CENTER FOR HORMONFORSTYRRENDE STOFFER

**Litteraturgennemgang for perioden marts 2017 – juni 2017**

# Indhold

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

Søgning er udført på PubMed og dækker perioden 14. marts - 20. juni 2017

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 frasorteret. De kommenterede artikler er highlightet.

Der er denne gang udvalgt artikler med fokus på BPA. Første artikel er et kinesisk studie, der undersøger niveauet af BPA, UV-filtre mm i børnetøj, hvilket kunne være interessant at efterprøve i en dansk kontekst. Derudover er en artikel om prænatalt BPA-niveau i relation til kognitiv funktion hos børn udvalgt. Fundene i studiet er ikke imponerende til trods for det store studiemateriale, men det giver anledning til at diskutere relevante eksponeringsvinduer. Slutteligt er en artikel om BPA-niveauet i graviditeten i relation til blodsukkerniveauet valgt til kommentering, da studiet inkluderer gentagne BPA-målinger og bidrager til diskussionen om timing af eksponering. Slutteligt vises abstractet på en fjerde artikel; der er tale om et case-control studie mellem gravide, der fødte for tidligt og gravide, der fødte til tiden, hvor det blev undersøgt om gentagne BPA-målinger var associeret til thyroidea-niveauet.

God læselyst

## Udvalgte artikler

**Bisphenols, Benzophenones, and Bisphenol A Diglycidyl Ethers in Textiles and Infant Clothing.**
Xue J, Liu W, Kannan K.
Environ Sci Technol. 2017 May 2;51(9):5279-5286. doi: 10.1021/acs.est.7b00701. Epub 2017 Apr 13.

**Abstract**
Little is known with regard to the occurrence of potentially toxic chemicals in textiles and clothes. In this study, 77 textiles and infant clothing pieces were analyzed for the determination of bisphenols including bisphenol A (BPA) and bisphenol S (BPS), benzophenones, bisphenol A diglycidyl ethers (BADGEs), and novolac glycidyl ethers (NOGEs). BPA and BPS occurred in 82% and 53% of the textile samples, respectively, and at mean concentrations of 366 and 15 ng/g, respectively. Benzophenone-3 (BP3) occurred in 70% of the samples at a mean concentration of 11.3 ng/g. Among 11 BADGEs and NOGEs analyzed, BFDGE was the predominant compound, with a mean concentration of 13.6 ng/g. Concentrations of target chemicals were assessed by fabric type, color, and uses. Socks contained the highest concentrations of BPA (mean: 1810 ng/g) with concentrations as high as 13 300 ng/g in a 97% polyester fabric marketed for infants. Calculated dermal exposure dose to BPA by infants via textiles was as high as 7280 pg/kg BW/d. This is the first study to report the occurrence of, and exposure to, BPA, BPS, BADGEs, and NOGEs in textiles and clothing.

**Associations of Prenatal Urinary Bisphenol A Concentrations with Child Behaviors and Cognitive Abilities**
Joseph M. Braun, Gina Muckle, Tye Arbuckle, Maryse F. Bouchard, William D. Fraser, Emmanuel Ouellet, Jean R. Séguin, Youssef Oulhote, Glenys M. Webster, and Bruce P. Lanphear
Environ Health Perspect; DOI:10.1289/EHP984

**Abstract**
BACKGROUND: Prenatal bisphenol A (BPA) exposure has been associated with adverse neurodevelopment in epidemiological studies. However, prior studies had limited statistical power to examine sex-specific effects, and few examined child cognition. OBJECTIVES: We estimated the association between prenatal BPA exposure and child neurobehavior at 3 y of age in a prospective cohort of 812 mothers and their children. METHODS: We measured BPA concentration in urine samples collected at ~12 wk gestation among women enrolled in a 10-city Canadian cohort study. At approximately 3 y of age, we assessed children's cognitive abilities with the Wechsler Primary and Preschool Scale of IntelligenceE–III (WPPSI-III) and two scales of the Behavior Rating Inventory of Executive Function–Preschool (BRIEF-P). Parents reported children's behavior using the Behavior Assessment System for Children–2 (BASC-2). We estimated covariate-adjusted differences in neurobehavioral outcomes with a doubling in BPA concentration and sex-specific associations. RESULTS: BPA was not associated with WPPSI-III scores; child sex did not modify these associations. The association between BPA and BRIEF-P scores was modified by child sex (BPA × sex p-values≤ 0:03). For example, a doubling of BPA concentration was associated with 1-point (95% CI: 0.3, 1.7) poorer working memory in boys and 0.5-point (95% CI: −1:1, 0.1) better scores in girls. BPA was not associated with most BASC-2 scales; however, it was associated with more internalizing and somatizing behaviors in boys, but not in girls (BPA × sex p-values≤ 0:08). A doubling of BPA concentration was associated with poorer SRS-2 scores [b= 0:3 ( 95% CI: 0, 0.7)]; this association was not modified by sex.

CONCLUSION: Prenatal urinary BPA concentration was associated with some aspects of child behavior in this cohort, and some associations were stronger among boys.

**Trimester-Specific Urinary Bisphenol A Concentrations and Blood Glucose Levels Among Pregnant Women From a Fertility Clinic.**

Chiu YH, Mínguez-Alarcón L, Ford JB, Keller M, Seely EW, Messerlian C, Petrozza J, Williams PL, Ye X, Calafat AM, Hauser R, James-Todd T; for EARTH Study Team.

J Clin Endocrinol Metab. 2017 Apr 1;102(4):1350-1357. doi: 10.1210/jc.2017-00022.

**Abstract**

CONTEXT: Women with a history of infertility are at increased risk of impaired glucose tolerance during pregnancy. Studies suggest higher urinary bisphenol A (BPA) concentrations are associated with diabetes in nonpregnant populations, but the association between BPA and glucose levels among pregnant women is unclear.
OBJECTIVE: To assess trimester-specific urinary BPA concentrations in relation to blood glucose levels among subfertile women.
DESIGN: Environment and Reproductive Health Study, an ongoing prospective cohort study.
SETTING: A fertility center in a teaching hospital.
PATIENTS: A total of 245 women contributed at least one urine sample during first and/or second trimesters, delivered a singleton or twin pregnancy, and had available blood glucose data (2005 to 2015).
MAIN OUTCOME MEASURE: Blood glucose levels after a nonfasting 50-g glucose challenge test at 24 to 28 weeks of gestation.
RESULTS: The specific gravity-adjusted geometric mean urinary BPA concentrations during first and second trimesters were 1.39 and 1.27 μg/L, respectively. Second-trimester BPA concentrations were positively associated with blood glucose (P, trend = 0.01). Specifically, the adjusted mean glucose levels (95% confidence interval) for women in the highest quartile of second-trimester BPA concentrations was 119 (112, 126) mg/dL compared with 106 (100, 112) mg/dL for women in the lowest quartile. No associations were observed between first-trimester BPA concentrations and glucose levels.
CONCLUSIONS: BPA exposure during the second trimester may have adverse effect on blood glucose levels among subfertile women. As the findings represent the first report suggesting a potential etiologically relevant window for BPA and glucose in humans, further studies are needed.

**Thyroid hormone parameters during pregnancy in relation to urinary bisphenol A concentrations: A repeated measures study.**

Aung MT, Johns LE, Ferguson KK, Mukherjee B, McElrath TF, Meeker JD.

Environ Int. 2017 Jul;104:33-40. doi: 10.1016/j.envint.2017.04.001. Epub 2017 Apr 13.

**Abstract**

BACKGROUND: Maternal supply of thyroid hormones during pregnancy serves a critical role in fetal development. Although animal and in vitro studies provide evidence for thyroid hormone disruption as a

result of bisphenol A (BPA) exposure, there is still a lack of evidence in human studies, particularly in the context of pregnancy.
OBJECTIVES: We aimed to explore the associations between urinary BPA concentrations and plasma thyroid hormone parameters during gestation in pregnant women, and also investigated potential windows of vulnerability during gestation.
METHODS: Our study population included 116 cases of preterm birth and 323 controls from a nested case-control study. We measured BPA in urine and thyroid hormone parameters in plasma samples collected at up to four study visits during pregnancy (median for each visit: 9.64, 17.9, 26.0, and 35.1weeks gestation). We used linear mixed models for repeated measures analyses, and multivariate linear regression models stratified by study visit to explore potential windows of susceptibility.
RESULTS: In our repeated measures analysis, BPA and thyrotropin (TSH) were inversely associated. An interquartile range (IQR) increase in BPA was associated with an 8.21% decrease in TSH (95% confidence interval [CI]: -14.2, -1.83), and a 4.79% increase in free T4 (95% CI: 0.82, 8.92). BPA and TSH were also inversely associated in our cross-sectional analyses at visits 3 and 4.
CONCLUSIONS: Our results suggest that TSH is inversely associated with urinary BPA in a consistent manner across pregnancy. Disruption of TSH levels during pregnancy can potentially impact child development and interfere with normal birth outcomes.

## Bruttoliste

**1. Associations of Prenatal Urinary Bisphenol A Concentrations with Child Behaviors and Cognitive Abilities**
**Joseph M. Braun, Gina Muckle, Tye Arbuckle, Maryse F. Bouchard, William D. Fraser, Emmanuel Ouellet, Jean R. Séguin, Youssef Oulhote, Glenys M. Webster, and Bruce P. Lanphear**
**Environ Health Perspect; DOI:10.1289/EHP984**

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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 i perioden april - medio juni.

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 28 artikler.

To artikler er vist med abstract fordi de beskriver henholdsvis metoder og resultater, der bidrager til yderligere viden vedrørende testning, samt effekter af hormonforstyrrende stoffer.

Den første artikel omhandler udvikling af en protokol, der kan bruges til at skelne mellem cytotoksiske effekter og reelle (AR)-antagonistiske effekter i forbindelse med *in vitro* testning og anvendelsen af reporter-gen assays.
Den anden artikel omhandler *in vitro* studier af 15 phthalater og 19 phthalat metabolitter med det formål at undersøge deres hormonforstyrrende effekter på endpoints relaterede til henholdsvis ERα, ERβ og AR aktivitet.

## Udvalgte publikationer

**Differentiating true androgen receptor inhibition from cytotoxicity-mediated reduction of reporter-gene transactivation in-vitro.**

Marin-Kuan M, Fussell KC, Riederer N, Latado H, Serrant P, Mollergues J, Coulet M, Schilter B.
Toxicol In Vitro. 2017 Apr 1. pii: S0887-2333(17)30085-1. doi: 10.1016/j.tiv.2017.03.014.

**Abstract**

In vitro effect-based reporter assays are applied as biodetection tools designed to address nuclear receptor mediated-modulation. While such assays detect receptor modulating potential, cell viability needs to be addressed, preferably in the same well. Some assays circumvent this by co-transfecting a second constitutively-expressed marker gene or by multiplexing a cytotoxicity assay. Some assays, such as the CALUX®, lack this feature. The cytotoxic effects of unknown substances can confound in vitro assays, making the interpretation of results difficult and uncertain, particularly when assessing antagonistic activity. It's necessary to determine whether the cause of the reporter signal decrease is an antagonistic effect or a non-specific cytotoxic effect. To remedy this, we assessed the suitability of multiplexing a cell viability assay within the CALUX® transcriptional activation test for anti-androgenicity. Tests of both well-characterized anti-androgens and cytotoxic compounds demonstrated the suitability of this approach for discerning between the molecular mechanisms of action without altering the nuclear receptor assay; though some compounds were both cytotoxic and anti-androgenic. The optimized multiplexed assay was then applied to an uncharacterized set of polycyclic aromatic compounds. These results better characterized the mode of action and the classification of effects. Overall, the multiplexed protocol added value to CALUX test performance.

**Agonistic and antagonistic effects of phthalates and their urinary metabolites on the steroid hormone receptors ERα, ERβ, and AR.**

Engel A, Buhrke T, Imber F, Jessel S, Seidel A, Völkel W, Lampen A.
Toxicol Lett. 2017 May 29;277:54-63. doi: 10.1016/j.toxlet.2017.05.028.

**Abstract**

Phthalate plasticizers have been reported to exert adverse effects via activation of the estrogen receptors ERα and ERβ and inhibition of the androgen receptor AR as molecular initiating events. After oral uptake, phthalates are metabolized to their corresponding monoesters and subsequently to oxidized phthalate monoester derivatives, which are in turn conjugated to glucuronic acid and finally excreted with the urine. In contrast to the parent phthalates, toxicological data regarding their primary and secondary metabolites are rare. The present study aimed at the characterization of potential endocrine effects of 15 phthalates and 19 phthalate metabolites by using reporter gene assays to monitor human ERα, ERβ, and AR activity. In these in vitro assays, the phthalates either stimulated or inhibited ERα and ERβ activity and inhibited AR activity, whereas the phthalate metabolites had no impact on the activity

of these human hormone receptors. In contrast, the metabolites of di-(2-ethylhexyl) phthalate (DEHP) stimulated transactivation of the human peroxisome proliferator-activated receptors PPARα and PPARγ in analogous reporter gene assays, although DEHP itself did not activate these nuclear receptors. Therefore, primary and secondary phthalate metabolites appear to exert different effects at the molecular level compared to the parent compounds.

## Bruttoliste

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**10. Differentiating true androgen receptor inhibition from cytotoxicity-mediated reduction of reporter-gene transactivation in-vitro.**
**Marin-Kuan M, Fussell KC, Riederer N, Latado H, Serrant P, Mollergues J, Coulet M, Schilter B.**
**Toxicol In Vitro. 2017 Apr 1. pii: S0887-2333(17)30085-1. doi: 10.1016/j.tiv.2017.03.014.**

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Rosenmai AK, Bengtström L, Taxvig C, Trier X, Petersen JH, Svingen T, Binderup ML, Barbara Medea Alice VV, Dybdahl M, Granby K, Vinggaard AM.
Food Chem Toxicol. 2017 May 29;106(Pt A):250-259. doi: 10.1016/j.fct.2017.05.061.

**23. Agonistic and antagonistic effects of phthalates and their urinary metabolites on the steroid hormone receptors ERα, ERβ, and AR.**
**Engel A, Buhrke T, Imber F, Jessel S, Seidel A, Völkel W, Lampen A.**
**Toxicol Lett. 2017 May 29;277:54-63. doi: 10.1016/j.toxlet.2017.05.028.**

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# *In vivo* studier ved DTU Fødevareinstituttet

**Søgning er udført på PubMed og dækker perioden April - ultimo Juni 2017**

Følgende søgeprofil er benyttet i PubMed: ((endocrine disrupt*) AND (rat OR mice OR mammal*)) OR ((endocrine disrupt*) AND (in vivo*))((endocrine disrupt*) AND (Paraben*)) OR ((endocrine disrupt*) AND (Phthalat*)) OR ((PFAS* OR Perfluor*) AND (endocrine disrupt*) OR ((Endocrine disrupt* AND (antiandrogen)) OR ((endocrine disrupt*) AND (behaviour OR behavior*)) OR ((Endocrine disrupt*) AND (Bisphenol A or BPA) OR ((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 34 artikler (Bruttolisten).

Fire artikler er udvalgt med abstract, fordi vi mener de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på et nyt alternativ til BPA (Soto et al. 2017) samt en ny artikel fra CLARITY-BPA studiet (Patel et al. 2017). De 2 sidste artikler hvor der er medtaget abstracts er to artikler fra DTU Fødevareinstituttet:
En der beskriver at en kombination af lave doser af pesticider forårsager nedsat fødselsvægt hos rotter (Hass et al. 2017) og en gennemgang at vores nuværende viden om miljøkemikalier og lægemidler og deres potentielle bidrag til udviklingen af "ovarie dysgenese syndrom" (ODS) (Johansson et al. 2017).

**Rigtig god sommer & læselyst.**

Ud fra bruttolisten (se længere nede i dokumentet) er udvalgt følgende 4 artikler til engelsk abstrakt.

## Udvalgte publikationer

**Evidence of Absence: Estrogenicity Assessment of a New Food-Contact Coating and the Bisphenol Used in Its Synthesis.**

Soto AM, Schaeberle C, Maier MS, Sonnenschein C, Maffini MV.

Environ Sci Technol. 2017 Feb 7;51(3):1718-1726. doi: 10.1021/acs.est.6b04704. Epub 2017 Jan 18.

**Abstract**

Consumer concerns about exposure to substances found in food contact materials with estrogenic activity (EA) have created substantial demand for alternatives. We assessed the potential EA of both a new bisphenol monomer used to synthesize polymeric coatings for metal food-contact applications and the nonintentionally added substances (NIAS) that may migrate into food. We evaluated tetramethyl bisphenol F (TMBPF) using *in vitro* and *in vivo* assays. We extracted the polymeric coating using food simulants ethanol (50% v/v) and acetic acid (3% w/v) and measured migration using tandem liquid chromatography (LC)/mass spectrometry (MS) and LC time-of-flight MS for TMBPF and NIAS, respectively. We also tested migrants for EA using the E-SCREEN assay. TMBPF did not show estrogenic activity in the uterotrophic assay and did not alter puberty in male and female rats or mammary gland development in female rats. Neither TMBPF nor the migrants from the final polymeric coating increased proliferation of estrogen-sensitive MCF7 cells. TMBPF did not show estrogen-agonist or antagonist activity in the estrogen receptor-transactivation assay. TMBPF migration was below the 0.2 parts per billion detection limit. Our findings provide compelling evidence for the absence of EA by TMBPF and the polymeric coating derived from it and that human exposure to TMBPF would be negligible.

**Bisphenol A Exposure, Ovarian Follicle Numbers, and Female Sex Steroid Hormone Levels: Results From a CLARITY-BPA Study.**

Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A, Flaws JA.

Endocrinology. 2017 Jun 1;158(6):1727-1738. doi: 10.1210/en.2016-1887

**Abstract**

Bisphenol A (BPA) is an industrial chemical found in thermal receipts and food and beverage containers. Previous studies have shown that BPA can affect the numbers and health of ovarian follicles and the production of sex steroid hormones, but they often did not include a wide range of doses of BPA, used a small sample size, focused on relatively short-term exposures to BPA, and/or did not examine the consequences of chronic BPA exposure on the ovaries or steroid levels. Thus, this study was designed to examine the effects of a wide range of doses of BPA on ovarian morphology and sex steroid hormone production. Specifically, this study tested the hypothesis that prenatal and continuous BPA exposure reduces ovarian follicle numbers and sex steroid hormone levels. To test this hypothesis, rats were dosed with vehicle, ethinyl estradiol (0.05 and 0.5 µg/kg body weight/d), or BPA (2.5, 25, 250, 2500, and 25,000 µg/kg body weight/d) from gestation day 6 until 1 year as part of the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA). Ovaries and sera were collected on postnatal days 1, 21, and 90, and at 6 months and 1 year. The ovaries were subjected to histological evaluation of follicle

numbers and the sera were subjected to measurements of estradiol and progesterone. Collectively, these data indicate that BPA exposure at some doses and time points affects ovarian follicle numbers and sex steroid levels, but these effects are different than those observed with ethinyl estradiol exposure and some previous studies on BPA.

**Combined exposure to low doses of pesticides causes decreased birth weights in rats.**

Hass U, Christiansen S, Axelstad M, Scholze M, Boberg J.

Reprod Toxicol. 2017 May 17. pii: S0890-6238(17)30128-4. doi: 10.1016/j.reprotox.2017.05.004. [Epub ahead of print]

**Abstract**

Decreased birth weight is a common effect of many pesticides in reproductive toxicity studies, but there are no empirical data on how pesticides act in combination on this endpoint. We hypothesized that a mixture of six pesticides (cyromazine, MCPB, pirimicarb, quinoclamine, thiram, and ziram) would decrease birth weight, and that these mixture effects could be predicted by the Dose Addition model. Data for the predictions were obtained from the Draft Assessment Reports of the individual pesticides. A mixture of equi-effective doses of these pesticides was tested in two studies in Wistar rats, showing mixture effects in good agreement with the additivity predictions. Significantly lower birth weights were observed when compounds were present at individual doses below their no-observed adverse effect levels (NOAELs). These results emphasize the need for cumulative risk assessment of pesticides to avoid potentially serious impact of mixed exposure on prenatal development and pregnancy in humans.

**Environmental influences on ovarian dysgenesis - developmental windows sensitive to chemical exposures.**

Johansson HKL, Svingen T, Fowler PA, Vinggaard AM, Boberg J.

Nat Rev Endocrinol. 2017 Jul;13(7):400-414. doi: 10.1038/nrendo.2017.36. Epub 2017 Apr 28. Review.

**Abstract**

A woman's reproductive health and ability to have children directly affect numerous aspects of her life, from personal well-being and socioeconomic standing, to morbidity and lifespan. In turn, reproductive health depends on the development of correctly functioning ovaries, a process that starts early during fetal life. Early disruption to ovarian programming can have long-lasting consequences, potentially manifesting as disease much later in adulthood. A growing body of evidence suggests that exposure to chemicals early in life, including endocrine-disrupting chemicals, can cause a range of disorders later in life, such as those described in the ovarian dysgenesis syndrome hypothesis. In this Review, we discuss four specific time windows during which the ovary is particularly sensitive to disruption by exogenous insults: gonadal sex determination, meiotic division, follicle assembly and the first wave of follicle recruitment. To date, most evidence points towards the germ cell lineage being the most vulnerable to chemical exposure, particularly meiotic division and follicle assembly. Environmental chemicals and pharmaceuticals, such as bisphenols or mild analgesics (including paracetamol), can also affect the somatic cell lineages. This Review summarizes our current knowledge pertaining to environmental chemicals and pharmaceuticals, and their potential

contributions to the development of ovarian dysgenesis syndrome. We also highlight knowledge gaps that need addressing to safeguard female reproductive health.

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**1. Combined exposure to low doses of pesticides causes decreased birth weights in rats.**
**Hass U, Christiansen S, Axelstad M, Scholze M, Boberg J.**
**Reprod Toxicol. 2017 May 17. pii: S0890-6238(17)30128-4. doi: 10.1016/j.reprotox.2017.05.004. [Epub ahead of print](abstract).**

**2. Environmental influences on ovarian dysgenesis - developmental windows sensitive to chemical exposures.**
**Johansson HKL, Svingen T, Fowler PA, Vinggaard AM, Boberg J.**
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**Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A, Flaws JA.**
**Endocrinology. 2017 Jun 1;158(6):1727-1738. doi: 10.1210/en.2016-1887 (valgt).**

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

Søgningen er udført på Web of Science (all databases) og dækker perioden 31/3 - 19/6 2017.

| | | |
|---|---|---|
| Søgeprofilen kombinerer: | ”endocrine disrupt*” and | fish*<br>amphibia*<br>bird* OR avia*<br>invertebrat*<br>mollus*<br>gastropod*<br>insect*<br>crustacea*<br>echinoderm*<br>ursus<br>reptil* OR alligator<br>whal* OR seal* OR dolphin* |

Fra bruttolisten (længere nede i dokumentet) er udvalgt tre artikler til inklusion af abstract. Kriterierne for udvælgelsen af publikationer 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 udvælges artikler, der omhandler ’nye’ stoffer, der har vist sig hormonforstyrrende; specielt hvis disse har relevans for danske forhold.

## Udvalgte publikationer

**Impaired Swim Bladder Inflation in Early-Life Stage Fathead Minnows Exposed to A Deiodinase Inhibitor, Iopanoic Acid.**

Cavallin JE, Ankley GT, Blackwell BR, Blanksma CA, Fay KA, Jensen KM, Kahl MD, Knapen D, Kosian PA, Poole S, Randolph EC, Schroeder AL, Vergauwen L, Villeneuve DL.

Environmental toxicology and chemistry. Accepted preprint. 2017.

**Abstract:**

Inflation of the posterior and/or anterior swim bladder are processes previously demonstrated to be thyroid-hormone regulated. We investigated whether inhibition of deiodinases, which convert thyroxine (T4) to the more biologically-active form, 3,5,3'-triiodothyronine (T3), would impact swim bladder inflation. Two experiments were conducted using a model deiodinase inhibitor, iopanoic acid (IOP). First, fathead minnow embryos were exposed to 0.6, 1.9, or 6.0 mg/L or control water until 6 days post-fertilization (dpf) at which time posterior swim bladder inflation was assessed. To examine anterior swim bladder inflation, a second study was conducted with 6 dpf larvae exposed to the same IOP concentrations until 21 dpf. Fish from both studies were sampled for T4/T3 measurements and gene transcription analyses. Incidence and length of inflated posterior swim bladders were significantly reduced in the 6.0 mg/L treatment at 6 dpf. Incidence of inflation and length of anterior swim bladder were significantly reduced in all IOP treatments at 14 dpf, but inflation recovered by 18 dpf. Throughout the larval study, whole body T4 concentrations increased and T3 concentrations decreased in all IOP treatments. Consistent with hypothesized compensatory responses, deiodinase-2 mRNA was up-regulated in the larval study, and thyroperoxidase mRNA was down-regulated in all IOP treatments in both studies. These results support the hypothesized adverse outcome pathways linking inhibition of deiodinase activity to impaired swim bladder inflation.

**Re-evaluation of thyroid hormone signaling antagonism of tetrabromobisphenol A for validating the T3-induced Xenopus metamorphosis assay.**

Wang Y, Li Y, Qin Z, Wei W.

Journal of Environmental Sciences. 52: 325-332. 2017.

**Abstract:**

We developed the T3-induced Xenopus metamorphosis assay, which is supposed to be able to sensitively detect thyroid hormone (TH) signaling disruption of chemicals. The present study aimed to validate the T3-induced Xenopus metamorphosis assay by re-evaluating the TH signaling antagonism of tetrabromobisphenol A (TBBPA), a known TH signaling disruptor. According to the assay we developed, Xenopus tadpoles at stage 52 were exposed to 10–500 nmol/L TBBPA in the presence of 1 nmol/L T3. After 96 hr of exposure, TBBPA in the range of 10–500 nmol/L was found to significantly inhibit T3-induced morphological changes of Xenopus tadpoles in a concentration-dependent manner in term of body weight and four morphological endpoints including head area (HA), mouth width (MW), unilateral brain width/brain length (ULBW/BL), and hind-limb length/snout-vent length (HLL/SVL). The results show that these endpoints we developed are sensitive for characterizing the antagonistic effects of TBBPA on T3-

induced metamorphosis. Following a 24-hr exposure, we found that TBBPA antagonized expression of T3-induced TH-response genes in the tail, which is consistent with previous findings in the intestine. We propose that the tail can be used as an alternative tissue to the intestine for examining molecular endpoints for evaluating TH signaling disruption. In conclusion, our results demonstrate that the T3-induced Xenopus metamorphosis assay we developed is an ideal in vivo assay for detecting TH signaling disruption.

**Development and validation of an OECD reproductive toxicity test guideline with the mudsnail Potamopyrgus antipodarum (Mollusca, Gastropoda).**

Ruppert K, Geiss C, Askem C, Benstead R, Brown R, Coke M, Ducrot V, Egeler P, Holbech H, Hutchinson TH, Kinnberg KL, Lagadic L, Le Page G, Macken A, Matthiessen P, Ostermann S, Schimera A, Schmitt C, Seeland-Fremer A, Smith AJ, Weltje L, Oehlmann J.

Chemosphere. 181: 589-599. 2017.

**Abstract:**

Mollusks are known to be uniquely sensitive to a number of reproductive toxicants including some vertebrate endocrine disrupting chemicals. However, they have widely been ignored in environmental risk assessment procedures for chemicals. This study describes the validation of the *Potamopyrgus antipodarum* reproduction test within the OECD Conceptual Framework for Endocrine Disrupters Testing and Assessment. The number of embryos in the brood pouch and adult mortality serve as main endpoints. The experiments are conducted as static systems in beakers filled with artificial medium, which is aerated trough glass pipettes. The test chemical is dispersed into the medium, and adult snails are subsequently introduced into the beakers. After 28 days the reproductive success is determined by opening the brood pouch and embryo counting. This study presents the results of two validation studies of the reproduction test with eleven laboratories and the chemicals tributyltin (TBT) with nominal concentrations ranging from 10 to 1000 ng TBT-Sn/L and cadmium with concentrations from 1.56 to 25 µg/L.

The test design could be implemented by all laboratories resulting in comparable effect concentrations for the endpoint number of embryos in the brood pouch. After TBT exposure mean EC10, EC50, NOEC and LOEC were 35.6, 127, 39.2 and 75.7 ng Sn/L, respectively. Mean effect concentrations in cadmium exposed snails were, respectively, 6.53, 14.2, 6.45 and 12.6 µg/L.

The effect concentrations are in good accordance with already published data. Both validation studies show that the reproduction test with *P. antipodarum* is a well-suited tool to assess reproductive effects of chemicals.

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**Ruppert K, Geiss C, Askem C, Benstead R, Brown R, Coke M, Ducrot V, Egeler P, Holbech H, Hutchinson TH, Kinnberg KL, Lagadic L, Le Page G, Macken A, Matthiessen P, Ostermann S, Schimera A, Schmitt C, Seeland-Fremer A, Smith AJ, Weltje L, Oehlmann J.**
**Chemosphere. 181: 589-599. 2017.**

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