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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Italian Triazine Cancer Epidemiology Studies,
HED Project No. 0-1573

TO: Jude Andreason
Special Review Branch
Special Review and Reregistration Division (H7508C)

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I. INTRODUCTION

As requested, two studies of Triazine exposure and ovarian cancer have been reviewed to determine the evidence for a causal association. The two studies reviewed are as follows:

1. Donna A., et al. Ovarian mesothelial tumors and herbicides: A case-control study. Carcinogenesis 5:941-942, 1984.

2. Donna A., et al. Triazine herbicides and ovarian epithelial neoplasms. Scandanavian Journal of Work, Environment and Health 15:47-53, 1989.

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II. CONCLUSION

Taken together, the two studies suggest a possible association between triazine exposure and ovarian cancer. Selection bias and lack of adjustment for reproductive risk factors cannot be ruled out as alternative explanations for the first study. Statistical significance appears to have been obtained in the second study by arbitrarily changing the size of the confidence interval, an unacceptable procedure. Had exposure categories been combined, which would have been acceptable, statistical significance might have been obtained. The use of electoral rolls as controls may be a source of bias, but it cannot be evaluated due to lack of information. Taken together, these two studies plus the animal evidence indicate the need for further epidemiologic studies to evaluate this potential association.

III. DETAILED CONSIDERATIONS - the 1984 Study

Synopsis

The first study, published in 1984, was based on 66 cases of ovary cancer which were diagnosed from 1974 to 1980 and histologically confirmed. Sixty of the 66 cases were traced and interviewed at home regarding occupational history and herbicide exposure. Next-of-kin were interviewed for 10 of the 60 cases that had already died at the time of the interview. A total of 135 controls were selected after the interviews with cases had been completed. Incident cases of cancer from sites other than ovary were selected from the same file as the cases. Of the initial 135 controls selected, 127 were interviewed. Interviewers were told that this was a new case series and were not aware that these subjects would be used as controls. Definite exposure was defined as a subject who described personal use of herbicides and was familiar with various brand names. Possible exposure applied to those who were farmers after 1960 and resided in known herbicide usage areas, but denied personal use.

The odds ratio for the association between herbicide exposure and ovarian cancer was 4.4 with a 95 percent confidence interval of 1.9 to 16.1. Risk was mainly seen in the younger subjects (less than 55 years old) who exhibited an odds ratio of 9.1 with a confidence interval of 3.0 to 28.3. The interview did not collect information on reproductive history. However, available records were searched to determine the number of live births and the frequency of childlessness which was similar in cases and controls.

Comment

The use of other cancers as controls resulted in 46 percent of them having breast cancer. A separate analysis excluding breast cancer resulted in a somewhat lower odds ratio of 3.5 with a confidence interval of 1.4 to 8.4. The possibility of selection bias from selection of other cancers is an important

consideration. NDEB's own analysis and that of other researchers show that female breast cancer rates are generally much lower in rural farm areas than in urban areas. Selecting breast cancers would result in a greater likelihood of getting an urban dweller who would be less likely to have herbicide exposure. This same situation may exist in Italy where the present study was conducted. The other cancer sites used as controls, predominantly uterus, cervix, and colon, are also cancers that tend to occur at lower rates in females from rural areas than in urban areas. Therefore, the separate analysis excluding breast cancer controls does not eliminate selection bias. More information, especially on farm residence, should have been collected to rule this possibility out.

Another concern in this study is that controls were not selected until after cases were interviewed. Typically, cases and controls are selected and interviewed at the same time. Even though the interviewers were told they were interviewing more cases in the present study, some may have figured out that they were really controls. In any case, interviewers may have been less diligent the second time around at eliciting herbicide exposure history.

A careful review of reproductive risk factors related to ovarian cancer was not performed at the time of the interview. Normally, case-control studies consider all likely risk factors at the time of interview. The fact that this was not done suggests a bias on the part of the investigators who had developed their suspicion of herbicides based on animal carcinogenicity tests. The investigators made up for this to some extent by subsequently determining the number of live births and the incidence of childlessness in their subjects. Childlessness, a risk factor, was three times more prevalent in cases than in controls (32% versus 12%, respectively).

Exposure ascertainment is always difficult to perform in studies of this kind. In this study, reports of use serve as a surrogate for exposure, and possible exposure is defined as residing in known herbicide use areas. The identity or amounts of different herbicides used are not reported in this study, although the later study indicates that triazines are the principal ones in use.

In summary, a relationship was found between herbicide use and ovarian cancer with the highest estimate of risk in the younger age group (less than 55). Possible selection bias in the controls and lack of adjustment for reproductive risk factors cannot be ruled out as alternative explanations. Specific herbicides that might account for the excess risk are not discussed.

IV. DETAILED CONSIDERATIONS - the 1989 Study

Synopsis

The second study, published in 1989, was a follow-up to the first study. The study was conducted in Alessandria province of Italy and confined to the 143 "municipalities" where corn is grown. Histologically confirmed cases of ovarian cancer were selected from an 8-hospital cancer registry and from 10 other hospitals in the region that served the 143 municipalities. In this study, cases were limited to women 20 to 69 years of age who were residents in the chosen area in the period July 1980 through June 1985. This time frame follows the incidence period of the previous study. Two controls were selected for each case from electoral rolls of the municipalities in the study area. Controls were matched with cases on age. Of the 69 cases eligible for participation in the study, 42 were alive and interviewed, and for 23 of the 27 deceased cases, next-of-kin were interviewed. On average, cases were interviewed 30 months after diagnosis. Of the 150 controls selected, 11 were eliminated due to having an operation to remove the ovaries, 4 refused, 6 could not be traced and 3 were unable to respond. Thus, there were a total of 65 cases and 126 controls included in the analysis. Definite exposure was defined as subjects who reported using triazines or worked in corn fields where herbicides were reported in use. According to agricultural experts in the area, any corn treated with herbicides would include use of triazines. Possible exposure was any subject who acknowledged exposure to herbicides, had a job possibly involving herbicide exposure, or worked in corn cultivation after 1964. Exposure was assessed blindly by looking at questionnaire results.

This study "was planned to have an 80% power of detecting a threefold risk with a 5% significance level." Among the reproductive factors assessed, parity (having had children) was found to be a protective factor with an odds ratio of 0.4 and 90% confidence limits of 0.2 to 0.8. After adjustment for age, parity and oral contraceptive use, no other reproductive factor yielded a statistically significant odds ratio. The odds ratio for subjects definitely exposed to triazines was statistically significant with an estimate of 2.7 and 90% confidence limits (CI) of 1.0 to 6.9. Those possibly exposed had an elevated risk that was not statistically significant (odds ratio 1.8, 90% CI 0.9-3.5). Stratified analysis based on years of exposure (more or less than 10 years) and exposure (definite or possible) did not yield statistically significant odds ratios, but the ratios were higher for the two categories that reflected increased exposure (definitely exposed and for more that 10 years). The authors conclude that these two risk trends favor the plausibility of an association. A separate stratified analysis considered only agricultural workers and found that definite exposure still yielded higher odds ratios than possible exposure, but more than 10 years exposure yield a lower odds ratio than less than 10 years exposure among those possibly exposed. It is

more important to note that none of these odds ratios are significantly different (statistically) from one another or from 1.0.

Comment

This article appears to display a serious disregard for the usual statistical protocol. The earlier study used 95% confidence intervals, the present study "was planned to have ... a 5% significance level," yet results are reported as 90% confidence levels rather than 95% levels. Furthermore, the only statistically significant result for triazines was just marginally significant. The lower confidence limit was 1.0. Had 95% limits been used, there would not have been any statistically significant results for triazines. It appears as though the investigators changed the significance level in order to obtain statistical significance. If so, it calls into question whether other aspects of the investigation were modified to obtain the desired result.

Shortcomings listed in the earlier study, including selection of cancer controls, interviewing controls after the cases, and lack of assessment of reproductive risk factors were not problems in the present study. In general, the study appears to be carefully designed to address the earlier shortcomings and thoughtfully analyzed. This makes the change in confidence levels all the more disappointing.

The analysis of just agricultural occupation was an important attempt to control for confounding factors likely to be associated with rural lifestyle. Unfortunately, this analysis did not yield any statistically significant odds ratios. Had the definitely exposed category been combined with the possibly exposed category, the larger sample size may have been produced significantly elevated estimates of risk (even at the 95% level). Combining exposure categories is an acceptable procedure which was employed in the earlier study. So, one wonders why it wasn't used here.

The analysis of reproductive factors was adjusted for age, parity, and use of oral contraceptives. The analysis of triazine exposure was adjusted for age, number of live births, and use of oral contraceptives. Parity (ever having children) is not the same as number of live births. No explanation is given for this change in adjustment.

Exposure, as in the earlier study, was based on the results of the interview. Presumably the interviewers were able to distinguish between cases and controls in this study and this is a potential source of bias. The fact that exposure "was established blindly" by the investigators reviewing the questionnaires does not remove this potential source of bias. Farmers are reported to use other herbicides besides triazines, but no quantitative estimates of the extent of this other usage is provided.

The authors state "Samples taken from electoral rolls were representative of the source population." With the exception of short discussion of migration and deaths in this population, there is very little evidence to back up this assertion. Without knowing more about how people get onto electoral rolls in Italy, it is impossible to assess what selection bias might be introduced. The use of the terminology "96% representativity" is unique to this study and misleading as to the potential for selection bias.

The authors note that there are two risk trends which favor the existence of an association between triazine exposure and ovarian cancer. These two trends result from the odds ratios for definite exposure being higher than for possible exposure and, similarly, the odds ratios for greater than 10 years exposure being more than for less than 10 years exposure. The probability of any one trend occurring by chance is 0.5. Therefore, the probability of the two trends occurring together is 0.25.

In summary, there is some suggestion of improperly adjusting the analysis to obtain significant results. Such results may properly have been obtained by combining exposure categories. The use of electoral rolls for controls may have been an important source of selection bias, but it is one that cannot be evaluated without more information.

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