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# Biomarkers of adult and developmental neurotoxicity

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**Abstract**

Neurotoxicity may be defined as any adverse effect on the structure or function of the central and/or peripheral nervous system by a biological, chemical, or physical agent. A multidisciplinary approach is necessary to assess adult and developmental neurotoxicity due to the complex and diverse functions of the nervous system. The overall strategy for understanding developmental neurotoxicity is based on two assumptions: (1) significant differences in the adult versus the developing nervous system susceptibility to neurotoxicity exist and they are often developmental stage dependent; (2) a multidisciplinary approach using neurobiological, including gene expression assays, neurophysiological, neuropathological, and behavioral function is necessary for a precise assessment of neurotoxicity. Application of genomic approaches to developmental studies must use the same criteria for evaluating microarray studies as those in adults including consideration of reproducibility, statistical analysis, homogenous cell populations, and confirmation with non-array methods. A study using amphetamine to induce neurotoxicity supports the following: (1) gene expression data can help define neurotoxic mechanism(s), (2) gene expression changes can be useful biomarkers of effect, and (3) the site-selective nature of gene expression in the nervous system may mandate assessment of selective cell populations.
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*Keywords:* Neurotoxicity; Nervous system; Biomarkers; Gene expression; Amphetamine

## Introduction

The appropriate selection and use of biological markers or biomarkers are fundamental for optimizing the assessment of the risk of neurotoxicants. Biomarkers may be defined as indicators signaling events in a biological system and are classified into three categories, those of exposure, effect, and susceptibility (Committee on Biological Markers of the National Research Council, 1987). Exposure biomarkers may include either the quantitation of exogenous agents or the complex of endogenous substances and exogenous agents within the system. Biomarkers of effect may be indicators of an endogenous component of a biological system or an altered state of the system that is recognized as an alteration or disease (Committee on Biological Markers, 1987). More specifically, biomarkers of neurotoxic effects or events may be either a significant alteration in the levels of endogenous component(s) of a biological system or an altered state of the system that is consistently detected during or after neurotoxic agent exposure. These biomarkers are more informative and predictive if they either result only from a neurotoxic exposure or occur with greater intensity and duration after a neurotoxic insult. A biomarker of susceptibility is an indicator that a biological system is especially vulnerable to toxic insult by an exogenous agent (Committee on Biological Markers, 1987).

Neurotoxicity may be defined as any adverse effect on the structure or function of the central and/or peripheral nervous system by a biological, chemical, or physical agent that diminishes the ability of an organism to survive, reproduce, or adapt to its environment. Neurotoxic effects

*Abbreviations:* AMPH, amphetamine; METH, methamphetamine; DA, dopamine; 5-HT, serotonin; RT-PCR, reverse transcription-polymerase chain reaction; PLCo, posteriolateral cortical amygdaloid nucleus; NPY, neuropeptide Y precursor; IGF-1, insulin-like growth factor; IGFBP-1, insulin-like growth factor binding protein.

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may be permanent or reversible, produced by neuropharmacological or neurodegenerative properties of a neurotoxicant, or the result of direct or indirect actions on the nervous system (Slikker, 1991). These effects can often be measured directly by neurochemical, neurophysiological, and neuropathological techniques, whereas others must be inferred from observed behavior. Extrapolation across species is feasible but must take into account the relative ontogeny of the nervous system among species. Insults to the nervous system may take various forms and may be quite subtle (Anger, 1986). Although its manifestations may change with age, neurotoxicity may occur at any time in the life cycle from gestation through senescence. The developing nervous system may be more or less susceptible to neurotoxic insult depending on the stage of development. Biomarkers used in adults are often applicable for use during development but developmental stage-specific differences must be considered (Ali et al., 1986a, 1986b; Annau and Eccles, 1986; Ecobichon et al., 1990; Ginsberg et al., 2003; Lipscomb et al., 1989; Paule et al., 1986; Pearson and Dietrich, 1985; Silbergeld, 1986; Slikker and Chang, 1998). While the adult nervous system may also be acutely susceptible to new insults, the effects of earlier injuries may be revealed as it ages (Weiss, 1990). Psychoactive substances may also indirectly impair health by inducing behaviors that decrease safety in the performance of numerous activities.

Ongoing studies conducted over the last 20 years in laboratory animals (Bowyer and Peterson, 2002; Davidson et al., 2001) and more recent human studies (Ernst et al., 2000; McCann et al., 1998; Volkow et al., 2001a, 2001b) have identified the substituted amphetamines METH and AMPH as neurotoxic agents. These studies have identified biochemical, anatomical, and behavioral biomarkers of neurotoxicity. An interesting facet of these studies is that they have identified both motor and cognitive functions, based on a diverse set of neural systems, which are adversely affected. As well, the relevance of laboratory animal studies that have predicted and defined specific regions of the brain in which AMPH and METH produce neurotoxicity is highlighted by these animal studies. Many of the brain regions identified as being adversely affected by METH in laboratory animal studies were later shown to be similarly affected in human studies. Several putative neurotoxic biomarkers for these systems have been identified in laboratory animals and will be discussed at length later.

The nervous system's complexity not only provides a multitude of mechanisms by which toxicants can produce injury, but also provides a considerable challenge in the development of risk assessment strategies. Unlike risk assessment for carcinogens where tumor yield is often considered a universal endpoint, neurotoxicity may manifest itself in many ways. A multidisciplinary approach is necessary to assess neurotoxicity because of the complex and diverse functions of the nervous system (Fig. 1). Once those effects are defined, dose-response studies can serve to indicate which of the effects stem from a common cause. Finally, as with all risk assessments that rely on animal data, the extrapolation to the human situation must be accomplished. Such issues are currently being addressed directly by using the same behavioral endpoints and assessment procedures in both laboratory animals and humans (Paule et al., 1988a; Slikker et al., 2000). Fortunately, as exemplified for lead (ATSDR, 1988; U.S. EPA, 1986), all the components necessary to conduct human risk assessments on other chemicals can be described and exercised. Risk assessment approaches have been developed and published for methylenedioxymethamphetamine (MDMA) (Gaylor and Slikker, 1990), for methanol (Slikker and Gaylor, 1998; for domoic acid (Slikker et al., 1998), and for neurotoxicity in general (Paule et al., 1988b; Sheehan et al., 1989; Slikker and Sobotka, 1997). Therefore, risk assessments based solely on the basis of the neurotoxicities of chemicals are feasible.

## Amphetamine case study

The therapeutic agent amphetamine (AMPH), which also has a high drug abuse potential, will be used as an example agent to demonstrate the various approaches to generate a neurotoxicity profile and to identify possible biomarkers of effect. AMPH and methamphetamine (METH) have the pharmacological effects of a psychomotor stimulant, enhancing wakefulness, increasing blood pressure, and inducing euphoria. However, they also produce adverse or toxicological effects including hyperthermia, tremor, panic states, paranoid hallucinations, and mental confusion (Hardman et al., 1996). As well, both AMPH and METH neurotoxicities are often associated with hyperthermia, convulsions, and stroke, all of which greatly enhance neurotoxic outcome (Bowyer and Holson, 1995; Bowyer et al., 1994, 1998; Davidson et al., 2001). In selected brain areas, METH exposure results in decreased levels of dopamine (DA) and serotonin (5-HT), decreased activity in the enzymes tyrosine hydroxylase and tryptophan hydroxylase, decreases in uptake/transporter sites for DA and 5-HT, and histological evidence of terminal damage (Hotchkiss and Gibb, 1980; Kogan et al., 1976; Schmidt et al., 1985; Seiden et al., 1975, 1988). Reports have indicated histological evidence for AMPH and METH, site-selective neuronal degeneration in both humans and animal models (Bowyer et al., 1994, 1998; Commins and Seiden, 1986; Davidson et al., 2001; Eisch and Marshall, 1998; Schmued and Bowyer, 1997; Volkow et al., 2001a, 2001b). Also, there are developmental differences with respect to the brain regional sensitivity to neurodegeneration produced by METH and AMPH that must be considered (Bowyer, 2000; Bowyer and Peterson, 2002).

The basic hypothesis is that either AMPH or METH administration will result in neurotoxicity as indicated by cell death via one or more related pathways involving