CORRESPONDENCE

Offspring sex ratio as an indicator of reproductive hazards associated with pesticides

Editor—De Cock et al[1] note that waiting times to conception are significantly longer in the wives of men exposed to pesticides. They cite the evidence that the nematocide dibromochloropropane (DBCP) is associated with diminished sperm counts.[2] It is worth noting that there is other evidence of hazard in these men. They reportedly have significantly high gonadotrophin concentrations although their testosterone concentrations remain normal.[3] I have hypothesised that the sexes of human offspring are associated with the hormone concentrations of their parents at the time of conception, high testosterone producing boys, and high gonadotrophin, girls.[4-7] In conformity with this hypothesis, there is a highly significant excess of daughters among the offspring of male DBCP applicators.[8]

So it would be interesting to know whether there was an excess of daughters among the 91 children sired by the pesticide workers studied by de Cock et al.[1] More generally, workers in industrial medicine might consider offspring sex ratios as a criterion of reproductive hazard: they are cheaply and painlessly ascertained, and are not subject to the measurement errors and biases that characterise assessments of sperm quality and hormone concentrations. It should be noted that although a bias towards daughters may be indicative of hazard in male workers, there are no grounds for supposing this in female workers. At any rate, in any such analysis, the sex of offspring should be categorised by sex of parent.

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1 de Cock J, Westveer K, Heederik D, Velde E te, Kooij R van. Time to pregnancy and occupational exposure to pesticides in fruit growers in the Netherlands. *Occup Environ Med* 1994;51:693–9.
2 Whorton D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. *Lancet* 1977;ii:1259–61.
3 Whorton D, Milby TH, Krauss RM, Stubbs HA. Testicular function in DBCP exposed pesticide workers. *J Occup Med* 1979;21:161–6.
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7 James WH. The hypothesized hormonal control of sex ratio at birth—a second update. *J Theor Biol* 1992;155:121-8.
8 Potashnik G, Yanai-Inbar I. Dibromochloropropane (DBCP): an 8-year re-evaluation of testicular function and reproductive performance. *Fertil Steril* 1987;47:317–23.

Author's reply:

Editor—We would like to thank James for his letter on offspring sex ratio among children of fruit growers in our study on time to pregnancy.[1] In his letter James refers to a highly significant and large excess of daughters among the offspring of male 1,2-dibromo-3 chloropropane (DBCP) applicators[2] and wonders if information on sex ratio is also available for the fruit growers in our study. In our initial survey, we did not gather these data. As data on sex ratio are easy to obtain, we gathered this information recently by telephone. We asked wives of fruit growers the outcomes of their pregnancies. Except for one pregnancy, we were able to gather all the information on 140 pregnancies. The total number of pregnancies was 127 (excluding 12 miscarriages for which the sex ratio was unknown and one pregnancy before the period of study). The overall sex ratio was 0·51 with a 95% confidence interval (95% CI) of 0·43–0·59 (based on a binomial distribution with expected population value for sex ratio (proportion of males) was 0·514[3]). For the 91 pregnancies in our time to pregnancy study, the overall sex ratio was also 0·51.

In a more detailed analysis we first related the sex ratio to the exposure variables that were also studied in the time to pregnancy study. A decrease in sex ratio was found when recent years of birth were compared with earlier pregnancies. Also, time to pregnancy increased with more recent years of birth (table 1).

The most recent period (1987–90) showed a lower sex ratio of borderline significance (0·33) compared with the previous periods (0·56) (Fisher's exact test, two sided, P = 0·08). A similar trend in sex ratio was found for the total group of 127 pregnancies. We also found a change in sex ratio dependent on gravidity. For the first, second, third, and subsequent children, sex ratios of 0·60, 0·57, 0·42, and 0·31 were found respectively. The first two pregnancies of a couple in comparison with next pregnancies showed a sex ratio of 0·58 and 0·38 respectively (Fisher's exact test, two sided, P = 0·08).

*Table 1 Offspring sex ratio and time to pregnancy according to year of birth (n = 85)**

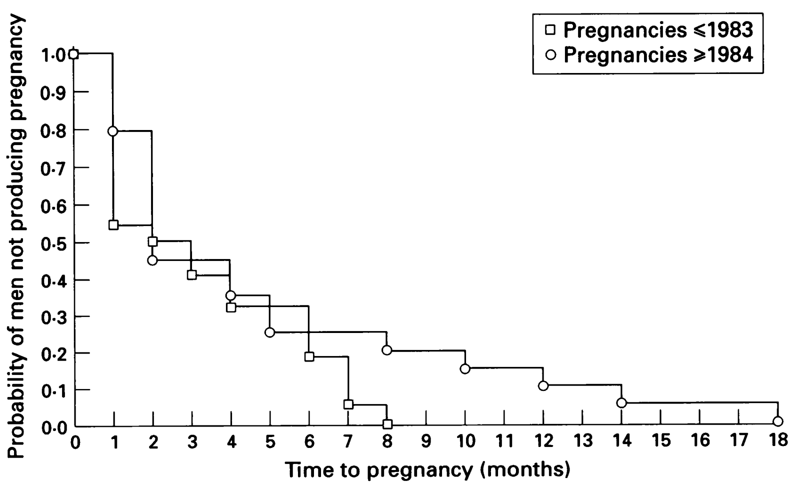
Year of birth	*Pregnancies n*	*Sex ratio*	*Time to pregnancy (months)*
1978–80	18	0·56	2·9
1981–83	22	0·55	3·5
1984–86	24	0·58	4·2
1987–90	21	0·33	4·1

*n = 91 pregnancies from the time to pregnancy study, excluding six miscarriages of unknown sex.

This raises the question whether gravidity acts as a confounder in this analysis, as does time to pregnancy. Because of small numbers, stratification of sex ratio according to gravidity and year of birth was not possible. Surprisingly, a difference in time to pregnancy according to year of birth was found for boys but not for girls. The figure is a Kaplan-Meier curve (PROC LIFETEST) by year of birth for boys. The curves, did not differ significantly. A univariate survival analysis with the PHREG SAS procedure as described in our study on time to pregnancy,[1] for the period of birth comparing pregnancies occurring in 1983 or before (1) with more recent pregnancies (0) as the independent variable, showed a fecundability ratio of 1·61 (95% CI 0·83–3·13) for boys and 1·13 (95% CI 0·59–2·15) for girls. No differences in age at the time of conception of men or women, or the age difference between both parents were found in our study. Therefore, a role of age dependent hormone concentrations of the parents on offspring sex ratio at the time of conception is not a very likely explanation for these findings.

In our study on time to pregnancy, we focused on seasonal effects of exposure of men. No significant differences according to season were detected in the sex ratios. Observed sex ratios for the quarter of a year in which conception took place were: 0·64 (January-March), 0·44 (April-June), 0·48 (July-September), and 0·52 (October-December).

As no relation between sex ratio and any of the exposure variables used in our study on time to pregnancy was found, other available information on exposure was considered as well. Because offspring sex ratio is a dichotomous variable, we studied outcome in a case-control like design with maximum likelihood logistic regression models by computing odds ratios (ORs) with SAS PROC LOGISTIC. As the odds



Time to pregnancy by year of birth for boys (n = 44).

Table 2 Odds ratios of 16 cases (more daughters than sons within a family) compared with 27 controls (43 sons) for exposure variables

Variable	Odds ratio (95% CI)
Spraying days 1990 (30 days)	18·4 (1·72–196)
Cross current sprayer (10 days/y)	1·7 (1·05–2·59)
Herbicide sprayer (5 days/y)	3·6 (1·10–11·6)
(Manual) knapsack sprayer (5 days/y)	2·2 (1·06–4·66)
Metiram (1 spraying/y)	1·3 (1·05–1·61)
Azinphos-methyl (0/1)	4·4 (1·11–17·3)
Paraquat (0/1)	>5·6* (>5·6–< 13·4)

*Paraquat was used by all families with more daughters compared with 74% in the control group.

ratio for gravidity (OR 1·35, 95% CI 0·92–1·99) was not significant and adjustment did not influence the regression coefficient or standard errors of variables of interest, only crude ORs are given.

No relation was found for most of the variables studied in our time to pregnancy study, but the results suggest a relation between use of a cross current airblast sprayer (days/y) (OR = 1·3, 95% CI 0·96–1·72) and use of a herbicide sprayer (days/y) (OR = 2·0, 95% CI 1·08–3·76) and production of daughters. Also we compared families with more daughters than sons with the other families. Instead of gravidity we corrected for the number of children within a family (OR = 1·23, 95% CI 0·66–2·31). As some families also conceived children before the time of the study (1978–90) number of children is a surrogate measure for gravidity. Because differences with or without adjustment were small, table 2 gives only crude ORs. For the 43 families a relation was found between the number of days with use of a herbicide sprayer (days/y), a cross current airblast sprayer (days/y), and a knapsack sprayer (days/y) and the number of daughters within a family. When comparing families with more daughters than sons (16 families), the number of spraying days a year was 37 compared with 25 for the other families, 7·6 *v* 3·3 days/y for the herbicide sprayer, 16 *v* 5·4 days/y for the cross current airblast sprayer, and 7·1 *v* 3·0 days/y for the knapsack sprayer. In families with more daughters use of the specific pesticides Azinphosmetyl (insecticide), Metiram (fungicide), and Paraquat (herbicide) was higher. Table 2 shows the results of the ORs.

For families with more sons than daughters (17) a significant OR was found for use of a cross current sprayer (yes or no, OR = 0·16, 95% CI 0·03–0·82), and number of spraying days with this type of sprayer (OR = 0·54 per 10 spraying days/y, 95% CI 0·30–0·98).

Discussion

Overall sex ratio was not different from the expected ratio of 0·51. James hypothesised that high concentrations of testosterone at the time of conception produce boys, and high concentrations of gonadotrophin produce girls. Among the offspring of DBCP applicators[3] a highly significant excess of daughters was found. As exposure to pesticides in fruit growing typically includes mixed exposure to many different compounds, similar to that found for DBCP, it is not possible to predict the direction of a shift in sex ratio induced by exposure to pesticides among this group. As the number of subgroups is small it is impossible to draw firm conclusions, but some of the results are of interest. From our results there are some indications that exposure to pesticides in agriculture may affect offspring sex ratio. The shift towards daughters in the most recent period (sex ratio of 0·33) was remarkable. Also the finding that spraying frequency, frequency of use of specific equipment, and use of some specific pesticides are related to a shift towards more daughters within a family may point to an exposure effect. One should be careful in interpreting these results. The fact that use of several pesticides is related to sex ratio does not necessarily imply that individual pesticides are causally related to sex ratio. Fruit growers use a complex mixture of agents and the use of one agent is often correlated with the use of another one. It is unlikely that the shift in sex ratio is caused by the introduction of particular pesticides as all pesticides have been applied to some extent during the study period. Because application techniques changed considerably over time, the introduction of certain techniques seems a more plausible explanation for this finding. It is possible that with certain underlying mechanisms, effects may not be caused by exposure of the male worker only, as most women live near the orchard and they often participate during particular activities like pruning, thinning, and harvesting. No seasonal trends were found in this analysis as was found for time to pregnancy. Our finding that there might be a difference in time to pregnancy for boys and girls as well, may indicate that both sex ratio and time to pregnancy are interlinked. We cannot explain why exposure variables associated with time to pregnancy are not related to sex ratio.

In conclusion, we think that the suggestion by James to analyse sex ratio is a useful one and should be explored further. To consider both sex ratio and time to pregnancy simultaneously may have advantages in elucidating occupational hazards of (pesticide) exposure. Our results do show that other variables such as families with predominantly daughters or sons, which are indicative of a shift in sex ratio, might be more powerful because they use another sampling unit (family instead of a crude stratification by exposure). Especially in this study among agricultural workers an analysis on a family level might be relevant because the exposure might be aggregated at the family level as well. In general, it seems useful to explore, after time to pregnancy and sex ratio, the presence of families with a predominance of one of the sexes as little is known about the underlying biological mechanisms as well as the statistical properties of these indices.

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1 Cock J de, Westveer K, Heederik D, Velde E te, Kooij R van. Time to pregnancy and occupational exposure to pesticides in fruit growers in The Netherlands. *Occup Environ Med* 1994;51:693–9.
2 Ptashnik G, Yanai-Inbar I. Dibromochloropropane (DBCP): an 8-year re-evaluation of testicular function and reproductive performance. *Fertil Steril* 1987;47:317–23.
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NOTICE

The Toxicity of Mixtures of Solvents. 13 June 1995. London.

A one day meeting organised jointly by the SCI Health and Safety Group, RSC Toxicology Subject Group, and the Institute of Occupational Hygiene. The meeting will examine the theoretical and practical difficulties in assessing the toxicity of solvent mixtures and effecting control of exposure. Data from experimental studies, occupational exposures and poisoning incidents will be reviewed with an aim of understanding the nature of mixed solvent toxicity. The second half of the meeting will concentrate on industrial experiences, and will consider techniques for assessing and controlling exposures to mixtures of solvents in the workplace.

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