CENTER FOR HORMONFORSTYRRENDE STOFFER

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Indhold

Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

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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

Udvalgte publikationer

Occupational Exposure to Endocrine-Disrupting Chemicals and Birth Weight and Length of Gestation: A European Meta-Analysis

Birks L, Casas M, Garcia AM, Alexander J, Barros H, Bergström A, Bonde JP, Burdorf A, Costet N, Danileviciute A, Eggesbø M, Fernández MF, González-Galarzo MC, Gražulevičienė R, Hanke W, Jaddoe V, Kogevinas M, Kull I, Lertxundi A, Melaki V, Andersen AN, Olea N, Polanska K, Rusconi F, Santa-Marina L, Santos AC, Vrijkotte T, Zugna D, Nieuwenhuijsen M, Cordier S, Vrijheid M.
Environ Health Perspect. 2016 May 6. [Epub ahead of print]

BACKGROUND: Women of reproductive age can be exposed to endocrine-disrupting chemicals (EDCs) at work and exposure to EDCs in pregnancy may affect fetal growth.
OBJECTIVES: We assessed whether maternal occupational exposure to EDCs during pregnancy as classified by application of a job exposure matrix was associated with birth weight, term low birth weight (LBW), length of gestation, and preterm delivery.
METHODS: Using individual participant data from 133,957 mother-child pairs in 13 European cohorts spanning births from 1994 to 2011, we linked maternal job titles with exposure to 10 EDC groups as assessed through a job exposure matrix. For each group, we combined the two levels of exposure categories (possible and probable) and compared birth outcomes with the unexposed group (exposure unlikely). We performed meta-analyses of cohort-specific estimates.
RESULTS: Eleven percent of pregnant women were classified as exposed to EDCs at work during pregnancy based on job title. Classification of exposure to one or more EDC group was associated with an increased risk of term LBW (OR 1.25, 95%CI 1.04, 1.49), as were most specific EDC groups; this association was consistent across cohorts. Further, the risk increased with increasing number of EDC groups (OR 2.11 95%CI 1.10, 4.06 for exposure to 4 or more EDC groups). There were few associations ($p < 0.05$) with the other outcomes; women holding job titles classified as exposed to bisphenol A or brominated flame retardants were at higher risk for longer length of gestation.
CONCLUSION: Results from our large population-based birth cohort design indicate that employment during pregnancy in occupations classified as possibly or probably exposed to EDCs was associated with an increased risk of term LBW.

Associations of individual characteristics and lifestyle factors with metabolism of di-2-ethylhexyl phthalate in NHANES 2001-2012

Yaghjyan L, Carlsson NP, Ghita GL, Chang SH
Environ Res. 2016 Aug;149:23-31

BACKGROUND: Previous studies suggest that a higher ratio of primary to secondary metabolites of di-2-ethylhexyl phthalate (DEHP), reflective of a slower DEHP conversion rate, is associated with a greater physiologic effect. We examined associations of several individual characteristics and lifestyle factors with the ratio of mono-2-ethylhexyl phthalate to mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHP:MEHHP) and %MEHP (the ratio of MEHP to the sum of the secondary metabolites).
METHODS: We used the data from the National Health and Nutrition Examination Survey, 2001-2012. The study included adults with BMI<30 and no diabetes. Pregnant women were excluded. We examined associations of age, race, gender, Body Mass Index, smoking, alcohol and caffeine consumption, medication use, cancer history, and menopausal status and postmenopausal hormone use (in women) with MEHP:MEHHP and %MEHP using multivariable linear regression. The values for %MEHP were log-transformed in the analysis.

RESULTS: In multivariable analysis, non-Caucasian individuals had higher %MEHP (non-Hispanic Blacks: β=0.114, 95% Confidence interval [CI]: 0.050, 0.177; Hispanic: β=0.089, 95% CI: 0.024, 0.154; other race: β=0.126, 95% CI: 0.033, 0.219). Age was inversely associated with MEHP:MEHHP (β=-0.001, 95% CI: -0.002, -0.001) and %MEHP (β=-0.006, 95% CI: -0.008, -0.004). Overweight individuals had lower MEHP: MEHHP and lower %MEHP (β=-0.035, 95% CI: 0.062, -0.008 and β=-0.104, 95% CI: -0.162, -0.046, respectively). Alcohol consumption was inversely associated with %MEHP among men (p-trend=0.03).
CONCLUSIONS: Individual and lifestyle characteristics are associated with differences in DEHP metabolism. Understanding underlying biological mechanisms could help to identify individuals at a greater risk of adverse effects from DEHP exposure.

Urinary Concentrations of Parabens and Other Antimicrobial Chemicals and Their Association with Couples' Fecundity.
Smarr MM, Sundaram R, Honda M, Kannan K, Buck Louis GM.
Environ Health Perspect. 2016 Jun 10. [Epub ahead of print]

BACKGROUND: Human exposure to parabens and other antimicrobial chemicals is continual and pervasive. The hormone disrupting properties of these environmental chemicals may adversely affect human reproduction.
OBJECTIVE: To prospectively assess couples' urinary concentrations of antimicrobial chemicals in the context of fecundity, measured as time-to-pregnancy (TTP).
METHODS: In a prospective cohort of 501 couples, we examined preconception urinary chemical concentrations of parabens, triclosan and triclorcarban in relation to TTP; chemical concentrations were modeled both continuously and in quartiles. Cox's proportional odds models for discrete survival time were used to estimate fecundability odds ratios (FORs) and 95% confidence intervals (CIs) adjusting for a priori defined confounders. In light of TTP being a couple-dependent outcome, both partner and couple-based exposure models were analyzed. In all models, FOR estimates < 1.0 denote diminished fecundity (longer TTP).
RESULTS: Overall, 347 (69%) couples became pregnant. The highest quartile of female urinary methyl paraben (MP) concentrations relative to the lowest reflected a 34% reduction in fecundity [aFOR= 0.66; 95% CI=(0.45, 0.97) and remained so when accounting for couples' concentrations [aFOR= 0.63; 95% CI=(0.41, 0.96)]. Similar associations were observed between ethyl paraben (EP) and couple fecundity for both partner and couple-based-models (p-trend=0.02 and p-trend=0.05, respectively). No associations were observed with couple fecundity when chemicals were modeled continuously.
CONCLUSIONS: Higher quartiles of preconception urinary concentrations of MP and EP among female partners were associated with reduced couple fecundity in partner-specific and couple-based exposure models.

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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*”,

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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.

Udvalgte publikationer

2 artikler er blevet udvalgt til nærmere beskrivelse baseret på, at de beskriver resultater der bidrager til ny eller yderligere viden om grupper af hormonforstyrrende stoffer.
Den første artikel omhandler *in vitro* studier af fungicidet prochloraz' evne til at inducere oxidativt stress og DNA skader i humane celler.
Den anden artikel omhandler et *in vitro* studie med det formål, at undersøge de hormonforstyrrende effekter af et udvalg af perfluorerede alkylerede stoffer, samt tre tekniske blandinger, der alle anvendes i forskellig grad i fødevareemballage af papir og pap.

Fungicide prochloraz induces oxidative stress and DNA damage in vitro.
Lundqvist J, Hellman B, Oskarsson A.
Food Chem Toxicol. 2016 May;91:36-41. doi: 10.1016/j.fct.2016.03.002.

Prochloraz is widely used in horticulture and agriculture, e.g. as a post-harvest anti-mold treatment. Prochloraz is a known endocrine disruptor causing developmental toxicity with multiple mechanisms of action. However, data are scarce concerning other toxic effects. Since oxidative stress response, with formation of reactive oxygen species (ROS), is a common mechanism for different toxic endpoints, e.g. genotoxicity, carcinogenicity and teratogenicity, the aim of this study was to investigate if prochloraz can induce oxidative stress and/or DNA damage in human cells. A cell culture based in vitro model was used to study oxidative stress response by prochloraz, as measured by the activity of the nuclear factor erythroid 2-related factor 2 (Nrf2), a key molecule in oxidative defense mechanisms. It was observed that prochloraz induced oxidative stress in cultured human adrenocortical H295R and hepatoma HepG2 cells at non-toxic concentrations. Further, we used Comet assay to investigate the DNA damaging potential of prochloraz, and found that non-toxic concentrations of prochloraz induced DNA damage in HepG2 cells. These are novel findings, contradicting previous studies in the field of prochloraz and genotoxicity. This study reports a new mechanism by which prochloraz may exert toxicity. Our findings suggest that prochloraz might have genotoxic properties.

Fluorinated alkyl substances and technical mixtures used in food paper-packaging exhibit endocrine-related activity in vitro.
Rosenmai AK, Taxvig C, Svingen T, Trier X, van Vugt-Lussenburg BM, Pedersen M, Lesné L, Jégou B, Vinggaard AM.
Andrology. 2016 May 6. doi: 10.1111/andr.12190.

Migration of chemicals from packaging materials to foods may lead to human exposure. Polyfluoroalkyl substances (PFAS) can be used in technical mixtures (TMs) for use in food packaging of paper and board, and PFAS have been detected in human serum and umbilical cord blood. The specific structures of the PFAS in TMs are often unknown, but polyfluorinated alkyl phosphate esters (PAPs) have been characterized in TMs, food packaging, and in food. PAPs can be metabolized into fluorotelomer alcohols (FTOHs) and perfluoroalkyl carboxylic acids (PFCAs). Some PFAS have endocrine activities, highlighting the need to investigate these effects. Herein, we studied the endocrine activity of less characterized PFAS, including short-chain PFCAs and FTOHs, PAPs, and TMs of unknown chemical composition. Long-chain PFCAs were

also included. We applied seven assays covering effects on estrogen, glucocorticoid, androgen, and peroxisome proliferator-activated receptor (PPAR) activity, as well as steroidogenesis in vitro and ex vivo. In general, PAPs, FTOHs, TMs, and long-chain PFCAs showed estrogenic activity through receptor activation and/or increasing 17β-estradiol levels. Furthermore, short- and long-chain PFCAs activated PPARα and PPARγ. Collectively, this means that (i) PAPs, FTOHs, and PFCAs exhibit endocrine activity through distinct and sometimes different mechanisms, (ii) two out of three tested TMs exhibited estrogenic activity, and (iii) short-chain FTOHs showed estrogenic activity and short-chain PFCAs generally activate both PPARα and PPARγ with similar potency and efficacy as long-chain PFCAs. In conclusion, several new and divergent toxicological targets were identified for different groups of PFAS.

Bruttolisten

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Herudover er der yderligere 1 artikel, som ikke blev fanget af de valgte søgekriterier:
Low-Dose Bisphenol-A Impairs Adipogenesis and Generates Dysfunctional 3T3-L1 Adipocytes
Ariemma F, D'Esposito V, Liguoro D, Oriente F, Cabaro S, Liotti A, Cimmino I, Longo M, Beguinot F, Formisano P, Valentino R.
PLoS One. 2016 Mar 4;11(3):e0150762. doi: 10.1371/journal.pone.0150762. eCollection 2016.

In Vivo studier ved DTU Fødevareinstituttet

Søgning er udført på PubMed og dækker perioden April - ultimo Juni 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 and (endocrine disrupt*) AND EOGRTS.

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 32 artikler (Bruttolisten).

Udvalgte publikationer

Tre artikler er blevet udvalgt til nærmere beskrivelse (abstrakt og konklusion) og to artikler er udvalgt blot til abstract. Disse artikler er valgt fordi vi mener de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på Bisphenol A (Hass et al. 2016 og Mandrup et al. 2016) samt blandinger og effekter på hunner (Johansson et al. 2016). De 2 artikler hvor der er medtaget abstracts omhandler OECD test guidelines TG 421/422 (Beekhuijzen et al. 2016) og undersøger "triggers" for kohorterne i TG 443 (Moore et al. 2016).

Rigtig God læselyst.

Ud fra bruttolisten (se længere nede i dokumentet) er udvalgt følgende 3 artikler til engelsk abstrakt og dansk resume og 2 artikler blot med deres abstract.

Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats.
Hass U, Christiansen S, Boberg J, Rasmussen MG, Mandrup K, Axelstad M.
Andrology. 2016 Apr 18. doi: 10.1111/andr.12176.

Bisphenol A (BPA) is widely used in food contact materials and other products and is detected in human urine and blood. BPA may affect reproductive and neurological development; however EFSAs opinion on BPA (EFSA 2015) concluded that none of the available studies were robust enough to provide a point of departure for setting a Tolerable Daily Intake (TDI) for BPA.
In the present study, pregnant Wistar rats (n=17-21) were gavaged from gestation day 7 to pup day 22 with BPA doses of 0; 25 µg; 250 µg; 5 mg or 50 mg/kg bw/day. In the offspring, growth, sexual maturation, weights and histopathology of reproductive organs, oestrus cyclicity, and sperm counts were assessed. Neurobehavioural development was investigated using a behavioural testing battery including tests for motor activity, sweet preference, anxiety and spatial learning.
Decreased sperm count was found at the lowest BPA dose, i.e. 25 µg /kg/day, but not at the higher doses. Reproductive organ weight and histology were not affected and no behavioural effects were seen in male offspring. In the female offspring, exposure to 25 µg /kg bw/day resulted in increased body weight late in life and altered spatial learning in a Morris water maze, indicating masculinization of the brain. Decreased intake of sweetened water was seen in females from the highest BPA dose group, also a possible sign of masculinization. The other investigated endpoints were not significantly affected.
In conclusion, the present study using a robust experimental study design, has shown that developmental exposure to 25 µg/kg bw/day BPA can cause adverse effects on fertility (decreased sperm count), neurodevelopment (masculinization of spatial learning in females) and lead to increased female body weight late in life. These results suggest that the new EFSA temporary TDI of 4 µg/kg bw/day is not sufficiently protective with regards to endocrine disrupting effects of BPA in humans.

Low-dose effects of bisphenol A on mammary gland development in rats.
Mandrup K, Boberg J, Isling LK, Christiansen S, Hass U.
Andrology. 2016 Apr 18. doi: 10.1111/andr.12193.

Bisphenol A (BPA) is widely used in food contact materials, toys and other products. Several studies have indicated that effects observed at doses near human exposure levels may not be observed at higher doses. Many studies have shown effects on mammary glands at low doses of BPA, however, due to small number of animals or few doses investigated these data have not been used by EFSA as point of departure for the newly assessed Tolerable Daily Intake (TDI).
We performed a study with perinatal exposure to BPA (0, 0.025, 0.25, 5 and 50 mg/kg bw/day) in rats (n=22 mated/group). One of the aims was to perform a study robust enough to contribute to the risk assessment of BPA and to elucidate possible biphasic dose-response relationships. We investigated mammary gland effects in the offspring at 22, 100 and 400 days of age.
Male offspring showed increased mammary outgrowth on pup day (PD) 22 at 0.025 mg/kg BPA, indicating an increased mammary development at this low dose only. Increased prevalence of intraductal hyperplasia was observed in BPA females exposed to 0.25 mg/kg at PD 400, but not at PD 100, and not at higher or lower doses.
The present findings support data from the published literature showing that perinatal exposure to BPA can induce increased mammary growth and proliferative lesions in rodents. Our results indicate that low dose exposure to BPA can affect mammary gland development in male and female rats, although higher doses show a different pattern of effects. The observed intraductal hyperplasia in female rats could be associated with an increased risk for developing hyperplastic lesions, which are parallels to early signs of breast neoplasia in women. Collectively, current knowledge on effects of BPA on mammary gland at low doses indicates that highly exposed humans may not be sufficiently protected.

Perinatal exposure to mixtures of endocrine disrupting chemicals reduces female rat follicle reserves and accelerates reproductive aging.

Johansson HK, Jacobsen PR, Hass U, Svingen T, Vinggaard AM, Isling LK, Axelstad M, Christiansen S, Boberg J. Reprod Toxicol. 2016 Jun;61:186-94. doi: 10.1016/j.reprotox.2016.03.045. Epub 2016 Apr 2.

Exposure to endocrine disrupting chemicals (EDCs) during development can have negative consequences later in life. In this study we investigated the effect of perinatal exposure to mixtures of human relevant EDCs on the female reproductive system. Rat dams were exposed to a mixture of phthalates, pesticides, UV-filters, bisphenol A, butylparaben, as well as paracetamol. The compounds were tested together (Totalmix) or in subgroups with anti-androgenic (AAmix) or estrogenic (Emix) potentials. Paracetamol was tested separately. In pre-pubertal rats, a significant reduction in primordial follicle numbers was seen in AAmix and PM groups, and reduced plasma levels of prolactin was seen in AAmix. In one-year-old animals, the incidence of irregular estrous cycles was higher after Totalmix-exposure and reduced ovary weights were seen in Totalmix, AAmix, and PM groups. These findings resemble premature ovarian insufficiency in humans, and raises concern regarding potential effects of mixtures of EDCs on female reproductive function.

Update of OECD DART guidelines with endocrine disruptor relevant endpoints: Practical considerations.
Beekhuijzen M, van Otterdijk F, Wieland W, van Tuyl M, Rijcken RP, Peter B, Emmen H. Reprod Toxicol. 2016 Apr 7. pii: S0890-6238(16)30052-1. doi: 10.1016/j.reprotox.2016.04.002. [Epub ahead of print]

In 1998, the OECD initiated a high-priority project aimed at revising existing test guidelines and developing new test guidelines for screening of potential endocrine disruptors. In 2011, OECD 443 was adopted, and in 2015 OECD 421 and OECD 422 were updated with endocrine disruptor relevant endpoints. A feasibility study for the enhancement of OECD 414 with endocrine disruptor relevant endpoints is currently ongoing. The addition of these endpoints is considered crucial for gaining more information on endocrine disruptor potency of tested chemicals, however it should be noted that these additions have a major impact on the study designs and give rise to several practical challenges. The aim of this review is to discuss important aspects of these challenging study designs and to share our knowledge on their implementation in our laboratory. Together, this review can be used as guidance for other laboratories, study monitors and registration officers

Guidance on the selection of cohorts for the extended one-generation reproduction toxicity study (OECD test guideline 443). Moore NP, Beekhuijzen M, Boogaard PJ, Foreman JE, North CM, Palermo C, Schneider S, Strauss V, van Ravenzwaay B, Poole A. Regul Toxicol Pharmacol. 2016 May 28;80:32-40. doi: 10.1016/j.yrtph.2016.05.036. [Epub ahead of print]

The extended one-generation reproduction toxicity study (EOGRTS; OECD test guideline 433) is a new and technically complex design to evaluate the putative effects of chemicals on fertility and development, including effects upon the developing nervous and immune systems. In addition to offering a more comprehensive assessment of developmental toxicity, the EOGRTS offers important improvements in animal welfare through reduction and refinement in a modular study design. The challenge to the practitioner is to know how the modular aspects of the study should be triggered on the basis of prior knowledge of a particular chemical, or on earlier findings in the EOGRTS itself, requirements of specific regulatory frameworks notwithstanding. The purpose of this document is to offer guidance on sciencebased triggers for these extended evaluations.

Bruttolisten

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Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Knowledge (all databases) og dækker perioden 29/3 – 8/7 2016.

Søgeprofilen kombinerer:	”Endocrine disrupt*” and	Fish* Amphibia* Bird* OR avia* Invertebrat* Mollus* Gastropod* Insect* Crustacea* Echinoderm* Ursus Reptil* OR alligator Whal* OR seal* OR dolphin*

Fra bruttolisten (længere nede i dokumentet) er udvalgt fire artikler til medtagelse af abstract og yderligere kommentarer. To af artiklerne omhandler effekten af BP-3 på dansemyg og kommenteres samlet.
Kriterierne for udvælgelsen af publikationer til kommentering er, at de bidrager til ny viden omkring effekter af og virkningsmekanismer for hormonforstyrrende stoffer i ’wildlife’ og/eller at de repræsenterer vigtig viden, som vurderes at have særlig interesse for Miljøstyrelsen bl.a. i forbindelse med styrelsens fokus på udvikling af testmetoder. Desuden kommenteres artikler, der omhandler ’nye’ stoffer og miljøfaktorer, der har vist sig hormonforstyrrende; specielt hvis disse har relevans for danske forhold.

Udvalgte artikler

Linking the response of endocrine regulated genes to adverse effects on sex differentiation improves comprehension of aromatase inhibition in a Fish Sexual Development Test.
Muth-Kohne E, Westphal-Settele K, Bruckner J, Konradi S, Schiller V, Schafers C, Teigeler M, Fenske M.
Aquatic toxicology (Amsterdam, Netherlands). 176: 116-127. 2016.

ABSTRACT:
The Fish Sexual Development Test (FSDT) is a non-reproductive test to assess adverse effects of endocrine disrupting chemicals. With the present study it was intended to evaluate whether gene expression endpoints would serve as predictive markers of endocrine disruption in a FSDT. For proof-of-concept, a FSDT according to the OECD TG 234 was conducted with the non-steroidal aromatase inhibitor fadrozole (test concentrations: 10 µg/L, 32 µg/L, 100 µg/L) using zebrafish (Danio rerio). Gene expression analyses using quantitative RT-PCR were included at 48 h, 96 h, 28 days and 63 days post fertilization (hpf, dpf). The selection of genes aimed at finding molecular endpoints which could be directly linked to the adverse apical effects of aromatase inhibition. The most prominent effects of fadrozole exposure on the sexual development of zebrafish were a complete sex ratio shift towards males and an acceleration of gonad maturation already at low fadrozole concentrations (10 µg/L). Due to the specific inhibition of the aromatase enzyme (Cyp19) by fadrozole and thus, the conversion of C19-androgens to C18-estrogens, the steroid hormone balance controlling the sex ratio of zebrafish was altered. The resulting key event is the regulation of directly estrogen-responsive genes. Subsequently, gene expression of vitellogenin 1 (vtg1) and of the aromatase cyp19a1b isoform (cyp19a1b), were down-regulated upon fadrozole treatment compared to controls. For example, mRNA levels of vtg1 were down-regulated compared to the controls as early as 48 hpf and 96 hpf. Further regulated genes cumulated in pathways suggested to be controlled by endocrine mechanisms, like the steroid and terpenoid synthesis pathway (e.g. mevalonate (diphospho) decarboxylase (mvd), lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase; lss), methylsterol monooxygenase 1 (sc4mol)) and in lipid transport/metabolic processes (steroidogenic acute regulatory protein (star), apolipoprotein Eb (apoEb)). Taken together, this study demonstrated that the existing Adverse Outcome Pathway (AOP) for aromatase inhibition in fish can be translated to the life-stage of sexual differentiation. We were further able to identify MoA-specific marker gene expression which can be instrumental in defining new measurable key events (KE) of existing or new AOPs related to endocrine disruption.

In Vivo Screening Using Transgenic Zebrafish Embryos Reveals New Effects of HDAC Inhibitors Trichostatin A and Valproic Acid on Organogenesis.
Li L, Bonneton F, Tohme M, Bernard L, Chen XY, Laudet V.
Plos One. 11(2): 2016.

ABSTRACT: The effects of endocrine disrupting chemicals (EDCs) on reproduction are well known, whereas their developmental effects are much less characterized. However, exposure to endocrine disruptors during organogenesis may lead to deleterious and permanent problems later in life. Zebrafish (Danio rerio) transgenic lines expressing the green fluorescent protein (GFP) in specific organs and tissues are powerful tools to uncover developmental defects elicited by EDCs. Here, we used seven transgenic lines to visualize in vivo whether a series of EDCs and other pharmaceutical compounds can alter organogenesis in zebrafish. We used transgenic lines expressing GFP in pancreas, liver, blood vessels, inner ear, nervous system, pharyngeal tooth and pectoral fins. This screen revealed that four of the tested chemicals have detectable effects on different organs, which shows that the range of effects elicited by EDCs is wider than anticipated. The endocrine disruptor tetrabromobisphenol-A (TBBPA), as well as the three drugs diclofenac, trichostatin

A (TSA) and valproic acid (VPA) induced abnormalities in the embryonic vascular system of zebrafish. Moreover, TSA and VPA induced specific alterations during the development of pancreas, an observation that was confirmed by in situ hybridization with specific markers. Developmental delays were also induced by TSA and VPA in the liver and in pharyngeal teeth, resulting in smaller organ size. Our results show that EDCs can induce a large range of developmental alterations during embryogenesis of zebrafish and establish GFP transgenic lines as powerful tools to screen for EDCs effects in vivo.

The effects of binary UV filter mixtures on the midge Chironomus riparius.
Ozaez I, Morcillo G, Martinez-Guitarte JL.
Science of the Total Environment. 556: 154-162. 2016.

ABSTRACT: Organic ultraviolet (UV) filters are used in a wide variety of products, including cosmetics, to prevent damage from UV light in tissues and industrial materials. Their extensive use has raised concerns about potential adverse effects in human health and aquatic ecosystems that accumulate these pollutants. To increase sun radiation protection, UV filters are commonly used in mixtures. Here, we studied the toxicity of binary mixtures of 4-methylbenzylidene camphor (4MBC), octyl-methoxycinnamate (OMC), and benzophenone-3 (BP-3), by evaluating the larval mortality of Chironomus riparius. Also molecular endpoints have been analyzed, including alterations in the expression levels of a gene related with the endocrine system (EcR, ecdysone receptor) and a gene related with the stress response (hsp70, heat shock protein 70). The results showed that the mortality caused by binary mixtures was similar to that observed for each compound alone; however, some differences in LC50 were observed between groups. Gene expression analysis showed that EcR mRNA levels increased in the presence of 0.1mg/L 4MBC but returned to normal levels after exposure to mixtures of 4MBC with 0.1, 1, and 10mg/L of BP-3 or OMC. In contrast, the hsp70 mRNA levels increased after exposure to the combinations tested of 4MBC and BP-3 or OMC mixtures. These data suggest that 4MBC, BP-3, and OMC may have antagonist effects on EcR gene transcription and a synergistic effect on hsp70 gene activation. This is the first experimental study to show the complex patterned effects of UV filter mixtures on invertebrates. The data suggest that the interactions within these chemicals mixtures are complex and show diverse effects on various endpoints.

UV filters induce transcriptional changes of different hormonal receptors in Chironomus riparius embryos and larvae.
Ozaez I, Aquilino M, Morcillo G, Martinez-Guitarte JL.
Environmental pollution (Barking, Essex : 1987). 214: 239-247. 2016.

ABSTRACT: Organic ultraviolet (UV) filters are emerging contaminants that are ubiquitous in fresh and marine aquatic systems due to their extensive use in cosmetics, plastics, paints, textiles, and many other industrial products. The estrogenic effects of organic UV filters have been long demonstrated in vertebrates, and other hormonal activities may be altered, according to more recent reports. The impact of UV filters on the endocrine system of invertebrates is largely unknown. We have previously reported that some UV filters may affect ecdysone-related genes in the aquatic insect Chironomus riparius, an ecotoxicologically important model organism. To further analyze other possible effects on endocrine pathways, we first characterized four pivotal genes related with hormonal pathways in insects; thereafter, these genes were assessed for alterations in transcriptional activity after exposure to 4-methylbenzylidene camphor (4MBC) or benzophenone-3 (BP-3), two extensively used sunscreens. We found that both chemicals disturbed the expression of all four genes analyzed: hormonal receptor 38 (HR38), methoprene-tolerant (Met), membrane-associate progesterone receptor (MAPR) and insulin-like receptor (INSR), measured by changes in mRNA levels by real-time PCR. An upregulatory effect at the genomic level was

detected in different developmental stages. Interestingly, embryos appeared to be more sensitive to the action of the UV filters than larvae. Our results suggest that the risk of disruption through different endocrine routes is not negligible, considering the significant effects of UV filters on key hormonal receptor and regulatory genes. Further effort is needed to develop environmental risk assessment studies on these pollutants, particularly for aquatic invertebrate model organisms.

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