Author’s response to reviews

Title: The influence of prenatal exposure to trans-fatty acids for development of childhood haematopoietic neoplasms (EnTrance): a natural societal experiment and a case-control study

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Author’s response to reviews:

Dear Editor

We thank the Editor for the opportunity to revise and resubmit our manuscript.

We respond below to Reviewer #2 for the additional questions and revised the manuscript further accordingly. We hope that these answers and changes clarify our plans satisfactorily. We would like to highlight that we use two designs investigating TFA and childhood cancer:

1) In the 1st design (objective 1) we investigate whether or not the Danish TFA legislation in 2004 had an influence on the incidence of haematopoietic cancers in children born after the legislation. We thus compare the incidence of haematopoietic cancers in all Danish children born in 1988-2004 (before the legislation) with the incidence of haematopoietic cancers in all Danish children born in 2004-2008 (after the legislation) using cox regression with splines, adjusted for co-variates. This objective does not include TFA from neonatal blood. The reviewer #2 is suggesting a comparison with the birth cohort 2009-2017, which we never mentioned. We believe that there is a misunderstanding and that the additional queries of this reviewer were already addressed in our last revision, as justified in our answers below.

2) In our 2nd design (objective 2) we investigate if neonatal levels of TFA differ among 700 cases and 700 controls that have been matched on the exact date of birth (both case and the matched control born on the same day, month and year). Neonatal iTFA will be analysed for all cases (n=700) of haematopoietic cancers in children until age 7 years,
born between 1988 and 2009 and 700 matched controls. We hope to have clarified this by our figure of design 1 and a flow chart of design 2 in the last revision, based on the previous suggestions by the reviewer.

We hope that our additional comments fully clarify our protocol.

Sincerely,

Ina Olmer Specht

Editor #

Please note that based on the review feedback the protocol as described in the current manuscript may not be sufficient to answer your hypothesis that TFA exposure during the critical phase of fetal development result in increased risk for haematopoietic cancers, Detailed suggestions have been provided below to assist in revising the manuscript, but this is not a guarantee that it will reach a sufficient standard fro acceptance. Please address these comment below

Reviewer #2:

1. Regarding Reply to query #2: Although measuring iTFA levels in the blood of neonates is a more direct exposure measure for the outcome in question than measuring the iTFA levels in the blood of mother (reply to query 2), it may give insufficient evidence to implicate any one prenatal factor (in this case exposure to TFA) in hematopoietic cancer aetiology, unless the levels in the mothers (antenatal) are also shown to be changed.

REPLY: In the study of TFA-levels from neonatal blood (objective 2) we are not looking at any changes in levels associated with the change in legislation. The premise for this study is rather, to examine if neonatal levels of TFA may be related to the subsequent risk of developing cancer before age 7 years, irrespectively of the law. By close matching on a one-to-one level of every cancer case with one control of same gender, born on the same date (meaning same day and month and year) and at the same hospital we wish to compare TFA levels at birth for children who developed cancer and matched controls. It is not useful for this part of the study to involve maternal blood.

2. There can be number of confounding factors that cannot be accounted for and can influence the results and cause spurious association if cases are selected among children between 1998 to 2008 and controls born between 2009 to 2017. To control for this variation, cases and controls should be selected equally among neonates born before and after the legislative ban.
Reply: We believe that the reviewer may be mixing the two objectives of our study, and as referred to above, acknowledge that we may not have described the two objectives sufficiently clear. We hope the above clarification, in addition to the figure and the flow chart included in the last revision of our manuscript, now makes the twofold aims of our study clear. E.g. in Objective 1 we examine cancer incidence among individuals before the TFA legislation with cancer incidence after the TFA legislation, as proxy of exposed to higher or lower levels of TFA during gestation, respectively. In Objective 2 we perform a matched case control study where for each case we have a closely matched control irrespectively of the date of the TFA ban.

We hope it is now easier to understand the two distinct designs we are using.

We are not investigating children born after 2008 (lines 160 and 171).

If the reviewer is referring to Objective 1 (the 1st design) we are aware that potential confounders can influence the results (lines 234-241). Comparison of incidence rates before and after the ban is a correct design to show any simultaneous changes in the levels of TFA exposure and that of incidence. Using this design, the role of the confounders is limited because we are investigating all children born in Denmark in that time period. However, if a potential confounder is changing over time, e.g. age of the mother is increasing over time, we might get confounded results due to the secular trend. We will thus incorporate the known potential confounders in our model and if so we will adjust for them.

If the reviewer is referring to the 2nd design (objective 2): The matched case-control study is a classical matched case-control study 1:1 with cases and controls selected in the same time period (born in 1988 to 2008), and with a follow-up of 7 years for cancer diagnoses as an end-point, for all cases and controls regardless when they were born. We will adjust for potential confounders (line 250). The cases and controls will be selected equally among neonates born before and after the legislative ban, irrespectively of the TFA legislation, but we will hopefully be able to see a decrease in TFA levels in serum in cases and controls born after the TFA legislation (line 211-213).

3. Regarding Reply to query #3: 'In Objective 2 we measure the exact TFA levels in neonatal blood regardless of the TFA law".- Does it mean that authors plan to measure TFA levels in only specific cases (children born between 1988-2008) and matching controls (children born between 2009-2017) or in all children born in the years 1988 to 2017 regardless of whether they got haematopoietic cancers or not?

Reply: We will not measure TFA in all children born in Denmark in the investigated time period (1988 to 2008). We performed power calculations to define the statistically required sample size that is economically responsible. We will only measure the TFA levels in the cases and their matched controls born in the time period. Our power calculation suggested 700 cases and 700 controls born in 1988 to 2008. Each case and control pair will be born in the same year (e.g. case: 1990 and control: 1990, or case: 2004 and control: 2004). As described in the paper we will not include children born after year 2008 in our study.
4. In line 176, the authors want to use cases and controls sharing the "exact date of birth". It is not clear if the year too will be matched. For example, for a case with a birth date of say January 1st, 1988, the control will be born on January 1st, 1988 or on January 1st falling in the years 2009 to 2017?

Reply: Date of birth also refers to the years of birth. This has now been specified in the manuscript.

We will not be investigating children born in the years 2009 to 2017. As written in line 154 The Cancer Registry is complete until December 31st 2015. To get 7 years follow-up from birth we are not able to include birth cohorts after 2008.