Commissioned by the Norwegian Scientific Committee for Food Safety 2005:

Requested review of Nordby K-C, Andersen A, Irgens LM, Kristensen P. Indicators of mancozeb exposure in relation to thyroid cancer and neural tube defects in farmers' families. Scand J Work Environ Health 2005;31(2):89–96.

This review uses the abstract and abbreviated text from the original publication to summarize the objectives, exposure and outcome measures, results and the authors´ conclusions. It will then discuss aspects of the exposure assessment, the design, the results, and the conclusions regarding the neural tube defects. Finally, some suggestions and concluding remarks are given.

Objectives The fungicide mancozeb has been suspected of causing thyroid cancer and neural tube defects. The aim of the study was to investigate associations of indicators of mancozeb exposure with thyroid cancer and neural tube defects in farmers' families.

Methods National registers in Norway, identifying 105 403 female and 131 243 male farmers, born in 1925–1971, and their 300 805 children available for analyses, born in 1952–1991, were cross-linked with national agricultural censuses, 1969–1989, and the population register. Neural tube defects (ICD-8 740–742) at birth among the 102 703 children conceived between May 1973 and April 1991 were identified in the medical birth register. Likewise thyroid cancer (ICD-7 194) was identified in the cancer register through 2000.
Mancozeb exposure was reflected by the combination of potato farming, pesticide use, and meteorologically based fungal forecasts. Potato farming was defined as potato cultivation in any census in 1969–1989. Pesticide use was defined as pesticide purchase in 1968 or pesticide application equipment on the farm in 1978. The number of fungal forecasts, broadcasted since the 1950´s, for each year between 1973 and 1990 from the nearest station was allocated to each farm in order to calculate the number of forecasts in the conception season applied in the analyses for neural tube defects. The farms were further categorized according to work input of more than 500 hours yearly by either parent, agricultural education of the farm holder, agricultural acreage, and geographic location
Conceptions were categorized according to the level of mancozeb sales in the year of conception and according to high potato crop loss due to dry rot in that year The first month after conception was considered as the relevant window of exposure for neural tube defects.
Potato production was regarded as "probably the best single parameter reflecting mancozeb application".
The adjusted rate ratio or prevalence ratio (PR) estimates with 95% confidence intervals were computed in a Poisson regression analysis.

Results Neural tube defects (131 cases, prevalence 12.8/10 000 births) were moderately associated with potato cultivation (PR 1.6, 95% CI 1.1–2.3) and paternal work of >500 hours/year (PR 1.6, 95% CI 1.1–2.5). Mancozeb exposure was not associated with thyroid cancer.

The authors´ conclusions A moderate association seems to exist between mancozeb exposure and neural tube defects, but not between mancozeb exposure and thyroid cancer.

Comments

The study is conducted within a cohort of farmers. The analysis essentially consists of comparisons of malformation rates across categories of variables that are assumed to be markers for mancozeb exposure. Several such proxy variables are used. Two of those show associations with neural tube defects (NTD), namely potato production and input of more than 500 hours of work on the farm by the father. The authors point out that the association with potato production is somewhat stronger when the analysis is restricted to births within three years of a census that is when misclassification can be assumed to be smaller. However, this statement is weakened by the fact that the PRRs are actually very close (1.55 vs 1.64 for potato farming).

The association for more than 500 hours of farm work is only present in the fathers. Given the hypothesis that any NTD effect would be due to foetus exposure during the first month after conception it should only be the mother's exposure that would be of direct relevance. A better understanding of what the 500 hour work variable actually implies would perhaps explain this, but at present it seems inconsistent with the hypothesis that this effect is only seen in the fathers. The risk estimate of 1.55 for potato farming versus no farming should also preferably have been calculated separately for women and men.

None of the other markers of mancozeb exposure that are reported in the article shows an association with NTB. These variables are number of fungal forecasts, summer conception, year of high mancozeb sales, and years of high crop damage. Thus, while one proxy variables, maybe the most basic one, does show an association that is consistent with the hypothesis of an effect of mancozeb exposure on NTBs, several other markers of exposure do not show such associations. From this point of view the results are somewhat inconsistent. However, from the information that is provided in the article it is not really possible to assess the significance of this inconsistency, because this would require information about how well the various exposure variables actually predict exposure to mancozeb. For a very poor predictor of true exposure one would not actually expect to observe an association with malformation rate. Yet the lack of association with NTB for these exposure indicators does weaken the overall results of an association between NTB risk and indicators of mancozeb exposure.

The authors correctly comment that "the major weakness of the study was the use of exposure indicators as proxies for real exposure" but that "the resulting misclassification of exposure is likely to be nondifferential, leading to a bias towards the null of any true associations between the exposure and outcome". A main effect of this is that internal consistency within the study is difficult to evaluate because the relative performance of the proxy variables is unknown.

Normally, assessment of internal consistency including dose-response analysis is a powerful tool when evaluating etiological data.

The anticipated route of exposure is not clearly stated in the article but it is assumed that the authors have had airborne exposure after application in mind rather than eating of contaminated potato. Mancozeb is not taken up from the soil by plants (Extoxnet) but is broken down to ethylene thiourea, a metabolite that has produced cancer in laboratory animals.

Mancozeb is available as dusts, liquids, water dispersible granules, as wettable powders, and as ready-to-use formulations. More information on the type of Mancozeb formulations and application methods used in Norway would have been warranted for a better understanding of the exposure conditions.

The authors have used several independent registries with proxy information on potential mancozeb exposure in a very clever and unbiased way. Of course direct, individual information would have been preferable and it is easy to agree with the comment in the accompanying editorial that "it would have been even more interesting if the authors had managed to do a validation study of exposure, and, more specifically, of the exposure classification used".

It might have been possible to compare data from potato growers on mancozeb usage and exposure with the estimates from the different registers if e.g. production diaries or notifications of purchases among the case farms had been kept. Sometimes, hygiene measurements can be performed after reconstruction of work place and environmental exposures from past working practices and compared with other estimates or proxies. Job-exposure matrices can sometimes be used to increase the precision from registry data. Obviously any such potentially possible procedures would have been costly in this case.

The finding of an association with fathers working more than 500 hours on the farm but not the mothers is intriguing. If there is a true association between the fathers´ exposure and NTD it has to be produced by a sperrmatogenic effect and would not necessarily be linked to exposure during the first month after conception. Other "windows of exposure" could have been tried.

The increased risk of children having NTD in the South region (2,04; CI: 1.14 – 3.65) is noteworthy and consistent with environmental factors playing a causal role but it is unclear if this finding is of any significance with respect to the mancozeb analysis. The authors do not discuss this and if potato cultivation is particularly common in the South or if there might be other characteristics, including other relevant exposures factors.

The observed excess risks are moderately high. For potato production it is 1.6 (95% CI: 1.1-2.3) and for the father putting in more than 500 hours of farm work it is 1.6 (1.1-2.5). While the null value is excluded from both these confidence intervals, it is certainly not inconceivable that the risk elevations are due to chance. The likelihood of chance being the explanation is reduced because the analyses were performed in response to a specific hypothesis. On the other hand, the findings are not entirely consistent. It is possible that mancozeb exposure is the explanation to the excess risks of NTB, but random variability is certainly a prominent alternative candidate for explanation of the findings.

Suggestions for further work up of the study

- clarify the formulation and application methods of the pesticide and try to find validation methods of the exposure estimates
- add information on other potential pesticides and chemicals applied
- prolong the follow-up if the exposure has continued
- try to explain the excess risk in the South
- discuss the finding that the excess seems to have been associated mainly with the fathers' working hours and not the mothers' and deliver sex specific rates for all relevant items
- possibly try other "windows of exposure" considering that the potential risk seems linked to exposure of the father rather than the mother
- look at and/or comment on other malformations in order to shed more light on random variability of the data (the previous articles from the same research team on this cohort have not been available to the reviewers and might contain relevant information on other malformations)

Concluding remarks

This is a well performed study and a well written paper. The research team is highly qualified and includes the relevant expertise. The study results are a cause of concern in that they offer limited support for the hypothesis that mancozeb exposure increases the risk of malformation in the offspring, as the authors quite accurately put it. Additional information as specified above (c.f. Suggestions for further work up of the study) would facilitate an improved evaluation of the findings in the study.

An attempt to confirm the results seems warranted. In doing so, an attempt should obviously be made to evaluate and if possible improve the exposure markers. However, even an attempt to replicate the finding that potato production is linked to neural tube defects in the offspring would be of great value if done with adequate statistical power and in a population where the same fungicides are being used.

Stockholm 1 December 2005

Anders Ahlbom Christer Hogstedt