REPRODUCTIVE RISKS OF BENZENE: NEED FOR ADDITIONAL STUDY

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INTRODUCTION

Critical processes of differentiation and growth that occur during gestation have often been the primary focus of reproductive toxicology. But it was not until 1932 that maternal consumption of vitamin A during gestation was shown to induce congenital defects in animals, and the first demonstration of a human teratogen (aminopterin) did not occur until the early 1950s. It has now been shown that reproductive hazards can be mediated through reproductive processes other than intrauterine development, including gametogenesis in both males and females, lactation, and ejaculation. The effects can be permanent or temporary, and they can result from single exposures to such agents as germ cell mutagens or from continuous exposure to such agents as synthetic sex hormones and inhibitors of steroid metabolism (Hunt, 1979).

The expanding knowledge of reproductive processes and recent improvements in testing have increased the number of reproductive end points that can be examined, and these now include such quantifiable effects as premature reproductive senescence and delayed puberty. But, although many methods have been developed for estimating human teratogenic potency and hazard, none is uniformly accepted, and existing standard test protocols are still inadequate.

The discovery of relationships between exposure to organic solvents and potential adverse reproductive effects, such as congenital malformations, chromosomal abnormalities, and altered fertility in experimental animals and man, has renewed the scientific interest in reproductive toxicology. In particular, the widespread occupational and environmental exposure to benzene—approximately 1.3 billion gallons of benzene were produced in the United States in 1984—constitutes adequate justification for concern and for reevaluation of reproductive effects.

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This paper briefly reviews the toxicologic evidence of reproductive effects of benzene, describes some recent suggestive epidemiologic studies on the effects of related solvents on human reproduction, and comments on the limits of previous scientific paradigms for studying these effects.

EXPERIMENTAL STUDIES

Some early animal studies indicated that exposure to benzene could produce testicular and ovarian degeneration. The results of most studies of the reproductive effects of benzene were considered negative, however, because fetal outcome was the end point of concern.

A 1981 review (Mehlman, 1981) of three injection and nine inhalation studies of benzene teratology in animals concluded that exposure to benzene at concentrations that induce overt maternal toxicity also causes embryotoxicity, reduced fetal weight, and malformation. Chronic exposure to benzene caused decreased body weight in dams and offspring and slight skeletal variations in the offspring. An additional review of some of these studies (Chatburn et al., 1981) concurred that doses of benzene that produce maternal toxicity produce measurable fetal effects. Although exposures were limited to the period of organogenesis (days 6–16 of gestation in rats and mice and days 6–18 of gestation in rabbits), retardation of fetal development (as measured by fetal weight and degree of ossification) was accompanied by and attributed to the toxic effect of benzene in the mother, which was evidenced as a decrease in maternal weight gain in all studies. This is the phenomenon of the coeffective teratogen, which occurs when a toxicant adversely affects both the maternal and fetal environments (Johnson et al., 1981). Although the studies in question were restricted to the effects of benzene on embryonic growth and development, the results suggested that benzene was weakly teratogenic at doses of 500 ppm and weakly fetotoxic at 100 and 50 ppm.

Increased numbers of malformations, including absence and delayed ossification of sternebrae after maternal exposure, have also been reported (Watanabe, 1970; Kimmel, 1973). It has been suggested, however, that these malformations be evaluated cautiously when they are the only signs of embryotoxicity.

Very few experimental studies have investigated the effects of maternal or paternal exposure to benzene before conception or after birth. The potential toxic effects before conception are not well understood, but early experimental studies suggested that toxic doses of benzene cause ovarian and testicular atrophy and degeneration. Vara and Kinnunen (1946) injected benzene into rabbits daily until leukopenia occurred and observed hypoplastic ovaries, chromosomal damage, and mitotic interruptions in ova. Hett and Maak (1938) found that inhalation of benzene vapor sufficient to induce blood dyscrasias produced degeneration of ovarian follicles, "multinucleate ova," and testicular atrophy in mice.

There is some evidence that males are more susceptible than females to the effects of benzene exposure. Results of the micronucleus test in mice (Siou et al., 1977) showed

that adult females were twice as resistant as males to the effects of intraperitoneally administered benzene. Castrated male mice acquired a resistance to these effects that could be reversed with testosterone treatment (Siou and Sourdeix, 1980). A more recent report corroborated these observations of increased susceptibility of males to the myeloclastogenic effects of benzene metabolites and supported the finding that females were consistently more resistant than males to benzene in the micronucleus test and metaphase analysis.

Some unanalyzed data on reproductive effects included in the 1983 results of the National Toxicology Program bioassay (1983) indicated a dose-response relationship between benzene exposure and a preneoplastic response in reproductive tissues. Ovarian epithelial hyperplasia in female mice [control group, 12 animals (25%); low-dose group, 39 (80%); mid-dose group, 31 (63%); and high-dose group, 29 (59%)] and dose-related interstitial cell hyperplasia in male rats (control group, 10%; low-dose group, 8%; mid-dose group, 22%; and high-dose group, 40%) were reported.

EPIDEMIOLOGIC STUDIES

In the last several years, a number of epidemiologic studies have reported that parental occupation can be a cancer risk factor for children. Fabia and Thuy (1974) found an excess of cases of cancer in children whose fathers worked in hydrocarbon-related occupations at the time of the children's birth. Hakulinen et al. (1976) noted an excess (statistically nonsignificant) of brain-tumor cases in children whose fathers were employed in hydrocarbon-related occupations at the time of conception. Kwa and Fine (1980) reported a significant excess of paternal employment as paper or pulp mill workers in children with tumors of the brain and nervous system. Hemminki et al. (1981) and Peters et al. (1981) reported similar relationships with parental exposures to toxic chemicals, including solvents and pesticides.

In contrast with the positive studies, Zack et al. (1980) reported no association between paternal hydrocarbon-related occupations and childhood cancer. Controls in this negative study were parents of children with noncancer blood disorders who were patients at Texas Children's Hospital Hematology Research Clinic; however, those parents were neighborhood residents selected by the cases' parents and might have had similar environmental exposures. A review of seven studies on this subject concluded that the evidence was not definitive (Gold et al., 1982).

Nervous disorders in children have also been associated with parental occupational exposures that might have included benzene. For example, compared with all other Parents of sick children not having congenital malformations, fathers working as painters had a relative prevalence ratio of 4.9 of having children with congenital malformations of the central nervous system. In addition, maternal exposure to organic solvents during the first trimester of pregnancy has been associated with malformations of the central nervous system (Holmberg, 1979).

In addition to studies associating benzene-related parental occupations with increased risk of cancer and congenital CNS defects in children, a study in Finland has indicated an association with spontaneous abortions in the rubber industry (Hemminki, **1983).** However, women employed in a rubber factory for shorter periods (3–23 months) were found to have a higher frequency of spontaneous abortions than women employed for longer periods. Several possible explanations were offered for that finding (none could be confirmed): newly employed people might do work that is more hazardous (with respect to benzene exposure), women with a tendency to spontaneous abortion might be likely to work for short periods, and women who have spontaneous abortions might choose to quit working. Although pinpointing the individual causative chemicals remains impossible, benzene remains one candidate because of the high potential for exposure to it in the rubber industry.

DISCUSSION

In most epidemiologic studies, information on exposure constitutes the weakest link in the analysis. Although the specific role of benzene exposures in the epidemiologic studies noted here remains a matter of speculation, it merits mention, in light of the results of animal studies.

The critical processes of differentiation and growth that occur during gestation have often been the primary focus of reproductive toxicology, but other processes in the reproductive cycle have recently begun to gain attention. The ages of menarche and menopause, for example, have recently been shown to be affected by exposure to some xenobiotics (Mattison, 1983). Effects of chemicals on paternal fertility and on the offspring are also of increasing interest.

With the expanding knowledge of quantifiable reproductive end points, it is increasingly evident that the mechanisms and mediators of reproductive effects include more than those present during gestation, and the effects can occur before conception and after birth.

Susceptibility to xenobiotics might be understood as a function of the rate of cell division—the more rapidly cells divide, the greater the chance for interference with their normal processes. Gametogenesis would thus be an especially vulnerable process. The mean number of spermatozoa produced daily is approximately 125×10^6, and they survive for months in the epididymis. Females, however, are born with approximately 2×10^6 primary oocytes, only **400** of which are ovulated as potential concepti (Biggers, 1979). Although the rate of cell division might make sperm cells more susceptible than ova, the potential duration of exposure is greater for the ova.

Postnatal maternal exposure can cause developmental impairment of the newborn through lactation, and transgenerational effects, such as diethylstilbestrol-induced clear-cell adenocarcinoma, can also become evident postnatally (Ritts et al., 1983).

CONCLUSION

Epidemiologic methods have limitations that require supplementation with experimental methods to permit evaluation of the reproductive hazards of chemicals. The epidemiologic and experimental studies reviewed here argue strongly for additional studies of the potential reproductive effects of benzene exposure. Recent experimental evidence indicates that males might be more susceptible than females to benzene. Older studies showed testicular and ovarian degeneration at toxic doses. Results of recent epidemiologic studies imply that benzene and some related solvents are associated with transgenerational cancer. Additional animal studies should provide valuable insight into the effects of benzene throughout the reproductive continuum.

In light of results of epidemiologic studies of children and their parents, additional experimental animal studies on preconception and postnatal maternally mediated effects of benzene should be undertaken. The absence of evidence of benzene's hazards to human reproduction offers no proof that it bears no such hazards.

Susceptibility to xenobiotics can be thought of as a function of the rate of cellular division, with more rapidly developing cells being more susceptible. Bioassay data (NTP, 1983) show dose-related interstitial-cell hyperplasia in male rats and ovarian epithelial hyperplasia in female mice exposed to benzene gavage.

Finally, the marked sex differences in susceptibility during germ cell formation should be considered. In the human female, for example, meiosis begings during fetal development, but is not complete until puberty, and the genetic material is relatively well protected during the "resting phase" from birth until puberty (Strobino, 1978). In contrast, the first meiotic division in human males does not occur until puberty, when diploid primary spermatocytes begin to undergo transformation (Ganong, 1977). These spermatocytes remain vulnerable to a host of exogenous influences, including temperature, drugs, and other xenobiotics.

In addition to the seemingly obvious potential for direct effects on maternal fertility, the potential for effects on paternal fertility and the transmissibility of these effects to Offspring should be considered.

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