

'ComPest' projektet

Kombinationseffekter af pesticider på fødselsvægt og metabolisme-programmering i rotter



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Beskyttelse af mennesker - Risikovurdering

- No Observed Adverse Effect Level (NOAEL)
- Deles med 10×10
- ADI, TDI eller DNEL ~ "sikkert" niveau
- Sammenlignes med eksponering af mennesker
- Ofte et stof ad gangen
- **MEN....**
- Cocktaileffekter ved NOAELs for de enkelte stoffer - for bl.a. stoffer, der forstyrrer kønshormoners virkning
- Coctaileffekter for stoffer med samme type effekter?



Formål med 'ComPest' projektet



- Fører eksponering for en blanding af 6 pesticider til lavere **fødselsvægt** ved doser under de enkelte pesticiders NOAELs?
- Kan **kombinationseffekten forudsiges** med modellen Dosis-Addition?
- Ses effekter på vækst og **metabolisk programmering** af ungerne ved brug af biomarkører for fedme og type 2 diabetes?
- At give **input til regulatoriske overvejelser**

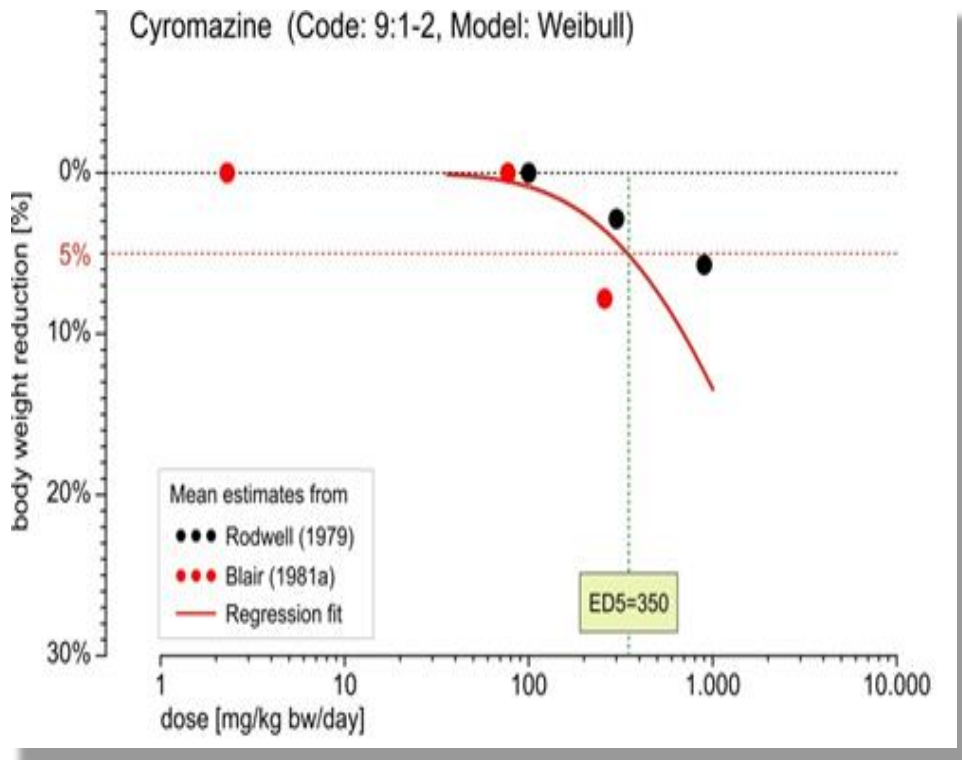
Mange stoffer og mange forskellige (ukendte) mekanismer

Link mellem lav fødselsvægt og bl.a. metaboliske sygdomme

Valg af pesticider



- Lavere fødselsvægt ved doser uden effekt på mødrene
- Dosis-response data analyseret med non-lineær regression
- 5% nedsat fødselsvægt for hvert pesticid -> Mix-100%



Pesticider og mix-doser

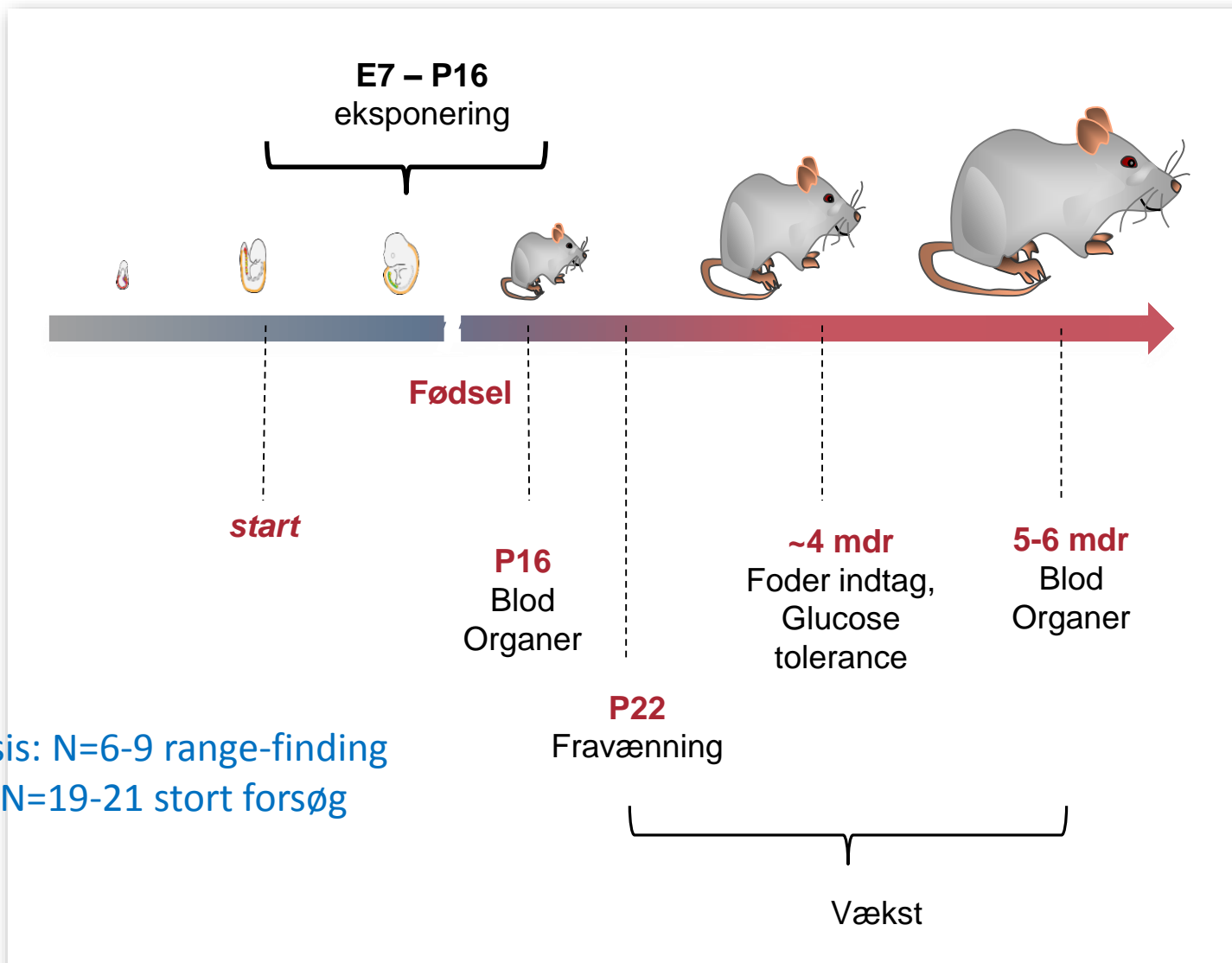


- Brugte ikke mix-100% - for højt!
- Pesticid-doser alene: under NOAEL

| Pesticid (renhed) | Mix-5% (mg/kg bw/day) | Mix-16% (mg/kg bw/day) | Mix-37.5% (mg/kg bw/day) |
|-----------------------------|------------------------------------|-------------------------------------|---------------------------------------|
| Cyromazine (97%) | 17.5 | 64,8 | 131.25 |
| MCPB (99.8%) | 5.0 | 18.5 | 37.50 |
| Pirimicarb (98.7%) | 3.0 | 11.1 | 22.50 |
| Quinoclamine (99.9%) | 0.5 | 1.9 | 3.75 |
| Thiram (99.9%) | 1.0 | 3.7 | 7.50 |
| Ziram (98.2%) | 1.0 | 3.7 | 7.50 |
| Total mix | 28 | 104 | 210 |

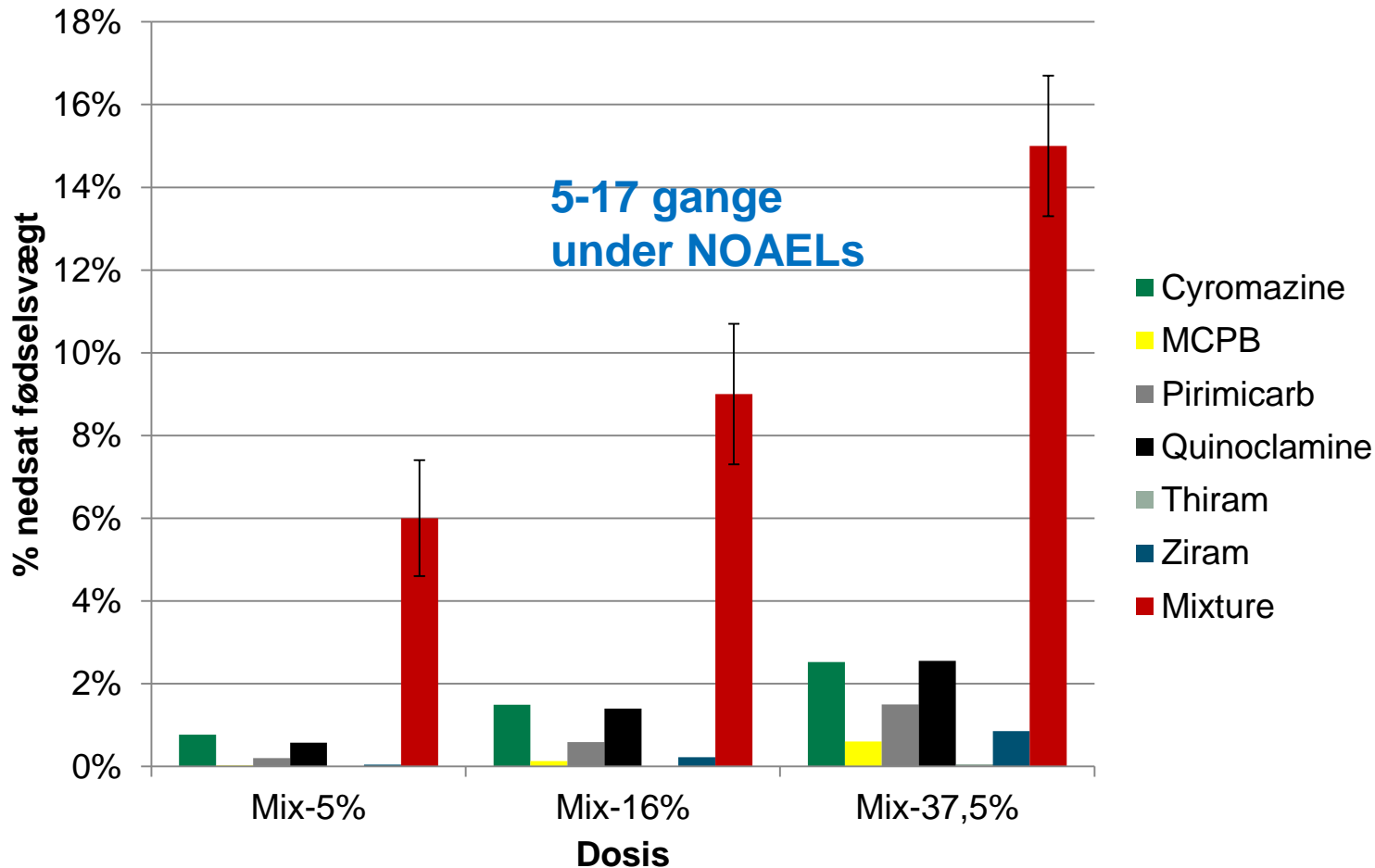
- Forventet cocktaileffekt beregnet med Dosis-Addition

Forsøgsdesign



Kuld/dosis: N=6-9 range-finding
forsøg; N=19-21 stort forsøg

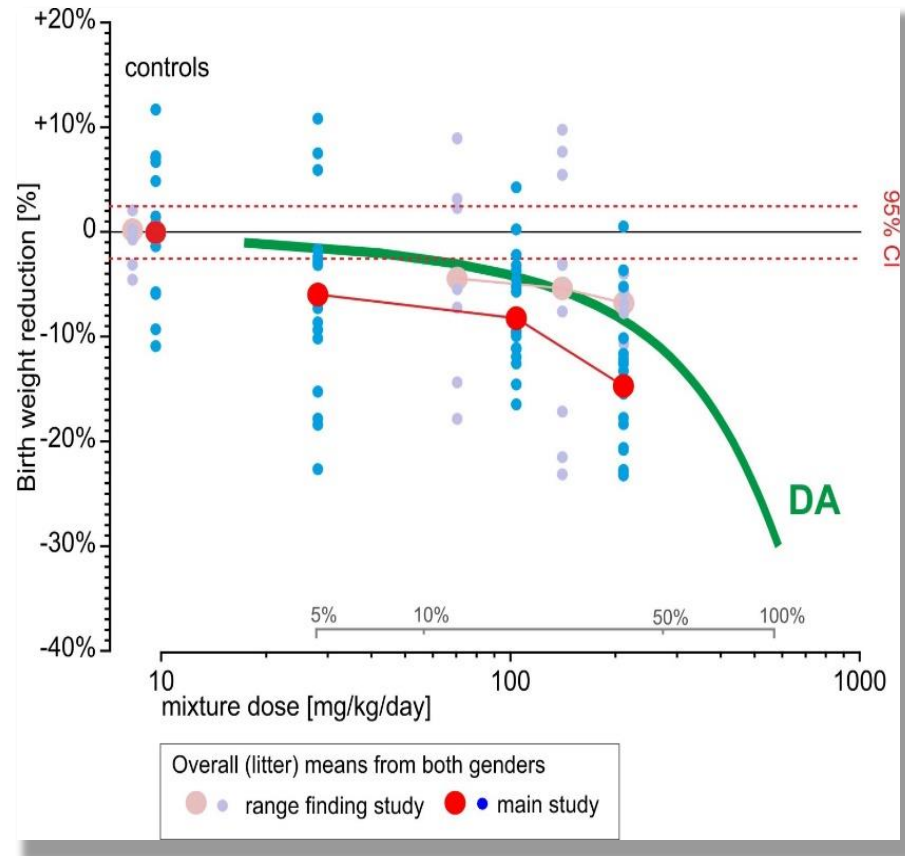
Nedsat fødselsvægt under NOAELs



Effekt af de enkelte pesticider – baseret på non-lineær regression

Kombinationseffekt - Dosis-Addition

Cyromazine
MCPB
Pirimicarb
Quinoclamine
Thiram
Ziram



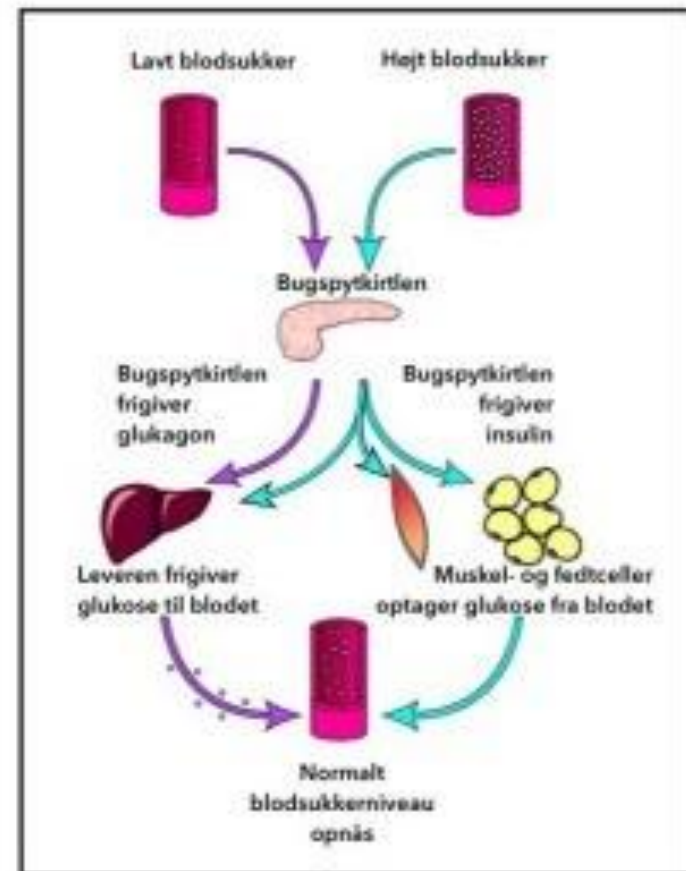
God forudsigelse af kombinationseffekten

Metabolisk programmering af ungerne og sen-effekter DTU

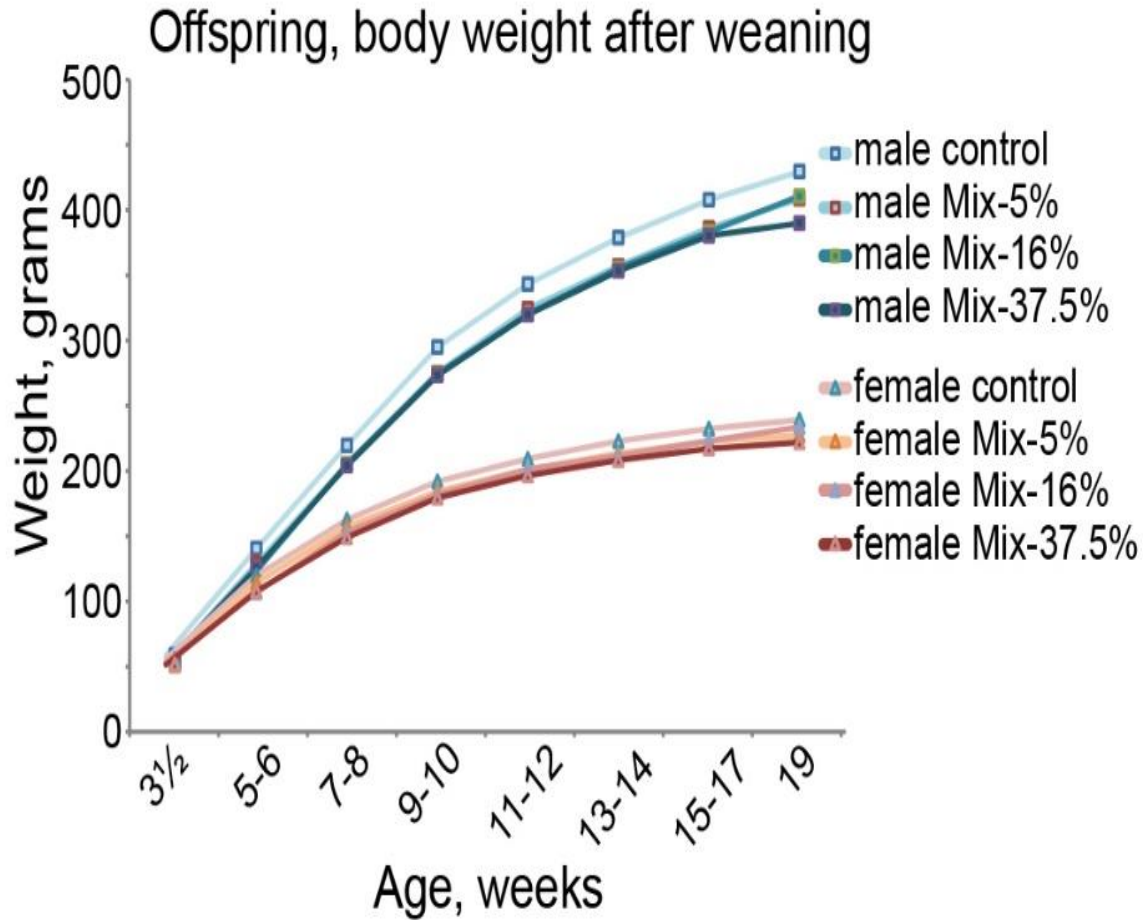


- Link mellem lav fødselsvægt og bl.a. metaboliske sygdomme
- Vækst hos ungerne?
- Glukose tolerance
- Biomarkører for fedme og type 2 diabetes?
- Effekter på metaboliske parametre?

Regulering af blodsukker



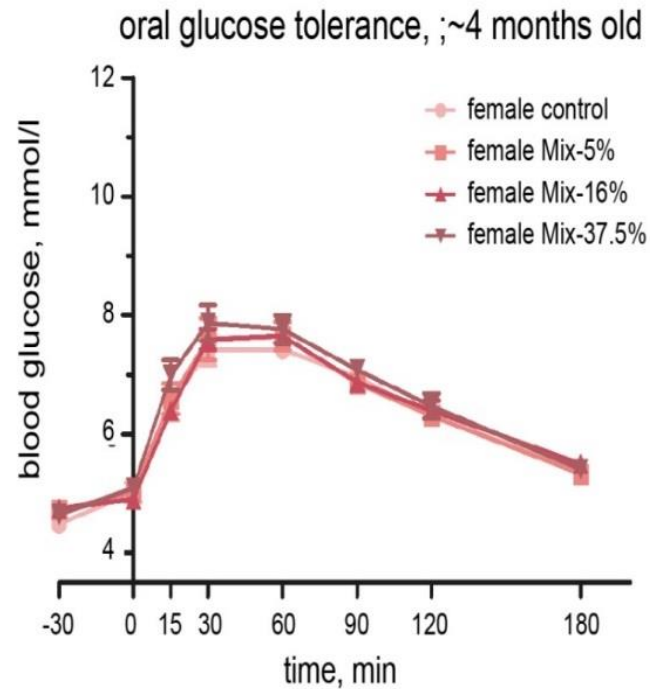
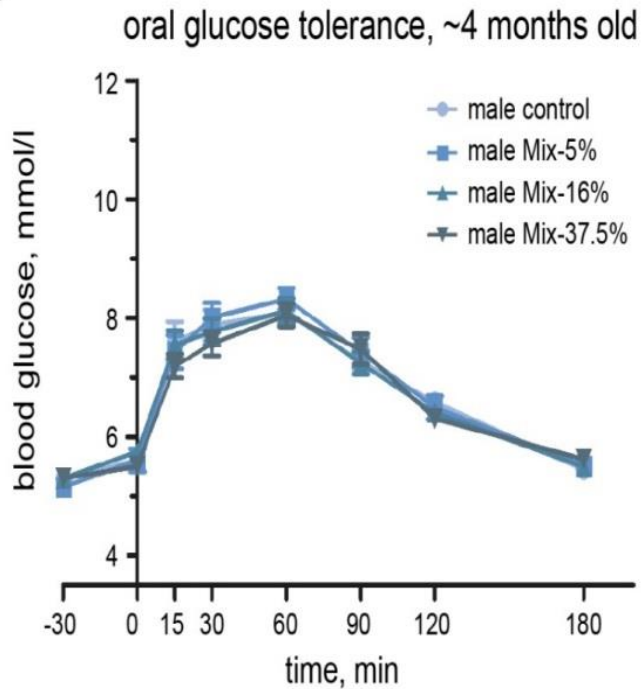
Nedsat vækst hos hanunger



N=18-20

Glucose tolerance, 4 mdr - ingen effekt

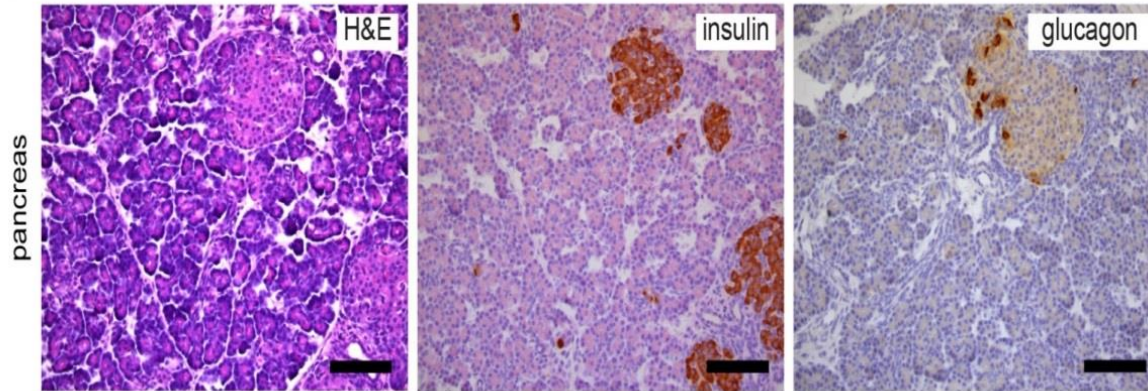
A)



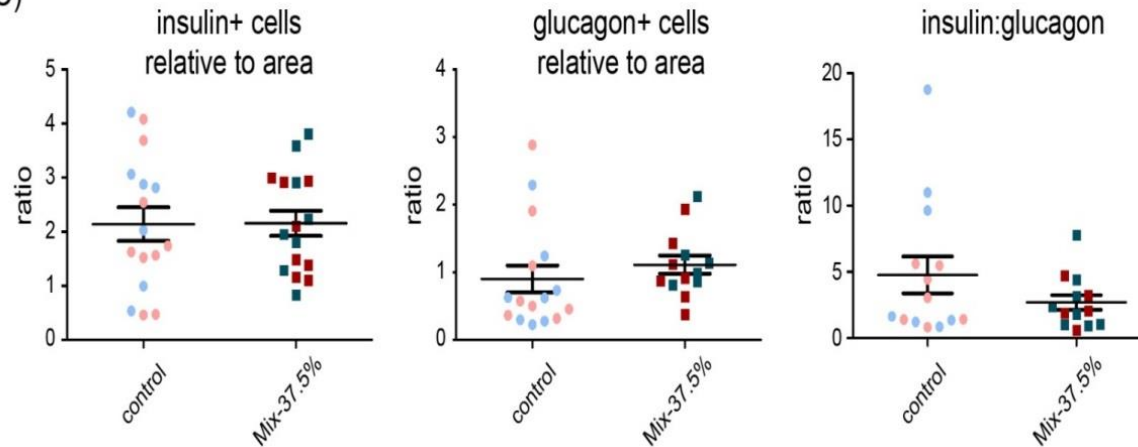
Mean +/- SEM; N=17-20

Ingen tegn på histopatologiske effekter i pankreas, P16

B)



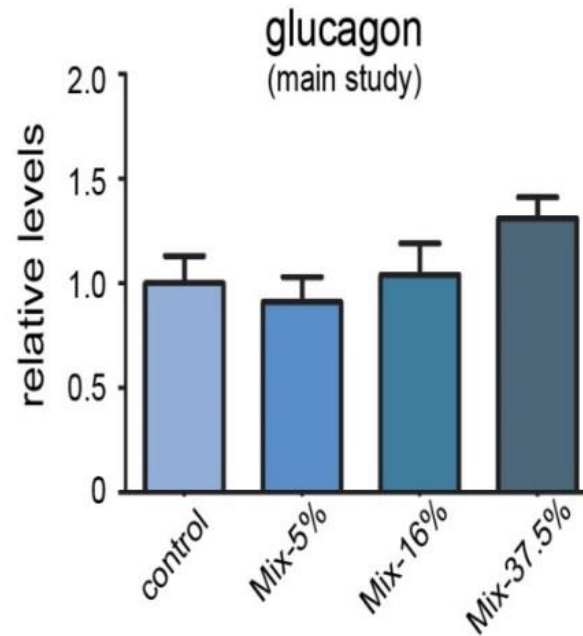
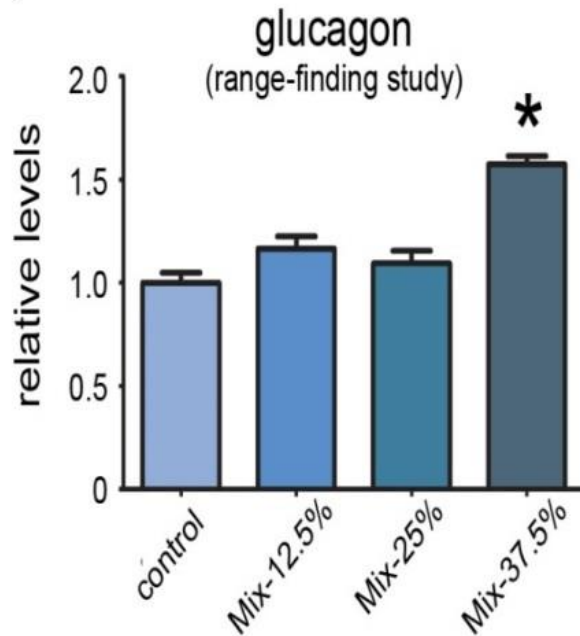
C)



N=13-14 per gruppe; Scale bars = 100 μ m

Øget glukagon hos hanunger??

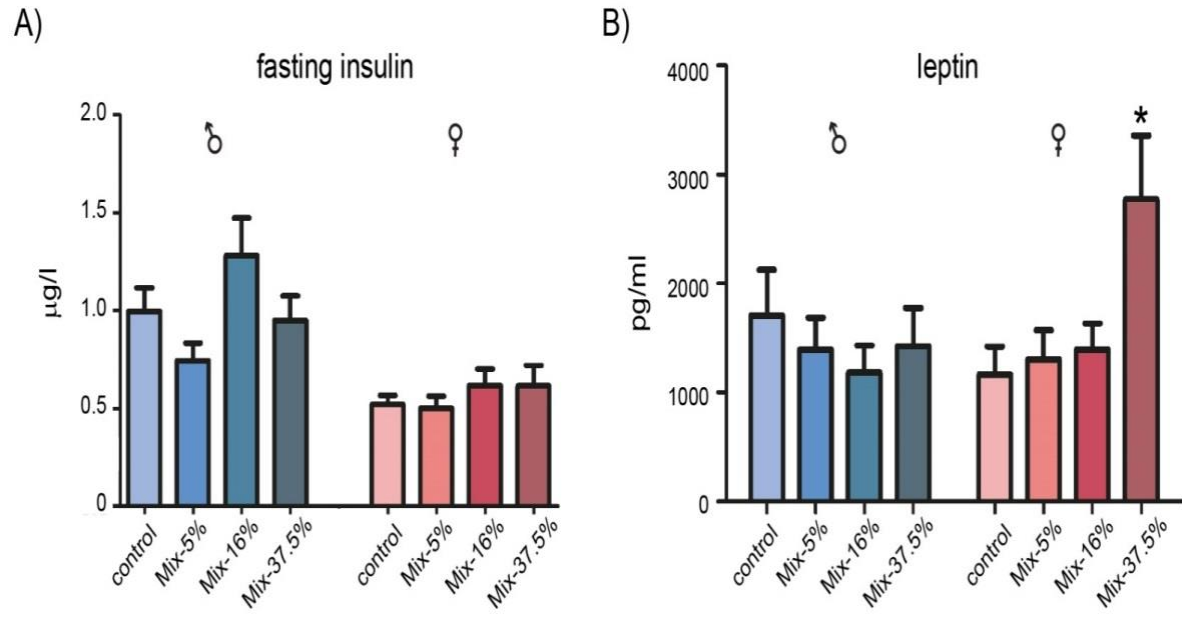
A)



$p < 0.05$ (ANOVA w/Dunnett's)

N=6-9 range-finding study; N=17-18 main study

Ingen effekt på insulin, leptin øget i højdosis-hunner



Leptin:
Fedtvæv
Mæthed
Øget stofskifte

Nedsat – fedme?

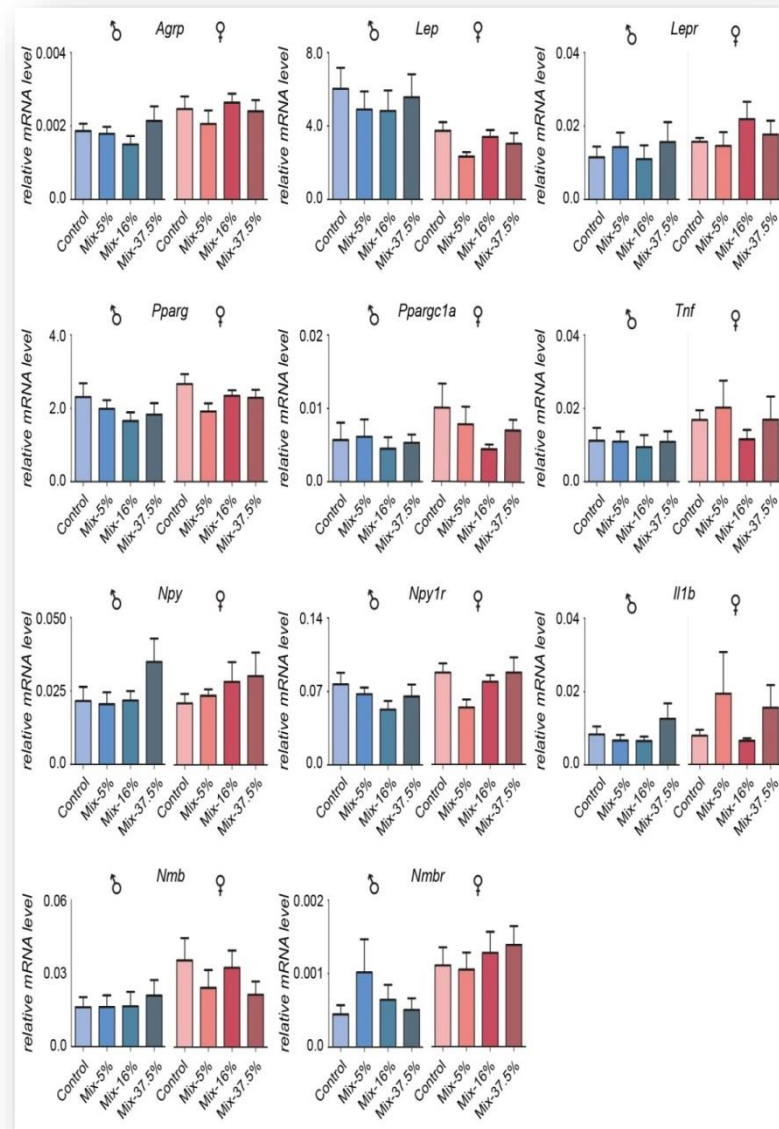
Mean+SEM; * $p < 0.05$ (ANOVA w/Dunnett's)

A) N=17-20 males; N=17-10 females

B) N=17-20 males; N=10-14 females

Genekspression profilering i fedtvæv

- Relativt upåvirket af eksponering
- Kønsforskel (kontrolgruppe)



Mean+SEM; N=8-9/group

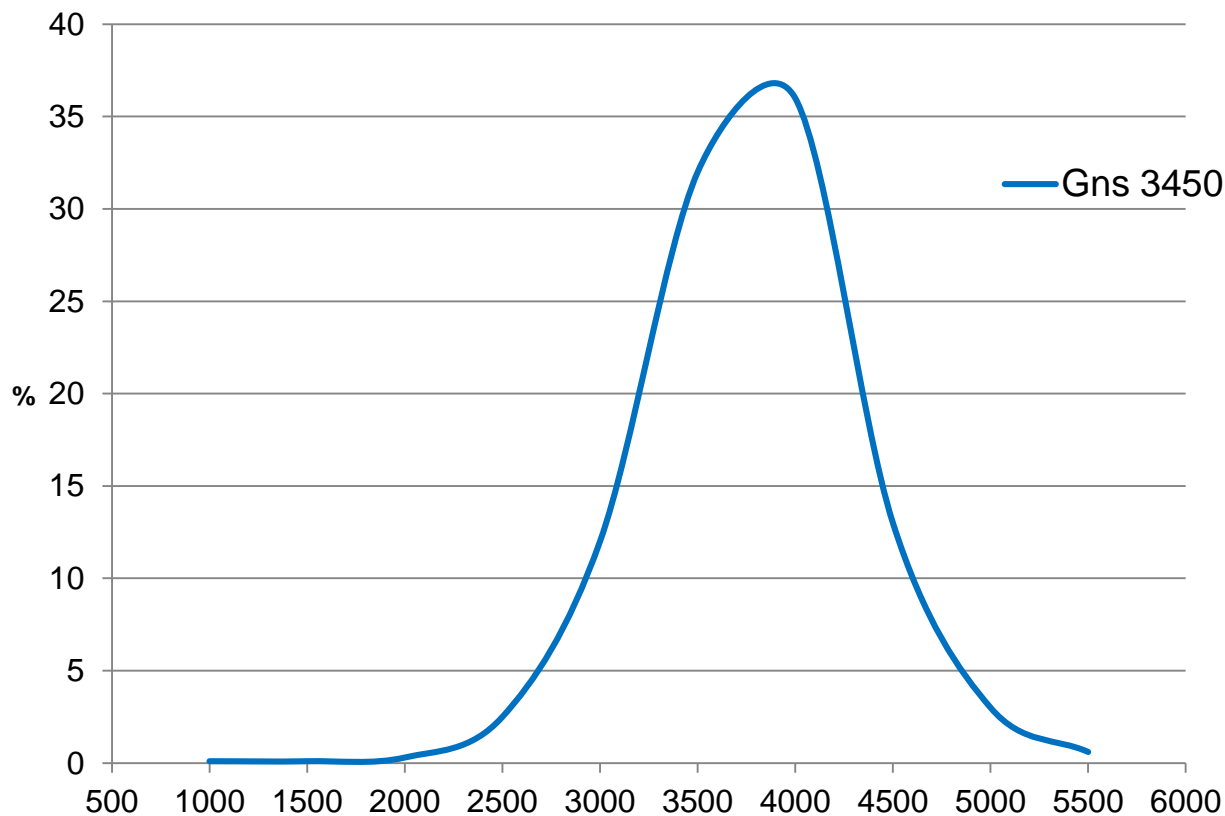
Konklusioner



- **Kombinationseffekt af pesticider på fødselsvægt**
- Nedsat fødselsvægt ved langt lavere doser end NOAEL for denne effekt for de enkelte pesticider
- Risikovurdering baseret på NOAEL for et pesticid ad gangen undervurderer risikoen for lav fødselsvægt
- Modellen dosis-addition gav en god forudsigelse af den observerede kombinationseffekt

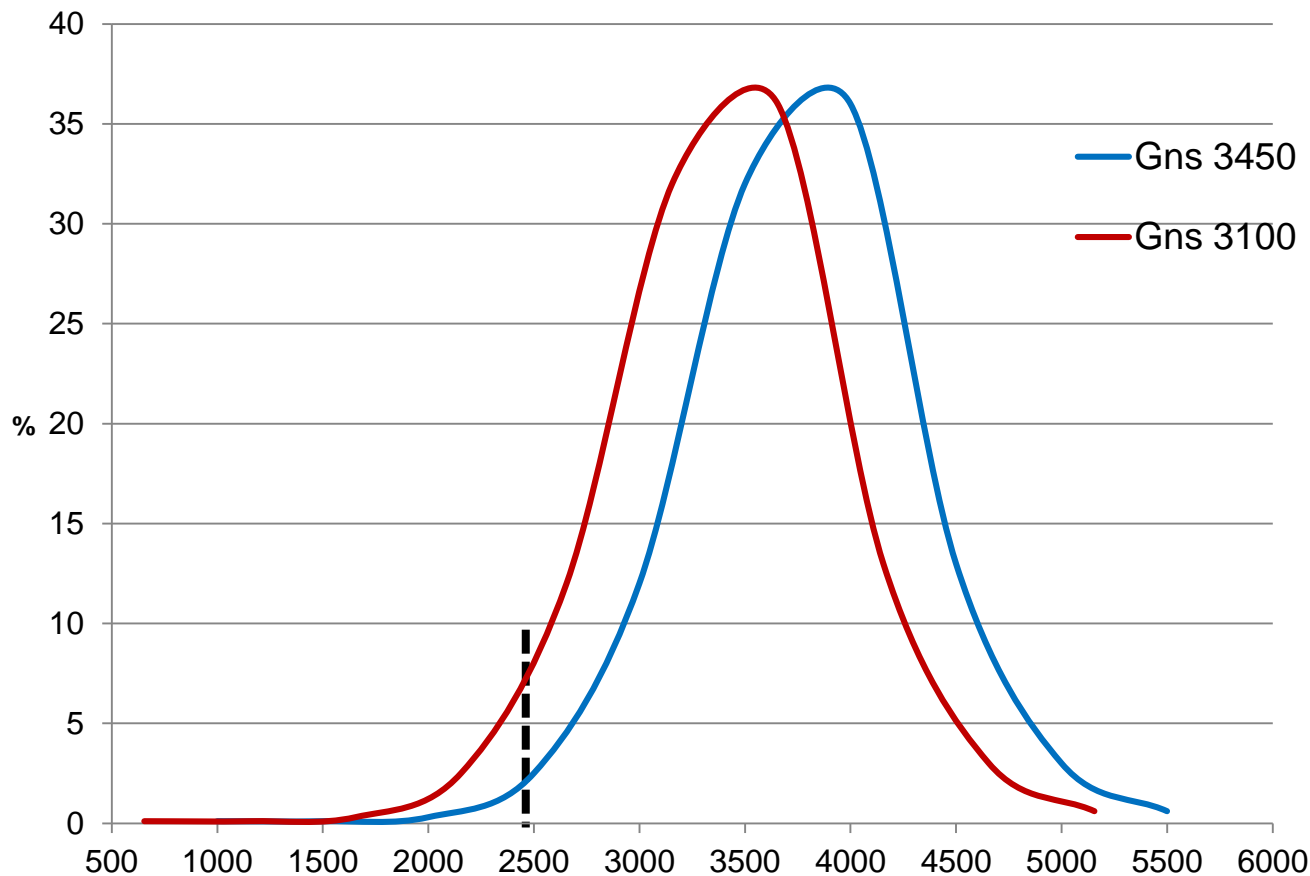
- Effekter på vækst og metaboliske programmering: ingen tydelige tegn på metabolisk syndrom eller fedme hos ungerne op til de var 4-5 måneder
- Men hvad med, når de er 1-1,5 år gamle? Fed mad?

Normalfordeling - fødselsvægt



Gennemsnit 3400-3500 g, ca. 3% under 2500 g
10% nedsat -> gns. ca. 3100 g

Skift i normalfordeling



10% nedsat, gns. 3100 -> mange flere under 2500 g
Øget risiko for mange alvorlige sygdomme

Anbefalinger til regulatoriske overvejelser



- Brug **kumulativ risikovurdering** for at tage højde for kombinationseffekter
- Gruppering af stoffer - samme type af effekt
- Inkludere alle typer af stoffer - andre stoffer end pesticider kan føre til nedsat fødselsvægt

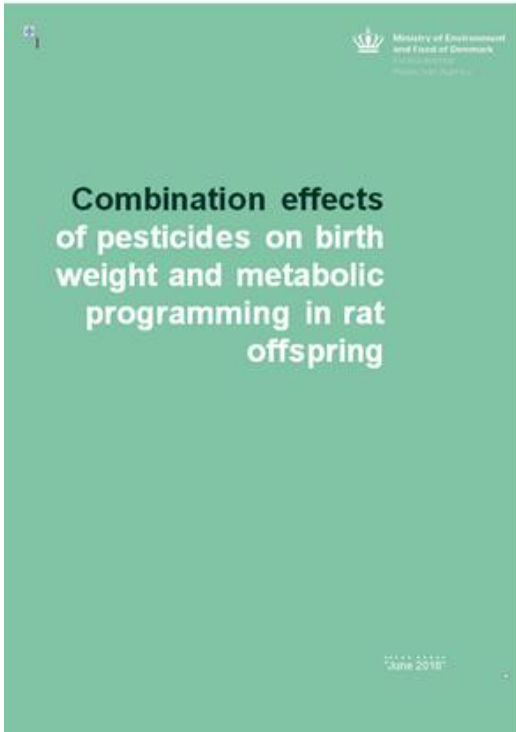
Praksis i dag i DK

- Kumulativ risikovurdering af restkoncentrationer af pesticider i fødevarer – **fremskridt**
- **Men** der tages ikke højde for
 - andre kilder
 - andre typer af stoffer med samme effekter
- **Forbedring anbefales!**

ComPest - Publikationer



Hass *et al* (2017) *Reprod Toxicol* 72:97-105.



Combined exposure to low doses of pesticides causes decreased birth weights in rats

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ABSTRACT

Decreased birth weight is a common effect of many pesticides in reproductive toxicity studies, but there are no empirical data on how pesticides act in combination on this endpoint. We hypothesized that a mixture of six pesticides (cyromazine, MCPB, pirimicarb, quinacrine, thiram, and ziram) would decrease birth weight, and that these mixture effects could be predicted by the Dose Addition model. Data for the predictions were obtained from the Draft Assessment Reports of the individual pesticides. A mixture of equal effective doses of these pesticides was tested in two studies in Wistar rats, showing mixture effects in good agreement with the additive predictions. Significantly lower birth weights were observed when compounds were present at individual doses below their no observed adverse effect levels (NOAELs). These results emphasize the need for cumulative risk assessment of pesticides to avoid potentially serious impact of mixed exposure on prenatal development and pregnancy in humans.

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Svingen *et al* (2018) *Sci Rep* 8:305.



OPEN Effects on metabolic parameters in young rats born with low birth weight after exposure to a mixture of pesticides

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Pesticide exposure during fetal life can lead to low birth weight and is commonly observed in reproductive toxicology studies. Associations have also been found in low birth weight babies born from pesticide-exposed gardeners. Since low birth weight is also linked to metabolic disorders, it can be speculated that early life exposure to pesticides could increase the risk of becoming obese or developing diabetes later in life. We have analyzed potential long-term effects of gestational and lactational exposure to a low dose mixture of six pesticides that individually can cause low birth weight: Cyromazine, MCPB, Pirimicarb, Quinacrine, Thiram, and Ziram. Exposed male offspring, who were smaller than controls, displayed some degree of catch-up growth. Insulin and glucose regulation was not significantly affected, and analyses of liver and pancreas did not reveal obvious histopathological effects. Efforts towards identifying potential biomarkers of metabolic disease risk did not result in any strong candidates, albeit leptin levels were altered in exposed animals. In fat tissues, the key genes *Lepr*, *Adip* and *Adipo* were altered in high dosed offspring, and were differentially expressed between sexes. Our results suggest that early-life exposure to pesticides may contribute to the development of metabolic disorders later in life.

MST rapport, maj 2018
(<https://mst.dk/service/publikationer/publikationsarkiv/2018/maj/combination-effects-of-pesticides/>)

Tak til:



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Støtte: Miljøstyrelsen, pesticidforskning



Tak fordi I lyttede - spørgsmål?



Research Group for Molecular and Reproductive Toxicology

