

# CENTER FOR HORMONFORSTYRENDE STOFFER

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Litteraturgennemgang for perioden august 2016 – september 2016

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## Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

Søgning er udført på PubMed og dækker perioden 27. juli 2016 – 25. september 2016

Følgende søgeprofil er benyttet:

**Bisphenol A**  
**Phthalat\***  
**Paraben\***  
**(perfluor\* OR polyfluor\*)**  
**Triclocarban**  
**Triclosan**  
**(Flame retardant)**  
**tributyltin**  
**endocrine disrupters**

kombineret med nedenstående tekst:

**AND expos\* AND (human OR men OR women OR child\* OR adult\* OR adolescen\* OR infan\*)**

Limits: title/abstract, English language

I den listede bruttoliste er dobbeltgængere fjernet, ligesom hits der hører under kategorierne in vivo studier, in vitro studier eller wildlife er frasortet. De kommenterede artikler er highlightet.

De udvalgte artikler er 1) kontrolleret høj eksponering for DBP og sædkvalitet, 2) perfluorstoffer og feber hos børn, 3) paracetamol og AGD og 4) phthalate eksponering og påvirket epigenetik i moderkagen

God læselyst

## Udvalgte artikler

### **A crossover-crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and semen quality in men with inflammatory bowel disease**

Nassan FL, Coull BA, Skakkebæk NE, Williams MA, Dadd R, Mínguez-Alarcón L, Krawetz SA, Hait EJ, Korzenik JR, Moss AC, Ford JB, Hauser R.

Environ Int. 2016 Oct;95:120-30. doi: 10.1016/j.envint.2016.08.006. Epub 2016 Aug 26.

#### **Abstract**

**BACKGROUND:** Phthalates are widely used chemicals with ubiquitous exposure. Dibutyl-phthalate (DBP), a male reproductive toxicant in animals, is understudied in humans. Some mesalamine medications used to treat inflammatory bowel disease (IBD) have DBP in their coating, whereas other mesalamine formulations do not.

**OBJECTIVES:** Taking advantage of differences in mesalamine formulations, we investigated whether high-DBP exposure from mesalamine medications was associated with decreased semen parameters.

**METHODS:** 73 men with IBD taking mesalamine participated in a crossover-crossback prospective study. Men taking non-DBP containing mesalamine at baseline i.e., background exposure, crossed-over for four months to high-DBP mesalamine and then crossed-back for four months to their non-DBP mesalamine (B1HB2-arm;Background1-High-Background2) and vice versa for men taking high-DBP mesalamine at baseline (H1BH2-arm;High1-Background-High2). Men provided up to six semen samples (2: baseline, 2: crossover and 2: crossback).

**RESULTS:** We estimated crossover, crossback and carryover effects using linear mixed models adjusted for abstinence time, age, season and duration on high-DBP mesalamine at baseline. Semen parameters in B1HB2-arm (26 men, 133 samples) decreased after high-DBP mesalamine exposure (crossover versus baseline), especially motility parameters, and continued to decrease further even after crossback to non-DBP mesalamine (crossback versus crossover). The cumulative carryover effect of high-DBP (crossback versus baseline) was a decrease of % total sperm motility by 7.61(CI:-13.1, -2.15), % progressive sperm motility by 4.23(CI:-8.05, -0.4) and motile sperm count by 26.0% (CI:-46.2%, 1.7%). However, H1BH2-arm (47 men, 199 samples) had no significant change during crossover or crossback.

**CONCLUSIONS:** Men newly exposed to high-DBP mesalamine for four months had a cumulative reduction in several semen parameters, primarily sperm motility, that was more pronounced and statistically significant even after exposure ended for four months.

### **Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort**

Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Høst A, Grandjean P, Jensen TK.

Environ Int. 2016 Sep 5;96:58-64.

#### **Abstract**

**INTRODUCTION:** Perfluorinated alkylated substances (PFAS) are persistent industrial chemicals that have resulted in global environmental exposures. Previous epidemiological studies have reported possible

effects on the immune system after developmental PFAS exposure, but the possible impact on childhood infectious disease is unclear.

**OBJECTIVES:** To investigate the association between prenatal exposure to PFAS and symptoms of infections at age 1-4years.

**METHODS:** The Odense Child Cohort is an on-going prospective study on children's health, where serum concentrations of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorodecanoic acid (PFDA) and perfluorononanoic acid (PFNA) were measured in 649 pregnant women before gestational week 16. Of these women, 359 reported on symptoms of infection in their child every two weeks for a one-year period. The association between prenatal exposure to PFAS and the symptoms was estimated using a logistic regression model and a negative binomial regression model. For the latter, the outcome was reported as an incidence rate-ratio (IRR), and all models were adjusted for maternal age, educational level, parity and child age.

**RESULTS:** On average, the children experienced symptoms of infection 23% of the time during one year. PFOS exposure in the high tertile compared to the low tertile was associated with a statistically significant increased proportion of days with fever (IRR: 1.65 (95% CI: 1.24, 2.18), P-trend<0.001) and an increased odds of experiencing days with fever above the median (OR: 2.35 (95% CI: 1.31, 4.11)). The latter tendency was also apparent for PFOA (OR: 1.97 (95% CI: 1.07, 3.62)). Further, higher concentrations of PFOS and PFOA tended to increase the number of episodes of co-occurrence of fever and coughing and fever and nasal discharge during the one-year study period.

**CONCLUSION:** We found a positive association between prenatal exposure to PFOS and PFOA and the prevalence of fever, which may be a sensitive marker of infection. This finding is in agreement with an immunotoxic effect of prenatal exposure to PFAS. The wider implications for childhood infectious disease deserve attention.

### **Prenatal paracetamol exposure is associated with shorter anogenital distance in male infants**

Fisher BG, Thankamony A, Hughes IA, Ong KK, Dunger DB, Acerini CL.

Hum Reprod. 2016 Sep 8. [Epub ahead of print]

#### **Abstract**

**STUDY QUESTION:** What is the relationship between maternal paracetamol intake during the masculinisation programming window (MPW, 8-14 weeks of gestation) and male infant anogenital distance (AGD), a biomarker for androgen action during the MPW?

**SUMMARY ANSWER:** Intrauterine paracetamol exposure during 8-14 weeks of gestation is associated with shorter AGD from birth to 24 months of age.

**WHAT IS ALREADY KNOWN:** The increasing prevalence of male reproductive disorders may reflect environmental influences on foetal testicular development during the MPW. Animal and human xenograft studies have demonstrated that paracetamol reduces foetal testicular testosterone production, consistent with reported epidemiological associations between prenatal paracetamol exposure and cryptorchidism.

**STUDY DESIGN, SIZE, DURATION:** Prospective cohort study (Cambridge Baby Growth Study), with recruitment of pregnant women at ~12 post-menstrual weeks of gestation from a single UK maternity unit

between 2001 and 2009, and 24 months of infant follow-up. Of 2229 recruited women, 1640 continued with the infancy study after delivery, of whom 676 delivered male infants and completed a medicine consumption questionnaire.

**PARTICIPANTS/MATERIALS, SETTING, METHOD:** Mothers self-reported medicine consumption during pregnancy by a questionnaire administered during the perinatal period. Infant AGD (measured from 2006 onwards), penile length and testicular descent were assessed at 0, 3, 12, 18 and 24 months of age, and age-specific Z scores were calculated. Associations between paracetamol intake during three gestational periods (<8 weeks, 8-14 weeks and >14 weeks) and these outcomes were tested by linear mixed models. Two hundred and twenty-five (33%) of six hundred and eighty-one male infants were exposed to paracetamol during pregnancy, of whom sixty-eight were reported to be exposed during 8-14 weeks. AGD measurements were available for 434 male infants.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Paracetamol exposure during 8-14 weeks of gestation, but not any other period, was associated with shorter AGD (by 0.27 SD, 95% CI 0.06-0.48,  $P = 0.014$ ) from birth to 24 months of age. This reduction was independent of body size. Paracetamol exposure was not related to penile length or testicular descent.

**LIMITATIONS, REASONS FOR CAUTION:** Confounding by other drugs or endocrine-disrupting chemicals cannot be discounted. The cohort was not fully representative of pregnant women in the UK, particularly in terms of maternal ethnicity and smoking prevalence. There is likely to have been misclassification of paracetamol exposure due to recall error.

**WIDER IMPLICATIONS OF THE FINDINGS:** Our observational findings support experimental evidence that intrauterine paracetamol exposure during the MPW may adversely affect male reproductive development.

**STUDY FUNDING/COMPETING INTERESTS:** This work was supported by a European Union Framework V programme, the World Cancer Research Fund International, the Medical Research Council (UK), the Newlife Foundation for Disabled Children, the Evelyn Trust, the Mothercare Group Foundation, Mead Johnson Nutrition, and the National Institute for Health Research Cambridge Comprehensive Biomedical Research Centre. The authors declare no conflict of interest.

### **Third trimester phthalate exposure is associated with DNA methylation of growth-related genes in human placenta**

Zhao Y, Chen J, Wang X, Song Q, Xu HH, Zhang YH

Sci Rep. 2016 Sep 22;6:33449. doi: 10.1038/srep33449

#### **Abstract**

Strong evidence implicates maternal phthalate exposure during pregnancy in contributing to adverse birth outcomes. Recent research suggests these effects might be mediated through the improper regulation of DNA methylation in offspring tissue. In this study, we examined associations between prenatal phthalate exposure and DNA methylation in human placenta. We recruited 181 mother-newborn pairs (80 fetal growth restriction newborns, 101 normal newborns) in Wenzhou, China and measured third trimester urinary phthalate metabolite concentrations and placental DNA methylation levels of IGF2 and AHRR. We found urinary concentrations of mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) were significantly inversely associated with placental IGF2 DNA methylation.

The associations were much more evident in fetal growth restriction (FGR) newborns than those in normal newborns. These findings suggest that changes in placental DNA methylation might be part of the underlying biological pathway between prenatal phthalate exposure and adverse fetal growth.

## Bruttoliste

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## ***In vitro* studier ved DTU Fødevareinstituttet**

### **Søgt i Pubmed med følgende kriterier:**

"Endocrine disrupt\* AND in vitro\*" samt "Endocrine disrupt\* AND expose\* AND in vitro\*",

"Paraben\* AND in vitro\*,"perfluor\* OR polyfluor\* AND in vitro\*" og "Phthalat\* AND in vitro\*".

Publiceret fra i perioden 2016/06/30 to 2016/12/31.

Efter at have fjernet genganger fra forrige litteraturopdateringslister, samt artikler der ikke hørte til under kategorien "*in vitro*" gav litteratursøgningen, med de angivne søgekriterier, tilsammen en liste med i alt 40 artikler.

## Udvalgte publikationer

2 artikler er blevet udvalgt til nærmere beskrivelse baseret på, at de beskriver resultater der bidrager til ny eller yderligere viden om grupper af hormonforstyrrende stoffer eller metoder til testning af stoffer med hormonforstyrrende egenskaber.

Den første artikel omhandler et studie, hvor man har haft til formål at udvikle et *in vitro* assay, der kan erstatte den såkaldte uterotrofiske test i gævner/*in vivo*.

Den anden artikel omhandler *in vitro* studier af mykotoksinet zeralenon (ZEN) (et såkaldt mycoestrogen), dets metabolit  $\alpha$ -zeralenol ( $\alpha$ -ZEL) og et andet mykotoksin, alternariol (AOH). Formålet med studiet var at undersøge de østrogene kombinations effekterne af de tre mykotoxiner.

### **Development of an *In vitro* Assay Measuring Uterine-Specific Estrogenic Responses for Use in Chemical Safety Assessment.**

Miller MM, Alyea RA, LeSommer C, Doheny DL, Rowley SM, Childs KM, Balbuena P, Ross SM, Dong J, Sun B, Andersen MA, Clewell RA.

Toxicol Sci. 2016 Aug 7. pii: kfw152.

A toxicity pathway approach was taken to develop an *in vitro* assay using human uterine epithelial adenocarcinoma (Ishikawa) cells as a replacement for measuring an *in vivo* uterotrophic response to estrogens. The Ishikawa cell was determined to be fit for the purpose of recapitulating *in vivo* uterine response by verifying fidelity of the biological pathway components and the dose-response predictions to women of child-bearing age. Expression of the suite of estrogen receptors that control uterine proliferation (ER $\alpha$ 66, ER $\alpha$ 46, ER $\alpha$ 36, ER $\beta$ , G-protein coupled estrogen receptor (GPER)) were confirmed across passages and treatment conditions. Phenotypic responses to ethinyl estradiol (EE) from transcriptional activation of ER-mediated genes, to ALP enzyme induction and cellular proliferation occurred at concentrations consistent with estrogenic activity in adult women (low picomolar). To confirm utility of this model to predict concentration-response for uterine proliferation with xenobiotics, we tested the concentration-response for compounds with known uterine estrogenic activity in humans and compared the results to assays from the ToxCast and Tox21 suite of estrogen assays. The Ishikawa proliferation assay was consistent with *in vivo* responses and was a more sensitive measure of uterine response. Because this assay was constructed by first mapping the key molecular events for cellular response, and then ensuring that the assay incorporated these events, the resulting cellular assay should be a reliable tool for identifying estrogenic compounds and may provide improved quantitation of chemical concentration response for *in vitro*-based safety assessments.

### **Synergistic estrogenic effects of Fusarium and Alternaria mycotoxins *in vitro*.**

Vejdovszky K, Hahn K, Braun D, Warth B, Marko D.

Arch Toxicol. 2016 Jul 11.

Mycotoxins are toxic secondary metabolites formed by various fungal species that are found as natural contaminants in food. This very heterogeneous group of compounds triggers multiple toxic mechanisms, including endocrine disruptive potential. Current risk assessment of mycotoxins, as for most chemical substances, is based on the effects of single compounds. However, concern on a potential enhancement of risks by interactions of single substances in naturally occurring mixtures has greatly increased recently. In this study, the combinatory effects of three mycoestrogens were investigated in detail. This includes the endocrine disruptors zearalenone (ZEN) and  $\alpha$ -zearalenol ( $\alpha$ -ZEL) produced by *Fusarium* fungi and alternariol (AOH), a cytotoxic and estrogenic mycotoxin formed by *Alternaria* species. For evaluation of effects, estrogen-dependent activation of alkaline phosphatase (AIP) and cell proliferation were tested in the adenocarcinoma cell line Ishikawa. The estrogenic potential varied among the single substances. Half maximum effect concentrations (EC50) for AIP activation were evaluated for  $\alpha$ -ZEL, ZEN and AOH as 37 pM, 562 pM and 995 nM, respectively. All three mycotoxins were found to act as partial agonists. The majority of binary combinations, even at very low concentrations in the case of  $\alpha$ -ZEL, showed strong synergism in the AIP assay. These potentiating phenomena of mycotoxin mixtures highlight the urgent need to incorporate combinatory effects into future risk assessment, especially when endocrine disruptors are involved. To the best of our knowledge, this study presents the first investigation on synergistic effects of mycoestrogens.



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*Toxicol In Vitro*. 2016 Oct;36:210-5. doi: 10.1016/j.tiv.2016.08.003. Epub 2016 Aug 7.

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*Sci Rep*. 2016 Aug 9;6:31281. doi: 10.1038/srep31281.

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*Toxicol Sci*. 2016 Jul 29. pii: kfw134. [Epub ahead of print]

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Luo S, Fang HQ, Yang H, Zhang LS, Jia XD.

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## ***In Vivo* studier ved DTU Fødevareinstituttet**

### **Søgning er udført på PubMed og dækker perioden Juli - ultimo September 2016**

Følgende søgeprofil er benyttet i PubMed: ((endocrine disrupt\*) AND (rat OR mice OR mammal\*)) OR ((endocrine disrupt\*) AND (in vivo\*)) OR ((endocrine disrupt\*) AND (Paraben\*)) OR ((endocrine disrupt\*) AND (Phthalat\*)) OR ((Endocrine disrupt\* AND (antiandrogen)) OR ((endocrine disrupt\*) AND (behaviour OR behavior\*)) OR ((Endocrine disrupt\*) AND (Bisphenol A or BPA) OR ((PFAS\* OR Perfluor\*) AND (endocrine disrupt\*) AND risk assessment

Efter at have fjernet gengangere fra dem vi havde med på den forrige litteraturopdateringsliste samt *in vitro*, human eller SDU relevante artikler, gav litteratursøgningen en liste med i alt 54 artikler (Bruttolisten).

## Udvalgte publikationer

To artikler er blevet udvalgt til nærmere beskrivelse (abstrakt og konklusion) og to artikler er udvalgt blot til abstract. Disse artikler er valgt fordi vi mener de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på Vinclozolin og NMDR (Flick et al. 2016) samt påvirkninger af anogenital afstand efter pre- og postnatale eksponeringer (Kita et al. 2016). De 2 artikler hvor der er medtaget abstracts fra en artikel der foreslår en systematisk gennemgang og integreret vurdering (SYRINA) af hormonforstyrrende kemikalier (Vandenberg et al. 2016) og et studie med Lavdosis eksponering for bisphenol A i rotter og påvirkning af knogle geometri (Lejonklou et al. 2016).

### Rigtig God læselyst.

Ud fra bruttolisten (se længere nede i dokumentet) er udvalgt følgende 2 artikler til engelsk abstrakt og dansk resume og 2 artikler blot med deres abstract.

#### **Investigations of putative reproductive toxicity of low-dose exposures to vinclozolin in Wistar rats.**

Flick B, Schneider S, Melching-Kollmuss S, Fussell KC, Gröters S, Buesen R, Strauss V, van Ravenzwaay B. Arch Toxicol. 2016 Sep 9.

The current investigation examines whether the fungicide vinclozolin, which has an anti-androgenic mode of action, is capable of disrupting endocrine homeostasis at very low doses. The data generated clarify whether a non-monotonic dose-response relationship exists to enhance the current debate about the regulation of endocrine disruptors. Moreover, it is part of a series of investigations assessing the dose-response relationship of single and combined administration of anti-androgenic substances. A pre-postnatal in vivo study design was chosen which was compliant with regulatory testing protocols. The test design was improved by additional endpoints addressing hormone levels, morphology and histopathological examinations. Doses were chosen to represent an effect level (20 mg/kg bw/d), the current NOAEL (4 mg/kg bw/d), and a dose close to the "ADI" (0.005 mg/kg bw/d) for the detection of a possible non-monotonic dose-response curve. Anti-androgenic changes were observable at the effect level but not at lower exposures. Nipple/areola counts appeared to be the most sensitive measure of effect, followed by male sex organ weights at sexual maturation, and finally gross and histopathological findings. The results indicate the absence of evidence for effects at low or very low dose levels. A non-monotonic dose-response relationship was not evident.

#### **Manipulation of pre and postnatal androgen environments and anogenital distance in rats.**

Kita DH, Meyer KB, Venturelli AC, Adams R, Machado DL, Morais RN, Swan SH, Gennings C, Martino-Andrade AJ.

Toxicology. 2016 Sep 14. pii: S0300-483X(16)30196-2. doi: 10.1016/j.tox.2016.08.021.

We examined the anogenital distance (AGD) plasticity in rats through the manipulation of the androgen environment in utero and during puberty. Dams were treated from gestation days 13-20 with vehicle, flutamide (20mg/kg/day), di-(2-ethylhexyl) phthalate (DEHP, 750mg/kg/day), or testosterone

(1.0mg/kg/day). After weaning, male pups were randomly assigned to one of four postnatal groups, which received the same treatments given prenatally. Sixteen treatment groups were established based on the combination of pre- and postnatal exposures. The postnatal treatments were conducted from postnatal days 23-53. In utero flutamide and DEHP exposure significantly shortened male AGD, although this effect was more pronounced in flutamide-exposed rats. Postnatal flutamide, DEHP, and testosterone induced slight but significant reductions in male AGD. Our study indicates that AGD is a stable anatomical landmark that reflects the androgen action in utero, although it can also be slightly responsive to changes in the androgen environment following pubertal exposure.

**A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals.** Vandenberg LN, Ågerstrand M, Beronius A, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS, Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ, Rudén C. *Environ Health*. 2016 Jul 14;15(1):74. doi: 10.1186/s12940-016-0156-6.

#### BACKGROUND:

The issue of endocrine disrupting chemicals (EDCs) is receiving wide attention from both the scientific and regulatory communities. Recent analyses of the EDC literature have been criticized for failing to use transparent and objective approaches to draw conclusions about the strength of evidence linking EDC exposures to adverse health or environmental outcomes. Systematic review methodologies are ideal for addressing this issue as they provide transparent and consistent approaches to study selection and evaluation. Objective methods are needed for integrating the multiple streams of evidence (epidemiology, wildlife, laboratory animal, in vitro, and in silico data) that are relevant in assessing EDCs.

#### METHODS:

We have developed a framework for the systematic review and integrated assessment (SYRINA) of EDC studies. The framework was designed for use with the International Program on Chemical Safety (IPCS) and World Health Organization (WHO) definition of an EDC, which requires appraisal of evidence regarding 1) association between exposure and an adverse effect, 2) association between exposure and endocrine disrupting activity, and 3) a plausible link between the adverse effect and the endocrine disrupting activity.

#### RESULTS:

Building from existing methodologies for evaluating and synthesizing evidence, the SYRINA framework includes seven steps: 1) Formulate the problem; 2) Develop the review protocol; 3) Identify relevant evidence; 4) Evaluate evidence from individual studies; 5) Summarize and evaluate each stream of evidence; 6) Integrate evidence across all streams; 7) Draw conclusions, make recommendations, and evaluate uncertainties. The proposed method is tailored to the IPCS/WHO definition of an EDC but offers flexibility for use in the context of other definitions of EDCs.

#### CONCLUSIONS:

When using the SYRINA framework, the overall objective is to provide the evidence base needed to support decision making, including any action to avoid/minimise potential adverse effects of exposures. This framework allows for the evaluation and synthesis of evidence from multiple evidence streams. Finally, a decision regarding regulatory action is not only dependent on the strength of evidence, but also the consequences of action/inaction, e.g. limited or weak evidence may be sufficient to justify action if consequences are serious or irreversible.

#### **Low-dose developmental exposure to bisphenol A alters the femoral bone geometry in wistar rats.**

Lejonklou MH, Christiansen S, Örborg J, Shen L, Larsson S, Boberg J, Hass U, Lind PM. *Chemosphere*. 2016 Sep 1;164:339-346. doi: 10.1016/j.chemosphere.2016.08.114.

Bisphenol A (BPA) is a chemical produced in large volumes for use in manufacturing of consumer products and industrial applications, and an endocrine disruptor known to affect several hormonal systems. Bone

produces hormones and is additionally a sensitive hormone target tissue, and is thus potentially sensitive to low doses of endocrine disruptors such as BPA, especially during development.

**METHODS:**

110 pregnant Wistar rats were gavaged with 0; 25 µg; 250 µg; 5000 µg or 50,000 µg BPA/kg bodyweight (bw)/day from gestational day 7 until weaning at postnatal day 22. The three-month-old offspring were sacrificed and right femurs collected for length measurements, geometrical measurements by peripheral quantitative computed tomography (pQCT), as well as for analyses of biomechanical properties using the three-point-bending method.

**RESULTS:**

The femur was elongated in female offspring of dams exposed to 25 or 5000 µg BPA/kg bw/day (1.8% and 2.1%, respectively), and increased cortical thickness (4.7%) was observed in male offspring of dams exposed to 25 µg BPA/kg bw/day, compared to controls ( $p < 0.005$ ). The biomechanical properties of the bone were not significantly altered.

**CONCLUSIONS:**

In utero and lactational exposure to the lowest BPA dose used in this study altered femoral geometry in both male and female offspring. This was observed at 25 µg BPA/kg bw/day, a dose lower than the Human Equivalent Dose (HED) applied by EFSA to set a temporary TDI (609 µg BPA/kg bw/day), and far lower than the No-Observed-Adverse-Effect-Level (NOAEL) (5000 µg BPA/kg bw/day) on which the US FDA TDI is based.



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## Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Science (all databases) og dækker perioden 8/7 - 23/9 2016.

Søgeprofilen kombinerer: "Endocrine disrupt\*" and

- Fish\*
- Amphibia\*
- Bird\* OR avia\*
- Invertebrat\*
- Mollus\*
- Gastropod\*
- Insect\*
- Crustacea\*
- Echinoderm\*
- Ursus
- Reptil\* OR alligator
- Whal\* OR seal\* OR dolphin\*

Fra bruttolisten (længere nede i dokumentet) er udvalgt tre artikler til medtagelse af abstract og yderligere kommentarer. Kriterierne for udvælgelsen af publikationer til kommentering er, at de bidrager til ny viden omkring effekter af og virkningsmekanismer for hormonforstyrrende stoffer i 'wildlife' og/eller at de repræsenterer vigtig viden, som vurderes at have særlig interesse for Miljøstyrelsen bl.a. i forbindelse med styrelsens fokus på udvikling af testmetoder. Desuden kommenteres artikler, der omhandler 'nye' stoffer, der har vist sig hormonforstyrrende; specielt hvis disse har relevans for danske forhold.

## Udvalgte publikationer

### **Impaired anterior swim bladder inflation following exposure to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole part II: Zebrafish**

Stinckens E, Vergauwen L, Schroeder AL, Maho W, Blackwell BR, Witters H, Blust R, Ankley GT, Covaci A, Villeneuve DL, Knapen D.

Aquatic Toxicology. 173: 204-217. 2016.

#### ABSTRACT:

Disruption of the thyroid hormone (TH) system, an important mode of action, can lead to ecologically relevant adverse outcomes, especially during embryonic development. The present study characterizes the effects of disruption of TH synthesis on swim bladder inflation during zebrafish early-life stages using 2-mercaptobenzothiazole (MBT), a thyroid peroxidase (TPO) inhibitor. Zebrafish were exposed to different MBT concentrations until 120/168 h post fertilization (hpf) and 32 days post fertilization (dpf), in two sets of experiments, to investigate the effects of TPO inhibition on posterior and anterior swimbladder inflation respectively, as well as whole body thyroid hormone concentrations (triiodothyronine (T3) and its prohormone, thyroxine (T4)). At 120 hpf, MBT did not directly impair posterior chamber inflation or size, while anterior chamber inflation and size was impaired at 32 dpf. As previously shown in amphibians and mammals, we confirmed that MBT inhibits TPO in fish. Whole-body T4 decreased after MBT exposure at both time points, while T3 levels were unaltered. There was a significant relationship between T4 levels and the anterior chamber surface at 32 dpf. The absence of effects on posterior chamber inflation can possibly be explained by maternal transfer of T4 into the eggs. These maternally derived THs are depleted at 32 dpf and cannot offset TPO inhibition, resulting in impaired anterior chamber inflation. Therefore, we hypothesize that TPO inhibition only inhibits swim bladder inflation during late development, after depletion of maternally derived T4. In a previous study, we showed that iodothyroninedeiodinase (ID) knockdown impaired posterior chamber inflation during early development. Our findings, in parallel with similar effects observed in fathead minnow (see part I, this issue) suggest that thyroid disruption impacts swim bladder inflation, and imply an important distinction among specific subtypes of TH disrupting chemicals. However, the existence of another – yet unknown – mode of action of MBT impacting swim bladder inflation cannot be excluded. These results can be helpful for delineating adverse outcome pathways (AOPs) linking TPO inhibition, ID inhibition and other TH related molecular initiating events, to impaired swim bladder inflation in fish during early life stages. Such AOPs can support the use of in vitro enzyme inhibition assays for predicting reduced survival due to impaired posterior and anterior chamber inflation.

### **Perchlorate Exposure Reduces Primordial Germ Cell Number in Female Threespine Stickleback.**

Petersen AM, Earp NC, Redmond ME, Postlethwait JH, von Hippel FA, Buck C, Cresko WA.

Plos One. 11(7): e0157792. 2016.

ABSTRACT: Perchlorate is a common aquatic contaminant that has long been known to affect thyroid function in vertebrates, including humans. More recently perchlorate has been shown to affect primordial sexual differentiation in the aquatic model fishes zebrafish and threespine stickleback, but the mechanism has been unclear. Stickleback exposed to perchlorate from fertilization have increased androgen levels in the embryo and disrupted reproductive morphologies as adults, suggesting that perchlorate could disrupt the earliest stages of primordial sexual differentiation when primordial germ cells (PGCs) begin to form the gonad. Female stickleback have three to four times the number of PGCs as males during the first weeks of development. We hypothesized that perchlorate exposure affects primordial sexual differentiation by reducing the number of germ cells in the gonad during an important window of stickleback sex

determination at 14–18 days post fertilization (dpf). We tested this hypothesis by quantifying the number of PGCs at 16 dpf in control and 100 mg/L perchlorate-treated male and female stickleback. Perchlorate exposure from the time of fertilization resulted in significantly reduced PGC number only in genotypic females, suggesting that the masculinizing effects of perchlorate observed in adult stickleback may result from early changes to the number of PGCs at a time critical for sex determination. To our knowledge, this is the first evidence of a connection between an endocrine disruptor and reduction in PGC number prior to the first meiosis during sex determination. These findings suggest that a mode of action of perchlorate on adult reproductive phenotypes in vertebrates, including humans, such as altered fecundity and sex reversal or intersex gonads, may stem from early changes to germ cell development.

**Comparative sensitivity of juvenile and adult *Potamopyrgus antipodarum* (Mollusca: Hydrobiidae) under chronic exposure to cadmium and tributyltin.**

Ruppert K, GeiSS C, Ostermann S, Theis C, Oehlmann J.

Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering. 51(9): 736-743. 2016.

ABSTRACT: In this study, we assessed the chronic effects of the two antimicrobial substances triclocarban (TCC) and triclosan (TCS) on reproduction of a mollusk species by using the reproduction test with the New Zealand mudsnail *Potamopyrgus antipodarum*. Snails coming from a laboratory culture were exposed for 28 days to nominal concentrations ranging from 0.1 up to 10 mg/L for both chemicals (measured 0.082–8.85 mg TCC/L; 0.068–6.26 mg TCS/L). At the end of the experiment, snails were dissected and embryos in the brood pouch were counted to assess the individualized reproductive success of adult snails. Exposure to TCC resulted in an inverted u-shaped concentration–response relationship, with a stimulation of reproduction at low concentrations followed by an inhibition at higher concentrations. The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were 0.082 and 0.287 mg/L, respectively. TCS caused significantly increased embryo numbers at all tested concentrations, except in the group of 0.170 mg/L. Therefore, the NOEC for TCS was 0.170 mg/L and the LOEC was 0.660 mg/L. These results indicate that TCC and TCS may cause reproductive effects at environmentally relevant concentrations indicating a potential risk for aquatic organisms in the environment.

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Environmental Pollution. 216: 591-598. 2016.
2. A review of reproductive toxicity of microcystins.  
Chen L, Chen J, Zhang X, Xie P.  
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