CENTER FOR HORMONFORSTYRRENDE STOFFER

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Indhold

Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

Søgning er udført på PubMed og dækker perioden 12. november – 14. marts 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. Endelig er rene metodeartikler til hvordan stoffer måles udeladt. De kommenterede artikler er highlightet.

De første to udvalgte artikler er baseret på samme studiemateriale og omhandler effekter af prænatal eksponering til perflourerede stoffer (artikel 1) og phthalater (artikel 2) i relation til androgen- og glucocorticoidniveauet målt i navlestrengsblod. Desuden er udvalgt en artikel omhandlende udsættelsen for phathalater og andre plastblødgørere i svenske børneinstitutioner med fokus på kildeeksponering (artikel 3), og slutteligt er en sidste artikel udvalgt med fokus på sammenhængen mellem PFAS-niveau og risikoen for brystkræft, hvor information om genetiske varianter relevante for brystkræft desuden er inkluderet (artikel 4). Desuden er en enkelt artikel (artikel 5) desuden vist med abstract.

God læselyst

Udvalgte artikler

The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study

Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, Nakazawa H, Nonomura K, Kishi R.
Environ Health Perspect. 2017 Jan;125(1):111-118. doi: 10.1289/EHP142. Epub 2016 May 24.

Abstract

BACKGROUND: Perfluorinated chemicals (PFCs) disrupt cholesterol homeostasis. All steroid hormones are derived from cholesterol, and steroid hormones such as glucocorticoids and androgenic hormones mediate several vital physiologic functions. However, the in utero effects of PFCs exposure on the homeostasis of these steroid hormones are not well understood in humans.

OBJECTIVES: We examined the relationship between prenatal exposure to perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) and cord blood levels of glucocorticoid and androgenic hormones.

METHODS: We conducted a hospital-based birth cohort study between July 2002 and October 2005 in Sapporo, Japan (n = 514). In total, 185 mother-infant pairs were included in the present study. Prenatal PFOS and PFOA levels in maternal serum samples were measured using liquid chromatography-tandem mass spectrometry (LC-MS-MS). Cord blood levels of glucocorticoid (cortisol and cortisone) and androgenic hormones [dehydroepiandrosterone (DHEA) and androstenedione] were also measured in the same way.

RESULTS: We found a dose-response relationship of prenatal PFOS, but not PFOA, exposure with glucocorticoid levels after adjusting for potential confounders. Cortisol and cortisone concentrations were -23.98-ng/mL (95% CI: -0.47.12, -11.99; p for trend = 0.006) and -63.21-ng/mL (95% CI: -132.56, -26.72; p for trend < 0.001) lower, respectively, in infants with prenatal PFOS exposure in the fourth quartile compared with those in the first quartile. The highest quartile of prenatal PFOS exposure was positively associated with a 1.33-ng/mL higher DHEA level compared with the lowest quartile (95% CI: 0.17, 1.82; p for trend = 0.017), whereas PFOA showed a negative association with DHEA levels (quartile 4 vs. quartile 1: -1.23 ng/mL, 95% CI: -1.72, -0.25; p for trend = 0.004). We observed no significant association between PFCs and androstenedione levels.

CONCLUSIONS: Our results indicate that prenatal exposure to PFCs is significantly associated with glucocorticoid and DHEA levels in cord blood.

Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study

Araki A, Mitsui T, Goudarzi H, Nakajima T, Miyashita C, Itoh S, Sasaki S, Cho K, Moriya K, Shinohara N, Nonomura K, Kishi R.
Sci Total Environ. 2017 Mar 1;581-582:297-304. doi: 10.1016/j.scitotenv.2016.12.124. Epub 2016 Dec 30.

Abstract

Di(2-ethylhexyl) phthalate (DEHP) is known for its endocrine disrupting properties. We previously demonstrated that prenatal DEHP exposure is associated with decreased progesterone levels and

testosterone/estradiol ratio in the cord blood. However, evidence of the effects of prenatal DEHP exposure on adrenal androgen and glucocorticoids in infants is scarce. Thus, the objectives of this study were to investigate the association between prenatal DEHP exposure and adrenal androgen and glucocorticoids, and to discuss its effects on steroid hormone profiles in infants. This is part of a birth cohort study: The Hokkaido Study on Environment and Children's Health, Sapporo Cohort. Among the 514 participants, 202 mother-infant pairs with available data on maternal mono(2-ethylhexyl) phthalate (MEHP), adrenal androgen (dehydroepiandrostenedione [DHEA] and androstenedione) and glucocorticoid (cortisol and cortisone) cord blood levels were included in this study. After adjusting for potential confounders, a linear regression analysis showed that maternal MEHP levels were associated with reduced cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio, whereas increased DHEA levels and DHEA/androstenedione ratio. In a quartile model, when comparing the adjusted least square means in the 4th quartile of MEHP with those in the 1st quartile, cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio decreased, whereas DHEA/androstenedione and cortisol/cortisone ratios increased. Significant p-value trends for cortisol and cortisone levels, cortisol/cortisone ratio, and glucocorticoid/adrenal androgen ratio were observed. In combination with the previous results of reduced progesterone levels and testosterone/estradiol ratio, prenatal exposure to DEHP altered the steroid hormone profiles of infants. Further studies investigating the long-term effects of DEHP exposure on growth, neurodevelopment, and gonad and reproductive function are required.

Phthalates, non-phthalate plasticizers and bisphenols in Swedish preschool dust in relation to children's exposure

Larsson K, Lindh CH, Jönsson BA, Giovanoulis G, Bibi M, Bottai M, Bergström A, Berglund M.
Environ Int. 2017 Mar 5. doi: 10.1016/j.envint.2017.02.006.

Abstract
Children are exposed to a wide range of chemicals in their everyday environments, including the preschool. In this study, we evaluated the levels of phthalates, non-phthalate plasticizers and bisphenols in dust from 100 Swedish preschools and identified important exposure factors in the indoor environment. In addition, children's total exposure to these chemicals was determined by urine analysis to investigate their relation with dust exposure, and to explore the time trends by comparing with children who provided urine fifteen years earlier. The most abundant plasticizers in preschool dust were the phthalates di-isononyl phthalate (DiNP) and di-(2-ethylhexyl) phthalate (DEHP) with geometric mean levels of 450 and 266μg/g dust, respectively, and the non-phthalate plasticizers bis(2-ethylhexyl) terephthalate (DEHT) and diisononylcyclohexane-1,2-dicarboxylate (DiNCH) found at 105 and 73μg/g dust, respectively. The levels of several substitute plasticizers were higher in newer preschools, whereas the levels of the strictly regulated phthalate di-n-butyl phthalate (DnBP) were higher in older preschools. The presence of foam mattresses and PVC flooring in the sampling room were associated with higher levels of DiNP in dust. Children's exposure from preschool dust ingestion was below established health based reference values and the estimated exposure to different phthalates and BPA via preschool dust ingestion accounted for 2-27% of the total exposure. We found significantly lower urinary levels of BPA and metabolites of strictly

regulated phthalates, but higher levels of DiNP metabolites, in urine from the children in this study compared to the children who provided urine samples fifteen years earlier.

Polymorphism in xenobiotic and estrogen metabolizing genes, exposure to perfluorinated compounds and subsequent breast cancer risk: A nested case-control study in the Danish National Birth Cohort

Ghisari M, Long M, Røge DM, Olsen J, Bonefeld-Jørgensen EC.

Environ Res. 2017 Apr;154:325-333. doi: 10.1016/j.envres.2017.01.020.

Abstract

In the present case-cohort study based on prospective data from Danish women, we aimed to estimate the main effect of polymorphisms in genes known to be involved in the steroid hormone metabolic pathway and xenobiotic metabolism on the risk of developing breast cancer. We also studied a possible effect measure modification between genotypes and levels of serum perfluoroalkylated substances (PFASs) on the risk to breast cancer. We have previously reported a weak association between serum PFASs levels and the risk of breast cancer for this study population of Danish pregnant nulliparous women as well as in a smaller case-control study of Greenlandic women. The study population consisted of 178 breast cancer cases and 233 controls (tabnulliparous and frequency matched on age) nested within the Danish National Birth Cohort (DNBC), which was established in 1996-2002. Blood samples were drawn at the time of enrollment (6-14 week of gestation). Serum levels of 10 perfluorocarboxylated acids (PFCAs), 5 perfluorosulfonated acids (PFSAs) and 1 sulfonamide (perflurooctane-sulfonamide, PFOSA) were measured. Genotyping was conducted for CYP1A1 (Ile462Val; rs1048943), CYP1B1 (Leu432Val; rs1056836), COMT (Val158Met; rs4680), CYP17A1 (A1→ A2; rs743572); CYP19A1 (C→T; rs10046) by the TaqMan allelic discrimination method. In overall, no significant associations were found between the investigated polymorphisms and the risk of breast cancer in this study among Danish women. The previously found association between PFOSA and risk of breast cancer did vary between different genotypes, with significantly increased risk confined to homozygous carriers of the following alleles: COMT (Met), CYP17 (A1) and CYP19 (C).

CONCLUSION: Our results indicate that polymorphisms in COMT, CYP17 and CYP19 which are involved in estrogen biosynthesis and metabolism can modulate the potential effects of PFOSA exposure on the development of breast cancer.

Ibuprofen results in **alterations** of **human fetal testis development**

Ben Maamar M, Lesné L, Hennig K, Desdoits-Lethimonier C, Kilcoyne KR, Coiffec I, Rolland AD1, Chevrier C, Kristensen DM, Lavoué V, Antignac JP, Le Bizec B, Dejucq-Rainsford N, Mitchell RT, Mazaud-Guittot S, Jégou B.
Sci Rep. 2017 Mar 10;7:44184. doi: 10.1038/srep44184.

Abstract

Among pregnant women ibuprofen is one of the most frequently used pharmaceutical compounds with up to 28% reporting use. Regardless of this, it remains unknown whether ibuprofen could act as an endocrine disruptor as reported for fellow analgesics paracetamol and aspirin. To investigate this, we exposed human fetal testes (7-17 gestational weeks (GW)) to ibuprofen using ex vivo culture and xenograft systems. Ibuprofen suppressed testosterone and Leydig cell hormone INSL3 during culture of 8-9 GW fetal testes with concomitant reduction in expression of the steroidogenic enzymes CYP11A1, CYP17A1 and HSD17B3, and of INSL3. Testosterone was not suppressed in testes from fetuses younger than 8 GW, older than 10-12 GW, or in second trimester xenografted testes (14-17 GW). Ex vivo, ibuprofen also affected Sertoli cell by suppressing AMH production and mRNA expression of AMH, SOX9, DHH, and COL2A1. While PGE2 production was suppressed by ibuprofen, PGD2 production was not. Germ cell transcripts POU5F1, TFAP2C, LIN28A, ALPP and KIT were also reduced by ibuprofen. We conclude that, at concentrations relevant to human exposure and within a particular narrow 'early window' of sensitivity within first trimester, ibuprofen causes direct endocrine disturbances in the human fetal testis and alteration of the germ cell biology.

Bruttoliste

1. Ibuprofen results in alterations of human fetal testis development.
Ben Maamar M, Lesné L, Hennig K, Desdoits-Lethimonier C, Kilcoyne KR, Coiffec I, Rolland AD1, Chevrier C, Kristensen DM, Lavoué V, Antignac JP, Le Bizec B, Dejucq-Rainsford N, Mitchell RT, Mazaud-Guittot S, Jégou B.
Sci Rep. 2017 Mar 10;7:44184. doi: 10.1038/srep44184.

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Larsson K, Lindh CH, Jönsson BA, Giovanoulis G, Bibi M, Bottai M, Bergström A, Berglund M.
Environ Int. 2017 Mar 5. pii: S0160-4120(16)30792-9. doi: 10.1016/j.envint.2017.02.006. [Epub ahead of print]

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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 2016/12/31 to 2017/03/31.

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 45 artikler.

2 artikler er blevet udvalgt til abstract, da de beskriver resultater, der bidrager til ny eller yderligere viden om grupper af hormonforstyrrende stoffer. Den første artikel omhandler et *in vitro* studie af BPA og ni strukturelle BPA analoger med det formål, at undersøge stoffernes evne til at påvirke thyroid-hormon systemet. Den anden artikel omhandler *in vitro* studier med det formål, at undersøge de cytotoksiske egenskaber af nogle af de plastblødgørere, som anvendes som alternativer til diethylhexyl phthalat (DEHP) i medicinsk udstyr.

Udvalgte publikationer

Thyroid hormone disrupting potentials of bisphenol A and its analogues - in vitro comparison study employing rat pituitary (GH3) and thyroid follicular (FRTL-5) cells.

Lee S, Kim C, Youn H, Choi K.

Toxicol In Vitro. 2017 Apr;40:297-304. doi: 10.1016/j.tiv.2017.02.004

Abstract

As adverse health effects of bisphenol A (BPA) become a growing public health concern, the chemicals substituting BPA have been increasingly used in everyday lives. BPA substitutes have been frequently detected in both environment and biota in increasing levels. However, very limited toxicological information is available for these chemicals. In the present study, thyroid disrupting effects of nine structural analogues of BPA were evaluated along with BPA, using rat pituitary (GH3) and thyroid follicular (FRTL-5) cells. Similar to BPA, its analogues caused significant down-regulation of tshβ, trα, trβ, dio1 or dio2 genes in GH3 cells, and some analogues, such as BPF, BPM or BPZ, showed even greater potency compared to BPA. In FRTL-5 cells, the genes responsible for hormone synthesis, e.g., pax8, nis, tg or tpo genes, exhibited over 1.5-fold up-regulation following exposure to BPA analogues, such as BPS. The effects on gene regulation was different by the cell line. Our results clearly show that the BPA substituting chemicals may influence thyroidhormone homeostasis by affecting thyroid regulation and hormone synthesis, often at lower doses compared to BPA. Thyroid effects of the BPA analogues deserve further investigations in experimental organisms and in human populations

In vitro cytotoxic effects of DEHP-alternative plasticizers and their primary metabolites on a L929 cell line.

Eljezi T, Pinta P, Richard D, Pinguet J, Chezal JM, Chagnon MC, Sautou V, Grimandi G, Moreau E.

Chemosphere. 2017 Apr;173:452-459. doi: 10.1016/j.chemosphere.2017.01.026.

Abstract

Phthalic acid esters have been widely used to improve the plasticity of PVC medical devices. They carry a high exposure risk for both humans and the environment in clinical situations. Our study focuses on the cytotoxicity of alternative plasticizers. Postulated primarymetabolites were synthesized, not being commercially available. Cytotoxicity assays were performed on L929 murine cells according to the ISO-EN 10993-5 standard design for the biocompatibility of medical devices. The tested concentrations of plasticizers (0.01, 0.05 and 0.1 mg/ml) covered the range likely to be found in biological fluids coming into direct contact with the medical devices. DEHP, DINP and DINCH were cytotoxic at the highest concentration (0.1 mg/ml) for 7 days of exposure. Their corresponding metabolites were found to be more cytotoxic, for the same concentration. By contrast, TOTM and its corresponding metabolite MOTM were not found to be cytotoxic. DEHA showed no cytotoxicity, but its corresponding monoester (MEHA)

produced a cytotoxic effect at 0.05 mg/ml. In clinical situations, medical devices can release plasticizers, which can come into contact with patients. In vivo, the plasticizers are quickly transformed into primary metabolites. It is therefore important to measure the effects of both the plasticizers and their corresponding metabolites. Standard first-line cytotoxicity assays should be performed to ensure biocompatibility.

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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 68 artikler (Bruttolisten).

Fire artikler er blevet udvalgt til. Disse artikler er valgt fordi vi mener, de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på Linuron (Ding et al. 2017) samt hormonforstyrrende potentiale af Deltamethrin (Slima et al. 2017). De 2 sidste artikler er dels fra en review artikel, der gennemgår testmetoder for hormonforstyrrende sundhedsskadelige virkninger (Manibusan & Touart 2017) og en artikel der gennemgår hvordan AOP kan hjælpe i brugen af forudsigelsesmodeller i regulatorisk toksikologi. (Wittwehr et al. 2017).

Udvalgte publikationer

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.

Reproductive toxicity of linuron following gestational exposure in rats and underlying mechanisms.
Ding H, Zheng W, Han H, Hu X, Hu B, Wang F, Su L, Li H, Li Y.
Toxicol Lett. 2017 Jan 15;266:49-55. doi: 10.1016/j.toxlet.2016.12.013.

Abstract
Linuron is a widely used herbicide in agriculture; its endocrine disruptive toxicity has recently received public attention. This study was designed to examine the developmental toxicity of linuron on the reproductive system of male offspring following maternal exposure. Mother rats received oral gavages of linuron, once daily, at the dose of 0, 50, 100, 150 or 200mg/kg, from gestational day (GD)13 to GD18; gonadal organs from GD20 fetuses were examined. Data indicated that exposed male offspring had a significantly shortened anogenital distance. Pathological examination further revealed a lack of fusion in the urogenital fold in treated fetuses, the damaged seminiferous tubules, and the injured Leydig cell ultrastructure. Analysis of serum testosterone concentrations at postnatal day (PND)2 showed a significant dose-related reduction (about 33.7-58.75%, r=-0.838, $p<0.05$) as compared to controls. Immunohistochemical results demonstrated a significantly reduced expression of enzymes pertinent to the testosterone production including P450scc, 3β-HSD, and PCNA in Leydig cells ($p<0.05$). qPCR studies confirmed decreased levels of mRNAs encoding P450scc, 3β-HSD and PCNA ($p<0.05$). Taken together, these data suggest that maternal exposure to linuron hampers the male gonadal organ development; this appears to be due to linuron's direct action on the production of testosterone in fetal and postnatal offspring.

Endocrine disrupting potential and reproductive dysfunction in male mice exposed to deltamethrin.
Ben Slima A, Chtourou Y, Barkallah M, Fetoui H, Boudawara T, Gdoura R.
Hum Exp Toxicol. 2017 Mar;36(3):218-226. doi: 10.1177/0960327116646617.

Abstract
Pesticide exposure may affect semen quality and male fertility in humans. The aim of the present work was to elucidate the adverse effects of deltamethrin (Delta), a synthetic pyrethroid, on exposed male mice and their offspring. Adult male Albino/Swiss mice received deltamethrin (5 mg/kg) daily for 35 days and mated with untreated females to produce offspring. Classical measurements of ejaculate and sperm quality and testicular histopathological changes were assessed. Deltamethrin treatment affects sperm quality and quantity in the ejaculated semen of mice that had also markedly impaired libido as measured by indices of mating and fertility and number of pregnant females housed with male mice exposed to this pesticide.

Exposure mice to deltamethrin significantly decreased their testosterone and inhibin B levels and affected reproductive performance. Testes of exposed mice showed marked histopathological alterations as compared to the control group. The mice exposed to 5 mg/kg body weight/day of deltamethrin showed severe alterations of the seminiferous tubules, sloughing of the germ cells, the vacuolization of germ cell cytoplasm, and the disruption of spermatogenic cells compared to the control group. Altered pregnancy outcomes were directly attributed to damage of sperm of male mice exposed to deltamethrin compared to the control group. We concluded that exposure to deltamethrin affected the reproductive system of male mice explored by altered total sperm density, motility, and morphology in mice spermatozoa.

A comprehensive review of regulatory test methods for endocrine adverse health effects M. K. Manibusan & L. W. Touart (2017): Critical Reviews in Toxicology, DOI: 10.1080/10408444.2016.1272095 http://dx.doi.org/10.1080/10408444.2016.1272095

Abstract

Development of new endocrine disruption-relevant test methods has been the subject of intensive research efforts for the past several decades, prompted in part by mandates in the 1996 Food Quality Protection Act (FQPA). While scientific understanding and test methods have advanced, questions remain on whether current scientific methods are capable of adequately addressing the complexities of the endocrine system for regulatory health and ecological risk assessments. The specific objective of this article is to perform a comprehensive, detailed evaluation of the adequacy of current test methods to inform regulatory risk assessments of whether a substance has the potential to perturb endocrine-related pathways resulting in human adverse effects. To that end, approximately 42 existing test guidelines (TGs) were considered in the evaluation of coverage for endocrine-related adverse effects. In addition to evaluations of whether test methods are adequate to capture endocrine-related effects, considerations of further enhancements to current test methods, along with the need to develop novel test methods to address existing test method gaps are described. From this specific evaluation, up to 35 test methods are capable of informing whether a chemical substance perturbs known endocrine related biological pathways. Based on these findings, it can be concluded that current validated test methods are adequate to discern substances that may perturb the endocrine system, resulting in an adverse health effect. Together, these test methods predominantly form the core data requirements of a typical food-use pesticide registration submission. It is recognized, however, that the current state of science is rapidly advancing and there is a need to update current test methods to include added enhancements to ensure continued coverage and public health and environmental protection.

How Adverse Outcome Pathways Can Aid the Development and Use of Computational Prediction Models for Regulatory Toxicology.

Wittwehr C, Aladjov H, Ankley G, Byrne HJ, de Knecht J, Heinzle E, Klambauer G, Landesmann B, Luijten M, MacKay C, Maxwell G, Meek ME, Paini A, Perkins E, Sobanski T, Villeneuve D, Waters KM, Whelan M. Toxicol Sci. 2017 Feb;155(2):326-336. doi: 10.1093/toxsci/kfw207. Review.

Abstract

Efforts are underway to transform regulatory toxicology and chemical safety assessment from a largely empirical science based on direct observation of apical toxicity outcomes in whole organism toxicity tests to a predictive one in which outcomes and risk are inferred from accumulated mechanistic understanding. The adverse outcome pathway (AOP) framework provides a systematic approach for organizing knowledge that may support such inference. Likewise, computational models of biological systems at various scales provide another means and platform to integrate current biological understanding to facilitate inference and extrapolation. We argue that the systematic organization of knowledge into AOP frameworks can inform and help direct the design and development of computational prediction models that can further enhance the utility of mechanistic and in silico data for chemical safety assessment. This concept was explored as part of a workshop on AOP-Informed Predictive Modeling Approaches for Regulatory Toxicology held September 24-25, 2015. Examples of AOP-informed model development and its application to the assessment of chemicals for skin sensitization and multiple modes of endocrine disruption are provided. The role of problem formulation, not only as a critical phase of risk assessment, but also as guide for both AOP and complementary model development is described. Finally, a proposal for actively engaging the modeling community in AOP-informed computational model development is made. The contents serve as a vision for how AOPs can be leveraged to facilitate development of computational prediction models needed to support the next generation of chemical safety assessment.

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66. A comprehensive review of regulatory test methods for endocrine adverse health effects M. K. Manibusan & L. W. Touart (2017): Critical Reviews in Toxicology, DOI: 10.1080/10408444.2016.1272095 http://dx.doi.org/10.1080/10408444.2016.1272095 valgt til Abstract

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Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Science (all databases) og dækker perioden 12/12 2016 - 30/3 2017.

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 fire artikler til medtagelse af abstract. Kriterierne for udvælgelsen 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

Frogs model man: In vivo thyroid hormone signaling during development.

Sachs LM, Buchholz DR.

Genesis. 55(1-2): 2017.

ABSTRACT:

Thyroid hormone (TH) signaling comprises TH transport across cell membranes, metabolism by deiodinases, and molecular mechanisms of gene regulation. Proper TH signaling is essential for normal perinatal development, most notably for neurogenesis and fetal growth. Knowledge of perinatal TH endocrinology needs improvement to provide better treatments for premature infants and endocrine diseases during gestation and to counteract effects of endocrine disrupting chemicals. Studies in amphibians have provided major insights to understand *in vivo* mechanisms of TH signaling. The frog model boasts dramatic TH-dependent changes directly observable in free-living tadpoles with precise and easy experimental control of the TH response at developmental stages comparable to fetal stages in mammals. The hormones, their receptors, molecular mechanisms, and developmental roles of TH signaling are conserved to a high degree in humans and amphibians, such that with respect to developmental TH signaling "frogs are just little people that hop." The frog model is exceptionally illustrative of fundamental molecular mechanisms of *in vivo* TH action involving TH receptors, transcriptional cofactors, and chromatin remodeling. This review highlights the current need, recent successes, and future prospects using amphibians as a model to elucidate molecular mechanisms and functional roles of TH signaling during post-embryonic development.

Exposure to a PBDE/OH-BDE mixture alters juvenile zebrafish (Danio rerio) development.

Macaulay LJ, Chernick M, Chen A, Hinton DE, Bailey JM, Kullman SW, Levin ED, Stapleton HM.

Environmental Toxicology and Chemistry. 36(1): 36-48. 2017.

ABSTRACT:

Polybrominated diphenyl ethers (PBDEs) and their metabolites (e.g., hydroxylated BDEs [OH-BDEs]) are contaminants frequently detected together in human tissues and are structurally similar to thyroid hormones. Thyroid hormones partially mediate metamorphic transitions between life stages in zebrafish, making this a critical developmental window that may be vulnerable to chemicals disrupting thyroid signaling. In the present study, zebrafish were exposed to 6-OH-BDE-47 (30 nM; 15 µg/L) alone, or to a low-dose (30 µg/L) or high-dose (600 µg/L) mixture of PentaBDEs, 6-OH-BDE-47 (0.5–6 µg/L), and 2,4,6-tribromophenol (5–100 µg/L) during juvenile development (9–23 d postfertilization) and evaluated for developmental endpoints mediated by thyroid hormone signaling. Fish were sampled at 3 time points and examined for developmental and skeletal morphology, apical thyroid and skeletal gene markers, and modifications in swimming behavior (as adults). Exposure to the high-dose mixture resulted in >85% mortality within 1 wk of exposure, despite being below reported acute toxicity thresholds for individual congeners. The low-dose mixture and 6-OH-BDE-47 groups exhibited reductions in body length and delayed maturation, specifically relating to swim bladder, fin, and pigmentation development. Reduced skeletal ossification was also observed in 6-OH-BDE-47–treated fish. Assessment of thyroid and osteochondral gene regulatory networks demonstrated significantly increased expression of genes that regulate skeletal

development and thyroid hormones. Overall, these results indicate that exposures to PBDE/OH-BDE mixtures adversely impact zebrafish maturation during metamorphosis.

Kinetic Determination of Vitellogenin Induction in the Epidermis of Cyprinid and Perciform Fishes: Evaluation of Sensitive Enzyme-Linked Immunosorbent Assays.

Allner B, Hennies M, Lerche CF, Schmidt T, Schneider K, Willner M, Stahlschmidt-Allner P.
Environmental Toxicology and Chemistry. 35(12): 2916-2930. 2016.

ABSTRACT:

Induction of vitellogenin (VTG) in male and immature fish is a standardized endpoint in endocrine-disruption testing. To establish a nondestructive swab sampling method, VTG induction in the epidermis of Cypriniformes and Perciformes species was investigated. Both VTG and estrogen receptor genes are expressed in epidermal cells. Immunoaffinity and mass fingerprint analyses show induction of identical VTG peptides in liver and epidermis. Induction of VTG by estradiol (E2) and bisphenol A (BPA) in the epidermis was quantified with homolog enzyme-linked immunosorbent assays. Initial values in juveniles and males were below 1 ng VTG/mL extraction buffer. Exposure to E2 led to values between 200 ng/mL and 4600 ng/mL in cyprinids and between 10 ng/mL and 81 ng/mL in perciforms. Exposure to BPA increased VTG amounts to 250 ng/mL in fathead minnows, 1360 ng/mL in goldfish, 100 ng/mL in zebrafish, and 12 ng/mL in bluegills. Serum VTG contents demonstrated a similar dose–response pattern in the epidermis and the blood. These results show that VTG induction may be reliably assessed in the skin mucus of fishes, demonstrating the suitability of this biological sample for investigating estrogenic activity in compliance with Organisation for Economic Co-operation and Development standard protocols. This broadens the perspectives in toxicological screening and environmental monitoring, reducing the number of tested animals and minimizing harmful effects for animals, allowing for follow-up of individual induction profiles.

Validation of the OECD reproduction test guideline with the New Zealand mudsnail Potamopyrgus antipodarum using trenbolone and prochloraz.

GeiSS C, Ruppert K, Askem C, Barroso C, Faber D, Ducrot V, Holbech H, Hutchinson TH, Kajankari P, Kinnberg KL, Lagadic L, Matthiessen P, Morris S, Neiman M, Penttinen OP, Sanchez-Marin P, Teigeler M, Weltje L, Oehlmann J.
Ecotoxicology. E-pub ahead of print. 2017.

ABSTRACT:

The Organisation for Economic Cooperation and Development (OECD) provides several standard test methods for the environmental hazard assessment of chemicals, mainly based on primary producers, arthropods, and fish. In April 2016, two new test guidelines with two mollusc species representing different reproductive strategies were approved by OECD member countries. One test guideline describes a 28-day reproduction test with the parthenogenetic New Zealand mudsnail *Potamopyrgus antipodarum*. The main endpoint of the test is reproduction, reflected by the embryo number in the brood pouch per

female. The development of a new OECD test guideline involves several phases including inter-laboratory validation studies to demonstrate the robustness of the proposed test design and the reproducibility of the test results. Therefore, a ring test of the reproduction test with *P. antipodarum* was conducted including eight laboratories with the test substances trenbolone and prochloraz and results are presented here. Most laboratories could meet test validity criteria, thus demonstrating the robustness of the proposed test protocol. Trenbolone did not have an effect on the reproduction of the snails at the tested concentration range (nominal: 10–1000 ng/L). For prochloraz, laboratories produced similar EC_{10} and NOEC values, showing the inter-laboratory reproducibility of results. The average EC_{10} and NOEC values for reproduction (with coefficient of variation) were 26.2 µg/L (61.7%) and 29.7 µg/L (32.9%), respectively. This ring test shows that the mudsnail reproduction test is a well-suited tool for use in the chronic aquatic hazard and risk assessment of chemicals.

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3. Kinetic Determination of Vitellogenin Induction in the Epidermis of Cyprinid and Perciform Fishes: Evaluation of Sensitive Enzyme-Linked Immunosorbent Assays.
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