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Principles of Neurobehavioral Teratology

Linda C. Mayes and Anna Ward

The first documented use of the word *teratology* was the title of a 1678 discourse of prodigies and wonders (OED, 1989). Taken from the Greek root, τέρασ, meaning prodigy, portent, omen, or wonder (Bauer, 1957, 1979), the original connotation in Homer and in the New Testament was of divine communication: "Unless you see signs and wonders you will not believe" (John 4:48). The word did not acquire its connotation of deformed or monstrous until the mid-nineteenth century, when it first appeared in 1842 in a dictionary of scientific terms indicating the study of monsters or anomalies. Shortly thereafter, the term *teratogenesis* appeared in Robley Dunglison's fifteenth edition of a medical lexicon to indicate the study of deformities in the organization in plants and animals. Nineteenth-century physicians and scientists were well schooled in the Attic Greek of Homer and likely would have known that for the Greeks, deformities in infants were taken as a sign of divine warning, displeasure, or retribution. Moreover, that monstrous births were portents of displeasure and disaster also influenced European thinking. During the Middle Ages, births of malformed infants were significant events thought to predict catastrophes and "signs of punishment at hand" (Pare's Chyrugery, 1579, quoted in Warkany, 1977). Hence, in their choice of the root τέρασ, nineteenth-century physicians brought together the notion of portent with the emphasis on monstrosity and horror to the study of unexpected and poorly understood malformations. The idea of a teratogen or an agent that might be associated with such malformations made its appearance relatively late in the medical literature in a 1959 citation in the *Journal of Chronic Disease* (OED, 2000).

To be sure, the field of teratology as we currently understand it is a very recent invention reflecting combinations of several different fields including pharmacology, pathology, embryology, genetics, anatomy, and cytology. *Teratology* is the study of birth defects, their etiology, pathophysiology, and epidemiology (Wilson, 1973). Most narrowly construed, teratology is the study of congenital physical malformations. In a

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broader interpretation, teratology is the study of perinatal developmental injury or abnormal development and of the factors including birth accidents and genetic mutations that increase the risk of developmental injury (Wilson, 1977). In this broader interpretation, the field covers a vast range encompassing the impact of exposures to exogenous agents or events during specific developmental phases on physical and functional outcomes. The outcomes may range from death, physical malformation, growth abnormalities, and disruption in function in all organ systems including the central nervous system. Among the agents or events of interest are both prescribed and illicit drugs, industrial chemicals, environmental pollutants, irradiation, viral infections, traumas such as preterm birth, and psychological conditions such as increased perinatal stress or maternal depression/deprivation.

An even more recent research specialty is neurobehavioral teratology, which investigates the developmental impact of exposure to similar exogenous agents or events during different critical periods on the developing brain, and hence, on the offspring's psychological development (Vorhees, 1986). Neurobehavioral teratology examines agents (or events) capable of producing deficits in cognitive functioning or other measures of neurobehavioral performance in the absence of gross malformations of the central nervous system. The impact of individual exposures is assessed at varying distances in time from the original fetal exposure. The field became a distinctive branch of teratology when behavioral effects were suggested as the subtler outcome in a continuum of prenatal insults (Spyker, 1975). For example, studies of the impact on infant neurodevelopmental functioning of human exposure to radiation or methyl mercury or preclinical animal models on the effects of early experiential differences in handling on behavior were early examples of neurobehavioral teratologic studies as methodological approaches distinct from general teratology (Butcher, 1985; Vorhees, 1986). Up until that point, teratology had focused on fetal death and malformations, but neurobehavioral teratology emphasized functional disruption at exposures far below those capable of causing structural, physical malformations. Neurobehavioral teratology also emerged with the increasing need to regulate new drugs, and environmental and industrial exposures after the identification of thalidomide as a teratogen in the early 1960s (Adams, 1999). With the recognition of thalidomide as a drug with no toxicity in the adult but severe teratogenic effects for the fetus, the USDA began to require the evaluation of new drugs in pregnant experimental animals. Necessarily then, standards for determining teratogenicity were required; and debate ensued as to whether the labeling of an agent as a teratogen would be restricted to lethal effects, morphological abnormalities, growth retardation, and observable functional impairments, or would more subtle and sometimes later-appearing neurobehavioral functional disruptions at lower doses also be considered in the standards for defining an agent as a teratogen, an issue still largely unsettled in the regulatory arena (Adams, 1999; Vorhees, 1986c).

Given this emphasis on effects at lower exposure doses, much of the methodology of the discipline is focused on understanding the probability for a given outcome or the assessment of risk, and studies are directed toward establishing dose-response relations or the level at which there are no discernable effects for a given agent. The phrase "no discernable" is key, for this of course varies with the outcome selected for study. As a field, neurobehavioral teratology adds developmental psychology and developmental neuroscience to the multidisciplinary mixture of fields studying birth defects and developmental injuries in general; and despite the title "neurobehavioral teratology,"

the field has grown far beyond a strictly neurobehavioral or cognitive emphasis with a focus primarily on mental retardation to include functional neuroimaging, neurophysiology, neurochemistry, and neuropsychology and an emphasis on a continuum of deficits in a number of developmental and behavioral domains. Indeed, interest in developmental injury from a psychological/behavioral point of view has provided a significant incentive to understanding the normal or expectable features of early perceptual, social-emotional, and cognitive processes and how to measure more accurately and specifically these developmental domains.

Not surprisingly, with such a diverse range of exposures from discrete pharamacologic treatments to psychosocial events such as overwhelming stress and an equally broad range of possible neurobehavioral outcomes, there is no one consensual focus or methodological standard in the field. Neurobehavioral teratologic questions about developmental injury are approached from two broad perspectives (Adams, 1999; Vorhees & Mollnow, 1987). One perspective examines a question by its presumed causes, that is, by grouping subjects into those with and without the presumed cause of injury or exposure. In this perspective, studies may examine the prevalence of neurobehavioral deficits in the exposed individuals and attempt to establish relationships between the amount and timing of exposure and the severity of the deficit.

Another perspective studies questions of development injury not through the putative cause but rather through the target organ, system, or function of injury. Hence, subjects of study are grouped by their functional impairment, and the programmatic study is to understand the various mechanisms and routes to that particular functional impairment. Investigations focused on this perspective may also use exposure models to understand the relationships between, for example, disruption in specific brain regions and impairments in related functional systems – again a focus on mechanism, but in this case mechanisms of normal ontogeny studied through exposure models. These two broad orientations may lead to different research questions, findings, and interpretations inasmuch as one focuses on outcome while the other emphasizes mechanism. The most productive approach to any investigation of developmental injury brings a combination of these two perspectives and a plurality of methods to the research questions.

The fundamental logic of questions from neurobehavioral teratology specifically and teratology in general is does exposure to A during a specific phase of development cause B, C, and/or D immediately or later in development. (Or in the language of mechanism, does disruption in process A during a specific phase of development lead to disruptions in functions B, C, and/or D later in development.) Importantly, one agent, A, may produce several different outcomes (e.g., outcomes B, C, and/or D) depending on dose or amount of exposure, and there may be different dose-response curves for the different outcomes. Functional behavioral or psychological changes may occur at lower doses than abnormal growth or major disruptions in organogenesis and the shape of the dose-response curves may also be different depending on the outcome (Vorhees & Mollnow, 1987; also see section below). There are several examples of these kinds of direct causative models for both functional and physical outcomes. These include prenatal rubella exposure and its association with deafness and mental retardation, or the classic example of prenatal thalidomide exposure and severe malformations of limb development. In these models, there is a clear association between a specific exposure to a discrete toxin and a clearly defined and easily identified endpoint or outcome.

However, many, if not all, of the more contemporary questions capturing much of the interest in neurobehavioral teratology are those that involve far more complex models of exposure, timing, and outcome assessment. These are not always clearly direct causality models and hence are not easily approached with standard research designs. The exposures are neither specific nor discrete, the outcomes are not uniformly present even with documented exposure, and the severity or extent of the deformation or developmental abnormality is variable. In these more complex models, interactions between the exposure agent or event and the environment are central. That is, does the environment in one way or another moderate the fetus's or child's risk of exposure as well as vulnerability to the potentially toxic effects of exposure and at the same time, determine other risk factors that may also mediate the severity of any exposure-related outcome.

For example, even the question of malnutrition and its effect on fetal outcome is not a straightforward question of exposure and effect. Malnutrition more often occurs among very poor or displaced populations who are usually considerably stressed and isolated from adequate medical and prenatal care. These conditions may further compound the impact of malnutrition on fetal development in a way that would not occur theoretically if malnutrition occurred in isolation or in the absence of social displacement and chronic stress. Similarly, a more contemporary question regarding the effects of maternal antidepressants on infant neurodevelopmental integrity is also made more complex by the possible relations between maternal depression, heightened perinatal stress, and altered maternal-infant care. Even in questions of exposure to industrial chemicals or environmental pollutants, there may be a number of mediating and moderating factors that diminish or increase the likelihood of exposure and the severity of the outcome. And there is perhaps no better (and no more confounded) illustration of interactive neurobehavioral teratology models in humans than those involving the putative teratogenic effects of drug abuse during pregnancy.

In this chapter, we shall discuss ten basic principles central to any neurobehavioral teratology investigation. In a classic paper, Wilson outlined the basic principles of teratological investigations and Vorhees later adapted these to neurobehavioral teratology studies in preclinical models (Vorhees & Mollnow, 1987; Wilson, 1973; Wilson, 1977). In this review, we will further adapt or expand upon these principles to underscore the particular application to human studies. These principles, also outlined in Table 1.1, include:

1. Delineating the possible mechanisms of teratogenic effect.
2. Defining the specific teratogenic agent.
3. Specifying the timing of the exposure.
4. Defining the nature of the exposure.
5. Delineating the range of susceptibility and response relationships.
6. Selecting those groups at greater or lesser risk for exposure.
7. Considering the environmental context and conditions most related to the exposure.
8. Defining the outcomes most likely related to the mechanism of action of the exposure agent or event.
9. Considering when exposure-related outcomes are most likely to be apparent.
10. Taking into account those conditions that ameliorate or exacerbate any exposure-related functional outcomes.

Table 1.1. Principles of Behavioral Teratology

1. **Delineating the Possible Mechanisms of Teratogenic Effect:** Agents that are behaviorally teratogenic should act on the developing CNS by specific mechanisms.
2. **Defining the Specific Teratogenic Agent:** Not all agents that produce malformations are necessarily behavioral teratogens. Only those agents that produce either teratogenic or psychoactive CNS effects are capable of producing behavioral teratogenic effects.
3. **Specifying the Timing of the Exposure:** Based on the principle of critical periods, the type and magnitude of the behavioral teratogenic effect will depend on the stage of CNS development when exposure occurs.
4. **Defining the Nature of the Exposure:** The type and magnitude of the behavioral teratogenic effect depends on the type of agent, frequency and amount of use, and route of administration.
5. **Delineating Dose-Response Relationships and the Range of Susceptibility:** The type and severity of the behavioral effects depends on the dose of the agent reaching the developing central nervous system. Behavioral teratogenic effects are usually demonstrable at levels of exposure below that causing other malformations if the exposure agent is capable of causing behavioral changes.
6. **Selecting Those Groups at Greater or Lesser Risk for Exposure and Susceptibility to Effects:** How exposed groups are identified influences the likelihood of finding greater or lesser behavioral teratogenic effects. Individual genetic differences in the exposed individual or organism also influence the type and magnitude of behavioral teratogenic effect.
7. **Considering the Environmental Context and Conditions Most Related to the Exposure:** The magnitude and type of a behavioral teratogenic effect (and the likelihood of finding such an effect) depends on environmental factors.
8. **Selecting Outcomes Most Likely Related to the Mechanism of Action of the Exposure Agent or Event:** Behavioral teratogenic effects are expressed as impaired cognitive, perceptual, or social-emotional function or delayed maturation of capacities in these domains and the chosen outcomes for study should be linked to the proposed mechanism of CNS teratogenesis rather than selecting "broad band" measures of CNS function.
9. **Considering When Exposure Related Outcomes Are Most Likely to Be Apparent:** Not all behavioral teratogenic effects are apparent in the perinatal period. Some are evident later in development when environmental demands on specific functional domains are higher or when periods of developmentally time CNS reorganization are occurring.
10. **Taking Into Account Those Conditions that Ameliorate or Exacerbate Any Exposure Related Functional Outcomes:** Some behavioral teratogenic effects may be exacerbated or ameliorated by other exposures or environmental conditions such as how the organism is handled or parented or other unexpected events such as illnesses that occur after the exposure period.

These principles do cut across animal and human models (Vorhees & Mollnow, 1987), although the methodological challenges are different and sometimes more complex in the human model. Throughout the discussion of these principles, we shall draw in particular on studies of the problem of prenatal cocaine exposure, which especially illustrates the principles of defining the independent variable (Table 1.1, principle 4), delineating dose-response relations (principle 5), defining cohorts based on risk of exposure (principle 6), and specifying the environmental context and conditions most

related to the exposure (principle 7). Studies of prenatal cocaine exposure in human models that are informed by preclinical investigations are paradigmatic of contemporary neurobehavioral teratology perspectives. In our concluding section, we will suggest future directions for these kinds of models of behavioral teratogenicity in humans.

CENTRAL PRINCIPLES IN NEUROBEHAVIORAL TERATOLOGY STUDIES

One of the important legacies of the early stages of the field of neurobehavioral teratology is its initial "applied" nature as in, for example, the issues regarding the establishment of regulatory standards for drugs and chemicals. Because of this applied, practical beginning, in many of the early toxicological studies in both animal and human models, little attention was given to some critical methodological issues that presented potential prenatal and postnatal confounding variables (Nelson, 1990). The thalidomide tragedy notwithstanding, in most instances, establishing in humans clear links between prenatal exposures and immediate physical, neurological, or later developmental and psychological outcomes is fraught with significant methodological problems. Animal models of exposure offer some solutions to a number of these methodological issues while at the same time preclinical models may not adequately model the complexity of the human exposure situation. Thus, that only a few human environmental neurobehavioral teratogens have been identified (e.g., methyl mercury, PCBs, lead, alcohol) is not necessarily evidence for human invulnerability to teratogenic effects but rather that very few agents have actually been rigorously evaluated with appropriate methodological standards. For example, of at least 70,000 chemicals in regular commercial use, perhaps only 10 percent (excluding pharmaceutical agents) have been studied for their neurotoxic or neurobehavioral teratogenic potential (Dietrich, 1999; Rees, Francis, & Kimmel, 1990). Similarly, despite the number of psychoactive drugs that may be used during pregnancy and despite concerns regarding their potential teratogenic effects, for most, data are inconclusive (Levy & Koren, 1990, 1992) at least in part because of significant methodological issues. The following principles play a role in essentially every question regarding potential neurobehavioral teratogens.

Delineating Possible Mechanisms of Effect. While it perhaps seems obvious that it is important to consider the possible mechanisms of teratogenic effects of any given agent, it is not always the case particularly in studies of the human model that mechanisms of effect are either specified or hypothesized beyond the general expectation or assumption that, for example, psychoactive drugs administered during active CNS neurogenesis should be potentially teratogenic (see also below). This assumption not only ignores consideration of specificity of effect on particular CNS regions and functions, but also does not permit a more hypothesis-driven consideration of the possible domains of outcome to study. The notion that specifying mechanisms of effect on CNS that will be manifest in behavioral/psychological function does make that presumption that there are neurochemical and/or neuroanatomical, structural changes in the brain as a result of exposure to a particular agent which are in turn manifest in postnatal behavior and psychological functions. Often these possible mechanisms of action are defined not through investigations of the specific teratogen but rather through studies of other agents with similar mechanisms of action in the brain. For example, the potential effects of cocaine on developing monoaminergic systems in the fetal brain

have been delineated more through in vitro and in vivo studies of monoaminergic regulation of neurogenesis, neuronal migration, and synaptogenesis rather than through direct study of disruptions in fetal brain structure-function relations with cocaine exposure (Mayes, 1999).

Parenthetically, because studies defining the mechanisms of action of a drug or of aspects of CNS ontogeny often are accomplished by investigators not strictly studying teratologic questions, it is paramount that any behavioral teratologic study be framed as an interdisciplinary endeavor and incorporate findings from investigations of basic mechanisms of action into the study's conceptual basis. It is on this point that the fault line between the two perspectives cited earlier is the most evident, that is, between teratologic studies focused on outcome and those focused on mechanisms of disruption leading to developmental injuries. Once mechanisms of effect become the focus, it is possible to think of groups of teratogens rather than considering each teratogen as unique. For instance, there may be a group of teratogens that affect primarily cortical layering or neuronal migration, others in which the mechanism of action is primarily at the level of second messenger systems in dopaminergic pathways. It is of course also possible that one teratogenic agent may share several different mechanisms, and the relevant outcomes vary according to the mechanism most active at a particular time in development. For example, despite our emphasis on monoaminergic systems in the prenatal cocaine exposure model, cocaine acts on several other systems in the adult and developing brain. These include glutamate receptors, neuropeptides including the opioids dynorphin and enkephalin and nonopioids such as substance-P, and at the level of ion-channel transmission (Kreek, 1996; Reith, 1988; Shippenberg & Rea, 1997; White, Hu, Zhang, & Wolf, 1995; Ye, Liu, Wu, & McArdle, 1997). Very little work has been done on these systems in the fetal animal model, but based on these other mechanisms of action in the CNS, it is possible that these may also be involved in mechanisms of teratogenesis for cocaine and other pharmacologically similar stimulants.

Another salient and related point regarding teratologic mechanisms is the notion that all types of embryopathy are expressed through a set of final common pathways (Wilson, 1973, 1977). For example, it has long been observed that there is a definable and finite, albeit long, set of birth defects because of disruptions in basic processes of embryogenesis. Some disruptions in embryonic processes are not compatible with fetal survival and result in spontaneous abortion (Warkany, 1978). Other disruptions in embryonic ontogenesis are compatible with survival, albeit with physical and functional malformations evident at delivery or shortly afterward. Embryonic events necessary for normal development include closure of the neural plates, rotation and fusion of the palate, or rotation of the heart tube, and there are many ways these processes may be disrupted. But for these and other examples, the final common pathway is disruption of the morphogenetic process that results in these closure or rotation processes. Failure of closure or rotation may produce neural tube defects, congenital heart malformations, or cleft palate. While there may be hundreds, if not thousands, of drugs or other exposures that may alter these morphogenetic processes and result in a neural tube or heart defect, these malformations are not unique to the particular drug or agent.

This same argument may well apply to behavioral teratogenic questions (Vorhees & Mollnow, 1987). There may also be a finite number of disruptions in neural ontogeny and hence a finite number of final common behavioral disruptions that occur in the infant and young child. Thus, agents that are chemically very different may produce

behavioral profiles that are quite similar based on similar mechanisms of effect on neural ontogenetic processes. For example, cocaine and commonly prescribed psychoactive drugs for anxiety and depression, the selective serotonin reuptake inhibitors, share in part a common mechanism of effect on monoaminergic systems described above and hence may show a similar effect on some aspects of fetal neural development. Of course, early neurobehavioral functional abnormalities may interact with environmental conditions to modify the behavioral profile which does make the range of behavioral outcomes broader and more complex; and better methods of assessment also improves the fidelity of behavioral profiles as the same teratogenic agent is studied over time. Nonetheless, it is still an important and parsimonious principle to think about the outcomes of exposures to different teratogens in terms of final common behavioral profiles that may be the same across different exposure agents.

One other mechanism of effect, preconceptional or transgenerational effects, deserves mention though it is not well studied in any behavioral teratologic investigations. First, there are effects on offspring behavior from paternal rather than maternal exposure (Adams, Fabricant, & Legator, 1981). These are transmitted through exposure effects on male sperm and hence exposure effects through mutagenesis. Such mechanisms have been suggested for prenatal alcohol effects (Abel, 1992). Others have also suggested that transgenerational effects on behavior, that is, effects transmitted across more than one generation, may be mediated again by mutagenesis (Vorhees & Mollnow, 1987), though it may also be that a vulnerability to, for example, addiction is transmitted across generations not because of mutagenesis but rather through a genetically conveyed vulnerability that also conveys a vulnerability to other behavioral outcomes or phenotypes also associated with the specific genetic polymorphisms (see below).

Defining the Specific Teratogenic Agent. Not all agents that produce teratogenic effects in organ systems other than the brain are necessarily behavioral teratogens, though with increasingly refined methods of study, behavioral effects may be apparent. Thalidomide may be a notable example of the latter (McBride, 1977). Despite the dramatic limb reduction deformity, evidence is less clear regarding psychological developmental effects, although a reduction in IQ appears present after allowing for the impact of physical disabilities (McFie & Robertson, 1973). Conversely, only those agents that produce either teratogenic or psychoactive CNS effects are capable of producing behavioral teratogenic effects. While this may seem obvious, it is worth considering for a moment. All evidence to date suggests that all agents that result in CNS structural malformations at higher doses are also behavioral teratogens at lower doses.

Whether or not all psychoactive drugs are also potential structural and behavioral teratogens in the developing fetus is less clear. There are certainly instances of psychoactive drugs that apparently produce behavioral teratogenic effects, even effects on growth, but not apparent structural malformations. Examples include diazepam (Kellogg, Tervo, Ison, Parisi, & Miller, 1980), phenobarbital (Middahugh, 1986), neuroleptics (Vorhees, Brunner, & Butcher, 1979), and some pesticides (Mactutus & Tilson, 1986). Cocaine may also be an example, though at high doses cocaine may contribute to cerebral vascular accidents in the fetus because of vasoconstriction (Moore, Sorg, Miller, Key, & Resnik, 1986; Woods, Plessinger, & Clark, 1987) and cause structural malformations based on a mechanism that is different from the one associated with behavioral teratogenic effects. Hence, the absence of any data suggesting structural

malformations for a given psychoactive drug cannot be taken as evidence against behavioral teratogenesis. Furthermore, since behavioral effects typically occur at doses lower than those effecting growth or morphogenesis (see below), safety studies need to consider the possibility of functional disruption even at low, and presumably safe, doses.

At the same time, it is an error to assume that every psychoactive agent necessarily produces behavioral teratogenic (or for that matter teratogenic) effects in the developing central nervous system. There are a number of examples of psychoactive drugs that do not appear to be behaviorally teratogenic, albeit with the accumulated evidence to date. Acetazolamide, an anticonvulsant, appears to be one such example (Butcher, Hawver, Burbacher, & Scott, 1975); acetazolamide is associated with limb deformities but apparently not behavioral teratogenic effects.

Specifying the Timing of Exposures. Defining the timing of exposure is critical because of differing windows of vulnerability in the developing fetus. These "windows" are based on differing phases of central nervous system development. CNS ontogeny reflects a complex interaction among genetic factors, neurochemical substrates, and environmental conditions (Kosofsky, 1991). At least eight stages describe the processes that occur in each part of the developing brain: neural plate induction, neuronal and glial cell proliferation, cell migration, cell aggregation, neuronal maturation, neuronal connectivity including synaptogenesis, cell death, and process elimination (pruning). Within each of these phases, there are parallel processes of metabolic differentiation and maturation, and genes taking their regulatory cues from the immediate neurochemical (and experiential) environment regulate the onset/offset of each phase. As a more general map in humans, cell proliferation occurs between two and four months gestation for neurons and five months gestation to one year postnatally for glia; neuronal migration takes place primarily between three and five months gestation. Between six months gestation to several years postnatally, the brain is in a protracted organizational phase establishing neuronal connectivity and pruning less utilized connections or synapses to enhance other patterns of connectivity (Volpe, 1987). The timing (gestational and/or postnatal) of potentially toxic exposures determines the possible developmental consequences. Exposures occurring during the first half of gestation affect cytogenesis and histogenesis, while those exposures occurring in the latter half of pregnancy and postnatally influence growth and structural/functional differentiation. Exposures throughout both periods have "interactive" effects inasmuch as altering early events during the cytogenesis phase will also alter events downstream that are regulated by the completion of earlier phases. Brain development in the second half of gestation and the early postnatal period is characterized by both *progressive* (e.g., synaptogenesis and neuronal maturation) and *regressive* (e.g., cell death and pruning) processes. Blocking, delaying, extending, or shortening either progressive or regressive events will have probable (and different) effects on immediate structure/function relations, on related genetic regulatory processes, and on neuromaturational events downstream that are dependent on earlier events.

Behavioral teratologic studies are implicitly based on the concept of critical periods or on defining periods of maximum or specific vulnerability to a specific insult. Ontogenetic processes (at the biochemical, cellular, structural, or functional level) are most vulnerable to disruption during their earliest and most active phases. And the window

of maximal vulnerability also depends on the mechanism of action of the potential teratogen. That is, if the primary mechanism of action of a given drug is disruption of thymidine and uridine incorporation as, for example, is suggested for cocaine (Garg, Turndorf, & Bansinath, 1993), then the period of maximum vulnerability for the human fetus will be during neurogenesis and neuronal proliferation in the first trimester. Some windows of vulnerability are relatively short, others longer. For instance, there is a relatively narrow window during fetal development during which some agents can sufficiently alter neural plate closure so that brain and spinal cord malformations such as spina bifida occur (Warkany, Lemire, & Cohen, 1981). Behavioral teratologic questions are typically focused on more subtle types of structural and functional deficits that may not be visible on gross or even general microscopic inspection. There may also be several critical windows of vulnerability for any one potential teratogen, for many drugs disrupt neural ontogeny at several different stages and thus, depending on the timing of the exposure, the developmental process that is primary at that time will be most disrupted. Cocaine, for example, may have direct effects on neurogenesis, on neuronal migration, and on synaptogenesis – processes that cut across several critical windows of neural ontogeny. As with understanding mechanisms of teratogenesis, understanding critical periods or windows of vulnerability is based as much in the basic developmental neuroscience as in studies of toxicology. Indeed, toxicological studies may inform basic models of neural ontogeny and provide data that can be used to refine and narrow windows of vulnerability. Broadly defined neurogenesis phases may at times be too inclusive to allow more accurate definition of period-specific vulnerabilities. For example, within the broad phase of neuronal migration, there are many smaller windows and phase-specific processes such as the generation of radial glial cells, that may define mechanism-specific critical periods. Of equal importance, early disruptions in certain ontogenetic processes may have functional effects long after the exposure has stopped inasmuch as the interactions among systems and functional organization of processes continues to be affected by the early disruption. Continued histogenesis, functional organization, and brain (as well as other organ) growth through synpatogenesis and synaptic pruning continue long after birth including well into puberty. Thus, early ontogenetic alterations may have long-lasting effects without continued exposure to the teratogen.

For behavioral teratologic studies, a distinction between experience-expectant and experience-dependent or -sensitive is also important (Greenough, 1991; Greenough, Black, Klintsova, Bates, & Weiler, 1999; Greenough, Black, & Wallace, 1987). Experience-expectant processes are those that require certain experiences or events at one or more critical windows during ontogeny to develop fully. The classic postnatal example is the failure of the visual apparatus to develop without proper light exposure. Experience-dependent processes are those developmental events or phases that respond to experience and activity with enhanced development such as experience-sensitive synaptogenesis that occurs with enriched stimulating environments. Experience-dependent refers to incorporation of learned environmental information that is unique to the individual. The neural basis of this process appears to involve active formation of new synaptic connections in response to the events providing the information to be stored. Given that a considerable amount of neural development occurs in the human infant postnatally and in the first years of life, the concept of experience-dependent periods is particularly relevant for teratogenic studies of human exposures. Additionally,

for humans, many exposures are not limited to the prenatal period, and exposure during the postnatal period may also occur during a phase-specific period of vulnerability. Postnatal teratogenic exposures include to some licit and illicit drugs such as nicotine, crack, or marijuana through passive inhalation, to prescribed psychoactive drugs through breast milk, or to parental stress or depression through caregiving interactions.

Defining the Nature of the Exposure. All questions regarding the effects of prenatal exposure to agents or events have the dilemma of defining the route, amount, and duration of exposure. For nonillicit, prescribed drugs, these definitions may be relatively straightforward. The dosage, frequency, and route of administration are prescribed and known and the patient's compliance with the prescribed regimen and individual pharmacokinetic variation in the metabolism of the drug are the sources of variance in the amount of exposure. However, for events such as exposure to maternal stress, the exposure issues are complex: when can the "exposure" be said to begin and end, what defines a potentially teratogenic level of maternal stress, and by what metric. Similarly, defining amount and duration of exposure for environmental toxins such as PCBs can be equally difficult and problematic.

For human models of prenatal illicit drug exposure, defining the independent exposure variable may be the single most problematic issue in neurobehavioral teratologic studies (Mayes & Fahy, 2001). Substance abusers typically do not report consistently or reliably the frequency or amount of their drug use (Babor, Brown, & delBoca, 1990; Chasnoff, Landress, & Barrett, 1990; Grissom, 1997; Weiss, et al., 1998). Various strategies have been devised to improve the reliability of self-reports of substance use including use of time-lines, careful training of interviewers, narrow windows for retrospective recall (Callahan, et al., 1992; Carey, 1997; Richardson & Day, 1994; Rogers & Kelly, 1997). Even with these more sophisticated interviewing strategies, self-reports of single or polydrug use typically though not uniformly (Richardson, Day, & McGauhey, 1993) underestimate the amount of exposure, particularly of illicit drugs.

Frequency of exposure obtained through self-report histories is usually expressed as a number of days per unit time (e.g., per month, use in last thirty days, use per week). Self-reports are typically though not universally augmented with toxicologic sampling of urine for drugs such as cocaine, marijuana, or opiates. Repeated toxicology screening through a pregnancy may provide some confirmation and/or identification of users and not uncommonly toxicologic screens are obtained from both infant and mother at the time of delivery. Urine toxicology provides a relatively narrow window on use. For example, for cocaine users, urine toxicology is typically positive no longer than thirty-six hours after use and that window varies for other drugs. For cocaine, infants' meconium and/or hair (infant's or mother's) have gained some support as particularly good samples to ascertain or confirm infant exposure because they provide a longer window for ascertaining exposure. Some data suggest that meconium or hair from the newborn may be a reliable measure of exposure as far back as mid-first trimester (Callahan, et al., 1992; Graham, Koren, Klein, Schneiderman, & Greenwald, 1989; Kline, Ng, Schittini, Levin, & Susser, 1997; Ostrea, 1995). However, despite early enthusiasm for these kinds of longer window measures and despite their obvious utility, they do not provide a reliable quantitative estimate of exposure.

Indeed, quantity or amount of exposure is particularly difficult to estimate reliably in studies of illicit drug exposure – or for that matter in industrial or environmental

toxin exposures. Estimates of amount of drug per time of use are as problematic as frequency of use when obtained by self-report. Toxicologic assays typically do not provide sufficiently accurate quantitative assays to permit the definition of a more quantitative exposure variable. But it is an important variable since between individuals and for any one person, "dose" or amount per use varies enormously. There are obviously no standards for how illicit drugs are sold – how pure or how diluted with other ingredients that may be active or inert. Thus, even if an addict presents a more or less accurate account of frequency and amount of use, there are few to no reliable indices of how concentrated the drug was and what the carrier or substance for cutting the pure drug might have been.

With these various problems in obtaining accurate estimates of frequency and amount of exposure, the majority of studies of prenatal exposure to date have defined the independent exposure variable as a dichotomous one – exposed or not exposed. Grouping all exposed infants and children together obscures potential dose-related effects inasmuch as including those only minimally may reduce the likelihood of detecting exposure effects in the exposed group. Thus, a growing number of studies are attempting to create some metric of heavy, moderate, and light use to examine dose-related effects that follow either linear or nonlinear models (for example, see Frank, Augustyn, & Zuckerman, 1998; King, et al., 1995; Tronick, Frank, Cabral, Mirochnick, & Zuckerman, 1996).

A third problem in the definition of exposure variables in substance abuse models is maternal polydrug use. Rarely do addicts use one drug only. While they may consider one drug of abuse their primary drug, polydrug use and exposure is the rule rather than the exception. For example, for cocaine users, a very typical combination is alcohol and tobacco in combination with cocaine. The same issues of defining frequency and amount of use for each drug pertain, but also there are questions of interactive effects among drugs such as alcohol with cocaine and the resulting metabolite cocethylene. And a related problem specific to studies of prenatal exposure is obtaining reliable estimates of frequency and amount of exposure by trimester. Different drugs have different effects during the three trimesters of pregnancy. For example, in the first trimester, prenatal cocaine exposure may have a direct effect on neuronal migration and brain structure formation, whereas in the third trimester the central nervous system effect may be on synaptogenesis in specific brain regions (Dow-Edwards, Freed, & Milhorat, 1988; Frank, et al., 1998; Mayes & Bornstein, 1995). Related to breaking down exposure by trimester is continued exposure postnatally. Particularly among agents that may be inhaled passively (e.g., crack, tobacco, marijuana), postnatal exposure is relatively common (for example, see Bender, et al., 1995; Kjarasch, Glotzer, Vinci, Wietzman, & Sargent, 1991; Lustbader, Mayes, McGee, Jatlow, & Roberts, 1998).

Route of use presents another consideration in defining the exposure. While total amount is always an important metric in defining severity of exposure, amount of time above a certain peak blood level may also be important in some models of teratogenicity. Stated another way, the teratogenic effect may not be carried by cumulative amount of exposure time but only by those times when the level of exposure is above a certain threshold. Certain aspects of fetal alcohol effects may follow this threshold rather than the linear dose-related model. Blood levels peak at different points following use, depending on the preferred route of use. In animal models, intraperitoneal administration results in a very different blood level compared to subcutaneous, and both are different

from gastric lavage. Furthermore, regardless of route of administration, fetal cocaine levels are different from the maternal one – lower for subcutaneous administration (Spear, Kirstein, & Frambes, 1989) but higher for intraperitoneal (DeVane, Simpkins, Miller, & Braun, 1989). In humans, intravenous use as with heroin or smoking crack with rapid absorption through the pulmonary vascular bed provides rapid and large peak blood levels to both mother and fetus. Few to no studies, particularly of cocaine where the routes of use may be quite varied, have examined differences in outcome depending on preferred method of use.

That route of use may influence the peak blood level to which the developing brain is exposed raises another issue regarding definitions of the independent variable, the pharmacokinetics of how the agent is handled in the body. Even knowing how much drug is administered or used over a given period of time and by what route does not fully define how much active drug reaches the fetal brain and over what period of time. Pharmacokinetic factors including absorption, metabolism, tissue uptake, protein binding, and excretion are each critical in determining the neural toxicity for any given agent. For example, some drugs do not readily or fully cross the placenta to the fetal circulation and thus, maternal blood levels, even if available, will not accurately reflect fetal blood levels. Some drugs are differentially metabolized by adults so that for one mother, a given dose results in a lower blood level than for another individual. Typically, in human studies, these individual pharmacokinetic factors are very difficult to take into account, particularly in the case of illicit drugs. A few studies examining the teratogenic effects of, for example, selective serotonin reuptake inhibitors during the immediate perinatal and postnatal period have examined maternal and infant cord blood, and breast milk drug levels (Kristensen, et al., 1999; Schmidt, Olesen, & Jensen, 2000), but far more consideration needs to be given to incorporating pharmacokinetic principles into human studies.

Delineating and Dose-Response Relationships and the Range of Susceptibility. Implicit in these considerations regarding dose, frequency, route of administration, and pharmacokinetics is establishing dose-response relations for the effects of the teratogen on the developing brain. What is the relationship between the appearance of teratogenic effects and the amount and duration of exposure? While only a few studies report clear dose-response relationships, there are a few teratogens for which these reports are consistent, including alcohol, vitamin A, and phenytoin (Abel, 1992; Vorhees, 1974; Vorhees, 1986d). Dose-response relationships are not always evident for behavior, though failure to detect such may be a reflection of study methods rather than the absence of a relationship (Nelson, 1981; Vorhees, 1986b). For many drugs such as dilantin (phenytoin; Vorhees, 1986a), the behavioral dose-response window is narrow and the curve steep. That is, the window of effect is so narrow that the model may seem one of an all-or-none effect – any exposure results in a teratogenic effect – and there is only a small difference between a dose at which only behavioral effects are evident and the dose at which structural malformations appear. Careful studies within this narrow window may nonetheless produce a graded model of severity of effect depending on dose. Other drugs that seem to act as behavioral teratogens such as diazepam produce behavioral effects far below the doses that are structurally toxic (Driscoll, Ferre, Fernandez-Teruel, & Levi de Stein, 1995; Kellogg, et al., 1980; Wee & Zimmerman, 1983).

For human models, establishing dose-response curves is problematic for all the reasons cited earlier. It is difficult to establish dosage and timing with any reliability, and the fetus may be concomitantly exposed to other agents, both pharmacologic and experiential (e.g., maternal malnutrition, depression, acute stress), that surely alter the dose-response relation for the target drug. Limits in behavioral assessment may also be a factor in the difficulties in establishing dose-response curves for both humans and preclinical models. Behavioral assessment techniques detect functional changes within the context of the experimental situation and the limits of the task. For example, tests of response inhibition usually reflect the child's performance in a quiet, structured, supportive laboratory setting and do not reflect how these capacities function in a real world setting such as a classroom. The converse is also true. An individual's performance on a certain task may be so poor because of impairments in related and necessary capacities that it is difficult to detect any effect on the function in question. For instance, tasks of short-term verbal memory, another executive function that may be impaired with prenatal cocaine exposure, may place demands on children's receptive and expressive language capacities that are in turn often markedly impaired in part because of severe environmental deprivation. The child's poor performance on verbal working memory tasks does not necessarily reflect a severe behavioral teratogenic effect of the drug.

Also, related to the issue of dose-response for behavioral effects is a point touched on earlier – the relation between dose-response curves for behavioral effects and those for other structural teratogenic effects from the same drug. It is important to note that this is not the same point as the common and probably erroneous assumption that behavioral teratogenic effects occur at lower doses than all other dose-response relationships for teratogenesis or embryotoxicity (Vorhees & Mollnow, 1987). It appears only the case that if a drug is a behavioral teratogen, the behavioral effects occur at lower doses than any structural effects, though for effects on growth, the two dose-response curves may be quite close, even superimposed. The latter appears to be true for example for cocaine, for which effects on growth and on behavior appear in preclinical models at essentially the same dose.

The dose-response relations for behavioral teratogenesis, growth retardation, and structural malformations have several other implications (Vorhees, 1986c; Wilson, 1973). Figure 1.1 (adapted from Vorhees, 1986c) shows a family of hypothetical dose-response curves for different outcomes A, B, & C at different doses. The X-axis shows dose of drug and the Y-axis the percentage of individuals with the outcome. For any single curve, the percentage increases with increasing dose. (An important caveat is that this figure shows essentially linear relations for the three outcomes. Different outcomes may have different curves both in slope and in shape.) For the family of curves taken together, as the dose increases, more individuals begin to show not only outcome A but also B and then C. At very high doses, 100 percent of exposed individuals may theoretically show outcome A, nearly 100 percent will show B, and 50 percent C. Of course, it may also be that outcome C is so devastating, that is, the structural malformations are so profound, that more subtle psychological functional disruptions are obscured. At lower doses in which only outcome A, the behavioral outcome, is evident, there will also be a proportion of unaffected individuals whether these studies are done in preclinical models using members of a litter or in human models using children matched on relevant characteristics. Thus, at sufficiently low doses to produce relatively "pure" behavioral