

Litteraturgennemgang for perioden juli 2015 – september 2015

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Humane studier ved Afd. for Vækst og Reproduktion, Rigshospitalet

Søgning er udført på PubMed og dækker perioden 1. juli 2015 - 20. september 2015

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 frasortet. De kommenterede artikler er highlightet.

De fem udvalgte artikler omhandler phthalater og sammenhæng med pubertetsudvikling og mandlig reproduktionsudvikling. For sidstnævnte er der både medtaget et metastudie og et originalstudie. Endelig er der en artikel, der handler om overførsel via modermælken af per- og polyflourerede stoffer.

God læselyst!

Udvalgte publikationer

Association Between Urinary Phthalates and Pubertal Timing in Chinese Adolescents

Shi H, Cao Y, Shen Q, Zhao Y, Zhang Z, Zhang Y

J Epidemiol. 2015 Sep 5;25(9):574-82

BACKGROUND: Phthalates are synthetic chemicals and ubiquitous environmental contaminants, with hormonal activity that may alter the course of pubertal development in children.

OBJECTIVES: To determine whether exposure to phthalate metabolites is associated with timing of pubertal development in a cross-sectional study of a school-based clustered sample of 503 children from a suburban district in Shanghai, China, who were 7-14 years of age at enrollment (2010 October to November).

METHODS: We analyzed six phthalate metabolites in urine samples by isotope-dilution liquid chromatography tandem mass spectrometry. The associations of exposures to phthalates with pubertal timing of testes, breast, and pubic hair development (represented as Tanner stages) were evaluated using an ordered logistic regression model adjusted for chronological age, body fat proportion (BF%), and parental education.

RESULTS: In boys, urinary mono-n-butyl phthalate (MBP) levels were negatively associated with testicular volume, and mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) levels were negatively associated with pubic hair stages. The odds of being in an advanced stage were decreased by 43%-51%. In girls, mono (2-ethylhexyl) phthalate (MEHP), MEHHP, and MEOHP levels, as well as the sum of these levels, were positively associated with breast stages, and the association was much stronger in girls with high BF%; the odds of being in an advanced stage were increase by 29% to 50%.

CONCLUSIONS: Phthalate metabolites investigated in this study show significant associations with pubertal timing both in boys and in girls, especially among girls with high BF%.

Human urinary/seminal phthalates or their metabolite levels and semen quality: A meta-analysis

Cai H, Zheng W, Zheng P, Wang S, Tan H, He G, Qu W.

Environ Res. 2015 Aug 10;142:486-494

Health concerns surrounding human exposure to phthalates include diminished semen quality. Epidemiological findings remain inconsistent. We have performed a quality appraisal and meta-analysis to quantitatively summarize evidence for associations between phthalate exposures and human semen quality. Pubmed and Web of Science were searched for pertinent studies through October 2014. Cited references were reviewed to identify secondary studies. Studies that reported quantitative estimates of the association between phthalates or their metabolite levels in humans and semen quality were eligible. Random effects models were used to calculate pooled effects estimates. Overall, 20 studies met our inclusion criteria. Subsequently, 14 studies were included in the meta-analysis. Urinary monobutyl phthalate (MBP) and monobenzyl phthalate (MBzP) were associated with reduced sperm concentration (MBP [7.4-25.3µg/L], pooled odds ratio [OR]=2.60, 95% confidence interval [CI]=1.32-5.15; MBzP [14.0-540.2µg/L], pooled OR=2.23, 95% CI=1.16-4.30). Both MBP (24.6-14,459.0µg/L) and MEHP (3.1-208.1µg/L) were inversely associated with straight line velocity (VSL; MBP, pooled β =-2.51, 95% CI=-4.44, -0.59; MEHP, pooled β =-1.06, 95% CI=-1.99, -0.12). An IQR increase in MBzP and MEP levels (MBzP, IQR=11.35µg/L; MEP, IQR=449.4µg/L) was associated with an increase in comet extent (CE; MBzP, pooled β =3.57, 95% CI=0.89-6.25; MEP, pooled β =4.22, 95% CI=1.66-6.77). No associations were observed between monomethyl phthalate and any semen parameters. Our meta-analysis strengthens the evidence that specific phthalates or their metabolite levels may affect semen quality.

Phthalate exposure and reproductive parameters in young men from the general Swedish population

Axelsson J, Rylander L, Rignell-Hydbom A, Jönsson BA, Lindh CH, Giwercman A.

Environ Int. 2015 Aug 25;85:54-60.

BACKGROUND: In animals, exposure to certain phthalates negatively affects the male reproductive function. Human results are conflicting and mostly based on subfertile males, in whom the association between exposure and reproductive function may differ from the general population.

OBJECTIVES: To study if levels of phthalate metabolites were associated with semen quality and reproductive hormones in general Swedish men.

METHODS: We recruited 314 young men delivering semen, urine and blood samples at the same visit. We analyzed reproductive hormones and several semen parameters including progressive motility and high DNA stainability (HDS)-a marker for sperm immaturity. In urine, we analyzed metabolites of phthalates, including diethylhexyl phthalate (DEHP). We studied associations between urinary levels of the metabolites and seminal as well as serum reproductive parameters, accounting for potential confounders.

RESULTS: DEHP metabolite levels, particularly urinary mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), were negatively associated with progressive sperm motility, which was 11 (95% CI: 5.0-17) percentage points lower in the highest quartile of MECPP than in the lowest. Further, men in the highest quartile of the DEHP metabolite monoethylhexyl phthalate had 27% (95% CI: 5.5%-53%) higher HDS than men in the lowest quartile.

CONCLUSIONS: DEHP metabolite levels seemed negatively associated with sperm motility and maturation.

Considerable exposure to the endocrine disrupting chemicals phthalates and bisphenol-A in intensive care unit (ICU) patients.

Huygh J, Clotman K, Malarvannan G, Covaci A, Schepens T, Verbrugghe W, Dirinck E, Van Gaal L, Jorens PG.

Environ Int. 2015 Aug;81:64-72.

Critical care medicine has largely benefited from plastic-containing medical devices. However, bisphenol-A (BPA) and phthalates present in the plastics can leach from such devices. We hypothesized that intensive care unit (ICU) patients are exposed to BPA and phthalates through (plastic) medical devices. Serum (n = 118) and urine (n= 102) samples of adult ICU patients (n = 35) were analyzed for total BPA and phthalate metabolites (PMs). Our results showed that adult ICU patients are continuously exposed to phthalates, such as di(2-ethylhexyl)phthalate (DEHP), as well as to BPA, albeit to a lesser extent. This exposure resulted in detectable high serum and urinary levels in almost every patient and at every studied time point. Moreover, these levels were significantly higher than in controls or compared to referenced literature. The chronology of exposure was demonstrated: pre-operative urinary and serum levels of the DEHP metabolites were often below the detection limit. Plastic-containing medical devices were the main source of DEHP exposure: post-operative patients on hemofiltration, extracorporeal membrane oxygenation or both showed serum levels 100-or 1000-fold higher than the levels in the general population reported in the literature. The serum and some of the urinary levels of the DEHP metabolites are the highest ever reported in humans; some at biologically highly relevant concentrations of ≥ 10 -50 μ M. Despite the continuously tightening regulations, BPA and DEHP appear to be still present in (some) medical devices. Because patient safety is a concern in the ICU, further research into the (possibly toxic and clinical) effects of these chemicals released from medical devices is imperiously necessary.

Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates

Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jørgensen E.

Environ Sci Technol. 2015 Sep 1;49(17):10466-73.

Perfluorinated alkylate substances (PFASs) are widely used and have resulted in human exposures worldwide. PFASs occur in breast milk, and the duration of breastfeeding is associated with serum-PFAS concentrations in children. To determine the time-dependent impact of this exposure pathway, we examined the serum concentrations of five major PFASs in a Faroese birth cohort at birth, and at ages 11, 18, and 60 months. Information about the children's breastfeeding history was obtained from the mothers. The trajectory of serum-PFAS concentrations during months with and without breastfeeding was examined by linear mixed models that accounted for the correlations of the PFAS measurements for each child. The models were adjusted for confounders such as body size. The duration of exclusive breastfeeding was associated with increases of most PFAS concentrations by up to 30% per month, with lower increases during partial breast-feeding. In contrast to this main pattern, perfluorohexanesulfonate was not affected by breast-feeding. After cessation of breastfeeding, all serum concentrations decreased. This finding supports the evidence of breastfeeding being an important exposure pathway to some PFASs in infants.

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 2015/06/30 to 2015/12/31 (Juli 2015 og fremefter)

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 35 artikler(bruttolisten).

Udvalgte publikationer

2 artikler er blevet udvalgt til nærmere beskrivelse baseret på, at resultaterne bidrager til ny eller yderligere viden om grupper af hormonforstyrrende stoffer, samt kilder til eksponering for disse.

Den første artikel omhandler et *in vitro* studie med det formål, at etablere et nyt link mellem faktiske interne niveauer af persistente organiske miljøgifte (POPs) i mennesker og den koncentration, hvor de udviser hormonforstyrrende aktivitet *in vitro*.

Den anden artikel omhandler et studie af de potentielle hormonforstyrrende egenskaber af ekstrakter fra tre forskellige såkaldt "medicinske planter": Ginkgo biloba, Elettaria cardamomum og Plantago ovata.

Endocrine activity of persistent organic pollutants accumulated in human silicone implants - Dosing in vitro assays by partitioning from silicone.

Gilbert D, Mayer P, Pedersen M, Vinggaard AM.

Environ Int. 2015 Nov;84:107-14. doi: 10.1016/j.envint.2015.07.008. Epub 2015 Aug 8.

Persistent organic pollutants (POPs) accumulated in human tissues may pose a risk for human health by interfering with the endocrine system. This study establishes a new link between actual human internal POP levels and the endocrine active dose *in vitro*, applying partitioning-controlled dosing from silicone to the H295R steroidogenesis assay: (1) Measured concentrations of POPs in silicone breast implants were taken from a recent study and silicone disks were loaded according to these measurements. (2) Silicone disks were transferred into H295R cell culture plates in order to control exposure of the adrenal cells by equilibrium partitioning. (3) Hormone production of the adrenal cells was measured as toxicity endpoint. 4-Nonylphenol was used for method development, and the new dosing method was compared to conventional solvent-dosing. The two dosing modes yielded similar dose-dependent hormonal responses of H295R cells. However, with the partitioning-controlled freely dissolved concentrations (C_{free}) as dose metrics, dose-response curves were left-shifted by two orders of magnitude relative to spiked concentrations. Partitioning-controlled dosing of POPs resulted in up to 2-fold increases in progesterone and corticosteroid levels at C_{free} of individual POPs in or below the femtomolar range. Silicone acted not only as source of the POPs but also as a sorption sink for lipophilic hormones, stimulating the cellular hormone production. Methodologically, the study showed that silicone can be used as reference partitioning phase to transfer *in vivo* exposure in humans (silicone implants) to *in vitro* assays (partition-controlled dosing). The main finding was that POPs at the levels at which they are found in humans can interfere with steroidogenesis in a human adrenocortical cell line.

Assessment of hormone-like activities in Ginkgo biloba, Elettaria cardamomum and Plantago ovata extracts using in vitro receptor-specific bioassays.

Real M, Molina-Molina JM, Jimenez J, Diéguez HR, Fernández MF, Olea N.

Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2015 Sep;32(9):1531-41. doi: 10.1080/19440049.2015.1071922. Epub 2015 Aug 4.

Medicinal plants are widely used for the treatment of diseases and for the development of new drugs. This study was designed to determine the presence of hormone-like activities dependent on the activation of human estrogen receptor alpha (hER α) and/or androgen receptor (hAR) in methanol extracts prepared from three medicinal plants historically and currently used for therapeutic purposes: Ginkgo biloba leaves (GBL), Elettaria cardamomum seeds (ECS) and Plantago ovata seeds (POS). After a solid-liquid extraction (SLE) step, their effects on hER α function were assessed in MCF-7 breast cancer cells using the E-Screen bioassay, and their ability to induce hAR-mediated reporter gene expression was evaluated using the androgen-sensitive stable prostatic PALM cell line. Unlike POS extracts, GBL and ECS extracts showed estrogenic (0.07 and 0.20 nM E2Eq mg⁻¹, respectively) and anti-estrogenic (0.01 and 0.02 μ M ICI182780Eq mg⁻¹, respectively) activities. ECS extracts evidenced androgenic activity (0.30 nM R1881Eq mg⁻¹) and POS extracts anti-androgenic activity (22.30 μ M ProcEq mg⁻¹). According to these findings, these plant extracts may interfere with the endocrine system via one or more hormonal receptors, and further investigation is warranted into their role as endocrine disruptors in humans.

Bruttolisten (in vitro)

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Garg A, Rai G, Lodhi S, Jain AP, Yadav AK.

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Herudover er der yderligere 1 artikel, som ikke blev fanget af de valgte søgekriterier:

Effects of Common Pesticides on Prostaglandin D2 (PGD2) Inhibition in SC5 Mouse Sertoli Cells, Evidence of Binding at the COX2 Active Site, and Implications for Endocrine Disruption.

Kugathas S, Audouze K, Ermler S, Orton F, Rosivatz E, Scholze M, Kortenkamp A.

Environ Health Perspect. 2015 Sep 11. [Epub ahead of print]

In vivo studier ved DTU Fødevareinstituttet

Søgning er udført på PubMed og dækker perioden juli - ultimo september 2015

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

Efter at have fjernet gengangere fra dem vi havde med på den forrige litteraturopdateringsliste samt *in vitro*, human eller SDU relevante artikler, gav litteratursøgningen en liste med i alt 44 artikler (Bruttolisten).

To artikler er blevet udvalgt til nærmere beskrivelse (abstrakt og konklusion). Disse artikler er valgt fordi vi mener de bidrager til ny viden om hormonforstyrrende stoffer og her er der særligt fokus på Anilins effekter (Holm et al. 2015) og Bisphenol A adfærd (Rebuli et al. 2015).

Rigtig God læselyst.

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

Udvalgte publikationer

Aniline is rapidly converted into paracetamol impairing male reproductive development.

Holm JB, Chalmey C, Modick H, Jensen LS, Dierkes G, Weiss T, Jensen B, Nørregård MM, Borkowski K, Styrihave B, Koch HM, Mazaud-Guittot S, Jegou B, Kristiansen K, Kristensen DM. Toxicol Sci. 2015 Aug 10. pii: kfv179. [Epub ahead of print]

Industrial use of aniline is increasing worldwide with production estimated to surpass 5.6 million metric tons in 2016. Exposure to aniline occurs via air, diet and water augmenting the risk of exposing a large number of individuals. Early observations suggest that aniline is metabolised to paracetamol/acetaminophen, likely explaining the omnipresence of low concentrations of paracetamol in European populations. This is of concern as recent studies implicate paracetamol as a disrupter of reproduction. Here we show through steroidogenic profiling that exposure to aniline led to increased levels of the $\Delta 4$ steroids, suggesting that the activity of CYP21 was decreased. By contrast, paracetamol decreased levels of androgens likely through inhibition of CYP17A1 activity. We confirm that aniline in vivo is rapidly converted to paracetamol by the liver. Intrauterine exposure to aniline and paracetamol in environmental and pharmaceutical relevant doses resulted in shortening of the anogenital distance in mice, a sensitive marker of fetal androgen levels that in humans is associated with reproductive malformations and later life reproductive disorders. In conclusion, our results provide evidence for a scenario where aniline, through its conversion into anti-androgenic paracetamol, impairs male reproductive development.

Impact of Low Dose Oral Exposure to Bisphenol A (BPA) on Juvenile and Adult Rat Exploratory and Anxiety Behavior: A CLARITY-BPA Consortium Study.

Rebuli ME, Camacho L, Adonay ME, Reif DM, Aylor DL, Patisaul HB. Toxicol Sci. 2015 Jul 23. pii: kfv163. [Epub ahead of print]

Bisphenol A (BPA) is a high volume production chemical and has been identified as an endocrine disruptor, prompting concern that developmental exposure could impact brain development and behavior. Rodent and human studies suggest that early life BPA exposure may result in an anxious, hyperactive phenotype, but results are conflicting and data from studies using multiple doses below the no-observed-adverse-effect level (NOAEL) are limited. To address this, the present studies were conducted as part of the CLARITY-BPA (Consortium Linking Academic and Regulatory Insights on BPA Toxicity) program. The impact of perinatal BPA exposure (2.5, 25, or 2500 $\mu\text{g}/\text{kg}$ body weight (bw)/day) on behaviors related to anxiety and exploratory activity was assessed in juvenile (pre-pubertal) and adult NCTR Sprague-Dawley rats of both sexes. Ethinyl estradiol (EE; 0.5 $\mu\text{g}/\text{kg}$ bw/day) was used as a reference estrogen. Exposure spanned gestation and lactation with dams gavaged from gestational day 6 until birth, and then the offspring gavaged directly through weaning ($n = 12/\text{sex}/\text{group}$). Behavioral assessments included open field, elevated plus maze, and zero maze. Anticipated sex differences in behavior were statistically identified or suggested in most cases. No consistent effects of BPA were observed for any endpoint, in either sex, at either age compared to vehicle controls; however, significant differences between BPA-exposed and EE-exposed groups were identified for some endpoints. Limitations of the present study are discussed and include sub-optimal statistical power and low concordance across behavioral tasks. These data do not indicate BPA-related effects on anxiety or exploratory activity in these developmentally exposed rats.

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Wildlife studier ved Biologisk Institut, Syddansk Universitet (SDU)

Søgningen er udført på Web of Knowledge (all databases) og dækker perioden 24/6 - 29/9 2015.

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 medtagelse af abstract og yderligere kommentarer.

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. Endelig medtages efter Miljøstyrelsens ønske artikler omhandlende parabener.

Udvalgte publikationer

In vivo and in silico analyses of estrogenic potential of bisphenol analogs in medaka (*Oryzias latipes*) and common carp (*Cyprinus carpio*).

Yamaguchi A, Ishibashi H, Arizono K, Tominaga N.
Ecotoxicology and Environmental Safety 120: 198-205.

ABSTRACT: Various studies have demonstrated the estrogenic effect of bisphenol A (BPA), a member of bisphenol analogs (BPs), in in vitro and in vivo assays. However, limited data are available on the estrogenic potentials and risks of other BPs in aquatic organisms. In addition, the estrogenic effect of chemicals is known to have species-specific responses in teleost fish. The objective of this study was to evaluate the potential estrogenic effects of BPs on the medaka (*Oryzias latipes*) and common carp (*Cyprinus carpio*) using in vivo and in silico assays. Our quantitative real-time PCR analyses revealed that the expression levels of several hepatic estrogen-responsive biomarker genes in male medaka responded to various types and concentrations of BPs in a dose-response manner. The order of in vivo estrogenic potencies of BPs was as follows: BPC≈BPAF>BPB>BPA>>>BPP. To further investigate the interaction potential of BPs with medaka estrogen receptor α (ER α) in silico, a three-dimensional model of the ER α ligand-binding domain (LBD) was built and docking simulations were performed. The docking simulation analysis revealed that BPC interaction potential for medaka ER α LBD was the most potent, followed by BPAF and BPA. Comparing this with carp ER α LBD revealed that the interaction potentials of these BPs to medaka ER α LBD were more stable than to carp ER α LBD. Furthermore, we identified key amino acid residues in medaka ER α LBD that interacted with BPC (Glu356, Arg397, and Cys533), BPAF (Thr350 and Glu356), and BPA (Glu356 and Met424), and found some differences in these key amino acid residues between medaka and carp ER α LBDs. These results of in vivo and in silico analyses showed potential estrogenic effects of BPs in teleost fish, and they also indicated that the differences in interaction potentials and key amino acid residues between medaka and carp ER α LBDs may be due to the differences between the species and estrogenic potencies of the selected BPs.

Suburbanization, estrogen contamination, and sex ratio in wild amphibian populations.

Lambert M, Giller G, Barber L, Fitzgerald K, Skelly D.
Proc Natl Acad Sci USA 112(38): 11881-11886.

ABSTRACT: Research on endocrine disruption in frog populations, such as shifts in sex ratios and feminization of males, has predominantly focused on agricultural pesticides. Recent evidence suggests that suburban landscapes harbor amphibian populations exhibiting similar levels of endocrine disruption; however the endocrine disrupting chemical (EDC) sources are unknown. Here, we show that sex ratios of metamorphosing frogs become increasingly female-dominated along a suburbanization gradient. We further show that suburban ponds are frequently contaminated by the classical estrogen estrone and a variety of EDCs produced by plants (phytoestrogens), and that the diversity of organic EDCs is correlated with the extent of developed land use and cultivated lawn and gardens around a pond. Our work also raises the possibility that trace-element contamination associated with human land use around suburban ponds may be contributing to the estrogenic load within suburban freshwaters and constitutes another source of estrogenic exposure for wildlife. These data suggest novel, unexplored pathways of EDC contamination in human-altered environments. In particular, we propose that vegetation changes associated with suburban neighborhoods (e.g., from forests to lawns and ornamental plants) increase the distribution of phytoestrogens in surface waters. The result of frog sex ratios varying as a function of human land use implicates a role for environmental modulation of sexual differentiation in amphibians, which are assumed

to only have genetic sex determination. Overall, we show that endocrine disruption is widespread in suburban frog populations and that the causes are likely diverse.

Intersex in the clam *Scrobicularia plana* (Da Costa): Widespread occurrence in English Channel estuaries and surrounding areas.

Pope N, Childs K, Dang C, Davey M, O'Hara S, Langston K, Minier C, Pascoe P, Shortridge E, Langston W. *Marine Pollution Bulletin* 95(2): 598-609.

ABSTRACT: Estuarine clams *Scrobicularia plana* were sampled from 108 intertidal locations around the English Channel and adjacent areas. Although *S. plana* is believed to be a strict gonochorist, 58% of the populations sampled included intersexed individuals (described as male clams exhibiting ovotestis). Over the entire region, on average, 8.6% of male clams exhibited intersex, although proportions of affected males ranged from 0% to 53% depending on location. The severity of intersex was assessed using a simple classification scale, with the majority of individuals showing low levels of impact. Sex ratios were significantly skewed at some sites. There were no significant relationships between incidence or severity of intersex; or with size or parasitism of individual clams. Intersex in *S. plana* is a useful tool to assess endocrine disruptive effects in estuaries, although mechanisms of impact and causative agents remain uncertain.

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