

Comment on "Sexual Maturation in Relation to Polychlorinated Aromatic Hydrocarbons: Sharpe and Skakkebaek's Hypothesis Revisited"

The paper of Den Hond et al. (2002) is very interesting, mainly because the reported work demonstrates the potential of biomarkers in environmental health programs. However, we argue with the authors' conclusion that their findings

suggest that, in line with the concept of endocrine disruption and Sharpe and Skakkebaek's hypothesis [Sharpe and Skakkebaek 1993], environmental exposure to PCAHs [polychlorinated aromatic hydrocarbons] may adversely interfere with the sexual maturation during the fetal and pubertal stages of development.

The description of the project suggests that the study was designed to investigate the effects of incinerator emissions on the local population in two suburbs of Antwerp, Belgium, Wilrijk and Hoboken. However, the study was part of a larger research project commissioned by the Flemish government to determine the feasibility and issues to be considered in developing environmental health monitoring. More particular, the study of Den Hond et al (2002) was designed to determine whether evaluation of biomarkers measured as part of the regular medical examination of adolescents at the end of their secondary education period would be feasible. We were asked by the Flemish government to review the results of this project (Cuijpers et al. 2000; Ghoetghebeur et al. 2000). We feel that the objectives and hypotheses underlying a feasibility study should not be convoluted with those relative to the hypotheses arising from specific concerns in certain *a priori*-defined regions.

A major shortcoming of the design is the lack of randomness regarding both the study areas and the recruitment of the participants. The study areas were well-known polluted regions within the conurbation of Antwerp. This knowledge may have influenced the choice of tests to be performed. Researchers should be cautious regarding *a priori* choices of study areas, whether or not the sponsor influences the choice.

The use of volunteers instead of randomly selected participants is another flaw, introducing an extra risk of confounding. As a consequence, for example, the proportion of boys to girls in the three study areas differed considerably: from 0.7 to 3.0. In this case there may have been a selection bias of adolescents in lesser physical condition who volunteered for examination.

Reported results were the outcome of analyzing a multitude of associations among

the empirical data; they did not emerge after a rigorous test of an *a priori*-formulated hypothesis, at least not to our knowledge. Such findings are valuable because they may generate hypotheses for further research. In this respect we agree with the authors' closing sentence (Den Hond et al. 2002):

... [F]urther studies should be undertaken to confirm or to refute our interpretation of the present findings.

The sexual development of the study subjects was judged by the examining physicians. The correspondence in ratings varied between fair and good (as validated by kappa coefficients) (Fleiss 1981). It is then important to exclude a systematic over- or underestimation by one of the examining physicians. This test was not been reported in the study.

Finally, we would like to remark on the reference the authors made (Den Hond 2002) to an earlier report on pregnancy outcome in Flanders (Aelvoet et al 1999):

... [I]n 1997 the Flemish government reported a higher percentage of medically assisted conceptions in the district around the waste incinerators compared with the rest of Flanders....

We feel that this report is incorrectly and selectively referenced, suggesting that the findings are in line with earlier results. Taking into account the mother's age, no significant differences in way of delivery were found in the earlier study (Aelvoet et al 1999). Furthermore, that report does not contain statements about the necessity of medical assistance at delivery. Part of the above criticism was outlined in the review reports (Cuijpers et al. 2000) but unfortunately not addressed in the article by Den Hond et al. (2002).

To conclude, we would like to reiterate that we commend the authors with their highly relevant and extensive research work. However, they should have placed their study more clearly in the perspective of the Flemish environmental health project. Their conclusions generate interesting hypotheses for further work but should not be viewed as the results of a cause-and-effect study.

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REFERENCES

Aelvoet W, Nelen V, Schoeters G, Vanoverloop J, Wallijn E, Vlietinck R. 1999. Gepercipieerd excess aan congenitale afwijkingen en niet gepercipieerd excess aan meerlingen in de buurt van twee huisvuilverbrandingsovens in Neerland. In: Gezondheidsindicatoren 1997 (Aelvoet W, Fortuin M, Hooft P, Vanoverloop J, eds). Brussels: Ministerie van de Vlaamse Gemeenschap, p. 130-141.

Cuijpers C, Molenberghs G, Passchier WF, Pieters J.J.L. 2000. De vinger aan de pols van een gezond Vlaams milieu. (2). Rapportage van een review-commissie van het Project 'Milieu en Gezondheid' [in Dutch]. Brussels:Ministerie van de Vlaamse Leefgemeenschap.

Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. 2002. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect 110:771-776.

Fleiss JL. 1981. Statistical Methods for Rates and Proportions. New York:John Wiley & Sons.

Ghoetghebeur EJT, Passchier WF, Pieters J.J.L. 2000. De vinger aan de pols van een gezond Vlaams milieu. Rapportage van een review-commissie van het Project 'Milieu en Gezondheid' [in Dutch]. Brussels:Ministerie van de Vlaamse Leefgemeenschap.

Sharpe RM, Skakkebaek NE. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1392-1395.

"Sexual Maturation in Relation to Polychlorinated Aromatic Hydrocarbons...": Den Hond et al.'s Response

The Flemish Government commissioned the Environment and Health Study after a competitive call for research proposals in 1998 (Onderzoek Milieu en Gezondheid 1998). From the outset, this research project included two distinct population-based surveys, one in adolescents (16-18 years of age) and another in adults (21-65 years of age). We assume scientific responsibility only for the project in adolescents.

Our study (which was carried out in 1999) sought to investigate whether, in adolescents, biomarkers can show exposure to and health effects of common environmental pollutants (Staessen et al. 2001). We planned to maximize the gradient in environmental exposure by recruitment of adolescents from a rural control area (Peer, Belgium) and a polluted industrial suburb. For logistic reasons, the adolescents were recruited from and were examined at local schools. Life-long residence in the study areas was a prerequisite for participation. The Flemish Government imposed the selection of the polluted area because of a

long-lasting controversy about the possible impacts on public health of two waste incinerators in Wilrijk and a large nonferrous smelter in Hoboken. Health authorities assumed that our study would settle the local concern about detrimental health outcomes associated with the waste incinerators in Wilrijk. In spite of the imposed selection of the polluted area, our study had the advantage of a clear-cut contrast in exposure between the control and polluted areas, as evidenced by all available records on environmental monitoring.

Molenberghs et al. found the lack of randomness a major shortcoming of our study design. However, they miscalculated the proportion of boys to girls, which was 1.5 (60/40) in Peer, 1.0 (21/21) in Wilrijk, and 2.1 (39/19) in Hoboken. We believe that, in the context of the imposed selection, we maximally exploited the remaining degrees of freedom to avoid serious selection bias. Indeed, participants and nonparticipants had similar age, sex distribution, parental social class, and regional residence. In the industrial suburb they resided at similar distances from the main sources of pollution, which strongly suggests random selection with regard to exposure (Staessen et al. 2001). Although we cannot extrapolate to the general Flemish population, our conclusions are applicable to the adolescents enrolled in our population-based sample.

After our Data Monitoring Committee—which was enlarged by three international experts for peer-review—had discussed the study outcomes on 12 May 2000, our results became available for disclosure to the public and subsequent scientific publication. The health administration then appointed Molenberghs and colleagues as the official external reviewers of our study (Goetghebeur et al. 2000; Cuijpers et al. 2000). These statisticians seem to imply that our conclusions should have been restricted to the feasibility of biomonitoring in adolescents and that we should not have disclosed the unexpected medical findings suggesting relevant environmental health risks. To what extent their criticisms are justified and independent remains open for discussion. In this context, it is interesting to note that, on 25 July 2000, the Flemish Minister of Public Health addressed a letter to the principal investigator of our study (J.A.S.) demanding that disclosure of the findings be postponed for 6 months until the study in adults would be completed. This embargo was lifted on 21 November 2000, a few weeks after the municipal elections.

In contrast to the criticism of Molenberghs et al., we actually based our study on *a priori* hypotheses associating defined biomarkers of exposure with relevant effect markers (Staessen et al. 2001). This is also the case for the specific

article on the endocrine effects of polychlorinated aromatic hydrocarbons (PAHs) (Den Hond et al. 2002) and other recent papers that emanated from the adolescent study, for example, those on the immune (Van Den Heuvel et al. 2002) and genotoxic (Verheyen et al. Unpublished data) effects. Because we were concerned about the reproducibility of the staging of sexual maturation according to Marshall and Tanner's scale (Marshall and Tanner 1969; Marshall and Tanner 1970) and the measurement of testicular volume using Prader's orchidometer (Dörnberger et al. 1987), we mounted a separate validation study of which the results have been reported and discussed elsewhere (Den Hond et al. 2001).

Molenberghs et al. confuse medically assisted conceptions with medically assisted deliveries. Nevertheless, we acknowledge that age is a major determinant of fertility in women. However, the officially published report did not include an age-adjusted analysis of the proportion of medically assisted conceptions (Advoet et al. 1999). The median age in women at first delivery was 27.3 (interquartile range 25.2–29.3) years in Wilrijk and 26.6 (24.2–29.0) years in Flanders. A difference of 0.7 years cannot explain a 65–75% increase in medically assisted conceptions (5.6% in Wilrijk vs. 3.4% in Flanders for singleton births and 59.0% vs. 33.4% for multiple births).

We hope that, with these clarifications, our study is more clearly placed in the perspective of the Flemish Environment and Health Study. We agree with Molenberghs et al. that our findings, although in line with the concept of endocrine disruption and Sharpe and Skakkebaek's hypothesis, require further research. As in all cross-sectional epidemiologic studies, we did not prove causation. However, numerous experimental studies support the causal association between exposure to PAHs and effects on sexual maturation.

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REFERENCES

- Aelvoet W, Bekaert Y, Dejonghe M. 1999. Gepercipieerd excès aan congenitale afwijkingen en niet-gepercipieerd excès aan meelingen in de buurt van twee huisvuilverbrandingsovens in Nederland. In: Gezondheidsindicatoren 1997 (Aelvoet W, Fortuin M, Hooft P, Vanoverloop J, eds). Brussels:Ministerie van de Vlaamse Gemeenschap, 130–141.
- Cuijpers C, Molenberghs G, Passchier W, Pieters J. 2000. De vinger aan de pols van een gezond Vlaams milieu (2) (Dutch). Brussels:Ministerie van de Vlaamse Gemeenschap. Available: http://www.wvc.vlaanderen.be/gezondmilieu/onderzoeken/koepel/rapport_review-commissie/pdf/eindrapport.PDF [cited 25 October 2002].
- Den Hond E, Staessen JA, Hoppenbrouwers K, Vanderschueren D, Roels H. 2001. Delayed sexual development in adolescents: author's reply [Letter]. *Lancet* 358:1817.
- Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. 2002. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. *Environ Health Perspect* 110:771–776.
- Dörnberger V, Dörnberger G. 1987. Vergleichende Volumetrie des menschlichen Hodens unter besonderer Berücksichtigung der Hodensonographie, Praderorchidometer, Schirrenzirkel und Schublehre [Comparative volumetry of human testes using special types of testicular sonography, Praders orchidometer, Schirren's circle and sliding caliper]. *Andrologia* 19:487–496.
- Goetghebeur E, Passchier W, Pieters J. 2000. De vinger aan de pols van een gezond Vlaams milieu. Brussels:Ministerie van de Vlaamse Gemeenschap (Dutch). Available: http://www.wvc.vlaanderen.be/gezondmilieu/onderzoeken/koepel/toxicologie/pdf/addenda_adolescenten/12/pr000607.PDF [cited 25 October 2002].
- Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. *Arch Dis Child* 1969; 44:291–303.
- Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970; 45:13–23.
- Onderzoek Milieu en Gezondheid. 1998. Ontwikkeling van een gestructureerde aanpak voor vermoedelijke aan het milieu gerelateerde ziekteclusters in Vlaanderen en van een leefmilieu- en epidemiologische monitoring (Dutch). Brussels:Ministerie van de Vlaamse Gemeenschap.
- Staessen JA, Nawrot T, Den Hond E, Thijs L, Fagard R, Hoppenbrouwers K, et al. 2001. Renal function, cytogenetic measurements and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. *Lancet* 357:1660–1669.
- Van Den Heuvel RL, Koppen G, Staessen JA, Den Hond E, Verheyen G, Nawrot TS. 2001. Immunologic biomarkers in relation to exposure markers of PCBs and dioxins in Flemish adolescents (Belgium). *Environ Health Perspect* 110:595–600.

Declaring Chemicals "Not Carcinogenic to Humans" Requires Validation, Not Speculation

Regulatory agencies should provide detailed guidelines on how to use mechanistic and epidemiologic data to dismiss positive cancer evidence obtained from studies in experimental animals. Roberts and Ashby (2002) bemoaned that the U.S. Environmental Protection Agency (EPA) does not provide opportunity to conclude that there is no evidence of carcinogenicity from well-conducted and adequately powered epidemiologic studies. In their letter, Roberts and Ashby provided examples for which they claimed that there is enough information to draw valid conclusions of "no effect." Our examination