

Neurobehavioral Epidemiology: Application in Risk Assessment

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Neurobehavioral epidemiology may contribute information to risk assessment in relation to *a*) characterization of neurotoxicity and its time course; *b*) the dose–effect relationship; *c*) the dose–response relationship; and *d*) predisposing factors. The quality of this information relies on the validity of the exposure data, the validity and sensitivity of neurobehavioral function tests, and the degree to which sources of bias are controlled. With epidemiologic studies of methylmercury-associated neurotoxicity as an example, the field of research involves numerous uncertainties that should be taken into account in the risk assessment process. — *Environ Health Perspect* 104(Suppl 2):397–400 (1996)

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Introduction

Risk assessment for neurotoxicants relies upon dose–effect and dose–response data, most of which are obtained from experimental animal studies. Although the difficulties in risk assessment for behavioral effects were appreciated 20 years ago, a report from the U.S. National Academy of Sciences suggested that solutions could be rapidly achieved (*1*). Progress has been much slower than the optimistic predictions, especially in neurobehavioral epidemiology.

For the purpose of risk assessment, studies of exposed human populations should contribute information in the following four areas: *a*) characterization of the types of neurotoxicity that may occur as a result of

the exposure, including progression or reversal of the changes, so that the effects can be evaluated with regard to their adverse implications for human health; *b*) the average degrees of the neurotoxic effect caused by different magnitudes and durations of the exposure (dose–effect relationship); *c*) the relative frequency of a specific effect in response to different degrees of exposure (dose–response relationship); and *d*) predisposing factors that may render particular individuals more susceptible to the effects of the neurotoxic exposure.

For the most obvious neurotoxicants such as several metals, solvents, and pesticides, epidemiologic studies were not needed to document that toxic effects occurred in humans. Also, acute neurotoxicity has often been documented only by case reports. However, the fact that a particular chemical is neurotoxic does not contribute much to the database needed for risk assessment. Rather, this finding raises the need for more advanced information.

Neurobehavioral epidemiology consists of the disease-oriented neuroepidemiology (which can be looked upon as a subdiscipline of chronic-disease epidemiology) and exposure-driven environmental epidemiology. These two branches of the field generally correspond to case–control studies and cohort studies, respectively.

Although neuroepidemiology has enjoyed much attention, it is sad that a recent monograph (*2*) mainly dealt with major neurological diseases, and only one chapter (on lead poisoning) focused on adverse effects of environmental exposures. Similarly, neurobehavioral epidemiology is often poorly represented within the environmental field. There are several reasons for this relative lack of attention.

Ideally for risk assessment purposes, epidemiological studies should provide unequivocal data on pathognomonic effects where both the outcomes and the risk factors are accurately determined. The real world offers few such occasions. Unfortunately, because the nervous system is the target organ most commonly affected by environmental chemicals, each of the clinical signs and symptoms produced tends to be nonspecific (*3*). In particular, the chronic effects tend to be vague in the early stages so that they fall within the normal range or are indistinguishable from other abnormalities. When evaluating such data, other information from case reports or experimental studies should be considered, e.g., neurophysiologic and neuropathologic evidence.

The neurobehavioral performance of an individual is affected by several factors (Table 1). The particular outcome may depend on characteristics of the exposure, i.e., the severity and chronicity as well as the question of whether other exposures occurred at the same time. Also, the effect depends on the vulnerability of the subject, as indicated by, e.g., age and premorbid status. Many neurotoxic effects are not stationary, and the outcome may therefore depend on the patient's capability to compensate for the damage, the extent of possible repair processes, and whether the patient develops emotional reactions in response to the impairment.

Although the types of problems encountered within neurobehavioral epidemiology are in many respects similar to those seen in environmental and occupational studies in general (*4–6*), the difficulties encountered in neurobehavioral epidemiology are particularly troublesome. This applies in particular to the operational choice of exposure and outcome variables and the methods used to determine them.

This paper will explore some of the major obstacles using methylmercury as an example. Although one of the best documented human neurotoxicants, the

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Table 1. Factors that affect the neurobehavioral performance of an individual exposed to a neurotoxicant.

Exposure	Magnitude Duration Concomitant factors
Individual	Age (developmental stage) Premorbid status Preexisting diseases
Succession	Compensation Repair Secondary reactions

evidence available on this chemical is far from complete. The deficiencies seem to be typical of the general field of neurobehavioral epidemiology.

Exposure Evaluation

The first step in risk assessment is hazard identification in which the specific chemical or chemicals most likely to have caused the neurobehavioral dysfunction should be determined. This choice may be difficult because exposures are often of a mixed type. Having specified the chemical, the magnitude of individual exposure levels must then be assessed for the time interval during which the toxic effect was produced. In acute intoxication cases, exposure evaluation can be relatively straightforward. However, serious problems occur, e.g., with developmental dysfunctions related to prenatal exposures or with degenerative neurological disease associated with exposures that perhaps occurred decades before diagnosis.

When an outbreak of Minamata disease was discovered in Japan in the 1950s, the cause of this serious neurological disorder that especially affected newborns was not known. No one knew which types of samples might be needed for later analysis. Fortunately, in this particular area of Japan, an ancient custom was to dry the umbilical cord from each child and save it in a box. When methylmercury eventually appeared as a likely cause of the disease, umbilical cords were collected from 12 patients who had been born with congenital Minamata disease and also from two control groups (7). The methylmercury concentrations were generally much higher in the cords of Minamata disease patients. Some misclassification may have occurred, which may explain the overlapping of the results. However, the results should at least indicate the magnitude of the causative exposure. Unfortunately, as mercury concentrations in umbilical cords are not routinely determined, the results are difficult to relate to

exposures in other populations. The correlation between mercury concentrations in cord tissue with those in maternal hair, the most commonly used exposure indicator, was therefore determined from samples obtained in the Faroe Islands (8). From the equation for the regression line, the median maternal hair-mercury concentration for the Minamata disease group could be calculated to be about 23 $\mu\text{g/g}$ (115 nmol/g). Although a regression equation using other analytical methods may not accurately gauge the correlation under the exposure conditions seen in Minamata, the order of magnitude is probably not incorrect. However, as the group of patients studied may represent only those with the most severe congenital Minamata disease, the hair-mercury level calculated should not be interpreted as a threshold level for methylmercury neurotoxicity. These data illustrate how attempts at evaluating exposure levels about 40 years ago may still be relevant for risk assessments today.

In the subsequent outbreak of subacute methylmercury poisoning in Iraq, researchers chose to relate neurobehavioral outcome to the peak hair-mercury concentrations, not taking into account when this peak occurred in relation to the gestation period (9,10). Whether the maximum concentration was the best indicator of an exposure lasting for several months, this parameter could be used with reasonable confidence because it could be measured or estimated in each of the patients. However, when comparing these data to hair-mercury concentrations from chronically-exposed individuals, the question of how to translate peak levels into long-term average concentrations emerged. The variability of mercury concentrations along the length of the hair could depend on local and seasonal factors. In hair samples from the Faroe Islands, for example, the average coefficient of variation for a series of 1.1-cm hair segments from six women was only 8.1 to 23.8% (11). Thus, in the Faroe Islands population, with a series of up to twenty segments, for example, the maximum would therefore be expected to remain well below 50% above the average.

It is doubtful whether the hair-mercury concentration is always an accurate indicator of the active amount of methylmercury that has reached the nervous system, perhaps over a long period of time. Thus, sex-related differences in whole-body clearance and tissue distribution of methylmercury have been demonstrated experimentally

(12,13). Interestingly, a recent study of blood-mercury concentrations in a random population sample showed significantly higher values in women than in men, even when adjusted for exposure factors (14). Thus, sex-related differences in retention may well affect currently used indicators of mercury exposure.

The retention of mercury in the body may also depend on other exposures. For example, ethanol intake will increase the formation of mercury vapor (Hg^0) from ionic mercury (Hg^{2+}) in the blood, and some of that Hg^0 will be exhaled (15). Accordingly, mercury-exposed human volunteers tend to show a decreased concentration of inorganic mercury in blood after alcohol ingestion (16). In a population study in the Faroe Islands, the mercury concentration in cord blood was significantly lower when the mother had ingested alcoholic beverages during pregnancy; the median blood-mercury levels were 17% lower in alcohol drinkers (11). Although dietary differences may explain some of this difference, a toxicokinetic interaction seems likely. A parameter such as alcohol intake, which may affect both the maternal exposure indicator and the neurobehavioral outcome in the children, could be an important confounding factor in studies in neurobehavioral epidemiology.

Assessment of Neurobehavioral Effects

Neurobehavioral function involves several domains that can be evaluated by a multitude of methods; the parameters of choice should be sensitive to subtle effects of the exposure but should not be affected by other factors to a degree that would obscure a relationship with the exposure under study. Also, the earliest signs of dysfunction should be assessed to provide the best possible basis for decisions on prevention. Guidance on which domains to evaluate should be obtained from previous epidemiologic studies as well as from experience with human poisoning cases and experimental evidence.

Especially with prenatal exposures, the time of the neurobehavioral assessment is of importance because the effects may not become apparent until the nervous system has matured sufficiently to express the dysfunctions (17). As mental sequelae can rarely be reliably assessed at the time of the neurotoxic exposure, the developmental stage (both at the time of exposure and at the time of examination) must be taken into consideration. Also, during the interval between the

exposure and the diagnostic testing, other factors could affect development.

Much information was gathered on the effects of prenatal methylmercury exposure on postnatal development from the episodes of methylmercury poisoning in Japan and Iraq (7,9,10). Although milestone data and early developmental tests are somewhat crude and have a limited predictive validity with regard to subsequent cognitive development, data collected in Iraq have been used to establish dose-response relationships. The peak mercury concentration in maternal hair during pregnancy was used as an exposure indicator, and the effect parameter included psychomotor retardation (delayed walking and talking) and an increased number of abnormal neurological signs (10,18). This information has been a cornerstone for the determination of safe exposure levels by the International Programme on Chemical Safety (19).

In the Faroe Islands where high prenatal exposures to methylmercury occur (11), the age at which the child reached a developmental milestone (sitting, creeping, rising) was not associated with indices of prenatal mercury exposure, i.e., the mercury concentrations in umbilical cord blood and in maternal hair. However, the milestone development was associated with the hair-mercury concentration at 12 months of age, but the correlation was in an unexpected direction, i.e., indicating an advantage associated with an increased exposure to mercury (20). However, increased hair-mercury concentrations at 12 months of age were significantly related to long duration of nursing (21); thus, the association that was found must therefore be due to confounding by breast-feeding. Given the substantial advantages of breast-feeding with regard to milestone development (22), this factor must be considered.

In patients with congenital Minamata disease, mental retardation emerged as the main sign of the disease in older children; motor functions improved somewhat with time. A study conducted in 1962 reported that, of 72 Minamata children born from 1953 to 1954 and in 1959 (i.e., just before and just after the highest pollution period), 21 children (29%) had an IQ below 70 (7). In 1971, schoolchildren born in the Minamata area from 1955 to 1959 were examined, and high prevalences of mental retardation, sensory disturbance, mild dysarthria, and adiadochokinesia were observed (7). Unfortunately, detailed information on the test methods that were used is not available.

In a more recent population-based study in New Zealand (23,24), 73 pregnant women were identified with hair-mercury concentrations above 6 $\mu\text{g/g}$ (30 nmol/g). A total of 31 of the children born were examined at 4 years of age along with matched controls who had low levels of prenatal mercury exposure. In the Denver Developmental Screening test, abnormal or questionable results occurred three times more often in the exposed group (23). At 6 to 7 years of age, 61 of the children and three control groups were tested by the Wechsler Intelligence Scale for Children (WISC); an average maternal hair-mercury concentration of about 15 $\mu\text{g/g}$ (75 nmol/g) was found to be associated with decreased test performance (24). While the methods used in this study are well documented, the omnibus tests chosen may be useful only for producing an overall picture of the developmental stage. These tests are not necessarily sensitive to the effects of methylmercury on the most vulnerable domains and could well be affected by various confounding factors (25).

Epidemiologic Strategies

Epidemiologic data are not a prerequisite for risk assessment. In fact, opportunities for epidemiologic studies of neurotoxicity may arise only when prevention has failed, whether a risk assessment has been carried out, and whether the origin of the exposure is natural or anthropogenic. Given the fact that neurotoxic exposures continue to occur, the best possible epidemiologic studies should be carried out so that the unfortunate incidents will at least result in useful information that can provide a better basis for intervention.

Due to the considerable imprecision in the measurement of both exposure and neurobehavioral effects, such studies are subject to high degrees of bias and of random misclassification. A major aim of obtaining better data in neurobehavioral epidemiology is therefore to limit the imprecision and to identify and measure the factors that may affect the exposure parameters and the outcome variables.

Frequently, several exposure factors must be determined because the exposure under study is associated with other chemical exposures originating from the same source. Accordingly, the hair-mercury concentration can be interpreted as a marker of seafood intake and may therefore also serve as an indicator of exposure to other neurotoxic contaminants, such as polychlorinated biphenyls, that are likely to occur

in marine food. Unfortunately, this potential source of bias has not yet been explored in detail. On the other hand, methylmercury from seafood may occur in combination with selenium, which may attenuate the toxic potential of the mercury.

In selecting the outcome variables, tests should be chosen that are feasible but still sensitive to dysfunctions of the domains deemed to be affected by the exposure. The results should be interpreted based on the knowledge about the factors that could affect the individual test and in relation to the expected changes resulting from the exposure to the neurotoxicant. However, given the fact that neurobehavioral function varies considerably within a population, even similar exposure circumstances may be associated with widely different performance results in a group of exposed subjects. Also, despite results of functional tests remaining well within the expected interval, differences can still be considerable between groups of individuals with different levels of exposure. Further, the individual may not be aware of any dysfunctions, but even minimal changes can, in some cases, have severe implications for daily life. These considerations are important to keep in mind when interpreting neurobehavioral data.

To design a study in neurobehavioral epidemiology, a proper source population and a feasible sampling frame must be chosen. An internal control group with minimal exposure may be available within the source population, but otherwise a suitable comparison group must be identified. These decisions apply to both case-control studies and cohort studies. A wise choice of source population and sampling strategy may well circumvent some of the serious bias problems.

The prospective cohort study is often recommended as the most reliable source of information. However, this design does not eliminate the need to control for the imprecision of exposure and outcome variables, and it may suffer from an additional weakness, i.e., selection bias. Thus, the study of prenatal methylmercury exposure carried out in New Zealand (23,24) suffered from considerable attrition during the follow-up period. Similar experiences have been recorded in prospective studies of lead neurotoxicity in children, in which the annual loss to follow-up may be about 10% (26). The children who remain in the study could well differ substantially from the original cohort. Likewise, prospective studies of exposed workers have to allow for the fact that few of the new employees

stayed with the job for very long (27,28). Whether those who remained in their jobs were the most hardy is difficult to assess, but they are not likely to be a random and representative selection of the original groups. Accordingly, prospective studies should not be considered less critically than other epidemiologic studies.

When evaluating the epidemiologic evidence, several factors should be considered that could potentially bias the results toward the null hypothesis (29). With the inherent biases and possibilities of misclassification, epidemiologic studies will always reveal shadows that must be interpreted from all available evidence as reflec-

tions of the truth. Statistical significance should be interpreted in the light of sample size and the ranges of variation. Thus, a study that fails to document a statistically significant association should not necessarily be considered as evidence that an association is absent, i.e., the study is nonpositive rather than negative. However, the main problem in risk assessment is that dose-relationships, whenever indicated by epidemiologic data, are usually associated with immense confidence limits. A main problem is whether regression equations should assume the existence of a threshold below which an exposure will not cause any discernible effect.

One final observation should be made. Risk assessment must necessarily include considerable amounts of subjectivity, e.g., in the choice of source population, the parameters to be assessed, and the inference made with regard to the significance of the observed changes to human health (30). This is true in neurobehavioral epidemiology as in other disciplines that provide data for risk assessment. While recognizing the fact that decision making in environmental health must take into account ethical, social, economic, and political aspects, neurobehavioral epidemiology can contribute important information that should be interpreted and used in the proper context.

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