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Same sex, no sex, and unaware sex in neurotoxicology

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INTRODUCTION

Chemical contaminants that are largely the contribution of industrial processes and their byproducts pervade the environment. Every human is exposed to a multiplicity of such agents and carries their signatures in their tissues. They include heavy metals, solvents, pesticides, cosmetics, plastic products, and other components contributed by a staggering array of manufacturing processes, manufactured items, and their wastes. They exert effects on virtually all organ systems and act through a wide variety of toxic mechanisms.

Researchers in toxicology and the environmental health sciences continue to produce a torrent of publications attesting to the health risks engendered by these agents. Despite this mountain of information, we remain relatively ignorant about one vital aspect: their differential impact on males and females. The Institute of Medicine (IOM) report, *Does Sex Matter* (Wizemann and Pardue, 2001) emphasized the need to examine differences between male and female responses in the research enterprise because of wide gaps between males and females in disease patterns, in response to therapeutic interventions such as drugs, and to external agents. For current neurotoxicology, the predominant question is how environmental chemicals modify brain function and behavior as a function of sex.

Questions about sex differences in disease, in environmental health, and in toxicology have come to assume an importance not accorded them a relatively short time ago. It was only in 1985 that NIH mandated the inclusion of women in clinical trials, followed by other government initiatives (Figure 1). The Society for Women's Health Research was founded in 1990 in an effort to include women in major medical research studies and to address "... the need for more information about conditions affecting women exclusively, disproportionately, or differently than men." The IOM report (pp. 1–2) described its theme and rationale in this way:

...Over the past decade new discoveries in basic human biology have made it increasingly apparent that many normal physiological functions—and, in many cases, pathological functions—are influenced either directly or indirectly by sex-based differences in biology. This realization, however, has been slow in coming... Historically, the research community assumed that beyond the reproductive system, such differences do not exist or are not relevant. Still, ...scientific evidence of the importance of sex differences throughout the life span abounds.

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In 2005, the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) held a workshop on *Gender Differences and Human and Ecological Risk* that extended the arguments in the IOM report to toxicology. The SGOMSEC report addressed in detail a number of topics especially pertinent to toxicology. These include sex-specific effects on germ cell mutagenesis, sex differences as an element in health risk assessment, and sex differences in how wildlife are affected by environmental chemicals. As in the IOM report, the authors stressed the fundamental role of sex at every level of biological organization. One of the issues seen as critical is the tactic, used in many investigations, to classify sex as a “confounder,” a tactic that essentially strips its significance from the analysis (Gochfeld, 2007).

Much the same lack of attention or even awareness is common in laboratory research. Wald and Wu (2010), reviewing ten research areas, entitled their article “Of Mice and Women” to underscore the persistent bias. Sometimes, It is only when regulations call for both sexes to be studied, as in cancer bioassays, that both males and females are included. In this instance, it would be ludicrous to not do so; the disparate vulnerabilities and biological substrates are too overwhelming, as in mammary cancers. Many other disciplines, however, often ignore sex differences. The IOM report (p. 1) remarks almost plaintively that, “...scientists have paid much less attention to the direct study of these differences at the basic *cellular* and *molecular* levels.” Allegedly mechanistic studies relying on *in vitro* models tend to ignore sex; few carry out such studies using a culture medium reproducing the *in vivo* environment of the intact organism. Take the example of how estrogen receptors are distributed in the brain. In weanling rats, females exhibited higher counts and densities of ER α than males (Schlenker and Hansen, 2006). In adult rats, Zhang et al (2002) observed clear differences in the amount of ER-beta immunoreactivity between males and females. Given the number of additional, similar findings, and sex differences in circulating hormones, is it reasonable to study neuronal phenomena *in vitro* when the hormones governing sex differences are lacking in its milieu?

Sex differences in the response to environmental endocrine disruptors (EEDs) were one of the factors generating discussion about these chemical classes. Endocrine disruption surfaced as a critical element in environmental health research about 15 years ago. During 2009, 37 articles centered on endocrine disruptors were published in *Environmental Health Perspectives*, which publishes articles from many disciplines. They exemplify the scope of questions currently being addressed, ranging from *in vitro*, to whole animal, to epidemiological, to exposure assessment in approach. One of the subjects that EED research has highlighted is the difference between male and female sensitivity to environmental chemicals and the forms in which they might emerge. It feeds on questions that have risen to the apex of public curiosity and discussion, such as why girls, by some criteria (though not all), seem to be outperforming boys academically.

This review examines aspects of sex differences critical to neurotoxicology (cf., Becker et al, 2005). Although it centers on questions and studies that have irrupted into intense public debates, such as sex differences in mathematical talent, analogous questions are equally meaningful for laboratory research. One aim of this article is to highlight the connections between the two arenas and to encourage laboratory researchers to consider the implications of their work for the wider issues that prompt changes in policy.

The Architecture of Sex

Sex differences are hardly a new topic for science. Darwin (1871) addressed it in these terms (Chapter XXI):

It would be superfluous here to repeat what I have so lately said on the manner in which sexual selection has apparently acted on both the male and female side, causing the two sexes of man to differ in body and mind...

The IOM report distinguishes *sex* from *gender*, a distinction often overlooked in popular discourse and sometimes even in scientific writing. The report defines sex as a biological classification of male or female based on reproductive organs and chromosomal complement. Gender is viewed as how the individual's self and social identity is presented, and, while rooted in biology, is shaped by environment and experience.

The genetic component

Biology fixes sex at conception by genetic mechanisms. Reinius and Jazin (2009) showed that expression patterns of a number of genes encoded on the Y-chromosome can be detected in the human male prenatal brain. They argued that such expression patterns invoke functional consequences during the course of brain development. Such data support the contention of Davies and Wilkinson (2006) that we should abandon the dogma that sex differences in brain development are solely the outcome of hormonal masculinization of the male brain; as they argue, "It is not all hormones." Arnold (2004) also pointed out that the conventional portrait of hormonally-guided brain development simplistically ignores the role of the sex chromosomes and their developmental legacy. When Dewing et al (2006) searched for the SRY gene (the sex-determining region Y gene) in mouse substantia nigra, they found the protein localized to both the cytoplasm and tyrosine hydroxylase neurons in male brains, but observed that staining was absent in female brains.

The hormonal component

Although genetic sex can influence sex differences, it plays a relatively minor role in defining brain structure and behavior. Hormonal mechanisms predominate. During development, gonadal steroids provide the foundations through which the brain undergoes sexual differentiation. It is a process elaborated on in an immense literature. In an introduction to a special journal issue on the topic, McEwen (2009) wrote, "...the entire nervous system appears to be a target of reproductive hormones, as well as being subject to developmentally programmed sex differences." As depicted in Figure 2, the SRY gene on the Y chromosome is in essence the switch that turns on the secretion of testosterone by the testis. It is the key in brain sexual differentiation. Although the female brain is often described as the "default" structure, the process is not at all passive; female genes play a vital role, as emphasized by Arnold (2004) and noted above.

Structural and functional outcomes

Numerous sex differences in structure are discernible in both rodent and human brains, and are the subject of extensive research, as are the ways in which they respond to steroid hormones and some endocrine disruptors (e.g., Patisaul and Polston, 2008; Weiss, 2002). Anatomical differences include a variety of structures and the relative distributions of cell types. In rodents for instance, the sexually dimorphic nucleus of the preoptic area is larger in males (Arnold and Gorski, 1984; Gorski et al, 1980), and the anteroventral periventricular nucleus is larger in females (Davis et al. 1996).

In humans, clear differences are apparent in several ways. Female brains have a higher percentage of gray matter, male brains a higher percentage of white matter (Gur et al, 1999). Newer imaging methods, such as voxel-based morphometry, have also revealed variations in humans between the sexes that previously had not been suspected (e.g., Goldstein et al, 2002). Distributions of gray matter, for example, are sexually dimorphic, and there are regional sex differences in gray matter thickness (Sowell et al. 2007). Distinctive sex

differences arise in brain volume and have been traced individually through adolescence by Lenroot et al (2007). Male volume at age 19 years is about 200 cc greater than female volume.

Male and female brains are neurochemically distinct as well (Cosgrove et al. 2007), displaying sex-specific differences in dopaminergic and serotonergic markers (Figure 3).

Although the brain is the focus of this article, it is far from the only organ or system displaying sex differences. The liver, too, is sexually dimorphic (Mode and Gustafsson 2006; Waxman and Holloway 2009). Note the scope of these differences:

- Male rats exhibit higher hydroxylating activity than females
- Females express higher 5 α -reductase activity
- Male metabolism depends on androgen exposure
- P450s are sexually dimorphic.
 - Female: **CYP2C12** (15 β -hydroxylase)
 - Male: **CYP2C11** (16 α -hydroxylase)

Growth hormone pulsing patterns are also sexually dimorphic (Waxman and O'Connor 2006), with males exhibiting a periodicity of about 3.3 hr, while females exhibit an almost continuous pulsing pattern.

Such marked differences in liver biochemistry are important for brain function because they imply marked differences in how chemicals are metabolized. They will determine the speed and character of these processes, meaning that administering numerically equivalent doses of a toxic compound to males and females will not determine the absorbed dose of the parent compound, its metabolites, or metabolite concentrations in the target tissues.

Neurobehavioral Indicators

The sex-based influence of environmental chemicals on endpoints such as reproductive structure and function is a fairly straightforward question. Brain anatomy and behavior offer far more subtle challenges. Anatomic and neurochemical sex differences are accompanied by, and in fact are the sources of, sex differences in behavior. Decades of research in neurobiology, especially with the availability of newer tools such as magnetic resonance imaging, has allowed us to peer into the human brain to detect and even quantify anatomical differences between males and females, differences we had already seen in laboratory animals. In parallel, psychologists have been able to define differences in performance; for example, superior performance on tests of language by females and superior performance by males on tests of spatial perception (e.g., Halpern and Tau, 2001; Kimura, 2002). Still, Halpern (2009) stresses the challenge of separating intrinsic biological predispositions from those contributed by the environment.

Behavior occupies a paramount role in assessing threats to health from exposure to environmental exposures. It is now the primary criterion for determining how PCBs, lead, and methylmercury affect human populations, and is the predominant metric employed there for quantifying risk. Even in those studies in which both sexes are included, sex differences may be assigned a secondary role and sex defined as a covariate. The information discarded by doing so is similar in scope to those investigations in which the influence of social class, say, is taken out of its biological niche and labeled as a covariate or confounder (Weiss and Bellinger 2006).

Among the measures employed to determine how environmental exposures influence health, those dependent on behavior require virtually obsessive attention to sex differences. Such differences in complex behavioral functions can be evasive, small in magnitude, and often require novel techniques for detection. For many measures, we find considerable overlap between sexes in performance (Weiss, 1997). Scores on intelligence tests, aptitude assessments (e.g., mathematics), and other cognitive measures show narrow differences, depending on the instrument used for the assay and the direction of the difference. Or, they may show no differences in mean scores, but substantial differences in variability. Because of such narrow differences, a significant change either in magnitude or direction would be difficult to ascertain without a clear focus on such details. Eagly (1995) and Wood and Eagly (2002) stress the point that the consistency of sex differences on various behavioral dimensions and across cultures argues for their fundamental significance, and note that their magnitudes are not notably different from most findings in psychological research.

Sex differences in neurobehavioral function are clear in humans. Summarizing numerous sources on the topic, they include:

- Emotional expression
- Memory function
- Vision
- Hearing
- Face processing
- Pain perception
- Navigation strategies
- Stress responses
- Social responsiveness

Emotional expression differences are detectable early in development. Infant boys display higher activity levels and approach behaviors (Gartstein and Rothbart, 2003). Other forms of emotional expression or temperament show sex differences in the response to perinatal exposures to lead (Wasserman et al. 1998), bisphenol A (Braun et al. 2009), and phthalates (Engel et al. 2010). One message from such findings is that even in situations aimed at other endpoints, such as learning, an underlying sex difference in temperament could play a complicating role (Connellan et al. 2000; Lutchmaya et al. 2004).

Other kinds of sex differences are observable during infancy as well. For example, using visual tracking technology, Alexander et al. (2009) found sex-specific preferences in toys as early as 3 and 8 months of age, with girls preferring more typically feminine toys-, and boys preferring typically masculine toys. van de Beek et al (2009) saw a similar difference at 13 months of age. And Servin et al. (1999) found young boys to spend significantly more time in play with masculine toys at one, three, and five years of age. At later ages, differences in play behaviors, including toy choice, are easier to measure. Among school-aged boys and girls, prenatal exposure to PCBs was associated with less masculine play in boys and more masculine play in girls, while prenatal dioxin levels were associated with more feminine behaviors in both boys and girls (Vreugdenhil et al. 2002). Swan et al. (2010) reported that prenatal exposure to phthalates, chemicals classified as anti-androgens, was associated with reduced masculine play among 4–7 year old boys. Data such as these, visible so early in life, underscore the biological origins of sex differences in behavior.

Clinical Implications

Sex differences are ubiquitous in neurological disorders, with examples, taken from diverse sources in the literature, are given in Figure 4. The prevalence of ADHD, for example, is twice as high in males as in females according to NHANES, but an analysis of telephone interviews by Ramtekka et al (2010) showed lower male:female ratios than reported in some clinic-based studies, suggesting that females are underdiagnosed in the community, a “silent minority” so to speak (Berry et al. 1985). Also, because ADHD females display less disruptive behavior than ADHD males (Achenbach and Ruffle, 2000), they are more likely to be ignored if they display mostly inattentive behaviors rather than excessive activity (Gaub and Carlson, 1997). And Rucklidge (2010) points out that, when girls are diagnosed with ADHD, they are more often diagnosed than boys with ADHD as predominantly inattentive. Relative ratios based on the most easily observable behaviors, then, may seriously distort the true incidence of such a clinical entity in females.

Schizophrenia is another disorder displaying sex differences in its manifestations. In males, onset peaks at around age 20–25 years. In women, it peaks at around age 25–30 years (Hafner 2003). The brains of men and women schizophrenics display a different pattern of sex differences than the brains of normal men and women (Goldstein et al. 2002). Women’s expressions of affect are also more pronounced (Goldstein, 2006). As observed there, to understand the etiology of the affective arousal features associated with this disease requires an understanding of the role of neuroendocrine dysfunction, and its roots in fetal development. Thus, although males and females may be diagnosed in identical proportions, the signs of illness are expressed differently.

These examples hardly exhaust the number of behavioral disorders that exhibit sex differences either in prevalence or major symptoms. They are also apparent in the incidence of anti-social conduct disorder, anxiety disorders, and addictive disorders. And, as the IOM document indicates, hardly any disease or disorder afflicts both sexes equally.

Challenges to neurotoxicology

Animal behavior

The ubiquity of sex differences in human behavior, including clinical disorders, tells experimenters that neglecting such differences diminishes, or even erodes, the significance of laboratory research for the human condition. In many laboratory studies, though, sex may not even achieve the status of a mere confounder. It can be illuminating to survey the experimental literature to observe how frequently sex differences are ignored. Often, only one sex is studied, usually males, even for questions where such a choice seems unsuitable. Despite the preponderance of women suffering clinical depression (twice the prevalence of men), and despite sex differences in symptoms and response to antidepressant drugs, laboratory experiments on antidepressants often rely solely on male subjects (e.g., Tamburella et al. 2009; Bantsiele et al. 2009; Cousins and Seiden, 2000). Antidepressant studies that take account of sex almost invariably find differences in response measures (e.g., Sun and Alkon, 2006; Leuner et al, 2004).

In Volume 29 of *Neurotoxicology* (2009), the male:female ratio of whole-animal, single sex studies was 40:1. Only four studies used both sexes. Such a bias is evident in the literature as a whole. The PubMed entry, *morris maze*, yields 3634 entries. The entry, *sex differences morris maze*, yields 111 entries. The entry, *morris maze toxicity*, yields 381 entries. The entry, *morris maze toxicity sex differences*, yields 14 entries.

These figures reflect what might be termed *Sex Unawareness* on the part of experimenters. Two examples from volume 29 (authors not identified):

”Fifty SPF mice (age 9 weeks) weighing 26.3–30.9 g were purchased from Experimental Animal Center----- University.” [*Male, female, both?*]

“The treatment groups were based on the daily dose of alcohol administered [to pregnant rats] and the frequency of administration. Each treatment group... consisted of seven [neonatal] subjects (n = 7 per treatment/genotype). Both males and females were included in each group.” [*How many of each in the different groups?*]

Model organisms

The resources demanded by research on mammalian species have generated wide interest in organisms that are cheap to maintain while they can be tested in large numbers. Zebrafish are among these model species. Their rationale was described by Selderslaghs et al (2010) in these terms: “Currently, neurotoxicity testing defined by OECD and FDA is based solely on in vivo experiments, using large numbers of animals and unsuitable for screening numerous chemicals. The great demand for thousands of chemicals yet to be evaluated, urges the development of alternative test methods which are cheaper, faster and highly predictive for developmental neurotoxicity.” Zebrafish investigators largely overlook sex differences, however. For example, of eight published studies on the insecticide chlorpyrifos, none examined sex differences. Of the 411 rat studies, 39 examined sex and found significant differences in response. Yet, expression bias values for male-enriched and female-enriched genes in zebrafish found 2612 female-enriched and 3387 male-enriched genes (Small et al. 2009). And Santos et al (2008), in a study of proportional chromosome distribution of sexually dimorphic genes in zebrafish brain, found 21 to show significant sex differences.

Drosophila melanogaster brains are also sexually dimorphic in a number of areas (Kimura et al. 2005). Worm brains, too, are sexually dimorphic, as are their behaviors. *C. elegans* male neuronal distributions differ from those of hermaphrodites (Jazin and Cahill, 2010) but are almost never considered in behavioral studies. One exception was a study by Lee and Portman (2007) of olfactory preferences that uncovered large differences between males and hermaphrodites. Sex differences in the response to neurotoxicants are readily observed in even more primitive invertebrates (McClellan-Green et al. 2007) and are depicted in Figure 5. If model organisms are to fulfill their promise in toxicology their proponents cannot afford to neglect sex as a variable.

In vitro studies

A major theme of the IOM report is summarized in the chapter, *Every Cell has a Sex*. It reflects its emphasis on the principle that sex differences exist at every level of biological organization. For example (pp. 28–44), it makes the following statements:

- There are multiple, ubiquitous differences in the basic cellular biochemistry of males and females that can affect an individual’s health.
- Determine and disclose the sex of origin of biological research materials.
- Useful information on the sex of origin of cell and tissue culture material is often lacking in the literature.
- Scientists have paid much less attention to the direct study of these differences at the basic *cellular and molecular levels*.

And yet, in Volume 29 of *Neurotoxicology*, not a single *in vitro* study of approximately 60 took account of sex or included gonadal hormones in the medium. Many were of

indeterminate sex (e.g., PC12), or other transformed cell lines that are not representative of native neural cells and of uncertain utility for evaluating risks or even mechanisms that can be applied with confidence to both sexes. Shouldn't they arouse doubts about their usefulness in studies aimed at the mechanisms, say, of Parkinson's disease, whose prevalence is so sexually dimorphic? Confrontation with this problem seems to be lacking in the NAS report on the future of toxicology (Committee on Toxicity Testing and Assessment of Environmental Agents, 2007), which envisages a scheme in which whole animal studies are rare. Instead, screening and risk assessment are conducted by batteries of *in vitro* assay systems. The implications of such a scheme for behavioral toxicology are discussed in a recent article (Bushnell et al. 2010). The problems cited there are multiplied if such systems ignore sex differences.

Societal and policy implications

The issue of sex differences propels neurotoxicology into realms of inquiry that are simultaneously difficult and yet closely scrutinized by an interested if not fascinated public. Disparities between males and females in cognitive function, temperament, communication style, and other facets of behavior are the theme of a voluminous popular literature. Testimony to that fascination is exemplified by the flood of books on gender issues, with titles such as *Pink Brain, Blue Brain; Taking Sex Differences Seriously; Cognition and Sex Differences* (Figure 6). Although sex differences have always been a common motif in our culture, they have taken on greater contemporary visibility, perhaps because of transformations in women's roles. The popularity of publications such as those in Figure 6 exemplifies the scope and persistence of such questions.

The IOM report only glancingly explored policy issues that apply to neurotoxicology. Some of these evoke fervent debates largely arising from their subtle nature. Their implications are profound and sensitive enough that they provoked the resignation of Harvard's president when he attempted to explore why women in academia were apparently underrepresented in science and mathematics (Singer, 2005). To a notable degree, his question is embedded in statistics. Gender (using that term to include social influences as well as biological sex) disparities in mathematical aptitude, perhaps the core question in the debate, are endlessly debated. Some of the most influential data came from a continuing project, the *Study of Mathematically Precocious Youth*, first described by Benbow and Stanley (1980). It showed a substantial difference in mathematical reasoning ability between boys and girls in junior high in favor of boys, with the greatest disparity appearing in the upper ranges of ability. The authors ascribed the difference to an innate superiority in spatial tasks.

I've pointed out (Weiss, 1997), as have others, that the difference at the upper ranges of achievement stems from the nature of the distributions of scores. In the report by Benbow and Stanley (1983), the mean gender difference amounted to about 0.5 standard deviation; the differences strongly influenced career choice, as seen 20 years later (Benbow et al, 2000). The crucial message is lodged in the extremes of the distribution, however. For scores ≥ 500 , about the median for 12th grade males, the boy:girl ratio came to 2:1. At scores ≥ 600 the ratio rose to 4:1 while at the upper ranges the differences were even more stark. For scores ≥ 700 , the 94th percentile, it rose to 12:1. Because the current literature shows that males tend to be more variable than females on such tests, they will show longer tails on the distributions and greater proportions of high-scoring (and low-scoring) individuals. The differences are hardly overwhelming, however. Halpern (2009) observed that mean sex differences on tests of mathematical aptitude have been diminishing, with some data indicating that variances have been declining as well. Nevertheless, such questions generate inflammatory public debates. Scientists cannot separate themselves from involvement, especially those who conduct research on neurobehavioral function.

As scientists, we prefer to see strikingly visible results rather than quantitative data whose interpretation requires subtle reasoning and analysis. But actual policy more often hinges on small effects rather than robust ones because the latter are relatively rare in human research. Lead neurotoxicity is one noteworthy example. Rather modest shifts in the IQ distribution (Weiss 1988; Lanphear et al, 2005) are responsible for marked reductions in the blood lead levels now deemed acceptable (Gilbert and Weiss, 2006). Arguments about gender differences in ability also pivot around percentages in the single digits.

Figure 7 charts four different possibilities reflecting gender differences in the distribution of an arbitrarily-defined ability. Two (A and B) depict situations in which one gender's variance is the greater, meaning that it will reflect both more superior and more inferior scores. Two (C and D) depict situations in which the variance is the same but in which the means differ. Any of these four situations could be molded by environmental agents because few of these induce gender-neutral effects. Of all the toxicological specialties that contend with sex differences and the environment and their policy and political implications, neurotoxicology is the one most likely to be questioned by the wider public about interpretations.

But it is not only the human data that require interpretation. Although this article is not framed as a statistical discussion, it cannot escape the problem that most animal research in neurotoxicology adopts statistical approaches that focus narrowly on mean differences and null hypothesis testing. Only rarely are distributions explicitly examined. But sometimes, as with mathematical aptitude, the message lies in the properties of the distributions themselves, especially at their extremes. The fixation on mean differences and null hypothesis testing by investigators may obscure the true significance of experimental data (Krantz, 1999) and impede their translation into policy and into terms that define human populations. Animal experimenters rarely present values such as confidence intervals, effect sizes, or variance ratios when comparing treatment groups or, especially, sex. We impose, on ourselves, another barrier to making our work relevant.

Coda

It is puzzling, at least to this observer, to find such a lack of awareness, in much of neurotoxicology, of the degree to which sex differences pervade virtually every aspect of our research. It is as if we had denied a core principle of evolution. Yet, almost without exception, whenever we include both sexes in a study, we find that they differ in one or more dimensions. If they don't separate on the basis of some numerical index, they may differ in more qualitative terms. ADHD investigators, as observed earlier, are now aware of how neglecting these latter aspects can distort prevalence data.

The research scene seems to be shifting, however. Two recent articles argue that the current sex bias in research is ethically irresponsible as well as a barrier to knowledge. One analyzes how gender inequalities in biomedical research serve to undermine patient care (Kim et al. 2010). The other points more directly at neuroscience (Beery and Zucker, 2010). These authors noted that, in this discipline, single-sex studies of male animals outnumber those of females 5.5 to 1, confirming the bias that prevails in neurotoxicology. They too observed that reporting the sex of tissues or cell lines is even more rare, another example of how investigators working at this biological level ignore the principle that, as the IOM report emphasized, every mammalian cell has a sexual signature and basic cell chemistry. These two papers, as well as Wald and Wu (2010), observe that much of the male sex bias, at least for whole-animal studies, is based on convenience; that is, on the assumption that females, with complications such as estrus cycles, are more difficult to study. They also cite data indicating that, in fact, they do not pose more complications.

In our own discipline, we should recognize that sexually dimorphic response profiles may also offer clues to toxic mechanisms. For example, only one sex may be sensitive to a specific exposure for a specific endpoint. The endocrine disruptor literature is a useful source of such instances. In one, Rubin et al. (2006) found that developmental exposure to Bisphenol A (BPA) produced masculinization of a specific structure in female mouse brains but no effect in male brains. Braun et al. (2009) observed increased internalizing behavior in young girls exposed prenatally to BPA but no change in boys. Swan et al. (2010) reported that prenatal phthalate exposure reduced masculine play behavior in young boys but was without effect in girls. Using the same play inventory, Vreugdenhil et al. (2002) showed feminization of play behavior in boys and masculinization of play behavior in girls as a consequence of prenatal exposure to PCBs, but feminization in both sexes by dioxins. All of these changes result from altered brain development, a prime target of neurotoxicology research.

Early development is the phase of the life cycle that has attracted the most attention from neurotoxicologists. But as a society we are facing a demographic storm in the shape of an aging population and neurodegenerative diseases. They are strongly sex-linked, as seen in Figure 4. The sexually-differentiated endocrine linkages are discussed in Weiss (2007). Trying to study and model such diseases without considering sex detaches the outcomes from reality.

A British theatre comedy, *No Sex Please, We're British*, enjoyed a long run on the West End in London. The scientific version, *No Sex Please, We're Neurotoxicologists*, does not deserve equivalent success.

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Federal Government Recognition of Sex Differences in Research Protocols

- 1985 NIH issues guidelines for including women in clinical research
- 1993 Congress mandates inclusion of both sexes in research funded by NIH
- 1993 FDA requires examination of sex differences in clinical trials of new drugs
- 2001 IOM issues report, "*Exploring the Biological Contributions to Human Health: Does Sex Matter?*"

FIGURE 1.
History of sex difference protocols.

FIGURE 2.
Human fetal sex differentiation

TRANSMITTER	BRAIN REGION	SEX
5-HT TRANSPORTER	BRAINSTEM	F>M
5-HT_{1A} RECEPTOR	AMYGDAL, CORTEX, HIPPOCAMPUS, CINGULATE	F>M
5-HT_{2A} RECEPTOR	CORTEX	F>M
DOPAMINE TRANSPORTER	STRIATUM, CAUDATE	F>M
DOPAMINE SYNTHESIS	CAUDATE, PUTAMEN	F>M
D₂ RECEPTOR	STRIATUM	F<M AFFINITY
D₂ RECEPTOR	FRONTAL CORTEX	F>M BINDING

FIGURE 3.
Neurochemical sex differences

Sex Differences in Neurological Disorders		
DISORDER	F/M RATIO	COMMENT
Parkinson's disease	1:3.5	E2 reduces female risk
Alzheimer's disease	2.4:1	E2 reduces female risk
Amyotrophic Lateral Sclerosis	1:1.6	Increases after menopause
Multiple Sclerosis	2.5:1	E2 possibly beneficial
Depression	2:1	Peak onset ages 30-44
ADHD	1:2.1	Features vary by sex
Autism	1:4	Features vary by sex
Tourette's syndrome	1:4	Dopamine mechanisms
Schizophrenia	1:1.4	Brain sex differences

FIGURE 4.
Neurological disorders by sex

PHYLA	FEMALE	MALE	CHEMICAL
Insecta	metabolism↑	Metabolism ↔	Permethrin
Crustacea	ovodeposition ↑	mating behavior↓	Aroclor 1254
Mollusca	male accessory sex organs↑	accessory sex organs↑↑	Organic tin

FIGURE 5.
Sex differences in invertebrates

FIGURE 6.
Examples of books on sex differences

FIGURE 7.
Influence of distributions on evaluation of sex differences