Continuous data (e.g., magnitude, rate, amplitude), if found to be normally distributed, can be analyzed with general linear models using a grouping factor of dose and, if necessary, repeated measures across time (Winer, 1971). Univariate analyses of dose, comparing dose groups to the control group at each time point, are performed when there is a significant overall dose effect or a dose-by-time interaction. Post hoc comparisons between control and treatment groups can be made following tests for overall significance. In the case of multiple end points within a series of evaluations, some type of correction for multiple observations is warranted (Winer, 1971)

d. In Vitro Data in Neurotoxicology. Methods and procedures that fall under the general heading of short-term tests include an array of in vitro tests that have been proposed as alternatives to whole-animal tests (Goldberg and Frazier, 1989). In vitro approaches use animal or human cells, tissues, or organs and maintain them in a nutritive medium. Various types of in vitro techniques produce data for evaluating potential and known neurotoxic substances, including primary cell cultures, cell lines, and cloned cells. While such procedures are important in studying the mechanism of action of toxic agents, their use in hazard identification in human health risk assessment has not been explored to any great extent.

Data from in vitro procedures are generally based on simplified approaches that require less time to yield information than do many in vivo techniques. However, in vitro methods generally do not take into account the distribution of the toxicant in the body, the route of administration, or the metabolism of the substance. It also is difficult to extrapolate in vitro data to animal or human neurotoxicity end points, which include behavioral changes, motor disorders, sensory and perceptual disorders, lack of coordination, and learning deficits. In addition, data from in vitro tests cannot duplicate the complex neuronal circuitry characteristic of the intact animal.

Many in vitro systems are now being evaluated for their ability to predict the neurotoxicity of various agents seen in intact animals. This validation process requires considerations in study design, including defined end points of toxicity and an understanding of how a test agent would be handled in vitro as compared to the intact organism. Demonstrated neurotoxicity in vitro in the absence of in vivo data is suggestive but inadequate evidence of a neurotoxic effect. In vivo data supported by in vitro data enhance the reliability of the in vivo results.

## B. Dose-Response Evaluation

Dose-response evaluation is a critical part of hazard characterization and involves the description of the dose response relationship in the available data. Human studies covering a range of exposures are rarely available and therefore animal data are typically used for estimating exposure levels likely to produce adverse effects in humans. Evidence for a dose-response relationship is an important criterion in establishing a neurotoxic effect, although this analysis may be limited when based on standard studies using three dose groups or fewer. The evaluation of dose-response relationships includes identifying effective dose levels as well as doses associated with no increase in incidence of adverse effects when compared with controls. Much of the focus is on identifying the critical effect(s) observed at the lowest-observed-adverse-effectlevel and the no-observed-adverseeffect-level associated with that effect. The NOAEL is defined as the highest dose at which there is no statistically or biologically significant increase in the frequency of an adverse neurotoxic effect when compared with the appropriate control group in a data base characterized as having sufficient evidence for use in a risk assessment (see section C). Although a threshold is assumed for neurotoxic effects, the existence of a NOAEL in an animal study does not prove or disprove the existence or level of a biological threshold. Alternatively, mathematical modeling of the dose-response relationship may be performed to determine a quantitative estimate of responses in the experimental range. This approach can be used to determine a BMD, which may be used in place of the NOAEL (Crump, 1994) (see Dose-Response Analysis, Section IV).

In addition to identifying the NOAEL/ LOAEL or BMD, the dose-response evaluation defines the range of doses that are neurotoxic for a given agent, species, route of exposure, and duration of exposure. In addition to these considerations, pharmacokinetic factors and other aspects that might influence comparisons with human exposure scenarios should be taken into account. For example, dose-response curves may exhibit not only monotonic but also Ushaped or inverted U-shaped functions (Davis and Svendsgaard, 1990). Such curves are hypothesized to reflect multiple mechanisms of action, the presence of homeostatic mechanisms,

and/or activation of compensatory or protective mechanisms. In addition to considering the shape of the doseresponse curve, it should also be recognized that neurotoxic effects vary in terms of nature and severity across dose or exposure level. At high levels of exposure, frank lesions accompanied by severe functional impairment may be observed. Such effects are widely accepted as adverse. At progressively lower levels of exposure, however, the lesions may become less severe and the impairments less obvious. At levels of exposure near the NOAEL and LOAEL, the effects will often be mild, possibly reversible, and inconsistently found. In addition, the end points showing responses may be at levels of organization below the whole organism (e.g., neurochemical or electrophysiological end points). The adversity of such effects can be contentious (e.g., cholinesterase inhibition), yet it is such effects that are likely to be the focus of risk assessment decisions. To the extent possible, this document provides guidance on determining the adversity of neurotoxic effects. However, the identification of a critical adverse effect often requires considerable professional judgment and should consider factors such as the biological plausibility of the effect, the evidence of a dose-effect continuum, and the likelihood for progression of the effect with continued exposure.

## C. Characterization of the Health-Related Data Base

This section describes a scheme for characterizing the sufficiency of evidence for neurotoxic effects. This scheme defines two broad categories: sufficient and insufficient (Table 8). Categorization is aimed at providing certain criteria for the Agency to use to define the minimum evidence necessary to define hazards and to conduct doseresponse analyses. It does not address the issues related to characterization of risk, which requires analysis of potential human exposures and their relation to potential hazards to estimate the risks of those hazards from anticipated or estimated exposures.

Table 8.—Characterization of the Health-Related Database

## Sufficient Evidence

The sufficient evidence category includes data that collectively provide enough information to judge whether or not a human neurotoxic hazard could exist. This category may include both human and experimental animal evidence.

## Sufficient Human Evidence

This category includes agents for which there is sufficient evidence from