# Looking for Causes of Neural Tube Defects: Where Does the Environment Fit In?

Lowell E. Sever

Health Risk Assessment Department, Battelle Pacific Northwest Laboratories, Richland, Washington

The neural tube defects anencephaly and spina bifida are important causes of infant mortality and morbidity. Recent studies suggest that many of these defects can be prevented by the periconceptional use of folic acid. At the same time, we do not know what causes most cases of neural tube defects and there is evidence to suggest that they are etiologically heterogeneous. Additional research needs to be directed toward the role of occupational and environmental exposures in the etiology of these defects. Importantly, studies need to examine embryologically and anatomically specific types of defects and develop accurate information on biologically relevant exposures. Exposures toward which attention needs to be directed include organic solvents; agricultural chemicals, including pesticides; water nitrates; heavy metals such as mercury; ionizing radiation; and water disinfection by products. We also recommend that additional attention be paid to mechanisms of neural tube closure and to the potential role of genetic heterogeneity in the absorption and metabolism of xenobiotics and in their effects on the neural tube. — Environ Health Perspect 103(Suppl 6):165–171 (1995)

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## Introduction

Among many populations, defects of neural tube closure—anencephaly, spina bifida cystica or meningomyelocele, and encephalocele—are significant causes of infant mortality and morbidity. Commonly considered as a group and currently most often referred to as neural tube defects (NTDs), these defects have been studied extensively; yet their causes remain unknown. There is overwhelming evidence supporting a multifactorial etiology for this group of defects. In addition, there is increasing evidence that these defects are heterogeneous. As discussed below, more attention needs to be paid to the possibility of etiologic differences among the defects than often has been the case in the past.

The determination of the role of periconceptional folic acid supplementation in reducing the risk of NTDs must be considered a major public health accomplishment (*1*). There is now convincing evidence that folic acid supplementation prior to conception and early in gestation reduces both the occurrence and recurrence of NTDs. Oakley (*1*) has recently introduced the term and concept of folic acid-preventable spina bifida and anencephaly. It has been estimated that approximately 50% of NTDs are preventable by taking a supplement containing 0.4 mg of folic acid prior to conception and early in gestation. It must be kept in mind that the success of this preventive intervention is likely to vary among populations. This is supported by findings from the case–control study of vitamin supplementation and NTDs in Illinois and California that did not show protective effects (*2*).

Although the evidence that folic acid supplementation reduces the risk of anencephaly and spina bifida is compelling, it must be emphasized that we do not know how folic acid works. In addition, it is important to realize that although there are no overwhelming data to support a role for environmental agents in the etiology of NTDs, there are data that suggest that extrinsic agents need to be evaluated further.

My thesis is that additional attention needs to be paid to the potential roles of environmental and occupational agents in the etiology of NTDs. As part of this process, it is necessary to increase the specificity of categorizing both outcomes and exposures. The key issues to be addressed in this presentation are the importance of recognizing etiologic heterogeneity—and examining specific types of defects—and improving assessment of exposure in attempting to evaluate the role of exogenous agents in disrupting neural tube closure.

## Heterogeneity of Neural Tube Defects

Because of their epidemiologic and embryologic similarities, anencephaly and spina bifida have commonly been referred to as a single etiologic entity. This has been reflected in the use of collective terms to categorize the defects: historically, central nervous system malformations and, more recently, neural tube defects. I believe that this approach obscures the possibility of important etiologic differences between anencephaly and spina bifida, the two major defects subsumed in those groupings. In addition, some studies of central nervous system malformations have included nonneural tube defects such as hydrocephaly and microcephaly among the defects that were so characterized. While for some purposes the grouping of defects may be useful, I maintain that in trying to determine etiology, it is important to look at defects by as specifically defined anatomic/embryologic categories as possible.

There is a growing literature on the etiologic heterogeneity of the NTDs. Early discussions focused on the importance of looking at defects associated with chromosomal or genetic factors separately from those expected to be due to multifactorial etiology (*3–5*). Later discussions focused on epidemiologic differences between anencephaly and spina bifida (*6*) and on potential etiologic differences between NTDs with and without malformations in other organ systems—often referred to as single versus multiple (*7–9*) and upper versus lower NTDs (*10,11*). While not all

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Address correspondence to Dr. Lowell E. Sever, Battelle Seattle Research Center, 4000 NE 41st Street, Seattle, WA 98105. Telephone (206) 528-3348. Fax (206) 528-3553.

investigators agree with the concept of etiologic heterogeneity characterized by the above discussion (*12,13*), it is my opinion that the recent paper by Van Allen et al. (*14*) presenting evidence for multisite neural tube closure clearly supports the importance of looking at individual categories of defects, as we stressed in an early paper (*6*).

## Environmental Factors and the Specificity of Neural Tube Defects

One particularly important point in looking at environmental agents and NTDs is that when clusters occur, rates most frequently increase for either anencephaly or spina bifida but not for both defects. We examined this topic several years ago and our findings are included in Table 1.

Of particular interest are two additional clusters with which I have been involved. The first of these is a cluster observed in a Los Angeles County hospital in 1976 to 1977. During a 3-month period, seven of 923 babies were born with NTDs and six of the cases were anencephaly; of those, three were craniorachischisis. All these babies were born to Hispanic mothers. Rates of anencephaly in the United States are usually less than one per 1000 births, with even lower rates in Los Angeles County, although rates among Hispanics are higher than those among non-Hispanics (*6*).

Of more contemporary concern is the cluster of anencephaly in Cameron County in south Texas. In April 1991, a nurse in Brownsville, Texas, recognized that something was wrong: in one 36-hr period, three babies were born with anencephaly. The Brownsville nurse's observation was the first indication of this cluster. A subsequent investigation by the Texas Department of Health and the Centers for Disease Control and Prevention (CDC) revealed a cluster of anencephaly cases in 1990 and 1991, along with endemically high rates of the defect in the predominantly Hispanic population (*15*).

**Table 1.** Anencephaly and spina bifida cases in reported clusters of neural tube defects.

| Location | Anencephaly | Spina bifida | Ratio |
|---|---|---|---|
| Jacksonville, FL | 2 | 12 | 1:6 |
| Kanawha County, WV | 23 | 15 | 1.5:1 |
| Pulaski County, KY | 7 | 3 | 2.3:1 |
| Pineville, KY | 1 | 6 | 1:6 |
| Fitchburg, MA | 1 | 5 | 1:5 |
| Antioch-Pittsburg, CA | 8 | 2 | 4:1 |

A key feature of the south Texas cluster is that the rate of anencephaly in particular was found to be increased. Information is lacking on the anatomic details of the cases. As shown in Table 2, the rate of other NTDs (spina bifida predominantly) appears to be more consistent over time (*15*). As noted earlier, anencephaly was also observed to have increased in several other clusters, suggesting the possibility of defect-specific etiologic agents (*6*). Valproic acid, for example, increases the risk of spina bifida but not anencephaly (*16*).

The Brownsville anencephaly cluster has triggered increased concern regarding the potential role(s) of environmental agents in the etiology of NTDs. Preliminary studies by the Texas Department of Health and the CDC examined the possible role of environmental contamination in the Cameron County cluster but failed to identify any significant associations between environmental contaminants and NTDs (*15*). The community continues to be concerned that the excess of anencephaly is related either to environmental pollution from the maquiladoras across the Rio Grande in Matamoros, Mexico, or to pesticides applied to agricultural fields in the area. It is my position that, while there is no compelling evidence for environmental pollutants causing anencephaly, additional research needs to be carried out in this area to more fully evaluate the possible role of environmental factors in this cluster (*17*).

## The Environment and Neural Tube Defects

Throughout much of the history of the epidemiologic study of NTDs a very broad concept of the environment was used. The "environment" was considered to embrace all nongenetic aspects of etiology. Thus, environmental factors included maternal age, parity, social class, metabolic diseases, etc. In addition, we often thought of the intrauterine environment and the extrasomatic (ambient) environment as heuristically appealing characterizations. Other than concern about a few specific agents, for example potato blight (*18*), soft water (*19*), selected occupational groups (*20*), or drugs as teratogens (*16*), little attention was paid to the possibility of agents from the ambient or occupational environments playing etiologic roles in these defects. To illustrate this, it is interesting to note that in many of the classic studies of NTDs, occupation, particularly a baby's father's occupation, was used as an indicator of social class or socioeconomic status (*21*). In earlier papers we discussed the importance of social class in the epidemiology of NTDs and a variety of variables were used to define it, including occupation, education, mean census track income, etc. (*21*). For an extensive review of the epidemiology of NTDs, see Elwood et al. (*22*).

**Table 2.** Rates of neural tube defects by time of conception, Cameron County, Texas (*15*).

| | Anencephaly[a] | | Other neural tube defects[b] | |
|---|---|---|---|---|
| Years | Cases | Rate[c] | Cases | Rate[c] |
| 1986-1989 | 23 | 9.6 | 12 | 5.0 |
| 1990-1991 | 24 | 19.7 | 12 | 7.4 |

[a]Anencephaly $p$-value = 0.01. [b]Other neural tube defects $p$-value = 0.5. [c]Rates are per 10,000 births.

During the last 10 to 15 years, there has been increasing concern about the possible role of occupational exposures and exposures to chemicals in the ambient environment in the etiology of adverse reproductive outcomes, including NTDs (*23*). While it is extremely unlikely that a specific chemical or physical agent in the environment is responsible for the majority of NTDs, evidence suggests that additional attention needs to be paid to this issue.

In the remainder of this article I will briefly review the studies that I believe suggest possible associations between occupational and environmental exposures and NTDs. I will then close by considering some of the research needs relevant to this topic, focusing on four issues: *a*) the importance of considering specific defects in etiologic studies; *b*) the importance of improving methods of exposure assessment; *c*) the need to explore common mechanisms through which multiple agents could contribute to the etiology of NTDs; and *d*) the need to pay additional attention to genetic variability in response to xenobiotic agents. I suggest that we need to develop a coordinated approach to these issues, encouraging multicenter, multidisciplinary studies.

## Occupational Studies

What evidence is there to suggest that exposure to specific chemicals or physical agents may play a role in the etiology of NTDs in general or anencephaly specifically? I will briefly consider studies that have examined maternal occupational exposures, next consider paternal occupational exposures, and then discuss exposures from the ambient environment. Some occupations and occupational exposures

suggested to be associated with increased NTD risks include:

- Maternal occupations such as nursing
- Paternal occupations such as
  - farmer and farmworker
  - painter
  - food and beverage processing
- Maternal occupational exposures include
  - solvents
  - ionizing radiation
  - anesthetic gases
- Paternal occupational exposures include
  - solvents
  - pesticides
  - ionizing radiation
  - mercury.

Space does not allow a thorough review of all studies of occupational exposures; the interested reader is referred to Elwood et al. (*22*) and Sever (*23*) for additional information.

Some early studies suggested associations between maternal solvent exposure and risk of central nervous system malformations (*24*). In a subsequent study in this same population, the association was no longer present (*25*).

Roeleveld et al. (*26*) state that there is little evidence that structural or functional defects of the central nervous system are related to parental occupational exposure to organic solvents but that they should be regarded as potentially hazardous to the developing brain. An important consideration is the grouping of defects, since often neural tube closure defects are combined with central nervous system abnormalities with different pathogenetic mechanisms. In a review of organic solvent exposure and adverse pregnancy outcomes, Taskinen (*27*) interprets the literature as suggesting that maternal exposure to organic solvents during pregnancy may have adverse effects on offspring.

A case–control study of congenital malformations and parental employment in health care occupations was published recently by Matte et al. (*28*). The cases and controls for this study came from the Atlanta Birth Defects Case–Control Study. Totals of 4915 case babies and 3027 controls were included in this analysis.

Mothers employed in nursing occupations had statistically significant excess risk of having a child with anencephaly or spina bifida. Possible exposures to the following specific agents were also evaluated: anesthetic gases, X-irradiation, and mercury. Among mothers potentially exposed to X-irradiation, there was a statistically significantly excess of NTDs based on only three cases. Potential maternal exposure to anesthetic gases was also significantly associated with spina bifida, again based on three cases.

The authors (*28*) discuss their findings in relation to earlier work and suggest that additional studies of maternal nursing occupations are indicated. They downplay the association they found between potential for maternal X-irradiation exposure and NTDs since it was based on a very small number of cases; this finding deserves further examination. In a study of congenital malformations and parental occupational exposure to ionizing radiation, statistically significant associations were observed between NTDs and parental preconception radiation dose (*29*). As in the Atlanta study, this was based on a small number of cases and these findings were not interpreted as causal. Since, however, statistically significant associations were found with both paternal preconception dose and combined parental doses on the basis of tests for trend, the issue of preconception occupational radiation exposure and NTDs requires further evaluation.

A potential role for male-mediated factors in the development of NTDs has been suggested recently. Studies of occupational exposures and birth defects often look at job title or occupation and industry in an attempt to identify associations. For example, in a study in Montreal, an excess of NTDs was observed among offspring of fathers employed in the processing of food and beverages (*30*). A similar but statistically nonsignificant association was observed in British Columbia (*31*). Olshan et al. (*31*) did observe a statistically significant association between paternal employment as a painter and spina bifida risk.

Polednak and Janerich (*32*) reported an increased risk for anencephaly related to paternal employment as a farmer or farmworker. Brender and Suarez (*33*) carried out a case–control study of paternal occupation and anencephaly in Texas. Cases were identified from vital records for 1981 to 1986 and were compared to a series of controls selected from live births during the same period and frequency matched to the cases by race, ethnicity, and year of birth. Parental occupations were obtained from the vital records, and occupations with potential for exposure to pesticides and solvents were identified. For the group of paternal occupations associated with solvent exposure there was a significantly increased odds ratio, as there was for painters. Taskinen and colleagues (*34*) noted that paternal exposure to organic solvents before conception may have adverse effects on pregnancy and offspring.

Brender and Suarez (*33*) also examined the association between anencephaly and paternal pesticide exposure. For the group of paternal occupations with estimated exposure to pesticides, the odds ratio was 1.28, which was not statistically significantly elevated. For farmers and ranch workers, the odds ratio was 1.73, again an increase that was not statistically significant. Brender and Suarez (*33*) review some of the studies that suggest possible associations between NTDs, including anencephaly, and pesticide exposure. I share their assessment that additional studies need to be carried out examining this possible association. The use of solvents in pesticide formulations and the suggestions of associations between both solvent and pesticide exposures and NTDs increases the importance of such studies, particularly in areas where NTD rates are high.

Louik and Mitchell (*35*) reported significant associations between anencephaly and paternal mercury exposure, basing exposure status on a job-exposure matrix. This finding was based on a small number of cases. Of greater interest is the fact that they also observed increased risks of NTDs associated with paternal solvent exposure. For spina bifida there was a statistically significant association with xylene, and for anencephaly and spina bifida there was a statistically significant association with benzene. These findings, contained in an unpublished report to NIOSH (*35*), are consistent with the associations observed between paternal solvent exposure and anencephaly reported by Brender and Suarez (*33*) and, similarly, with an association between paternal employment as a painter and spina bifida, as observed by Olshan et al. (*31*).

Occupational studies suggest that pesticide exposure and exposure to solvents may increase the risk of having a child with an NTD. I emphasize that these studies are only suggestive. Of particular interest is the fact that the observed associations are not restricted to gestational exposures with direct effects on the embryo but include paternally mediated effects.

## Ambient Exposures

The possibility of effects on neural tube closure by exposure of both males and females to chemical agents is important as

we turn from the occupational environment to the ambient environment. Environmental exposure levels are usually lower than occupational exposure levels. Thus, it is important to realize that an increased risk may be present but not demonstrable because of inadequate statistical power to detect a low risk and also because of the increased probability of exposure misclassification at low exposure levels. Suggested associations between environmental contaminants and NTD risks include:

- Vinyl chloride
- Environmental pollution
- Hazardous waste sites
  - Solvents
  - Metals
- Agricultural chemicals
- Water nitrates
- Organic solvents
- Water disinfection by-products.

Important exposure pathways include air and water.

Ambient air and drinking water are the most common sources of environmental exposure. From the perspective of NTDs, relatively more attention has been paid to a potential role for drinking water. This has included consideration of water hardness and mineral constitution, water nitrates, organic solvents, and water disinfection by-products. We will briefly consider an airborne pollutant—vinyl chloride—and then discuss the latter three categories of drinking water contamination.

The possible importance of airborne pollutants in NTDs can be illustrated by studies of vinyl chloride. Several studies have been conducted of potential exposure to vinyl chloride monomer from industrial sources and NTDs. These studies grew out of suggestions that central nervous system malformation rates were high in communities with polyvinyl chloride polymerization plants (*36*). Studies in West Virginia (*37*) and Quebec (*38*) tested the suggested relationship using case–control studies and failed to demonstrate statistically significant associations. A more recent study in New Jersey found increased odds ratios for central nervous system defects in proximity to two vinyl chloride polymerization facilities, but the increases were not statistically significant (*39*). These studies can be used to illustrate some of the epidemiologic approaches and problems in studying environmental reproductive hazards (*40*).

High rates of anencephaly in Cubatao, Brazil, were suggested to be associated with environmental pollution in the area (*41*). To our knowledge, this is the only report regarding general environmental pollution and NTDs. There may be parallels with the occurrence of anencephaly in Brownsville, Texas.

A recent case–control study in upstate New York found increased odds ratios for NTDs associated with several indicators of potential exposure to hazardous waste sites (*42*). This study was based on cases from the New York State Congenital Malformation Registry and information on toxic waste sites from the New York State Hazardous Waste Site Inspection Program. A statistically significant odds ratio was observed for nervous system malformations and residential proximity to selected hazardous waste sites. In addition, significantly elevated odds ratios were observed between nervous system malformations and sites containing solvents and metals. The category "nervous system malformations" included IDC-9 rubrics 740–742, which is more inclusive than NTDs. The potential associations between solvents and metals and NTDs need to be evaluated further, since, as noted, there are occupational studies that suggest these exposures may be related to NTD risk (*23*).

Increased prevalence of NTDs has been observed in areas with a high use of agricultural chemicals (*29,43*). White et al. (*44*) studied associations between environmental chemical exposures and NTDs in New Brunswick. No associations were found between anencephaly or spina bifida and pesticides used in forestry. Since no information was available on the specific application of agricultural chemicals, the authors developed an agricultural chemical exposure opportunity index. No associations were observed between anencephaly or all spina bifida cases combined and potential exposure to agricultural chemicals. A significant association was observed, however, between the exposure index and spina bifida without hydrocephalus. The exposure assessment in this study was quite crude, with a strong potential for exposure misclassification. While the authors felt that the fact that only spina bifida without hydrocephalus was associated with the exposure index detracted from the plausibility of biological meaningfulness for the association, the recent evidence regarding multiple neural tube closure sites (*14*) puts this finding into a different context. Thus, these data may be more suggestive of a biologically meaningful association than has been thought to be the case (*44*).

Probably more attention has been paid to a possible role for water contaminants in the etiology of NTDs than to any other environmental factor. One of the first areas of concern was nitrates in water. Nitrate contamination of water supplies may result from agricultural (fertilizer) runoff, sewerage, or industrial waste.

Epidemiologic studies do not provide conclusive evidence that pregnant women who consume low levels of nitrates in drinking water are at an increased risk for having adverse reproductive outcomes. In South Australia an excess of birth defects led to a case–control study examining the relationship between maternal drinking water source (groundwater versus rainwater) and risk of congenital malformations (*45*). The risk of having a malformed infant was increased among women who drank groundwater. Risks for central nervous system malformations and oral clefts were particularly increased. A dose–response relationship was found using estimated nitrate concentrations. Strengths of the study include the completeness of case ascertainment and the monitoring of water nitrates during the study period. Limitations include the assumption that water concentrations were constant during monitoring intervals and that the subjects used the same drinking water source throughout pregnancy. Another assumption was that nitrates rather than some unmeasured drinking water contaminant were responsible. There could be unrecognized confounding by a third variable. For example, seasonal variation in malformation risks suggests that dietary, nutritional, or other environmental factors may have contributed to the increased malformation rates. The authors did not believe the association was related to chlorination by-products, a topic that we discuss below.

A Canadian case–control study found an increased risk for delivering an infant with a central nervous system malformation associated with exposure to nitrates through water from private wells (*46*). The opposite was found with drinking water obtained from other sources. To assess exposure, the investigators analyzed nitrates in water samples collected at addresses where study subjects lived at the time of delivery. This raises the important issue of determining exposures at relevant stages in pregnancy; recent studies have shown a high degree of residential mobility during pregnancy. The study was also limited by the lack of information about other

possible water contaminants. The opposite risks associated with drinking water source, independent of nitrate concentration, also suggest that other factors contributed to the observed effects.

Relationships between congenital malformations and water contamination were recently studied in New Jersey (*47–49*). Based on cases from the New Jersey Birth Defects Registry and databases with information on environmental pollutant levels, a number of statistically significant associations were demonstrated. Exposure assessments in the studies were based on analysis of existing environmental databases (*47,49*) and maternal interviews (*48*). A complete review of these studies is beyond the scope of this article, but these studies suggest the need for additional attention to water contamination and NTDs. No associations were found between NTDs and toxic air emission data, agricultural pesticide applications, or proximity to hazardous waste sites (*49*). Extensive studies of drinking water quality and birth defects, however, showed associations between several drinking water contaminants and NTDs. On the basis of a cross-sectional study, significant associations were observed between carbon tetrachloride concentrations and total trihalomethanes and NTDs (*47*). Weaker associations were observed for trichloroethylene, nitrates, and mixed water sources. More specific information on the breakdown by types of NTDs is not included. Trihalomethanes are by-products of water disinfection by chlorination (*50*).

A case–control study was carried out that obtained more specific information on water exposures and other risk factors (*48*). Statistically significant associations were found for total trihalomethanes and with perchloroethylene and nitrates, when the data were adjusted for the confounding effects of total trihalomethanes. Craun (*51*) has reviewed the New Jersey studies and suggested that the association between NTDs and trihalomethanes "appears questionable." In addition, the studies have been critiqued by a panel for the U.S. EPA and the International Life Sciences Institute Risk Sciences Institute (*50*). A number of important points regarding exposure assessment methods and classification of outcomes are raised in the latter review.

Little (*20*) cites an unpublished PhD dissertation by Rausch, who studied pregnancy outcome in upstate New York communities served by different types of water supplies. A highly significant association between anencephaly and chlorinated surface water was observed, but there was no association with use of chlorinated groundwater compared to use of nonchlorinated groundwater. The issue of potential associations between water disinfection by-products and NTDs deserves further research.

## Research Needs

The studies described above suggest that additional attention needs to be directed toward a potential role for occupational and environmental exposures to specific agents in the etiology of NTDs in some populations. As part of the research agenda to address this important public health topic, I want to emphasize the importance of considering specific types of defects, rather than lumping together all neural tube or central nervous system defects, in searching for etiology. The recent seminal paper by Van Allen and colleagues (*14*), supported by earlier discussions of the heterogeneity of NTDs (*3,4,6*), establishes clearly the necessity of, at a minimum, assessing anencephaly and spina bifida separately when attempting to identify environmental etiologic agents. If, in fact, these two defects have distinct embryologic bases, as the evidence for the multisite closure model suggests (*14*), then lumping them together would represent outcome misclassification that would tend to reduce any association between an environmental agent and one of the defects toward the null. Since clusters of anencephaly seem to be more common than clusters of spina bifida, it may be that environmental or occupational chemicals play potentially greater roles in the etiology of these defects than of spina bifida.

The second research need is for additional attention to specificity in exposure assessment. Exposure misclassification is recognized as a potential problem in studies of both occupational and ambient exposures. While what we are actually interested in is a biologically effective dose to either the conceptus or the gametes of the parents, depending on whether we are evaluating a direct teratogenic effect or a mutagenic effect, usually at best what we have is some indication of parental exposure. In most cases, we do not even have a direct measure of parental exposure but a surrogate such as job title, occupation, or residential location, perhaps tied to an environmental database. Thus, we are often dealing with a surrogate of a surrogate. Some approaches to exposure assessment commonly used in occupational studies and for ambient exposures include:

- Occupational studies
  - Job titles
  - Work histories
  - Industrial hygiene monitoring
  - Personal dosimetry
  - Biomonitoring
  - Biological markers
- Ambient studies (population level based on geographic location)
  - History of releases/applications
  - Environmental monitoring
  - Environmental modeling
- Ambient studies (individual level)
  - Questionnaires
  - Biomonitoring
  - Biological markers

It is important to emphasize that many of the limitations of current studies relate to the fact that it is often necessary to use available exposure information that is nonspecific with regard to an individual agent of concern. New approaches to exposure assessment using exposure biomarkers are needed in developmental toxicity, and informative studies are now under way (*52*). An additional issue of concern is the ability to estimate a biologically effective dose at a biologically relevant time. There is a great need for additional research support for efforts in retrospective exposure assessment (*53*).

Questions of the specificity of exposure are particularly pertinent to considerations of associations between defects and hazardous waste sites (*42*) or water contamination (*48*). Severe criticisms have been leveled at some of the studies discussed earlier with regard to the adequacy of the exposure assessment methods used.

Implicit in the discussion of exposure is the issue of exposure to whom. While much of the concern about occupational reproductive hazards in particular has tended to focus on exposures of pregnant women during sensitive periods early in embryogenesis, the growing evidence for paternally mediated developmental effects strongly suggests that exposures of both parents need to be taken into account (*54*). The studies we have reviewed suggest a role for male-mediated effects in the etiology of NTDs. If this is based on heritable effects, as the studies of radiation exposure and neural tubes suggest, then this indicates that effects are not necessarily sex-specific.

It is my opinion that additional attention needs to be paid to both maternal and paternal exposures to solvents and agricultural chemicals, including pesticides. Studies should include approaches to estimated biologically relevant doses to both

parents and to the embryo using biologic markers. In addition, concerns regarding the possible effects of solvents, agricultural chemicals, and water disinfection by-products in the ambient environment indicate the need for developing and applying more sophisticated approaches to exposure assessment than have been possible using environmental monitoring data routinely collected for other purposes. To avoid exposure misclassification in epidemiologic studies, the specificity and accuracy of exposure assessment must be considered a priority in future epidemiologic research.

A third area for research priority is attention to mechanisms of neural tube closure. The work of Van Allen and colleagues (*14*) provides insight into some of the embryologic and mechanistic issues that need attention. An important question is, what can we learn from the effect of folic acid supplementation on neural tube closure that may be relevant to environmental and occupational agents? While our hypothesis is that anencephaly and spina bifida are more etiologically distinct than has been appreciated in the past—and that different agents may play varying roles in affecting closure—it is also logical to try to understand common anomalies at the cellular or intracellular level.

Recent interest in a potential role for free radicals and free radical scavengers may be one place to search for common mechanisms. For example, Graf and Pippenger (*55*) have recently published the results of a preliminary study that showed increased rates of glutathione peroxidase deficiency in families with neural tube defects. Glutathione peroxidase is a free radical scavenger, and this study suggests that there may be an inherited vulnerability to peroxidation stress or reduced antioxidative protection mechanisms at the time of embryogenesis. There is growing interest in the potential relationship between the development of birth defects and free radical utilization. Interestingly, in light of the discussion of trihalomethanes, trihalomethane chloroform frequently is found in drinking water and leads to the production of free radicals (*56*).

Finally, additional attention needs to be paid to issues of genetic heterogeneity in maternal absorption and metabolism of xenobiotics and in embryonic response. As part of the consideration of mechanisms of action of both protective agents such as folic acid and teratogenic agents such as valproic acid, it is important to evaluate genetic heterogeneity. This applies to environmental teratogens as well, and the preceding discussion of glutathione peroxidase is relevant here. Since there is a genetic component in the etiology of both anencephaly and spina bifida and evidence is suggested for male-mediated developmental effects that imply a genetic mechanism, it is important that additional research emphasis be placed on issues such as genetic heterogeneity in the metabolism of xenobiotic agents.

## Conclusion

Birth defects are the single leading cause of infant mortality in the United States, and the two major defects of neural tube closure, anencephaly and spina bifida, make a significant contribution to this total (*57*). The periconceptional use of folic acid is anticipated to have a dramatic impact on the incidence of these two defects. At the same time, some data suggest an etiologic role for occupational and environmental agents. It is essential that while public health programs for increasing folic acid intake by potentially pregnant women are initiated, additional research be directed toward this latter possibility. This should include large-scale multicenter case–control studies that pay particular attention to examining the role of agents such as solvents, agricultural chemicals, and water disinfection by-products in the development of specific types of defects. Attention must be directed toward reducing misclassification of both outcomes and exposures and toward mechanisms through which agents affect neural tube closure.

As we try to reach our objectives of reducing infant mortality and improving the health of children, it is essential that we expand our study of the role of the environment in the etiology of birth defects. For too long those concerned with children's health have ignored the problem of birth defects. The need is clear, the need is great, and the time is now for a commitment to these issues.

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