

CENTER FOR HORMONFORSTYRENDE STOFFER

Litteraturgennemgang for perioden juli 2017 – oktober 2017

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Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

Søgning er udført på PubMed og dækker perioden 20.juni - 20. september 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 til dette kvartals litteraturgennemgang fokus på kildeeksponering blandt sårbare grupper, specifikt børn og gravide. De første tre artikler, der er udvalgt, fokuserer på flammehæmmere. Eksponeringen til flammehæmmere hos mennesker sker hovedsageligt via kontakt med støv og gennem kosten, og børn er en særligt udsat gruppe. I første studie er der fokus på små børns eksponering til flammehæmmere via støv, i anden artikel er der fokus på eksponeringen til flammehæmmere via kosten og i tredje artikel undersøges effekterne af eksponeringen for flammehæmmere i relation til intelligens og koncentrationsevner hos børn. De to efterfølgende artikler har fokus på gravide kvinders eksponering til plastblødgørere i hospitalsregi; første studie er et pilotstudie, hvor udsættelsen for phtalater og parabener efter ultralydsundersøgelse estimeres, og sidste studie har fokus på indholdet af forskellige plastblødgørere i medicinsk udstyr som fx katetere, slanger etc., der anvendes til gravide kvinder.

Udvalgte artikler

Brominated and organophosphorus flame retardants in body wipes and house dust, and an estimation of house dust hand-loadings in Dutch toddlers

Sugeng EJ, Leonards PEG, van de Bor M.

Environ Res. 2017 Oct;158:789-797. doi: 10.1016/j.envres.2017.07.035. Epub 2017 Jul 27.

Abstract

Children generally have higher Flame Retardant (FR) concentrations in serum compared to other age groups. Toddler behavior enhances direct contact with house dust since their frequent presence proximate to the floor, and their mouthing behavior. This study aimed to thoroughly investigate FR levels in body wipes of toddlers (8-18 months old) and in indoor dust using a noninvasive sampling technique. In this cross-sectional study, body wipes from hands, mouth and back, and indoor household dust samples were collected in twenty-one families and analyzed for one brominated- and seven organophosphorus FRs (polybrominated diphenyl ether 209 (BDE209), tris(2-chloroisopropyl) phosphate (TCIPP), tris(chloroethyl) phosphate (TCEP), tris(1,3-dichloroisopropyl) phosphate (TDCIPP), tris(phenyl) phosphate (TPHP), tris(methylphenyl) phosphate (TMPP), resorcinol bis(diphenyl phosphate) and bisphenol A bis(diphenyl phosphate)). Accelerated solvent extraction was used for extraction and the extract was measured with liquid chromatography combined with mass spectrometry. Non-parametric correlation analyses were performed to assess associations. All FRs were detected in body- and indoor dust samples (median range: 1.0ng/hand wipe (BDE209) to 65ng/hand wipe (TCIPP)) and were mostly correlated with each other. We estimated that approximately 260mg dust (range 50-880mg) accumulated on toddler's hands per day. Hand-to-mouth frequency was negatively associated with FR levels in wipes ($\tau = -0.38$, $p = 0.04$). With increasing age FR concentrations (BDE209, TCEP, TDCIPP, TPHP and TMPP) on hands decreased significantly ($p = 0.01-0.03$). Girls had significantly less FRs (TCEP, TCIPP, TPHP and TMPP) on the hands ($p = 0.01-0.03$) than boys. This is to the best of the authors' knowledge the first study in Europe that measured brominated- as well as organophosphorus FRs in several types of body wipes from toddlers and that estimated the amount of house dust that accumulates on toddler's hands.

Demographic and dietary risk factors in relation to urinary metabolites of organophosphate flame retardants in toddlers

Thomas MB, Stapleton HM, Dills RL, Violette HD, Christakis DA, Sathyanarayana S.

Chemosphere. 2017 Oct;185:918-925. doi: 10.1016/j.chemosphere.2017.07.015.

Abstract

Organophosphate flame retardants (OPFRs), including Tris (1,3-dichloro-isopropyl) phosphate (TDCPP), triphenyl phosphate (TPP), and isopropylated triphenyl phosphate (ITP), are increasingly used in consumer products because of the recent phase out of polybrominated diphenyl ether (PBDE) flame retardants. OPFRs have been widely detected in adults and have been linked to reproductive and endocrine changes in adult males. Carcinogenicity and damage to immunologic, neurologic and developmental systems have been observed in human cell lines. Young children are especially vulnerable to OPFR exposure, but little is known about exposure levels or exposure risk factors in this population. We examined parent-

reported demographic and dietary survey data in relation to OPFR urinary metabolite concentrations in 15- to 18-month old toddlers (n = 41). OPFR metabolites were detected in 100% of subjects. The metabolite of TPP, diphenyl phosphate (DPP) was detected most commonly (100%), with TDCPP metabolite, bis(1,3-dichloro-2-propyl) phosphate (BDCPP), detected in 85-95% of samples, and ITP metabolite, monoisopropylphenyl phenyl phosphate (ip-DPP), detected in 81% of samples (n = 21). Toddlers of mothers earning <\$10,000 annually had geometric mean DPP concentrations 66% higher (p = 0.05) than toddlers of mothers earning >\$10,000/year (7.8 ng/mL, 95% CI 5.03, 12.11 and 4.69 ng/mL, 95% CI 3.65-6.04, respectively). While no dietary factors were significantly associated with OPFR metabolite concentrations, results suggested meat and fish consumption may be associated with higher DPP and BDCPP levels while increased dairy and fresh food consumption may be associated with lower DPP, BDCPP, and ip-DPP levels. Research with larger sample sizes and more detailed dietary data is required to confirm these preliminary findings.

Developmental PBDE Exposure and IQ/ADHD in Childhood: A Systematic Review and Meta-analysis

Lam J, Lanphear BP, Bellinger D, Axelrad DA, McPartland J, Sutton P, Davidson L, Daniels N, Sen S, Woodruff TJ.

Environ Health Perspect. 2017 Aug 3;125(8):086001. doi: 10.1289/EHP1632.

Abstract

BACKGROUND: In the United States, one in six children are affected by neurodevelopmental disorders, and polybrominated diphenyl ethers (PBDEs) in flame-retardant chemicals are measured ubiquitously in children.

OBJECTIVE: We conducted a systematic a systematic review regarding developmental exposure to PBDEs and intelligence or Attention Deficit/ Hyperactivity Disorder (ADHD) and attention-related behavioral conditions in humans.

METHODS: We searched articles published up to 26 September 2016, and included original studies that quantified exposures to PBDEs incurred any time in proximity to conception or during in utero, perinatal, or childhood time periods. We evaluated the risk of bias of individual studies and the overall quality and strength of the evidence according to the Navigation Guide systematic review methodology. We established criteria in advance to identify studies that could be combined using random effects meta-analyses (DerSimonian-Laird method).

RESULTS: Fifteen studies met the inclusion criteria; 10 studies met the criteria for intelligence and nine for attention-related problems. We rated studies generally with “low” to “probably low” risk of bias and rated the overall body of evidence as “moderate” quality with “sufficient” evidence for an association between Intelligence Quotient (IQ) and PBDEs. Our meta-analysis of four studies estimated a 10-fold increase (in other words, times 10) in PBDE exposure associated with a decrement of 3.70 IQ points (95% confidence interval: 0.83, 6.56). We concluded the body of evidence was of “moderate” quality for ADHD with “limited” evidence for an association with PBDEs, based on the heterogeneity of association estimates reported by a small number of studies and the fact that chance, bias, and confounding could not be ruled out with reasonable confidence.

CONCLUSION: We concluded there was sufficient evidence supporting an association between developmental PBDE exposure and reduced IQ. Preventing developmental exposure to PBDEs could help prevent loss of human intelligence.

Ultrasound gel as an unrecognized source of exposure to phthalates and phenols among pregnant women undergoing routine scan

Messerlian C, Mustieles V, Wylie BJ, Ford JB, Keller M, Ye X, Calafat AM, Williams PL, Hauser R
Int J Hyg Environ Health. 2017 Aug 14. pii: S1438-4639(17)30333-4. doi: 10.1016/j.ijheh.2017.08.003.

Abstract

Systemic absorption of phthalates and parabens has been demonstrated after dermal application of body lotion, and medical devices such as intravenous bags and tubing have been identified as a source of exposure to di(2-ethylhexyl) phthalate (DEHP). However, use of products during medical procedures such as aqueous gel applied during obstetrical ultrasound in pregnancy has not been investigated as a potential source of endocrine disrupting chemical (EDC) exposure. Human studies have associated EDCs with various adverse pregnancy outcomes. There is a need to identify sources of inadvertent exposure to EDCs especially during vulnerable developmental periods such as pregnancy.

We conducted a pilot study to determine whether use of gel during routine obstetrical ultrasound increased urinary concentrations of phthalate and phenol biomarkers.

We recruited 13 women from the Massachusetts General Hospital who provided spot urine samples at the time of their second trimester anatomic survey. The first sample was collected prior to the procedure (pre-exposure, time 1), and two additional samples were obtained at approximately 1-2h (time 2) and 7-12h (time 3) post-exposure following the scan.

Urinary concentrations of several DEHP metabolites and metabolite of diisononyl cyclohexane-1,2-dicarboxylate (DINCH) increased across time. For example, the geometric mean concentrations of mono(2-ethyl-5-hydroxyhexyl) phthalate increased from 3.1ng/ml to 7.1ng/ml (p-value=0.03) between time 1 and time 3. We also observed significant differences in concentrations of metabolites of butylbenzyl phthalate (BBzP), di-n-butyl phthalate (DnBP), and di-isobutyl phthalate (DiBP). For example, mono-n-butyl phthalate (metabolite of DnBP) decreased from 3.5ng/ml to 1.8ng/ml (p-value=0.04) between time 1 and time 2, but then increased to 6.6ng/ml (p-value=0.002) at time 3. Propylparaben concentrations increased from 8.9ng/ml to 33.6ng/ml between time 1 and time 2 (p-value=0.005), followed by a decrease to 12.9ng/ml at time 3 (p-value=0.01). However, we cannot rule out the possibility that some of the observed differences are due to other sources of exposure to these compounds.

While additional research is needed, this pilot study potentially identifies a previously unknown source of phthalate and paraben exposure among pregnant women undergoing routine ultrasound examination.

Exposure of hospitalised pregnant women to plasticizers contained in medical devices

Marie C, Hamlaoui S, Bernard L, Bourdeaux D, Sautou V, Lémerly D, Vendittelli F, Sauvart-Rochat MP.
BMC Womens Health. 2017 Jun 20;17(1):45. doi: 10.1186/s12905-017-0398-7.

Abstract

BACKGROUND: Medical devices (MDs) in polyvinyl chloride (PVC) are not a well-known source of exposure to plasticizers, in particular during pregnancy. Because of its toxicity, the di-(2-ethylhexyl) phthalate (DEHP) has been replaced by other plasticizers such as di (isononyl)-cyclohexane-1,2-dicarboxylic acid (DINCH), tri-octyltrimellitate (TOTM) and di-(isononyl) phthalate (DiNP). Our study aimed to quantify the plasticizers (DEHP and alternative plasticizers) contained in PVC medical devices used for hospitalised pregnant women and to describe which these MDs had been used (type, number, duration of exposure).

METHODS: The plasticizers contained in the MDs used for daily care in the Obstetrics Department of a French University Hospital were extracted from PVC (after contact with a chloroform solution), identified and quantified by gas-chromatography-mass-spectrometry analysis. A total of 168 pregnant women hospitalised in the Obstetrics Department with at least one catheter were included in the observational study. The median number of MDs containing plasticizers used and the daily duration of exposure to the MDs were compared in three groups of pregnant women: "Pathology group" (women hospitalised for an obstetric disorder who did not give birth during this hospitalisation; n = 52), "Pathology and delivery group" (hospitalised for an obstetric disorder and who gave birth during this stay; n = 23) and "Delivery group" (admitted for planned or spontaneous delivery without obstetric disorder; n = 93).

RESULTS: DiNP, TOTM and DINCH were the predominant plasticizers contained in the MDs at an amount of 29 to 36 g per 100 g of PVC. Women in the "Pathology group" (preterm labour or other pathology) were exposed to a median number of two MDs containing TOTM and one MD containing DiNP, fewer than those in the "Pathology and delivery group" ($p < 0.05$). Women in the "Pathology group" had a median exposure of 3.4 h/day to MDs containing DiNP and 8.2 h/day to MDs containing TOTM, longer than those in the "Delivery group" ($p < 0.01$).

CONCLUSIONS: Our study shows that the medical management of pregnant women in a hospital setting entails exposure to MDs containing alternative plasticizers (DiNP, TOTM and DINCH).

Bruttoliste

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186. Estimating the health benefits of environmental regulations - Changes needed for complete benefits assessment

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Lam J, Lanphear BP, Bellinger D, Axelrad DA, McPartland J, Sutton P, Davidson L, Daniels N, Sen S, Woodruff TJ.

Environ Health Perspect. 2017 Aug 3;125(8):086001. doi: 10.1289/EHP1632.

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 fra 2017/06/30 til 2017/12/31.

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 28 artikler.

To artikler er blevet udvalgt, da de beskriver henholdsvis nye metoder og resultater, der bidrager til yderligere viden vedrørende testning, samt effekter af hormonforstyrrende stoffer. Den første artikel omhandler udvikling af en metode, der kan bruges til *high-throughput screening* for hormonforstyrrende effekter af miljø-, fødevareprøver, samt fremmedstoffer baseret på en human celle-model. Denne kan give information om hormonforstyrrende stoffers mere komplekse cellulære effekter på protein niveau. Den anden artikel beskriver et studie, hvor man har haft til formål at undersøge nogle af de mulige mekanismer bag de *in vitro* anti-androgene effekter af BPA og visse BPA analoger som tidligere studier har rapporteret.

Udvalgte publikationer

A Chip for Estrogen Receptor Action: Detection of Biomarkers Released by MCF-7 Cells through Estrogenic and Anti-Estrogenic Effects

Gier K, Preininger C, Sauer U.

Sensors (Basel). 2017 Aug 1;17(8). pii: E1760. doi: 10.3390/s17081760.

Abstract

The fluorescence-based multi-analyte chip platform for the analysis of estrogenic and anti-estrogenic substances is a new in vitro tool for the high throughput screening of environmental samples. In contrast to existing tools, the chip investigates the complex action of xenoestrogens in a human cell model by characterizing protein expression. It allows for the quantification of 10 proteins secreted by MCF-7 cells, representing various biological and pathological endpoints of endocrine action and distinguishing between estrogen- and anti-estrogen-dependent secretion of proteins. Distinct protein secretion patterns of the cancer cell line after exposure to known estrogen receptor agonists β -estradiol, bisphenol A, genistein, and nonylphenol as well as antagonists fulvestrant and tamoxifen demonstrate the potential of the chip. Stimulation of cells with Interleukin-1 β shifts concentrations of low abundant biomarkers towards the working range of the chip. In the non-stimulated cell culture, Matrix Metalloproteinase 9 (MMP-9) and Vascular Endothelial Growth Factor (VEGF) show differences upon treatment with antagonists and agonists of the estrogen receptor. In stimulated MCF-7 cells challenged with receptor agonists secretion of Monocyte Chemoattractant Protein (MCP-1), Interleukin-6 (IL-6), Rantes, and Interleukin-8 (IL-8) significantly decreases. In parallel, the proliferating effect of endocrine-disrupting substances in MCF-7 cells is assessed in a proliferation assay based on resazurin. Using ethanol as a solvent for test substances increases the background of proliferation and secretion experiments, while using dimethyl sulfoxide (DMSO) does not show any adverse effects. The role of the selected biomarkers in different physiological processes such as cell development, reproduction, cancer, and metabolic syndrome makes the chip an excellent tool for either indicating endocrine-disrupting effects in food and environmental samples, or for screening the effect of xenoestrogens on a cellular and molecular level.

Binding of bisphenol A, bisphenol AF, and bisphenol S on the androgen receptor: Coregulator recruitment and stimulation of potential interaction sites

Perera L, Li Y, Coons LA, Houtman R, van Beuningen R, Goodwin B, Auerbach SS, Teng CT.

Toxicol In Vitro. 2017 Oct;44:287-302. doi: 10.1016/j.tiv.2017.07.020. Epub 2017 Jul 24.

Abstract

Bisphenol A (BPA), bisphenol AF (BPAF), and bisphenol S (BPS) are well known endocrine disruptors. Previous in vitro studies showed that these compounds antagonize androgen receptor (AR) transcriptional activity; however, the mechanisms of action are unclear. In the present study, we investigated interactions of coregulator peptides with BPA, BPAF, or BPS at the AR complexes using Micro Array for Real-time Coregulator Nuclear Receptor Interaction (MARCoNI) assays and assessed the binding of these compounds on AR by molecular dynamics (MD) simulations. The set of coregulator peptides that are recruited by BPA-bound AR, either positively/or negatively, are different from those recruited by the agonist R1881-bound AR. Therefore, the data indicates that BPA shows no similarities to R1881 and suggests that it may recruit other coregulators to the AR complex. BPAF-bound AR recruits about 70-80% of the same coregulator peptides as BPA-bound AR. Meanwhile, BPS-bound AR interacts with only few peptides compared to BPA or BPAF-bound AR. MD results show that multiple binding sites with varying binding affinities are available on AR for BPA, BPAF, and BPS, indicating the availability of modified binding surfaces on AR for coregulator interactions. These findings help explain some of the distinct AR-related toxicities observed with bisphenol chemicals and raise concern for the use of substitutes for BPA in commercial products.

Bruttoliste

1. In Vitro Evaluation of Mitochondrial Function and Estrogen Signaling in Cell Lines Exposed to the Antiseptic Cetylpyridinium Chloride.

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Schlottz N, Kim GJ, Jäger S, Günther S, Lamy E.
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5. Brominated and organophosphate flame retardants target different neurodevelopmental stages, characterized with embryonic neural stem cells and neuronotypic PC12 cells.

Slotkin TA, Skavicus S, Stapleton HM, Seidler FJ.
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Zhang Y, Han L, Yang H, Pang J, Li P, Zhang G, Li F, Wang F.
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Lee GA, Hwang KA, Choi KC.
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***In vivo* studier ved DTU Fødevareinstituttet**

Søgning er udført på PubMed og dækker perioden Juli - ultimo September 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 30 artikler (Bruttolisten).

Fire artikler er blevet udvalgt, da vi mener, de bidrager til ny viden om hormonforstyrrende stoffer. De to første artikler har særligt fokus på kombinationseffekter af hormonforstyrrende stoffer (bl.a. pesticider) i et nyt assay "the Fetal Gonad Assay"(FEGA) (Gaudriault et al. 2017) samt en ny artikel fra Science der beskriver ny forskning mht. kønsdifferentieringen hos pattedyr (Zhao et al. 2017). Derudover er to artikler inkluderet omhandlende implementering af 3R i udviklings- og reproduktions-toksicitetstest (DART) (Beekhuijzen, 2017) og en artikel om et alternativ til phthalater, DINCH (Campioli et al. 2017).

Udvalgte publikationer

Endocrine Disruption in Human Fetal Testis Explants by Individual and Combined Exposures to Selected Pharmaceuticals, Pesticides, and Environmental Pollutants

Gaudriault P, Mazaud-Guittot S, Lavoué V, Coiffec I, Lesné L, Dejuçq-Rainsford N, Scholze M, Kortenkamp A, Jégou B. *Environ Health Perspect.* 2017 Aug 4;125(8):087004. doi: 10.1289/EHP1014..

Abstract

BACKGROUND: Numerous chemicals are capable of disrupting androgen production, but the possibility that they might act together to produce effects greater than those of the most effective component in the mixture has not been studied directly in human tissues. Suppression of androgen synthesis in fetal life has been associated with testis maldescent, malformations of the genitalia at birth, and poor semen quality later in life.

OBJECTIVES: Our aim was to investigate whether chemicals can act together to disrupt androgen production in human fetal testis explants and to evaluate the importance of mixture effects when characterizing the hazard of individual chemicals.

METHODS: We used an organotypic culture system of human fetal testes explants called FEtal Gonad Assay (FEGA) with tissue obtained at 10 and 12 gestational wk (GW 10-12), to screen 27 chemicals individually for their possible anti-androgenic effect. Based on the results of the screen, we selected 11 compounds and tested them as mixtures.

RESULTS: We evaluated mixtures composed of four and eight antiandrogens that contained the pharmaceuticals ketoconazole and theophylline and several previously untested chemicals, such as the pesticides imazalil and propiconazole. Mixtures of antiandrogens can suppress testosterone synthesis in human fetal testicular explants to an extent greater than that seen with individual chemicals. This revealed itself as a shift towards lower doses in the dose-response curves of individual antiandrogens that became more pronounced as the number of components increased from four to eight.

CONCLUSIONS: Our results with the FEGA provide the foundations of a predictive human mixture risk assessment approach for anti-androgenic exposures in fetal life.

Elimination of the male reproductive tract in the female embryo is promoted by COUP-TFII in mice.

Zhao F, Franco HL, Rodriguez KF, Brown PR, Tsai MJ, Tsai SY, Yao HH. *Science.* 2017 Aug 18;357(6352):717-720. doi: 10.1126/science.aai9136.

Abstract

The sexual differentiation paradigm contends that the female pattern of the reproductive system is established by default because the male reproductive tracts (Wolffian ducts) in the female degenerate owing to a lack of androgen. Here, we discovered that female mouse embryos lacking Coup-tfII (chicken ovalbumin upstream promoter transcription factor II) in the Wolffian duct mesenchyme became intersex-possessing both female and male reproductive tracts. Retention of Wolffian ducts was not caused by ectopic androgen production or action. Instead, enhanced phosphorylated extracellular signal-regulated kinase signaling in Wolffian duct epithelium was responsible for the retention of male structures in an androgen-independent manner. We thus suggest that elimination of Wolffian ducts in female embryos is

actively promoted by COUP-TFII, which suppresses a mesenchyme-epithelium cross-talk responsible for Wolffian duct maintenance.

The era of 3Rs implementation in developmental and reproductive toxicity (DART) testing: Current overview and future perspectives.

Beekhuijzen M.

Reprod Toxicol. 2017 Sep;72:86-96. doi: 10.1016/j.reprotox.2017.05.006. Epub 2017 May 25. Review.

Abstract

Since adoption of the first globally implemented guidelines for developmental and reproductive toxicity (DART) testing for pharmaceuticals, industrial chemicals and agrochemicals, many years passed without major updates. However in recent years, significant changes in these guidelines have been made or are being implemented. These changes have been guided by the ethical drive to reduce, refine and replace (3R) animal testing, as well as the addition of endocrine disruptor relevant endpoints. Recent applied improvements have focused on reduction and refinement. Ongoing scientific and technical innovations will provide the means for replacement of animal testing in the future and will improve predictivity in humans. The aim of this review is to provide an overview of ongoing global DART endeavors in respect to the 3Rs, with an outlook towards future advances in DART testing aspiring to reduce animal testing to a minimum and the supreme ambition towards animal-free hazard and risk assessment.

Effect of prenatal DINCH plasticizer exposure on rat offspring testicular function and metabolism.

Campoli E, Lee S, Lau M, Marques L, Papadopoulos V.

Sci Rep. 2017 Sep 11;7(1):11072. doi: 10.1038/s41598-017-11325-7.

Abstract

In 2002, the plasticizer 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) was introduced in the European market as a substitute for endocrine-disrupting phthalates. We found that in utero exposure of rats to DINCH from gestational day 14 until parturition affected reproductive organ physiology and reduced circulating testosterone levels at post-natal day 60, indicating a long-term effect on Leydig cells of the testis. Metabolically, animals exhibited randomly increased serum glucose concentrations not associated with impaired glucose utilization. Analysis of liver markers in the serum showed a hepatic effect; e.g. reduced bilirubin levels and albumin/globulin ratio. At post-natal day 200, random appearance of testicular atrophy was noted in exposed offspring, and limited changes in other reproductive parameters were observed. In conclusion, DINCH exposure appears to directly affect Leydig cell function, likely causing premature aging of the testes and impaired liver metabolic capacity. These effects might be attenuated with physiologic aging.

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- 21. Endocrine Disruption in Human Fetal Testis Explants by Individual and Combined Exposures to Selected Pharmaceuticals, Pesticides, and Environmental Pollutants.**
Gaudriault P, Mazaud-Guittot S, Lavoué V, Coiffec I, Lesné L, Dejuqc-Rainsford N, Scholze M, Kortenkamp A, Jégou B. *Environ Health Perspect.* 2017 Aug 4;125(8):087004. doi: 10.1289/EHP1014. (Valgt)
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Liu KH, Sun XF, Feng YZ, Cheng SF, Li B, Li YP, Shen W, Li L.

Toxicol Appl Pharmacol. 2017 Aug 15;329:9-17. doi: 10.1016/j.taap.2017.05.024. Epub 2017 May 26.

29. The era of 3Rs implementation in developmental and reproductive toxicity (DART) testing: Current overview and future perspectives.

Beekhuijzen M.

Reprod Toxicol. 2017 Sep;72:86-96. doi: 10.1016/j.reprotox.2017.05.006. Epub 2017 May 25. Review. (Abstract)

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Song Q, Zhang Y, Yan L, Wang J, Lu C, Zhang Q, Zhao M.

J Hazard Mater. 2017 Sep 15;338:57-65. doi: 10.1016/j.jhazmat.2017.05.015. Epub 2017 May 11.

Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Science (all databases) og dækker perioden 19/6 - 18/9 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 tre artikler. 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 kommenteres artikler, der omhandler 'nye' stoffer, der har vist sig hormonforstyrrende; specielt hvis disse har relevans for danske forhold.

Udvalgte publikationer

A multi-tiered, in vivo, quantitative assay suite for environmental disruptors of thyroid hormone signaling

Mengeling BJ, Wei Y, Dobrawa LN, Streekstra M, Louisse J, Singh V, Singh L, Lein PJ, Wulff H, Murk AJ, Furlow JD.

Aquatic Toxicology. 190: 1-10. 2017.

Abstract

The essential role of thyroid hormone (TH) signaling in mammalian development warrants the examination of man-made chemicals for its disruption. Among vertebrate species, the molecular components of TH signaling are highly conserved, including the thyroid hormone receptors (TRs), their heterodimer binding partners the retinoid-X receptors (RXRs), and their DNA recognition sequences (TREs). This molecular conservation allows examination of potential TH disruption in the tractable, in vivo model system of amphibian metamorphosis. Metamorphosis requires TH signaling for both instigation and progression, and it provides dramatic and well characterized phenotypes involving different cell fates. Here we describe a quantitative, precocious-metamorphosis assay suite we developed using one-week post-fertilization (PF) *Xenopus laevis* tadpoles in order to assess disruption of TH signaling. Tadpoles at this developmental stage (Nieuwkoop-Faber (NF)-48) are competent to respond to TH hormone, although not yet producing TH, along many metamorphic pathways, and they are uniform in size. This allowed us to quantify changes in morphology associated with natural metamorphosis (e.g. gill and tail resorption, brain expansion, and craniofacial remodeling) after five days of treatment. Using the same tadpoles from morphological measurements, we quantified a 20-fold increase in TH-induced cellular proliferation in the rostral head region by whole-mount immunocytochemistry. At the molecular level, we used F3-generation tadpoles from a transgenic *X. laevis* line, which expresses luciferase under the control of a native TRE, to assess the ability of compounds to disrupt TR function. The luciferase reporter showed over 10-fold activation by physiologic concentrations of TH. We used the synthetic TR antagonist NH-3 to demonstrate the feasibility of our assay suite to measure inhibition of TH activity at the level of the receptor. Finally, we assessed the capabilities of suspected TH-disrupting chemicals tetrabrominated diphenyl ether 47 (BDE-47) and tetrabromobisphenol A (TBBPA). We found that BDE-47 displays general toxicity rather than TH disruption, as it did not increase brain width nor affect the TRE-luciferase reporter. However, TBBPA, a suspected TR antagonist, although not effective in antagonizing cell proliferation, significantly inhibited the TRE-luciferase reporter, suggesting that it bears closer scrutiny as a TH disruptor. Overall the assay suite has important advantages over the classical tadpole metamorphosis assays with respect to the uniformity of animal size, small test volume, reproducibility, and short test period. The assays are performed before endogenous TH production and free feeding start, which further reduces complexity and variability.

Different effects of bisphenol a and its halogenated derivatives on the reproduction and development of *Oryzias melastigma* under environmentally relevant doses

Huang QS, Chen YJ, Lin LF, Liu YY, Chi YL, Lin Y, Ye GZ, Zhu HM, Dong SJ.

Science of the Total Environment. 595: 752-758. 2017.

Abstract

Bisphenol A (BPA) and its halogenated compounds (H-BPAs) are widely detected in the environmental media and organisms. However, their toxicological effects, especially chronic exposure at low doses, have not been fully compared. In this study, the effects of BPA and H-BPAs on the reproduction and development of *Oryzias melastigma* were systematically assessed and compared at various developmental stages. BPA and its derivatives tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA) elicited the acceleration of embryonic heartbeat. BPA did not show any significant impact on the hatching time and rate of embryos. In contrast, both TBBPA and TCBPA led to the delayed hatching and decreased hatching rate. Accordingly, the expressions of hatching enzyme significantly decreased upon exposure and TCBPA was found to be more toxic than TBBPA. The body weight and gonadosomatic index (GSI) of the treated fish were relatively lower than the control fish upon long term (four months from larvae to adult) exposure to BPA rather than H-BPAs. Slowed oocyte development occurred in the ovary, and the estrogen level decreased after exposure to BPA rather than H-BPAs. In male fish, no significant alteration was observed in the testis for all groups. The concentration of testosterone significantly decreased upon exposure to BPA rather than H-BPAs. The effects of these three chemicals on the estrogen-related gene expressions were different under various developmental stages. Our study indicated the importance of considering both the exposure stages and structure-activity relationship when assessing the eco-toxicological impact of pollutants.

Waterborne exposure to BPS causes thyroid endocrine disruption in zebrafish larvae

Zhang D, Zhou E, Yang Z.

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Abstract

Bisphenol S (BPS) is widely used as a raw material in industry, resulting in its ubiquitous distribution in natural environment, including the aqueous environment. However, the effect of BPS on the thyroid endocrine system is largely unknown. In this study, zebrafish (*Danio rerio*) embryos were exposed to BPS at 1, 3, 10, and 30 µg/L, from 2 h post-fertilization (hpf) to 168hpf. Bioconcentration of BPS and whole-body thyroid hormones (THs), thyroid-stimulating hormone (TSH) concentrations as well as transcriptional profiling of key genes related to the hypothalamic-pituitary-thyroid (HPT) axis were examined. Chemical analysis indicated that BPS was accumulated in zebrafish larvae. Thyroxine (T4) and triiodothyronine (T3) levels were significantly decreased at ≥ 10 and 30 µg/L of BPS, respectively. However, TSH concentration was significantly induced in the 10 and 30 µg/L BPS-treated groups. After exposure to BPS, the mRNA expression of corticotrophin releasing hormone (*crh*) and thyroglobulin (*tg*) genes were up-regulated at ≥10 µg/L of BPS, in a dose-response manner. The transcription of genes involved in thyroid development (*pax8*) and synthesis (sodium/iodide symporter, *slc5a5*) were also significantly increased in the 30 µg/L of BPS treatment group. Moreover, exposure to 10 µg/L or higher concentration of BPS significantly up-regulated genes related to thyroid hormone metabolism (deiodinases, *dio1*, *dio2* and uridinediphosphate glucuronosyltransferases, *ugt1ab*), which might be responsible for the altered THs levels. However, the transcript of transthyretin (*ttr*) was significantly down-regulated at ≥ 3 µg/L of BPS, while the mRNA levels of thyroid hormone receptors (*trα* and *trβ*) and *dio3* remained unchanged. All the results indicated that exposure to BPS altered the whole-body THs and TSH concentrations and changed the expression profiling of key genes related to HPT axis, thus triggering thyroid endocrine disruption.

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