for thyroid hormone, T_3, and for earlier pubertal development, addressed in both toxicological and epidemiological studies. The decrease in the weight of vas deferens was addressed only in a toxicological study, and the increase of BMI points only in an epidemiological study.

Table 1. Comparison of results obtained from the two methods*

Adverse effect	Number of cases	
	Dose-response modeling	PAF
Decrease in the vas deferens weight	1 192 617	--------
Increased T_3 levels	0	1 649 748
Earlier pubertal development	929 789	74 627
Obesity	--------	7 199 228

** These are preliminary results, currently being verified*

Conclusions

The economic costs could be calculated for only one endpoint, i.e. obesity, by multiplying the number of cases by the cost per case provided in the literature. The result is of 154 billion euro per year, for the European Union. It was not possible to associate a specific disease (and consequently public health costs) to the other endpoints.

The results obtained from both methods could be compared only for one endpoint, i.e. earlier pubertal development, for which both toxicological and epidemiological studies were available.

If these preliminary results are confirmed, we could estimate that the choice of one of the two methods can have a very significant impact on the estimated health costs and subsequent decision-making. However, the obtained results and the new method based on dose-response modeling require further verification and validation.

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