CENTER FOR HORMONFORSTYRRENDE STOFFER

Litteraturgennemgang for perioden september 2017 – december 2017

Indhold

Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

Søgning er udført på PubMed og dækker perioden 20. september – 5. december 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 markeret.

Første artikel udvalgt til dette kvartals litteraturgennemgang har fokus på niveauerne af flammehæmmere i elektroniske produkter som smartphones og laptops og viser markante forskelle i niveauerne alt efter hvilke mærke produkterne er fra. Anden artikel er et nyt stort dansk studie om prænatal phthalatudsættelse og sproglæring hos børn, der er en vigtig markør for børns senere skolepræstation og desuden arbejdsmæssige succes.
Slutteligt er der i dette kvartal udvalgt to spanske artikler baseret på det samme studiemateriale, der er en del af EU projektet 'HELIX' (Human Early-Life Exposome), der overordnet har formålet at undersøge sammenhængen mellem en række hormonforstyrrende stoffer i relation til forskellige helbredseffekter i de første år af livet. I nærværende to studier er der specifikt fokus på udsættelserne for perflourerede stoffer føtalt i relation til vækst i de første leveår.

Udvalgte artikler

Flame retardants on the surface of phones and personal computers

Zheng X, Sun R, Qiao L, Guo H, Zheng J, Mai B.

Sci Total Environ. 2017 Dec 31;609:541-545. doi: 10.1016/j.scitotenv.2017.07.202. Epub 2017 Jul 27.

Abstract

Mobile phones and personal computers (PCs) are essential products that are frequently contacted in daily life. Thus, phones and computers containing flame retardants (FRs) may play vital roles in human exposure to FRs. We measured several FRs, including polybrominated diphenyl ethers (PBDEs), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), tetrabromobisphenol (TBBPA), and phosphate flame retardants (PFRs), on the surfaces of phones and PCs (laptop keyboards and mice). Triphenyl phosphate (TPHP, 228pg/cm2) and tris(chloroisopropyl) phosphate (TCIPP, 43pg/cm2) were the most abundant chemicals on the surfaces of phones, while TPHP (65pg/cm2), TCIPP (48pg/cm2), and DBDPE (22pg/cm2) were dominant on the surfaces of PCs. The usage time and time after the production of the electronics were not significantly correlated with the FR concentrations, except for that of BDE 209. The concentrations of FRs differed on the surfaces of different brands of electronics. Dermal contact with the surface of electronics may contribute to human exposure to FRs, which should be of concern.

Prenatal phthalate exposure and language development in toddlers from the Odense Child Cohort

Olesen TS, Bleses D, Andersen HR, Grandjean P, Frederiksen H, Trecca F, Bilenberg N, Kyhl HB, Dalsager L, Jensen IK, Andersson AM, Jensen TK.

Neurotoxicol Teratol. 2017 Nov 30. pii: S0892-0362(17)30134-4. doi: 10.1016/j.ntt.2017.11.004.

Abstract

BACKGROUND: Phthalates are a group of chemicals found in a variety of consumer products. They have anti-androgenic properties and human studies have reported associations between prenatal phthalate exposure and neuropsychological development in the offspring despite different cognitive tests, different ages and varying timing of exposure.

OBJECTIVES: To investigate the association between prenatal phthalate exposure and language development in children aged 20-36months.

METHODS: In the Odense Child Cohort, we analyzed 3rd trimester urine samples of 518 pregnant women for content of metabolites of diethyl, di-n-butyl, diisobutyl, butylbenzyl, di(2-ethylhexyl), and diisononyl phthalate, adjusted for osmolality. Language development was addressed using the Danish version of the MacArthur-Bates Communicative Development Inventories "Words and Sentences". Associations were assessed using logistic regression models comparing children below and above the 15th percentile while stratifying by sex and adjusting for maternal age and educational level.

RESULTS: Phthalate metabolites were detectable in all samples although in lower levels than previous studies. Among boys, increased prenatal phthalate exposure was associated with lower scores in language development; odds ratios for vocabulary score below the 15th percentile with doubling in monoethyl phthalate, and summed di-(2-ethylhexyl) phthalate metabolites were respectively 1.24 (95% confidence interval: 1.05,1.46), and 1.33 (1.01,1.75). Similar associations were found for language complexity. No associations were found for girls.

CONCLUSIONS: Our findings are notable, as adverse associations were suggested even in this low-level exposed population, with only one spot urine sample for exposure assessment and control for confounders. Lower scores in early language development are of relevance to health as this test predicts later educational success.

Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort

Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iñiguez C, Martinez D, Costa O, Santa-Marina L, Pereda-Pereda E, Schettgen T, Sunyer J, Vrijheid M.

Environ Int. 2017 Nov;108:278-284.

Abstract

BACKGROUND: Prenatal perfluorooctanoate (PFOA) exposure has been associated with reduced birth weight but maternal glomerular filtration rate (GFR) may attenuate this association. Further, this association remains unclear for other perfluoroalkyl substances (PFAS), such as perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA). We estimated associations between prenatal PFAS exposure and birth outcomes, and the influence of GFR, in a Spanish birth cohort.

METHODS: We measured PFHxS, PFOS, PFOA, and PFNA in 1st-trimester maternal plasma (years: 2003-2008) in 1202 mother-child pairs. Continuous birth outcomes included standardized weight, length, head circumference, and gestational age. Binary outcomes included low birth weight (LBW), small-for-gestational-age, and preterm birth. We calculated maternal GFR from plasma-creatinine measurements in the 1st-trimester of pregnancy (n=765) using the Cockcroft-Gault formula. We used mixed-effects linear and logistic models with region of residence as random effect and adjustment for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy.

RESULTS: Newborns in this study weighted on average 3263g and had a median gestational age of 39.8weeks. The most abundant PFAS were PFOS and PFOA (median: 6.05 and 2.35ng/mL, respectively). Overall, PFAS concentrations were not significantly associated to birthoutcomes. PFOA, PFHxS, and PFNA showed weak, non-statistically significant associations with reduced birth weights ranging from 8.6g to 10.3g per doubling of exposure. Higher PFOS exposure was associated with an OR of 1.90 (95% CI: 0.98, 3.68) for LBW (similar in births-at-term) in boys. Maternal GFR did not confound the associations.

CONCLUSIONS: In this study, PFAS showed little association with birth outcomes. Higher PFHxS, PFOA, and PFNA concentrations were non-significantly associated with reduced birth weight. The association between PFOS and LBW seemed to be sex-specific. Finally, maternal GFR measured early during pregnancy had little influence on the estimated associations.

Prenatal Exposure to Perfluoroalkyl Substances and Cardiometabolic Risk in Children from the Spanish INMA Birth Cohort Study

Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iñiguez C, Martinez D, Romaguera D, Fernández-Barrés S, Santa-Marina L, Basterretxea M, Schettgen T, Valvi D Vioque J, Sunyer J, Vrijheid M.

Environ Health Perspect. 2017 Sep 20;125(9):097018.

Abstract

BACKGROUND: Perfluoroalkyl substances (PFAS) may affect body mass index (BMI) and other components of cardiometabolic (CM) riskduring childhood, but evidence is scarce and inconsistent.

OBJECTIVES: We estimated associations between prenatal PFAS exposures and outcomes relevant to cardiometabolic risk, including a composite CM-risk score.

METHODS: We measured perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) in maternal plasma (first trimester). We assessed weight gain from birth until 6 mo. At 4 and 7 y, we calculated the age- and sex-specific z-scores for BMI, waist circumference (WC), and blood pressure (BP) (n≈1,000). At age 4, we calculated the age-, sex-, and region-specific z-scores for cholesterol, triglycerides (TGs), high-density (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (n=627). At age 4, we calculated a CM-risk score (n=386) as the sum of the individual age-, sex-, and region-specific z-scores for WC, BP, HDL-C, and TGs. We used the average between the negative of HDL-C z-score and TGs z-score to give similar weight to lipids and the other components in the score. A higher score indicates a higher cardiometabolic risk at age 4.

RESULTS: PFOS and PFOA were the most abundant PFAS (geometric mean: 5.80 and 2.32 ng/mL, respectively). In general, prenatal PFAS concentrations were not associated with individual outcomes or the combined CM-risk score. Exceptions were positive associations between prenatal PFHxS and TGs z-score [for a doubling of exposure, β=0.11; 95% confidence interval (CI): 0.01, 0.21], and between PFNA and the CM-risk score (β=0.60; 95% CI: 0.04, 1.16). There was not clear or consistent evidence of modification by sex.

CONCLUSIONS: We observed little or no evidence of associations between low prenatal PFAS exposures and outcomes related to cardiometabolic risk in a cohort of Spanish children followed from birth until 7 y.

Bruttoliste

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In vitro studier ved DTU Fødevareinstituttet

Søgt i Pubmed med følgende kriterier:

” Endocrine disrupt* AND in vitro*” samt “Endocrine disrupt* AND expose* AND in vitro*”,

”Paraben* AND in vitro*,”perfluor* OR polyfluor* AND in vitro*” og “Phthalat* AND in vitro*”.

Publiceret fra i perioden 2017/09/30 to 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 29 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 beskriver studier, der har haft til formål at undersøge den metaboliske kapacitet i U2-OS celle baseret CALUX assays, samt beskriver en metode til at introducere metabolisk aktivitet i ERα CALUX og anti-AR CALUX assays.
Den anden artikel beskriver et studie, hvor man har haft til formål at udvikle en 3D in vitro co-kultur testikel model, for blandt andet at imødekomme det stigende behov, der er inden for reproduktionstoksikologi, for at få udviklet in vitro tests, der kan anvendes som alternativ til de nuværende dyreforsøg.

Udvalgte publikationer

Incorporation of metabolic enzymes to improve predictivity of reporter gene assay results for estrogenic and anti-androgenic activity.

van Vugt-Lussenburg BMA, van der Lee RB, Man HY, Middelhof I, Brouwer A, Besselink H, van der Burg B.

Reprod Toxicol. 2017 Nov 21;75:40-48. doi: 10.1016/j.reprotox.2017.11.005.

Abstract

Identification and monitoring of so-called endocrine-disrupting compounds has received ample attention; both the OECD and the United States Environmental Protection Agency (US EPA) have designed tiered testing approaches, involving in vitro bioassays to prioritize and partly replace traditional animal experiments. Since the estrogen (ER) and androgen (AR) receptor are frequent targets of endocrine disrupting chemicals, bioassays detecting interaction with these receptors have a high potential to be of use in risk assessment of endocrine active compounds. However, in many bioassays in vivo hepatic metabolism is not accounted for, which hampers extrapolation to the in vivo situation. In the present study, we have developed a metabolic module using rat liver S9 as an add-on to human cell-based reporter gene assays. The method was applied to reporter gene assays for detection of (anti-) estrogens and (anti-) androgens, but can be extended to cell-based reporter gene assays covering a variety of endpoints related to endocrine disruption.

From the Cover: An Animal-Free In Vitro Three-Dimensional Testicular Cell Coculture Model for Evaluating Male Reproductive Toxicants.

Yin L, Wei H, Liang S, Yu X.

Toxicol Sci. 2017 Oct 1;159(2):307-326. doi: 10.1093/toxsci/kfx139.

Abstract

Primary testicular cell coculture model has been used to evaluate testicular abnormalities during development, and was able to identify the testicular toxicity of phthalates. However, the primary testicular cell coculture model has disadvantages in employing animals for the isolation of testicular cells, and the complicated isolation procedure leads to inconsistent results. We developed an invitro testicular coculture modelfrom rodent testicular cell lines, including spermatogonial cells, Sertoli cells, and Leydig cells with specified cell density and extracellular matrix (ECM) composition. Using comparative high-content analysis of F-actin cytoskeletal structure between the coculture and single cellculture models, we demonstrated a 3D structure of the coculture, which created an invivo-like niche, and maintained and supported germ cells within a 3D environment. We validated this model by discriminating between reproductive toxicants and nontoxicants among 32 compounds in comparison to the single cell culture models. Furthermore, we conducted a comparison between the invitro (IC50) and invivo reproductive toxicity testing (lowest observed adverse effect level on reproductive system). We found the invitro coculture model could classify the tested compounds into 4 clusters, and identify the most toxic reproductive substances, with high concordance, sensitivity, and specificity of 84%, 86.21%, and 100%, respectively. We observed a strong correlation of IC50 between the invitro coculture model and the invivo testing results. Our results suggest that this novel invitro coculture model may be useful for screening testicular toxicants and prioritize chemicals for further assessment in the future.

Bruttoliste

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van Vugt-Lussenburg BMA, van der Lee RB, Man HY, Middelhof I, Brouwer A, Besselink H, van der Burg B.
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In vivo studier ved DTU Fødevareinstituttet

<u>Søgning er udført på PubMed og dækker perioden september - primo december 2017</u>

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 36 artikler (Bruttolisten).

To artikler er blevet udvalgt. Disse artikler er valgt fordi vi mener de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på kombinationseffekter af hormonforstyrrende stoffer (Schneider et al. 2017) samt en artikel om effekter af DINCH eksponering (Campioli et al. 2017).

Rigtig god læselyst.

Udvalgte publikationer

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

Investigations on the dose-response relationship of combined exposure to low doses of three anti-androgens in Wistar rats.

Schneider S, Fussell KC, Melching-Kollmuss S, Buesen R, Gröters S, Strauss V, Jiang X, van Ravenzwaay B.

Arch Toxicol. 2017 Sep 6. doi: 10.1007/s00204-017-2053-3.

Abstract

The current investigation examines whether combined exposure to three anti-androgens (flutamide, prochloraz, vinclozolin) result in interference with endocrine homeostasis when applied at very low dose levels, and whether the results of combined exposure are more pronounced than to the individual compounds. A pre-post-natal in vivo study design was chosen with more parameters than regulatory testing protocols require (additional endpoints addressing hormone levels, morphology and histopathological examinations). Dose levels were chosen to represent the lowest observed adverse effect level (LOAEL), the no observed adverse effect level (NOAEL), and the acceptable daily intake for each individual substance. Anti-androgenic changes were observable at the effect level (LOAEL) but not at lower exposures. Nipple/areola counts appeared to be a sensitive measure of effect, in addition to male sex organ weights at sexual maturation, and finally gross findings. The results indicate the absence of evidence for effects at low or very low dose levels. No (adverse) effects were seen at the NOAEL dose. A non-monotonic dose-response relationship was not evident. Combined exposure at LOAEL level resulted in enhanced responses for anogenital index, number of areolas/nipples, delayed preputial separation and reduced ventral prostate weight in comparison to the individual compounds.

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

Campioli 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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**4. Investigations on the dose-response relationship of combined exposure to low doses of three anti-androgens in Wistar rats.Schneider S, Fussell KC, Melching-Kollmuss S, Buesen R, Gröters S, Strauss V, Jiang X, van Ravenzwaay B.
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Arambula SE, Fuchs J, Cao J, Patisaul HB. Neurotoxicology. 2017 Dec;63:33-42. doi: 10.1016/j.neuro.2017.09.002.

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Gong M, Huai Z, Song H, Cui L, Guo Q, Shao J, Gao Y, Shi H. Chemosphere. 2017 Nov;187:140-146. doi: 10.1016/j.chemosphere.2017.08.119.

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35. Data describing lack of effects of 17α-ethinyl estradiol on mammary gland morphology in female mice exposed during pregnancy and lactation.
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Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Science (all databases) og dækker perioden 18/9 - 5/12 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 til inklusion af abstract og yderligere kommentarer. 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

Thyroid disrupting effects of halogenated and next generation chemicals on the swim bladder development of zebrafish.

Godfrey A, Hooser B, Abdelmoneim A, Horzmann KA, Freemanc JL, Sepulveda MS.

Aquatic Toxicology. 193: 228-235. 2017.

ABSTRACT:

Endocrine disrupting chemicals (EDCs) can alter thyroid function and adversely affect growth and development. Halogenated compounds, such as perfluorinated chemicals commonly used in food packaging, and brominated flame retardants used in a broad range of products from clothing to electronics, can act as thyroid disruptors. Due to the adverse effects of these compounds, there is a need for the development of safer next generation chemicals. The objective of this study was to test the thyroid disruption potential of old use and next generation halogenated chemicals. Zebrafish embryos were exposed to three old use compounds, perfluorooctanoic acid (PFOA), tetrabromobisphenol A (TBBPA) and tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and two next generation chemicals, 9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxdie (DOPO) and perfluorobutyric acid (PFBA). Sub-chronic (0–6 days post fertilization (dpf)) and chronic (0–28 dpf) exposures were conducted at 1% of the concentration known to kill 50% (LC_{50}) of the population. Changes in the surface area of the swim bladder as well as in expression levels of genes involved in the thyroid control of swim bladder inflation were measured. At 6 dpf, zebrafish exposed to all halogenated chemicals, both old use and next generation, had smaller posterior swim bladder and increased expression in the gene encoding thyroid peroxidase, *tpo* and the genes encoding two swim bladder surfactant proteins, *sp-a* and *sp-c.* These results mirrored the effects of thyroid hormone-exposed positive controls. Fish exposed to a TPO inhibitor (methimazole, MMI) had a decrease in *tpo* expression levels at 28 dpf. Effects on the anterior swim bladder at 28 dpf, after exposure to MMI as well as both old and new halogenated chemicals, were the same, i.e., absence of SB in ~50% of fish, which were also of smaller body size. Overall, our results suggest thyroid disruption by the halogenated compounds tested via the swim bladder surfactant system. However, with the exception of TBBPA and TDCPP, the concentrations tested (~5–137 ppm) are not likely to be found in the environment.

Knotting nets-molecular junctions of interconnecting endocrine axes identified by application of the adverse outcome pathway (AOP) concept.

Brüggemann M, Licht O, Fetter E, Teigeler M, Schafers C, Eilebrecht E.

Environmental Toxicology and Chemistry. 2017. DOI: 10.1002/etc.3995.

ABSTRACT:

In order to be defined as endocrine disruptor, a substance has to meet several criteria, including the induction of specific adverse effects, specific endocrine mode-of-action and a plausible link between both. Especially the latter criterion might not always be unequivocally determined, particularly as the endocrine system consists of diverse endocrine axes. The axes closely interact with each other, and manipulation of one triggers effects on the other.

This review aimed at identifying some of the many interconnections between these axes. This study focusses on fish, but also considers data obtained in studies on amphibians and mammals if these assist in closing data gaps, as most of the endocrine mechanisms are evolutionary conserved.

The review comprises data of ecotoxicological studies, as well as data on physiological processes. The gathered information delivers data on hormone/hormone receptor interactions or gene transcription

regulation. The identified key events (KE) and KE relationships (KER) provide explanations for unexpected effects on one axis, exerted by substances suspected to act specifically on another axis.

Based on these data, several adverse outcome pathway (AOP) segments were identified, describing connections between the HPG- and HPT-axes, the HPG- and HPA/I-axes, and the HPT- and HPA/I-axes. Central KEs identified across axes were altered aromatase activity, and altered expression and function of the proteins 11β-hydroxysteroid dehydrogenase (11β-HSD) and steroidogenic acute regulatory (StAR) protein. Substance classes, which act on more than one endocrine axis were for example goitrogens or aromatase inhibitors.

Despite the wealth of gathered information, it only provides a small insight into the molecular nets of endocrine axes, demonstrating the complexity of the interconnections between endocrine axes.

Vitellogenin concentrations in feral Danish brown trout have decreased: An effect of improved sewage treatment in rural areas?

Morthorst JE, Mathiesen KK, Holbech H, Pedersen KL, Bjerregaard P.

Environmental Toxicology and Chemistry. 2017. DOI: 10.1002/etc.4016.

ABSTRACT:

Feminization of male and juvenile fish caused by exposure to estrogens or estrogenic chemicals in effluents from central wastewater treatment plants (WWTP) is a worldwide issue of concern. Intersex and induction of the female yolk protein, vitellogenin, in male and juvenile fish are robust biomarkers for estrogenic exposure, and feminized fish have been observed downstream WWTP outlets in many countries. Danish central WWTPs reduce effluent estrogenicity effectively by advanced sewage treatment, and feminizations have not been observed downstream central WWTP outlets. However, between 2000 and 2004 investigations of Danish streams not receiving sewage from central WWTPs revealed a high variation in vitellogenin concentrations of male juvenile brown trout (*Salmo trutta*); some individuals had high concentrations probably due to point sources, and the plasma concentration was >50 ng mL^{-1} in 79 % of the juvenile males. The streams were re-investigated in 2010-2016, and the average male level had decreased to a hitherto unseen baseline level; in 2010 only 0.7% (one individual) of the males had a vitellogenin concentration >50 ng mL^{-1}, and could indicate that the estrogenicity of the streams decreased after 2004. We examined possible estrogenic sources in streams unaffected by central WWTP effluents, and found that the reduced vitellogenin levels are most likely explained by a national effort to improve on-site wastewater treatment in scattered houses not connected to central WWTPs.

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