

Prenatal exposure to
acetaminophen and phthalates:
Alterations in sexually dimorphic
development

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Newly emerging data suggest that exposure during pregnancy to analgesics such as acetaminophen (Tylenol, or APAP) is *widespread* and may impact outcomes altered by one or more phthalates by acting through similar mechanisms.

Outline

- Introduction to two classes of endocrine disruptors
 - Mild analgesics [e.g. acetaminophen (APAP)]
 - Phthalates [e.g. dibutyl phthalate (DEHP and DBP)]
- Reproductive toxicity [e.g. male AGD, cryptorchidism]
 - APAP
 - DEHP
- Neurotoxicity [e.g. Language development (LD)]
 - APAP
 - DBP
 - APAP + DBP

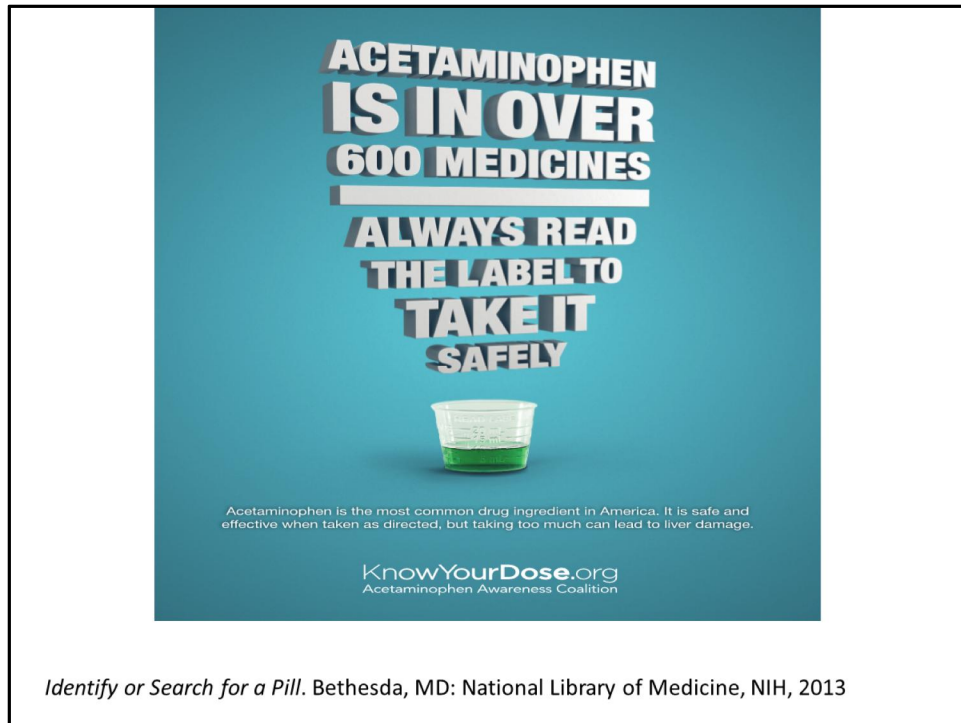
Today I will focus on *one analgesic (APAP), two phthalates (DEHP and DBP)* and their associations with male reproductive endpoints (*AGD and cryptorchidism*) and one neurodevelopmental endpoint (*language development*)



MILD ANALGESICS: ACETAMINOPHEN (APAP)

“Acetaminophen has not been formally assigned to a pregnancy category by the FDA but it is believed to be safe in pregnancy when used intermittently for short durations” Drugs.com.

Liver toxicity is a serious concern but I will not discuss this today



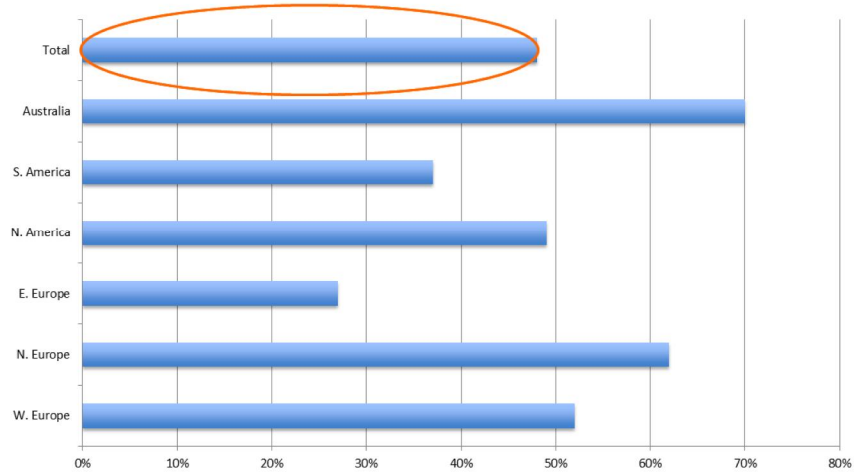
Because acetaminophen is a “silent partner” in many OTC and prescription drugs (including meds for pain, sleep, colds, coughs), when women are asked about their exposure they will likely underreport their APAP use.

Acetaminophen/Paracetamol/APAP

- N-acetyl-4-aminophenol (acetaminophen/paracetamol, NA4AP, or APAP):
 - One of the most commonly used over-the-counter analgesics worldwide (Modick et al 2015).
 - Not contra-indicated for use in pregnancy

Acetaminophen (APAP for short) is one of the most commonly used analgesics worldwide and because its use is not contraindicated in pregnancy, it is likely the analgesics *most widely used by pregnant women*

50% of women report using APAP in pregnancy



(Luccatelli et al 2014)

Based on questionnaire data (which likely underestimates exposure) 50% of women report using APAP while pregnant worldwide. The allowable daily dose is 4,000mg/day



- 2,200 pregnant women recruited in Karlstad, Sweden 2007-20100
- At recruitment (mean 10 weeks gestation) mothers:
 - Gave a urine sample
 - Reported on medication use from start of pregnancy to recruitment

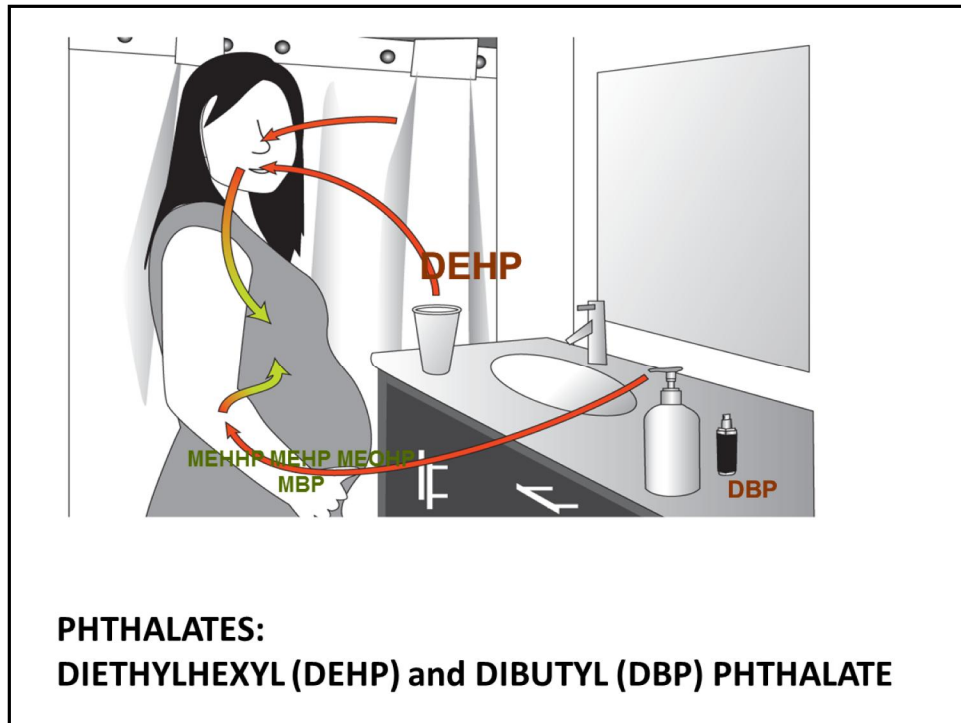
We looked at APAP exposure in SELMA, a large (2,000 moms and children) Swedish pregnancy cohort study. At recruitment (at median 10 weeks of pregnancy) we asked how much APAP women had taken since becoming pregnant. Women also gave a urine sample and in a pilot sample (140) we measured urinary concentration of APAP metabolites.

SELMA compared two methods of estimating APAP exposure

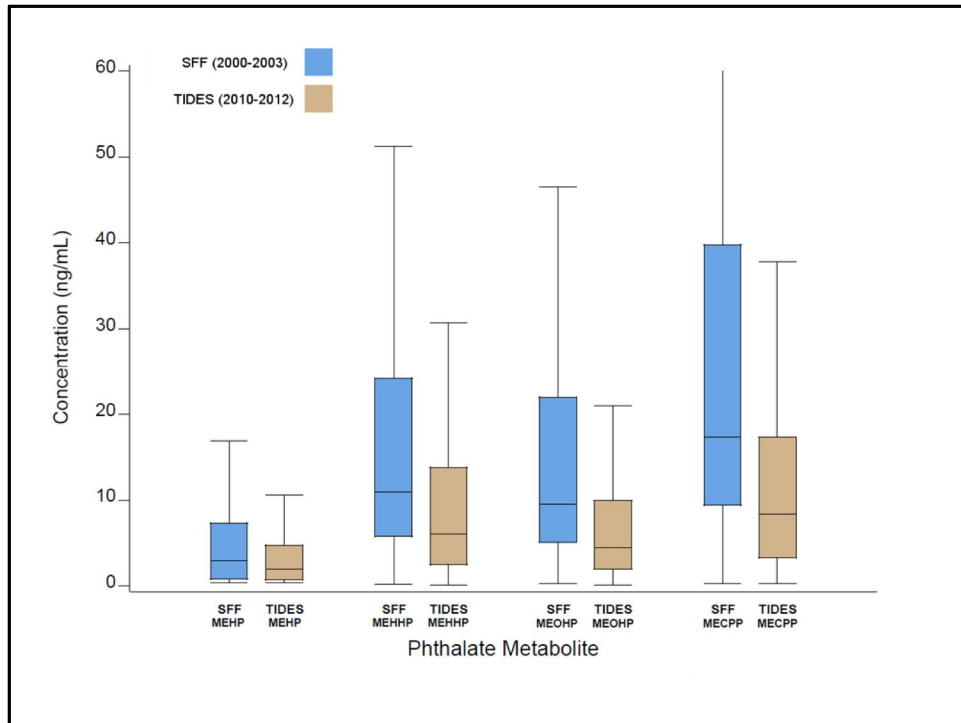
- Self-report :
 - Until now studies exposure has been based on mothers' self reports (assessed during pregnancy or after)
 - In SELMA mothers reported use of acetaminophen from the start of pregnancy to recruitment
- Bioassay:
 - APAP metabolites were measured in urine collected at study entry (plot study)

Exposure estimates based on these two methods were significantly correlated

No epidemiological study of APAP till now has examined biomarkers of APAP exposure. We found in SELMA that exposure estimates by self-report and by urinary biomarkers were significantly correlated (though both likely underestimate exposure, because of APAP's short half-life and mother's underreporting).



Let's turn to phthalates, a class of chemicals that enter our bodies without our knowledge when we eat foods and drink beverages and breathe air containing them. To stay within time constraints, I will talk about two phthalates DEHP and DBP, both known to decrease male fetal testosterone



Biomonitoring has shown that most pregnant women in the US population are exposed to these phthalates. 99% of US population is exposed to DEHP and DBP.

However, we showed in two pregnancy cohorts, recruited 10 years apart, that concentrations of these phthalates are decreasing in the US

APAP and Phthalates

REPRODUCTIVE TOXICITY

Turning to developmental outcomes, we will first consider reproductive outcomes associated with prenatal exposure to APAP and phthalates.

To date the reproductive endpoints linked to phthalates and APAP are in the male (consistent with their ability to decrease male fetal testosterone)

APAP and reproductive toxicity (1)

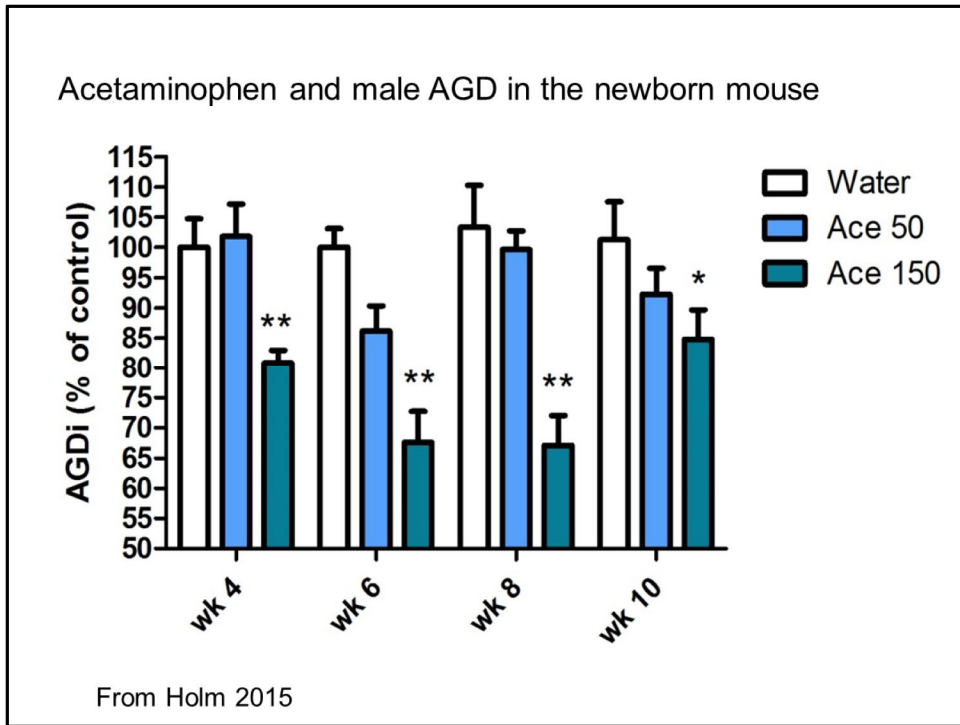
In lab studies APAP decreases testosterone and shortens AGD:

- Six of 7 *in vitro* and *ex vivo* studies found a **significant decrease in T production** with APAP exposure.
- Five of 6 *in vivo* studies 5 found a **significant reduction in male AGD** following prenatal APAP exposure.

Anderson and Swan 2017

APAP has been shown (in 6 of 7 laboratory studies that looked at this) to decrease testosterone production (a property shared by DEHP and DBP).

In 5 of 6 rodent studies male pups born to moms prenatally exposed to APAP showed significantly *increased rates of the genital defect cryptorchidism.*

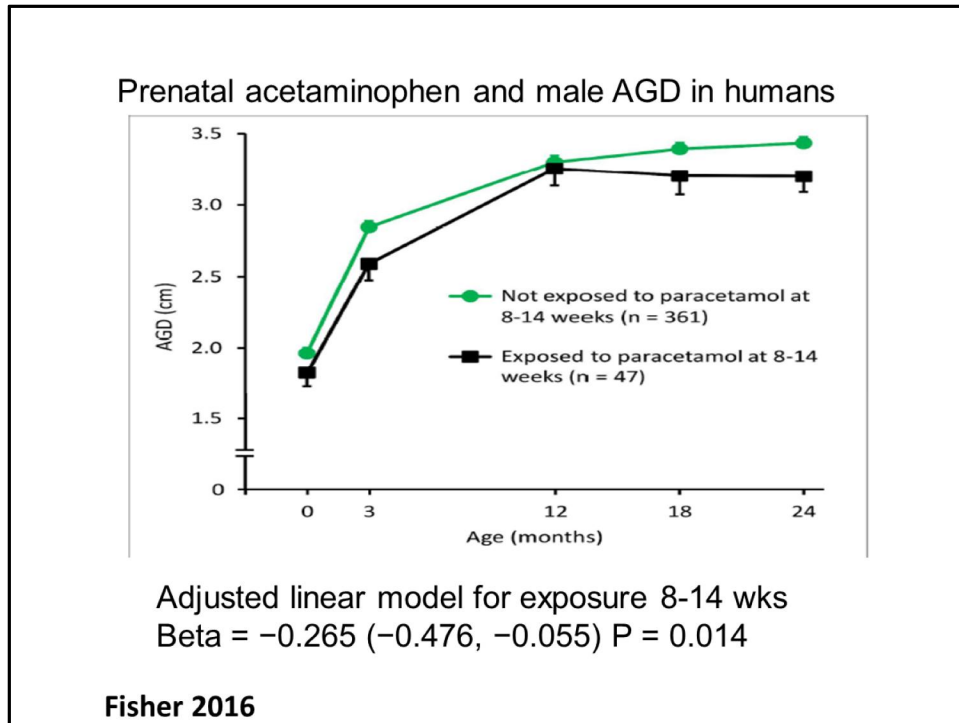


Prenatal APAP exposure also shortens the anogenital distance in rodents (another effect shared by DEHP and DBP)

APAP and reproductive outcomes (2)

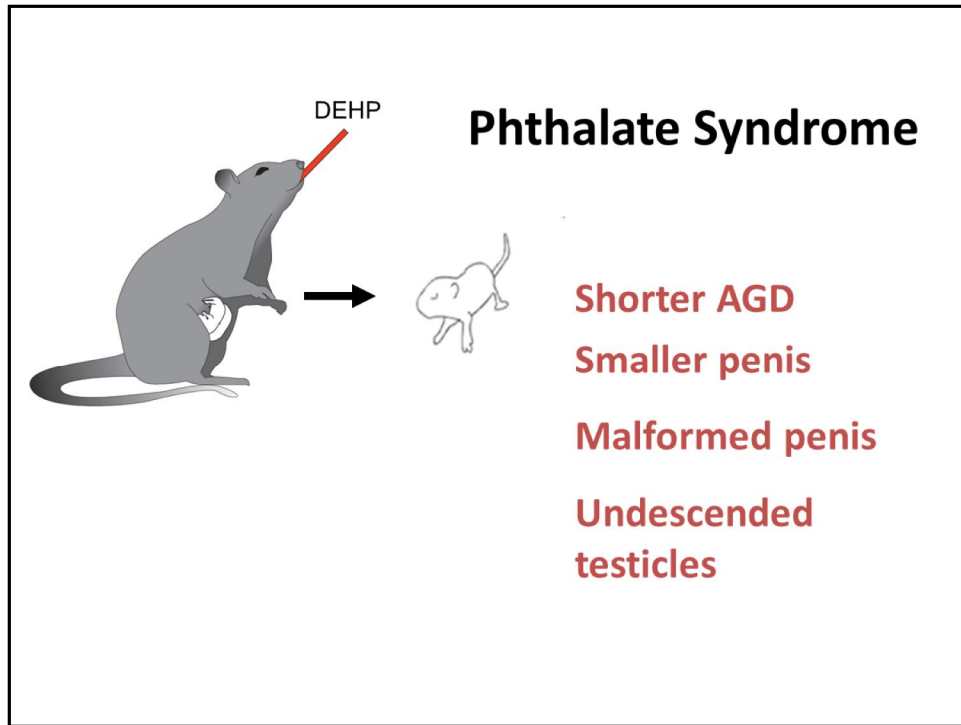
- APAP exposure and cryptorchidism in humans
 - ***Cryptorchidism significantly associated with self-reported prenatal APAP*** in 3 of 4 pregnancy cohorts [Jansen 2010, Kristensen 2011 (2 cohorts), Snijder 2012]
- APAP exposure and male AGD
 - ***Male AGD significantly inversely*** associated with APAP exposure in ***weeks 8-14*** (but not <8 or >14 weeks) (Fisher 2016) and mild analgesics <28 weeks (Lind 2016)

In human studies APAP has been significantly associated with both cryptorchidism and shorter male AGD



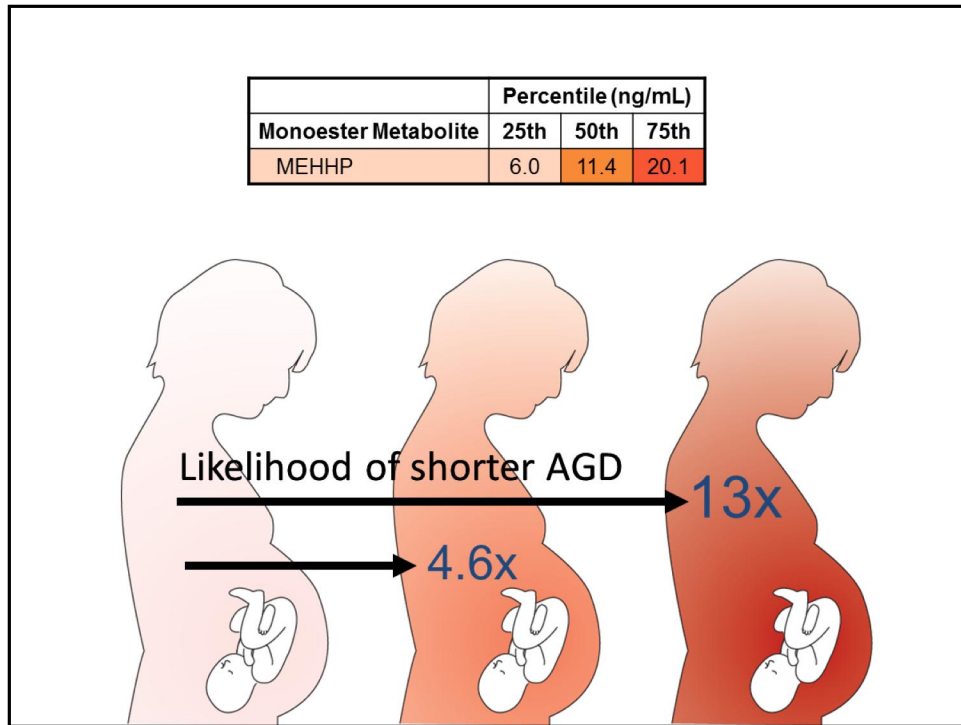
In the Cambridge Birth Cohort male infants had a shorter AGD throughout the first two years of life. (Other studies have linked shorter male AGD to semen quality, infertility and male genital anomalies).

Together these in vitro, in vivo and human studies demonstrate that APAP is an endocrine disruptor and a male reproductive toxicant



Turning to phthalates: There is a cluster of changes occurring in male rodents following *in utero* phthalate exposure, called the “phthalate syndrome”.

We asked, “Does prenatal exposure to DEHP and DBP cause the phthalate syndrome in humans?”



In multiple pregnancy cohorts DEHP was associated with shorter male AGD. In one the odds ratio for a boy having a short AGD (below median, adjusted) among mothers who had urinary MEHHP (a DEHP metabolite), that was high (13 times greater) or medium (4.6 times greater) compared to low (lower quartile).

DEHP (and DBP), like APAP, are endocrine disruptors and reproductive toxicants

APAP and Phthalates

NEUROTOXICITY: LANGUAGE DELAY

Testosterone is critical for neurodevelopment as well as reproductive tract development. Therefore, we examined neurodevelopment (here limited to one endpoint –language development) in relation to prenatal APAP and phthalate exposure.

Language delay in SELMA

- When children were 30 months old mothers were asked how many words their children used
- Those with <50 words were considered “language delayed”
- Language delay was <10% and higher in boys than girls

In Sweden all children have language development assessed at 30 months of age. Parents are asked how many words the child uses and those using <50 words are considered “language delayed” (LD). We looked at LD in relation to APAP and phthalate exposure in SELMA. We found <10% of children with LD, with a higher rate in boys than girls.

APAP exposure and Language Delay (LD) in SELMA

- Maternally reported APAP use was significantly associated with greater LD in girls but not boys
- Suggestion of a “protective effect”; the female male difference disappeared at highest dose
- Urinary APAP was also associated with greater LD in girls but not boys

Because data are unpublished I will only describe results.

We found that APAP exposure (whether assessed by maternal report or urinary biomarker) was significantly associated with LD in girls. In boys there was a suggestion of less LD, so that sex differences seen in the unexposed nearly disappeared at high APAP exposure.

Prenatal phthalate exposure and LD

In SELMA metabolites of two phthalates (DBP and BBzP) were associated with LD in both boys and girls

In The Infant Development and the Environment Study (TIDES) in the USA, the same LD questions were used:

- Rates of LD were similar to those in SELMA
- Concentration of DBP (not BBzP) in first trimester urine was associated with LD in boys and girls

We also looked at prenatal phthalate exposure in relation to language development in two studies.

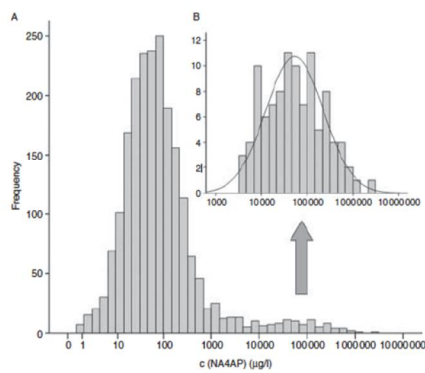
In SELMA metabolites of two anti-androgenic phthalates (DBP and BBzP) were associated with LD in both boys and girls.

In the US TIDES study, where rates of LD were similar to those in Sweden, DBP was associated with LD in both boys and girls

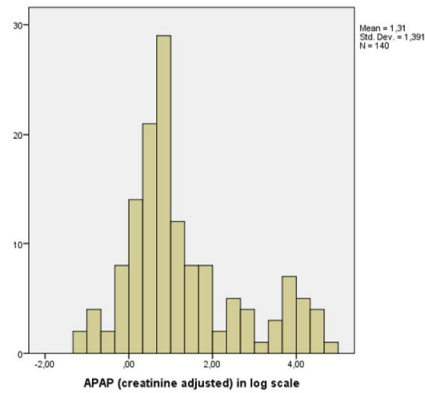
Aniline and other sources

**APAP IS ALSO AN ENVIRONMENTAL
EXPOSURE**

APAP exposure is ubiquitous



Sample of adult German population
(N=2098) (Modick et al 2014)



SELMA (Gestational week 10)
(N=140) (Bornehag unpublished)

There appears to be widespread *environmental exposure* to APAP which was detected in 100% of German population, SELMA and TIDES. In the distribution we see the low background exposure and a second peak from pharmaceutical exposure.

APAP: Not just a pharmaceutical exposure

- Environmental sources of APAP:
 - Surface and ground water (Mc Eachran et al 2015)
 - Fruit and vegetables, meat production (European Commission 2010)
 - Aniline (Holm et al 2015)

APAP has been found in surface and group water, fruits, vegetables and meats and it is an important component of aniline, a dye.

Aniline

- One of the most produced industrial chemicals; worldwide production likely to surpass 5.62 million tons in 2016 (Aster, 2014).
- An essential building block in modern day chemical production of compounds such as urethane polymers (e.g., synthetic fibers), dyes, rubbers and pesticides

Aniline is a high production chemical, used in many products including dyes, polymers and even pesticides. Data suggest it is hormonally active.

Colorants used in food, cosmetics, inks
and textiles including blue jeans



Aniline makes our blue jeans blue



Aniline Is Rapidly Converted Into Paracetamol Impairing Male Reproductive Development

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Lars Skovgaard Jensen,[§] Georg Dierkes,[‡] Tobias Weiss,[‡]
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David Møbjerg Kristensen*^{†,1}

“Aniline in vivo is rapidly converted to paracetamol by the liver”.

Surprisingly, in the body aniline is converted to APAP

This is clearly an environmental source of APAP!

Proposal

- Consider hormonally active pharmaceuticals as potential EDCs
- Examine endocrine disrupting effects of hormonally active pharmaceuticals across species
- Recommend minimal exposure to acetaminophen and other analgesics in pregnancy

Three points in closing

Hormonally active pharmaceuticals, like APAP should be considered as endocrine disrupting chemicals (EDCs)

Consider combined effects of APAP and other EDCs (like phthalates)

Reduce use of APAP and other analgesics by pregnant women (“as needed”)