NOTAT

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EFSA's vurdering af mulig sammenhæng mellem ADHD-symptomer og metabolitter af pyrethroider, baseret på Odense Børne Kohorten

Problemstilling

En forskningsartikel, hvor der rapporteres om en mulig sammenhæng mellem ADHD-symptomer og metabolitter af pyrethroider baseret på Odense Børne Kohorten blev offentliggjort i 2019[1].

Miljø- og Fødevareministeriet har bedt om en yderligere kvalificering af forskningsartiklen om en mulig sammenhæng mellem ADHD-symptomer og urinmetabolitter af pyrethroider og chlorpyrifos i henhold til den fastlagte praksis for reguleringsmæssig brug af befolkningsundersøgelser om effekter af sprøjtemidler, jf. https://www.ft.dk/samling/20191/almdel/MOF/bilag/386/index.htm , idet artiklen falder i mellemgruppen, hvor der er et middelstærkt grundlag for reguleringsmæssige indgreb. DTU's vurdering af artiklen er fremsendt til departementet den 9. januar 2020. Vurderingen viste, at undersøgelsen var af "lav/middel pålidelighed" og med "nogen evidens for en årsagssammenhæng". Miljøstyrelsen har efterfølgende bedt forfatteren til artiklen om kommentarer til den faglige vurdering fra DTU. Miljøstyren har modtaget forfatterens kommentarer og sendt dem til DTU med henblik på at vurdere, om kommentarerne giver anledning til at ændre vurderingen af artiklen. DTU's opdaterede faglige vurdering af artiklen fra den 29. juni 2020, indeholdt meget få tilretninger. DTU's opdaterede vurdering af artiklen er fremsendt til departementet den 3. juli 2020.

Miljøstyrelsen har endvidere sendt artiklen til EFSA mhp. at vurdere studiet og informere om evt. igangværende EU-arbejde vedrørende aktivstoffer, som danner de pågældende nedbrydningsprodukter.. MST har endvidere anmodet om, at EFSA vurderede statistikken og robustheden af fundene. EFSA har vurderet studiet på deres 108. PPR Panel møde den 19. november 2020, hvor Miljøstyrelsen og DTU også deltog. EFSAs fulde vurdering samt svar på uddybende spørgsmål fra Miljøstyrelsen fra 14. december 2020 og 22 januar 2021 følger herunder.

Det fremgår, at PPR panelet vurderer, at de statistiske metoder, som er anvendt er hensigtsmæssige. PPR Panelet vurderer endvidere, at der er en mulig sammenhæng mellem prænatal eksponering for pyrethroider og forældrevurderet ADHD-problemscore, men at der ikke er vist årsagssammenhæng.

Samlet set er det EFSA's vurdering, at de eksisterende epidemiologiske beviser for sammenhængen mellem urin-niveauer af 3-PBA (og trans-DCCA) og ADHD er ret begrænsede og, da modstridende

[1] Dalsager L, Fage-Larsen B, Bilenberg N, Jensen TK, Nielsen F, Kyhl HB, Grandjean P, Andersen HR. *Maternal urinary concentrations of pyrethroid and chlorpyrifos metabolites and attention deficit hyperactivity disorder (ADHD) symptoms in 2-4-year-old children from the Odense Child Cohort*. Environ Res. 2019; 176: 108533. doi: 10.1016/j.envres.2019.108533

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resultater også er set i litteraturen for 3-PBA (hovedmetabolitten), at der er behov for flere undersøgelser med optimalt design og udført i forskellige populationer for at underbygge fundene.

EFSA vurderer, at resultaterne for Trans-DCCA (en mindre metabolit, fundet i 11%), både kan repræsentere en ægte effekt eller alternativt en tilfældig effekt på grund af det relativt lille antal børn, der havde en score for ADHD symptomer større end 90-percentilen, og hvis mødre samtidig havde trans-DCCA niveauer over detektionsgrænsen. Sandsynligheden for at et statistisk signifikant fund afspejler en reel effekt påvirkes negativt af lav statistisk styrke i et studie, som det er tilfældet i dette studie.

EFSA's Vurdering af Dalsager et al, 2019 artiklen fra EFSA PPR Panelet, modtaget pr mail den 9. december 2020:

" Study under evaluation:

Dalsager L, Fage-Larsen B, Bilenberg N, Jensen TK, Nielsen F, Kyhl HB, Grandjean P, Andersen HR. *Maternal urinary concentrations of pyrethroid and chlorpyrifos metabolites and attention deficit hyperactivity disorder (ADHD) symptoms in 2-4-year-old children from the Odense Child Cohort*. Environ Res. 2019; 176: 108533. doi: 10.1016/j.envres.2019.108533

Question raised by Pernille Rosenskjold Jacobsen (Ministry of Environment and Food of Denmark)

We kindly ask EFSA to consider the paper (Dalsager et al, 2019) and evaluate the associations (reliability and causality) observed for pyrethroids. Especially relating to the robustness of the association observed between ADHD symptoms and the different metabolites considering the size of the increases (odds ratio and relative change ratio) observed, the quality of the methods and the statistical analysis used in the study.

Summary of the study

This study assessed the association between prenatal exposure to pyrethroids and chlorpyrifos and traits of ADHD in 2-4-year-old children from the Odense Child Cohort (Denmark). The main results showed that every doubling in maternal 3-PBA concentration was associated with a 3% higher than expected ADHD score (adjusted ratio: 1.03; 95% CI: 1.00–1.07). Also, a doubling in maternal 3-PBA concentration was associated with 13% higher odds of having an ADHD score ≥ the 90th percentile (adjusted OR: 1.13; 95% CI 1.01–1.25). The odds of an ADHD score ≥ the 90th percentile was 1.76-fold higher in children whose mothers had trans-DCCA concentrations above LOD compared to children whose mothers had concentrations below the LOD (adjusted OR: 1.76; 95% CI: 1.08–2.86). Concurrent concentrations of 3-PBA and the chlorpyrifos metabolite TCPY above their medians were associated with higher ADHD score (adjusted relative change ratio: 1.20; 95% CI 1.04–1.38) and higher odds of scoring ≥ the 90th percentile (adjusted OR: 1.98; 95% CI 1.26–3.11). The study concluded that prenatal exposure to pyrethroids was associated with ADHD related traits at 2–4 years of age.

Assessment of the study

Overall, the study of Dalsager et al. (2019) only supports a *possible* association between prenatal exposure to pyrethroid insecticides metabolites and the prevalence of ADHD traits in childhood. The strengths, limitation and risk of bias of the study are briefly addressed below.

1. Main *strengths* of the study:

- The prospective follow-up design (birth cohort) and the large sample size (n=948) are the main strengths of this study. The advantage of cohort studies is that exposure is assessed before the occurrence of the outcome measured. Accordingly, the reliability of this study is higher than that of a few other studies addressing the association between urinary levels of 3-PBA and ADHD traits, such as Lee et al. (2020)[2], Wagner-Schuman et al. (2015)[3] and Richardson et al. (2015)[4], which used a cross-sectional design.
- The use of biomonitoring for metabolites of the exposure of interest is generally considered to be the most relevant and reliable way to assess exposure in epidemiology studies. In this study, a number of specific and non-specific pyrethroid metabolites were measured, albeit with varying analytical sensitivity.

2. The major *limitations* of this study are the following:

- Possible misclassification of the exposure over pregnancy as only a single urinary sample was obtained at gestational week (GW) 28. Although the study claims that the exposure level is assumed to be more stable in populations continuously exposed to low concentrations of insecticides from residues in the diet, this assumption has not been demonstrated.
- Potential for misclassification of ADHD scores as these were parent-reported and the severity of symptoms may be systematically under- or overestimated.
- The size of the effect when analysing for the expected relative change (ratio) in ADHD score was rather low (3% and 13%) when 3-PBA was expressed as a continuous variable. Furthermore, the results were significant only for girls (relative change ratio 1.06, 95% CI 1.00–1.12) but not for boys (1.02; 95% CI 0.98–1.06). Effect sizes were similar but slightly larger for the OR for scoring ≥ the 90th percentile on the ADHD scale. However, only 18% of assessed children fell into this category.

3. The internal validity or *risk of bias* (RoB) of the study was appraised using a customised version of the OHAT/NTP RoB assessment tool (Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, 2015[5]). This tool was developed to provide an approach to the evaluation of RoB in the context of hazard identification for human risk assessment of chemicals. The following seven questions were assessed and rated as for RoB

- ***Q1: Did the selection of study participants result in appropriate comparison groups?***

  Rated as definitively low RoB. The study participants (mother-child pairs) were derived from a prospective birth cohort study, pregnant women were recruited from the same eligible population, using the same method and inclusion and exclusion criteria

[2] DOI: 10.1016/j.envres.2020.109739
[3] DOI: 10.1186/s12940-015-0030-y
[4] DOI: 10.1096/fj.14-260901
[5] Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. Office of Health Assessment and Translation (OHAT) Division of the National Toxicology Program National Institute of Environmental Health Sciences. https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf

- ***Q2: Did the study design or analysis account for important confounding and modifying variables?***

  Rated as probably low RoB. Factors (covariates) that may influence either gestational maternal exposure to insecticides or ADHD symptom score in the children were included in a directed acyclic graph DAG[6]. Negative regression models were adjusted for maternal educational level, parental psychiatric diagnosis, child age and sex, 3-PBA and TCPY concentrations, and urinary creatinine. However, potential co-exposure to developmental neurotoxicants (e.g., organophosphorus compounds, lead, mercury, among others) was not measured with the exception of urinary TCPY (a relatively specific metabolite of chlorpyrifos).

- ***Q3: Were outcome data complete without attrition or exclusion from analysis?***

  Rated as probably low RoB, particularly because of attrition. The CBCL: 1½-5 questionnaire was mailed to 2551 parents and a total of 1942 (76.6%) answered it. Only 1515 pregnant women provided a urine sample during pregnancy (N=1515). Finally, the study population consisted of 948 participants. Potential selection bias was assessed and it was found that study participants were generally older and more often non-smokers as compared to both groups of non-participants. Also, participants providing a urine samples generally had a higher educational level as compared to non-participants.

- ***Q4: Can we be confident in the exposure characterisation?***

  Rated as probably high RoB. Exposure was assessed by monitoring for the common pyrethroid metabolite (3-PBA) and other more specific metabolites in a single urine sample collected at GW 28. Analyses were performed by LC-MS/MS with the analytical method being based on a slightly modified version of a previously reported method.

  Only a single spot urine sample was collected in GW 28 and considered as a proxy for the pesticide exposure level during pregnancy, which is not necessarily representative of pyrethroid exposure over the entire pregnancy. Furthermore, a potential exposure after birth up to the age of assessment at 27 months cannot be excluded.

  Although the study determined 3-PBA, 4-F-3PBA, cis-DCCA, trans-DCCA, cis-DBCA in urine, only 3-PBA was detected in a high percentage of maternal samples (94.4%) and the remaining metabolites were detected in a very small proportion of samples (trans-DCCA in 11.4% and the rest in less than 5%). 3-PBA is a common metabolite of many synthetic pyrethroids, including allethrin, cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, permethrin, resmethrin, tralomethrin, and their isomers.[7] However, the use of 3-PBA as urinary biomarker to assess exposure to these compounds is limited by the possibility that measured concentrations may represent direct exposure to 3-PBA formed in food items from the degradation of these pesticides in the environment or metabolisation by plants rather than exposure to the parent compounds themselves. Hence, biomonitoring of these biomarkers in urine will lead to overestimation and misclassification of exposure for risk assessments and epidemiologic studies, particularly in scenarios of low level exposure such as occur in dietary and other non-occupational settings (Chen et al., 2012[8]).

[6] Directed acyclic graph (DAG) models are popular tools for describing causal relationships and for guiding attempts to learn them from data. They appear to supply a means of extracting causal conclusions from probabilistic conditional independence properties inferred from purely observational data.

[7] doi:10.1289/ehp.0901275

[8] doi: 10.1021/jf303116p

- ***Q5: Can we be confident in the outcome assessment?***

  Rated as probably low RoB since the Child Behaviour Checklist for ages 1½-5 years (CBCL: 1½-5) is considered a validated tool. It is a parent-report online questionnaire on which the child is rated on various behavioural and emotional problems. However, from 100 questions of the CBCL that addressed behavioural, emotional, and social problems, only 6 correspond to an ADHD problem scale. Furthermore, CBCL can be used to screen for ADHD disorders in preschool children population, but it is not a specific diagnostic tool for ADHD. Finally, the questionnaire was not administered by experienced neuropsychologists.

- ***Q6: Were all measured outcomes reported?***

  Rated as probably low RoB. The ADHD score was analyzed as ordinal data from CBCL:1½-5 results, and also as a dichotomized variable (scoring ≥ 90th percentile on the CBCL:1½-5) using appropriated regression analysis. Besides, 94 questions of the CBCL questionnaire were not reported as they were not directly related to ADHD.

- ***Q7: Were there other potential threats to internal validity? Were the statistical methods appropriate?***

  Rated as definitely low RoB. The statistical methods used were appropriate. Bivariate and multivariate (binomial and logistic regression analyses) were used. Crude and adjusted sizes of effects were obtained.

When the algorithms of OHAT/NTP were applied to the aforementioned ratings, the study was categorised as tier 3 (high RoB). The main driver for this categorization was Q4 (exposure characterization).

Overall appraisal with regard to the questions raised by the Ministry of Environment and Food of Denmark

Human observational studies are not designed to provide direct evidence of causality. Instead, the observed associations must be critically examined with regard to the likelihood of a cause-effect relationship. There are several aspects of this study which make it difficult to assess the observed associations with regard to causality. First and foremost, a spot urine sample taken at only a single time-point during pregnancy provides very little information about the overall exposure to pyrethroids during pregnancy and none about exposure during the first two years of life. Secondly, although confounders were adjusted for, relevant co-exposures to other potential developmental neurotoxins were not assessed with the exception of chlorpyrifos. Thirdly, the CBCL:1½-5 instrument is parent-administered and contains only a small number of questions related to behaviour possibly associated with ADHD. Fourthly, the observed relative change (ratio) in the primary model (negative binomial regression) was very small (only 3% higher than expected). Although the auxiliary model (logistic regression) showed similar and somewhat stronger associations when applied to those children scoring ≥ the 90th percentile on the ADHD scale, this only applied to a relatively small subset of children. It must therefore be concluded that an association between prenatal exposure to pyrethroids and parent-assessed ADHD problem score is possible but no causality can be determined.

Overall, the available epidemiological evidence in humans on the association between urinary levels of 3-PBA and ADHD is rather limited (Dalsager et al., 2019; Lee et al., 2020; Wagner-Schuman et al., 2015; and Richardson et al., 2015). Thereby, more studies with optimal designs and conducted in

different populations are needed to provide consistency to the available findings, as contradictory results are also available in the literature (Quirós-Alcalá et al., 2014[9]).”

Miljøstyrelsen sendte EFSA yderligere uddybende spørgsmål den 14. december 2020. Miljøstyrelsens spørgsmål efterfølges af EFSAs svar fra 22. januar 2021, markeret med rød tekst.

Thank you for this very thorough assessment it is indeed very helpful. We just have a couple of questions for clarification for Trans-DCCA, which was also discussed at the meeting.

Under Q4 it is stated “Although the study determined 3-PBA, 4-F-3PBA, cis-DCCA, trans-DCCA, cis-DBCA in urine, only 3-PBA was detected in a high percentage of maternal samples (94.4%) and the remaining metabolites were detected in a very small proportion of samples (trans-DCCA in 11.4% and the rest in less than 5%).”

Does this mean that Trans-DCCA (as the other metabolites mentioned) is considered detected in so few samples/few subjects that it is difficult to use the result?

Re: According to the study, trans-DCCA was detected in 11.4% of the study population (which consisted of 948 mothers) whereas 3-PBA was detected in 94.4%. There are two possible reasons underlying this large difference in the percentage of detection of both metabolites in urine samples:

- The analytic sensitivity of the methodology was different for the metabolites studied. The LOD was 0.03 ng/ml for 3-PBA and 0.4 ng/ml for trans-DCCA. Because pyretroids yielding trans-DCCA are hydrolysed to one molecule of 3-PBA and another of trans-DCCA, the analytical sensitivity of trans-DCCA was 13.64 times lower than that of 3-PBA on a molar basis.
- While many type I and type II pyrethroids share 3-PBA as a common metabolite, trans-DCCA only originates from a few pyrethroids (permethrin, cypermethrin and cyfluthrin). It is possible that the study population had higher exposure to pyrethroids other than those yielding trans-DCCA. Both metabolites have a similar apparent elimination half-life, 5.4 h for 3-PBA and 5.7 h for trans-DCCA in volunteers following ingestion (doi:10.1093/annhyg/mev059).

Based on the lower number of mothers with trans-DCCA levels above the LOD, the study categorized exposure as a dichotomous variable, i.e. above and below the LOD. In contrast to 3-PBA, the study may be underpowered to detect a potential significant association of trans-DCCA with ADHD traits due to a smaller sample size. In other words, the statistical power to detect significant associations was much lower as compared to 3-PBA.

Otherwise trans-DCCA is only mentioned in the initial “Summary of the study” where results from the Dalsager et al. paper, are presented. The effects associated with detecting Trans-DCCA above LOD does not seem to be considered in the overall appraisal although they were discussed during the meeting?

Re: The appraisal paid more attention to 3-PBA because the study addressed the association of pyrethroids (in general) with traits of ADHD. Based on the reasons mentioned above (i.e., a low detection rate for trans-DCCA and potential for underpowered analysis) this metabolite was only briefly considered for the study appraisal.

[9] DOI: 10.1289/ehp.1308031

Miljøstyrelsen bad om yderlige uddybning af svaret den 22. januar 2020. EFSAs svar kom den 1. Februar 2021 markeret med rød tekst:

The result of Trans-DCCA in Table 6 in the paper, show the odds of an ADHD score ≥the 90th percentile was 1.76-fold higher in children whose mothers had trans-DCCA concentrations above LOD compared to children whose mothers had concentrations below the LOD (statistically significant and also seen in boys and girls separately). Based on the answers in red is it correctly understood that, because of the low power to detect significant associations for Trans-DCCA, the observed statistically significant result in table 6 may actually not reflect a true effect?

There is a risk of over-interpreting single findings from observational studies, which should not be taking out of context in order to rationalise them. No firm conclusions should be drawn from an individual finding either, but the latter rather should be seen in the context of the whole study. That finding may therefore be related to the inherent limitations of the study, which may have a greater impact on individual parts thereof.

Having said that, the finding under consideration may represent a true effect observed in the study or, alternatively, a random effect due to the relatively small number of children who had a CBCL- ADHD score greater than the 90th percentile and whose mothers had trans-DCCA levels above the LOD. A low sample size, a small effect size, or both may negatively affects the likelihood that a nominally statistically significant finding of a study actually reflects a true effect as a result of low statistical power (Button et al., 2013).