SCIENTIFIC ABSTRACTS ON CHEMICAL ENDOCRINE DISRUPTION AND ITS EFFECTS ON THE REPRODUCTIVE SYSTEM

Scientific abstracts on Chemical endocrine disruption and its effects on the reproductive system

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Review

Disruption of androgen receptor signaling in males by environmental chemicals

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ABSTRACT

Androgen-disruptors are environmental chemicals in that interfere with the biosynthesis, metabolism or action of endogenous androgens resulting in a deflection from normal male developmental programming and reproductive tract growth and function. Since male sexual differentiation is entirely androgen-dependent, it is highly susceptible to androgen-disruptors. Animal models and epidemiological evidence link exposure to androgen disrupting chemicals with reduced sperm counts, increased infertility, testicular dysgenesis syndrome, and testicular and prostate cancers. Further, there appears to be increased sensitivity to these agents during critical developmental windows when male differentiation is at its peak. A variety of *in vitro* and *in silico* approaches have been used to identify broad classes of androgen disrupting molecules that include organochlorinated pesticides, industrial chemicals, and plasticizers with capacity to ligand the androgen receptor. The vast majority of these synthetic molecules act as anti-androgens. This review will highlight the evidence for androgen disrupting chemicals that act through interference with the androgen receptor, discussing specific compounds for which there is documented *in vivo* evidence for male reproductive tract perturbations.

This article is part of a Special Issue entitled 'Endocrine disruptors'.

1. Introduction

Male reproductive health is defined by both the proper development of the reproductive system and maintenance of function throughout adult life, including the capacity to reproduce. While female sexual differentiation, considered the default developmental pathway, is largely independent of estrogens and androgens, male sexual differentiation is driven by androgens produced by the fetal testes and is entirely androgen-dependent [1,2]. Consequently, it is expected that endocrine-disrupting chemicals (EDCs) that interfere with androgen action will have a greater impact on male developmental programming and reproductive tract maturation.

In contrast to estrogenic modes of action, relatively little is known about how androgenic/antiandrogenic EDCs at environmentally relevant concentrations affect male reproductive tract health. Androgens mediate a wide range of developmental and physiological responses in the male and are crucial for testicular and accessory sex gland development and function, pubertal sexual maturation in multiple organs, maintenance of spermatogenesis and maturation of sperm, male gonadotropin regulation through feedback loops and various male secondary characteristics such as bone mass, musculature, fat distribution and hair patterning [2,3]. Testosterone and its metabolite 5-α-dihydrotestosterone (DHT), the primary androgenic hormones, mediate their biological effects predominantly through binding of the androgen receptor (AR), which is expressed in many end-organs including the hypothalamus, pituitary, liver, prostate, and testes [3]. There are multiple sites whereby EDCs can interfere with androgen-dependent mechanisms and affect male reproductive tract health and these include androgen synthesis, metabolism and clearance, feedback regulation, AR expression in target organs, and direct AR binding [4–9]. This review will focus on EDCs that ligand the AR and in so doing, behave *in vitro* as AR antagonists and/or, in a few cases, as AR agonists. Further, we will highlight the *in vivo* evidence that some of these man-made chemicals interfere with biological processes and in so doing, disrupt male reproductive tract health and well-being.

2. Androgen receptor

The actions of androgens within target cells are transduced by the low abundance intracellular AR, the number 4 member of the NR3C subgroup of a nuclear receptor superfamily that mediates the action of steroid hormones [10]. The human *AR* cDNA was first cloned in 1988 [11,12] and an AR has since been described in a number of species including, mouse [13], rat [14], rabbit [15] monkey [16] and fish [17,18]. The single-copy androgen receptor gene is localized on the human X chromosome between q11–q13 [19] and contains 8 exons with a total length of 90 kb. As schematized in Fig. 1, the large AR gene encodes a 115–120 kDa modular protein

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Gonadal Hormones and Brain Development: Cellular Aspects of Sexual Differentiation

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SYNOPSIS. Sexual differentiation of the neural control of reproductive function with respect to both gonadotropin secretion and sexual behavior is thought to result from exposure of the brain to testicular androgens during a very restricted or critical period of CNS differentiation and development, when the tissue is competent to respond to the hormone, and after which it is refractory or responds in a reversible manner. This paper reviews the cellular aspects of sexual differentiation with particular emphasis on the morphological expression of the gonadal hormonal effects in the adult brain. It presents experimental evidence for the morphogenetic basis for the observed steroid effects by showing how the addition of steroid to undifferentiated hypothalamic cultures produces a selective neuritic response that is steroid-dependent. These results suggest that since afferent axonal input and temporal factors are critical for dendritic and synaptic differentiation, steroid-induced variations in neuritic development could result in gender-specific responses seen in sexual differentiation of reproductive function.

INTRODUCTION

Sexual differentiation of the neural control of reproductive function in mammals with respect to both gonadotropin secretion and sexual behavior is thought to result from exposure of the brain to testicular androgens during a very restricted or critical period of Central Nervous System (CNS) differentiation and development, when the tissue is competent to respond to the hormone, and after which it is refractory or responds in a reversible manner. Although most of the studies on sexual differentiation of the brain have been carried out in rodents, the basic concepts have been shown to exist in many other species, including primates. It must always be kept in mind, however, that although the underlying principles of hormonal action are probably valid for all of them, the physiological functions involved, the neural sites affected, the timing of the critical period and even the very hormone(s) responsible may vary widely among species.

For the rodent in whom the influence of gonadal hormones on sexual differentiation of the brain has been most extensively studied (see Gorski, 1971, 1973, for review), the critical period for steroid sensitivity occurs perinatally, extending into the early neonatal period. In the rat, sensitivity of the brain to steroid may be shown to extend from the 18th day of gestation to the 11th postnatal day (Barraclough, 1971; Lobl and Gorski, 1974). Single subcutaneous steroid injections or neonatal castration, however, are maximally effective only during the first five postnatal days—the period which may thus be considered as the physiological one.

During the critical period, androgens masculinize the neonatal male rodent with respect to the post-pubertal development of masculine behavior and the non-cyclic or tonic patterns of gonadotropin release. It is generally presumed that in the neonatal female, the absence of testicular secretions results in the development of the female pattern of sexual behavior and the

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Review

Estrogenic environmental chemicals and drugs: Mechanisms for effects on the developing male urogenital system

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ABSTRACT

Development and differentiation of the prostate from the fetal urogenital sinus (UGS) is dependent on androgen action via androgen receptors (AR) in the UGS mesenchyme. Estrogens are not required for prostate differentiation but do act to modulate androgen action. In mice exposure to exogenous estrogen during development results in permanent effects on adult prostate size and function, which is mediated through mesenchymal estrogen receptor (ER) alpha. For many years estrogens were thought to inhibit prostate growth because estrogenic drugs studied were administered at very high concentrations that interfered with normal prostate development. There is now extensive evidence that exposure to estrogen at very low concentrations during the early stages of prostate differentiation can stimulate fetal/neonatal prostate growth and lead to prostate disease in adulthood. Bisphenol A (BPA) is an environmental endocrine disrupting chemical that binds to both ER receptor subtypes as well as to AR. Interest in BPA has increased because of its prevalence in the environment and its detection in over 90% of people in the USA. In tissue culture of fetal mouse UGS mesenchymal cells, BPA and estradiol stimulated changes in the expression of several genes. We discuss here the potential involvement of estrogen in regulating signaling pathways affecting cellular functions relevant to steroid hormone signaling and metabolism and to inter- and intra-cellular communications that promote cell growth. The findings presented here provide additional evidence that BPA and the estrogenic drug ethinylestradiol disrupt prostate development in male mice at administered doses relevant to human exposures.

Contents

Abbreviations: AR, androgen receptor; Ar, androgen receptor gene; Bmp4, bone morphogenetic protein 4; BPA, bisphenol A; Capn6, Calpain; Cyp7b1, cytochrome P450, family 7, subfamily b, polypeptide 1; DES, diethylstilbestrol; DHT, 5α-dihydrotestosterone; EGF, epidermal growth factor; ERα, estrogen receptor α; ERβ, estrogen receptor β; ERR, estrogen related receptor; Esr1, estrogen receptor; Fgf10, fibroblast growth factor 10; IGF-1, insulin-like growth factor 1; Nkx3.1, NK3 homeobox 1; Q-PCR, quantitative reverse transcriptase-polymerase chain reaction; SERM, selective estrogen receptor modulator; SFRP, secreted frizzled-related protein; Sfrp4, secreted frizzled-related protein 4; Shh, sonic hedgehog; TGF-β, transforming growth factor-β; Thbs2, Thrombospondin 2; UGS, urogenital sinus; Wnt, Wingless-related MMTV integration site family; Wnt 7b, Wingless-related MMTV integration site family member 7b; Wnt11, Wingless-related MMTV integration site family, member 11.

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Steroid Hormones and Brain Development: Some Guidelines for Understanding Actions of Pseudohormones and Other Toxic Agents

by Bruce S. McEwen*

Gonadal, adrenal, and thyroid hormones affect the brain directly, and the sensitivity to hormones begins in embryonic life with the appearance of hormone receptor sites in discrete populations of neurons. Because the secretion of hormones is also under control by its neural and pituitary targets, the brain-endocrine axis during development is in a delicately balanced state that can be upset in various ways, and any agent that disrupts normal hormone secretion can upset normal brain development. Moreover, exogenous substances that mimic the actions of natural hormones can also play havoc with CNS development and differentiation.

This paper addresses these issues in the following order: First, actions of glucocorticoids on the developing nervous system related to cell division dendritic growth and neurotransmitter phenotype will be presented followed by a discussion of the developmental effects of synthetic steroids. Second, actions of estrogens related to brain sexual differentiation will be described, followed by a discussion of the actions of the nonsteroidal estrogen, diethylstilbestrol, as an example of exogenous estrogenic substances. The most important aspect of the potency of exogenous estrogens appears to be the degree to which they either bypass protective mechanisms or are subject to transformations to more active metabolites. Third, agents that influence hormone levels or otherwise modify the neuroendocrine system, such as nicotine, barbiturates, alcohol, opiates, and tetrahydrocannabinol, will be noted briefly to demonstrate the diversity of toxic agents that can influence neural development and affect personality, cognitive ability, and other aspects of behavior.

Because of the growth of neuroscience as a discipline and the increasing recognition of pervasive influences of hormones on brain development and adult brain function, many opportunities exist for expanding our knowledge regarding the actions of environmental toxicants.

Introduction

The brain is a target organ for the actions of hormones secreted by the gonads, adrenals, and thyroid gland, and this sensitivity to hormones begins in embryonic life with the appearance of hormone receptor sites in discrete populations of neurons. Because the secretion of hormones is also under control by its neural and pituitary targets, the brain-endocrine axis during development is in a delicately balanced state that can be upset in various ways. Thus, any agent that disrupts normal hormone secretion can upset normal brain development. Likewise, exogenous substances that mimic the actions of natural hormones can also play havoc with CNS development and differentiation. This article will examine both types of effects, but we shall place special emphasis on the actions of synthetic or natural substances that mimic actions of natural hormones. However, let us first consider the hormone receptors.

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Hormone Receptors in Brain

The brain responds to all six classes of steroid hormones (androgens, estrogens, progestins, glucocorticoids, mineralocorticoids, and vitamin D) and contains receptors for them, as well as for thyroid hormone (*1*). All of these hormone receptors are proteins that contain a hormone-recognizing domain and a domain that binds to specific DNA sequences (*2*). Thus, these receptors exert their effects by binding to specific enhancerlike elements of the genome and modulating (increasing or decreasing) gene expression. Because most of these receptors begin to be expressed in neurons during embryonic life, their presence allows hormones or other molecules that mimic hormone actions (pseudohormones) to affect brain development. Generally speaking, such effects involve induced growth or inhibition of growth of selected groups of neurons, as well as promotion of differentiation of neurotransmitter phenotype or regulatory phenotype (*3*).

The brain does not remain responsive in the same

An Extensive New Literature Concerning Low-Dose Effects of Bisphenol A Shows the Need for a New Risk Assessment

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Bisphenol A (BPA) is the monomer used to manufacture polycarbonate plastic, the resin lining of cans, and other products, with global capacity in excess of 6.4 billion lb/year. Because the ester bonds in these BPA-based polymers are subject to hydrolysis, leaching of BPA has led to widespread human exposure. A recent report prepared by the Harvard Center for Risk Analysis and funded by the American Plastics Council concluded that evidence for low-dose effects of BPA is weak on the basis of a review of only 19 studies; the report was issued after a delay of 2.5 years. A current comprehensive review of the literature reveals that the opposite is true. As of December 2004, there were 115 published *in vivo* studies concerning low-dose effects of BPA, and 94 of these report significant effects. In 31 publications with vertebrate and invertebrate animals, significant effects occurred below the predicted "safe" or reference dose of 50 µg/kg/day BPA. An estrogenic mode of action of BPA is confirmed by *in vitro* experiments, which describe disruption of cell function at 10^{-12} M or 0.23 ppt. Nonetheless, chemical manufacturers continue to discount these published findings because no industry-funded studies have reported significant effects of low doses of BPA, although > 90% of government-funded studies have reported significant effects. Some industry-funded studies have ignored the results of positive controls, and many studies reporting no significant effects used a strain of rat that is inappropriate for the study of estrogenic responses. We propose that a new risk assessment for BPA is needed based on *a*) the extensive new literature reporting adverse effects in animals at doses below the current reference dose; *b*) the high rate of leaching of BPA from food and beverage containers, leading to widespread human exposure; *c*) reports that the median BPA level in human blood and tissues, including in human fetal blood, is higher than the level that causes adverse effects in mice; and *d*) recent epidemiologic evidence that BPA is related to disease in women. *Key words:* bisphenol A, dose response, endocrine disruptors, low dose, nonmonotonic, risk assessment scientific integrity. *Environ Health Perspect* 113:926–933 (2005). doi:10.1289/ehp.7713 available via *http://dx.doi.org/* [Online 13 April 2005]

Bisphenol A (BPA) is a known environmental estrogen that is used as the monomer to manufacture polycarbonate plastic, the resin that is used as linings for most food and beverage cans, as dental sealants, and as an additive in other widely used consumer products. BPA is one of the highest-volume chemicals produced worldwide; global BPA capacity in 2003 was 2,214,000 metric tons (> 6.4 billion lb), with 6–10% growth in demand expected per year (Burridge 2003). Heat and contact with either acidic or basic compounds accelerate hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins. Specifically, heating of cans to sterilize food, the presence of acidic or basic food or beverages in cans or polycarbonate plastic, and repeated washing of polycarbonate products have all been shown to result in an increase in the rate of leaching of BPA (Brotons et al. 1995; Consumers Union 1999; Howdeshell et al. 2003; Kang and Kondo 2002; Kang et al. 2003; Olea et al. 1996; Raloff 1999). In addition, another potential source of human exposure is water used for drinking or bathing. Studies conducted in Japan (Kawagoshi et al. 2003) and in the United States (Coors et al. 2003) have shown that BPA accounts for most estrogenic activity that leaches from landfills into the surrounding ecosystem.

Convincing evidence that there is widespread exposure to BPA is shown by the finding of Calafat et al. (2005) that 95% of urine samples from people in the United States examined by the Centers for Disease Control and Prevention (CDC) have measurable BPA levels [range, 0.4 ppb (10th percentile) to 8 ppb (95th percentile); median = 1.3 ppb]. As described by Calafat et al. (2005), these levels are consistent with findings from other countries. For example, levels of unconjugated (parent) BPA in human blood and tissues are also in the same 0.1–10 ppb range (Ikezuki et al. 2002; Schonfelder et al. 2002) detected by Calafat et al. (2005) in urine. Because there is evidence that BPA is rapidly metabolized (Volkel et al. 2002), these finding suggest that human exposure to significant amounts of BPA must be continuous and via multiple sources. A relationship between blood levels of BPA and body fat in women has been reported (Takeuchi et al. 2004).

In this commentary, we document for the scientific, public health, and regulatory communities that exposure of experimental animals to "low doses" of BPA, which result in tissue levels within and even below the range of human exposure, has been related to adverse effects in a large number of recently published studies. A recent case–control study reporting that blood levels of BPA are related to ovarian disease in women (Takeuchi et al. 2004) adds to our concern. A large number of *in vitro* studies show that effects of BPA are mediated by both genomic and nongenomic estrogen-response mechanisms, with disruption of cell function occurring at doses as low as 1 pM or 0.23 ppt (Wozniak et al. 2005). Although the focus of most studies of effects of BPA has been on its estrogenic activity, recent reports indicating the potential to disrupt thyroid hormone action (Moriyama et al. 2002; Zoeller et al. 2005) mean other modes of action must also be considered. Very low part-per-trillion doses of BPA also cause proliferation of human prostate cancer cells via binding to a mutant form of the androgen receptor expressed in a subpopulation of prostate cancer cells (Wetherill et al. 2002), although BPA acts as an androgen antagonist in the presence of the wild-type androgen receptor (Lee et al. 2003; Paris et al. 2002) and can also block testosterone synthesis (Akingbemi et al. 2004). A comprehensive document containing all of the low-dose BPA references, as well as information concerning mechanisms of action, pharmacokinetics, sources of exposure, and exposure levels in humans, is available online (Endocrine Disruptors Group 2005).

Our current conclusion that widespread exposure to BPA poses a threat to human health directly contradicts several recent reports from individuals or groups associated with or funded by chemical corporations [Association of Plastics Manufacturers in

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ABSTRACT

Bisphenol A (BPA) is the monomer used to manufacture polycarbonate plastic, the resin lining of cans, and other products, with global capacity in excess of 6.4 billion pounds per year. Because the ester bonds in these bisphenol A-based polymers are subject to hydrolysis, leaching of BPA has led to widespread human exposure. A recent report prepared by the Harvard Center for Risk Analysis (HCRA) and funded by the American Plastics Council (APC) concluded that evidence for low-dose effects of BPA is weak based on a review of only 19 studies, and the report was issued after a delay of 2.5 years. A current comprehensive review of the literature reveals that the opposite is true. As of December 2004, there were 115 published studies concerning low-dose effects of BPA, and 94 of these report significant effects. In 31 publications with vertebrate and invertebrate animals, significant effects occurred below the predicted "safe" or reference dose of 50 μg/kg/day BPA. An estrogenic mode of action of BPA is confirmed by *in vitro* experiments, which describe disruption of cell function at 10^{-12} M or 0.23 parts per trillion (ppt). Nonetheless, chemical manufacturers continue to discount these published findings, since no industry-funded studies have reported positive effects of low doses of BPA, while over 90% of government-funded studies have reported positive effects. Some industry-funded studies have ignored the results of positive controls, and many studies reporting negative results used a strain of rat that is inappropriate for the study of estrogenic responses. We propose that a new risk assessment for BPA is needed based on: 1.The extensive new literature reporting adverse effects in animals at doses below the current reference dose, 2. The high rate of leaching of BPA from food and beverage containers, leading to widespread human exposure, 3. Reports that the median BPA level in human blood and tissues, including in human fetal blood, is higher than the level that causes adverse effects in mice, and 4. Recent epidemiological evidence that BPA is related to disease in women.

Bisphenol A (BPA) is a known environmental estrogen that is used as the monomer to manufacture polycarbonate plastic, the resin that lines most food and beverage cans, and as an additive in other widely used consumer products. BPA is one of the highest volume chemicals produced worldwide; global BPA capacity in 2003 was 2,214,000 metric tones (over 6.4 billion pounds), with 6-10% growth in demand expected per year (Burridge, 2003). Heat and contact with either acidic or basic compounds accelerate hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins. Specifically, heating of cans to sterilize food, the presence of acidic or basic food or beverages in cans or polycarbonate plastic, or repeated washing of polycarbonate products have all been shown to result in an increase in the rate of leaching of BPA (Brotons et al. 1995; Olea et al. 1996; CR 1999; SNO 1999; Kang et al. 2002; Howdeshell et al. 2003; Kang et al. 2003). In addition, another potential source of human exposure is in water used for drinking or bathing. Studies conducted in Japan (Kawagoshi et al. 2003) and in the USA (Coors et al. 2003) have shown that BPA accounts for the majority of estrogenic activity that leaches from landfill into the surrounding ecosystem.

Convincing evidence that there is widespread exposure to BPA is shown by the finding that 95% of urine samples from people in the USA examined by the Centers for Disease Control (CDC) have measurable BPA levels [range, 0.4 (10^{th} percentile) – 8 (95^{th} percentile) parts per billion (ppb); median = 1.3 ppb]. As described in the CDC report, these levels are consistent with findings from other countries (Calafat et al. 2005). Levels of unconjugated (parent) BPA in human blood and tissues are also in the same 0.1 – 10 part-per-billion range detected in urine (Ikezuki et al. 2002; Schonfelder et al. 2002). Since there is evidence that BPA is rapidly metabolized (Volkel et al. 2002), these finding suggest that human exposure to significant amounts of BPA must be continuous and via multiple sources. A relationship between blood levels of BPA and body fat in women has been reported (Takeuchi et al. 2004), suggesting that similar to other lipophilic compounds, BPA is stored in fat.

In this commentary we document for the scientific, public health and regulatory communities that exposures of experimental animals to "low doses" of BPA, that result in tissue levels within and even below the range of human exposure, has been related to adverse effects in a large number of recently published studies. A recent case-control study reporting that blood levels of BPA are related to ovarian disease in women adds to our concern (Takeuchi et al. 2004). There are a large number of *in vitro* studies showing that effects of BPA are mediated by both genomic and non-genomic estrogen-response mechanisms, with disruption of cell function occurring at doses as low as 1 pM or 0.23 parts per trillion (ppt) (Wozniak et al. 2005). While the focus of most studies of effects of BPA has been on its estrogenic activity, recent reports indicating the potential to disrupt thyroid hormone action (Moriyama et al. 2002; Zoeller et al. 2005) means other modes of action must also be considered. Very low part-per-trillion doses of BPA also cause proliferation of human prostate cancer cells via binding to a mutant form of the androgen receptor expressed in a sub-population of prostate cancer cells (Wetherill et al. 2002), although BPA acts as an androgen antagonist in the presence of the wild-type androgen receptor (Paris et al. 2002; Lee et al. 2003). A comprehensive document containing all of the low-dose BPA references, as well as information concerning mechanisms of action, pharmacokinetics, sources of exposure, and exposure levels in humans can be accessed at:

http://endocrinedisruptors.missouri.edu/vomsaal/vomsaal.html.

Our current conclusion that widespread exposure to BPA poses a threat to human health directly contradicts several recent reports from individuals or groups associated with or funded by chemical corporations (Gray et al. 2004; Kamrin 2004; Purchase 2004; APM 2005). For example, a recently published report on BPA prepared by a panel convened by the Harvard Center for Risk Analysis (HCRA), and funded by the American Plastics Council (APC), concluded that "the weight of the evidence for low-dose effects is very weak" (Gray et al. 2004). However, the charge to the HCRA panel, which was to perform a weight-of-the-evidence evaluation of available data on the

developmental and reproductive effects of exposure to bisphenol A in laboratory animals, led to an analysis of only 19 out of 47 available published studies on low-dose effects of BPA. The deliberations of the HCRA were in 2001-2002, and accordingly, a cut-off date of April 2002 was selected for consideration of the published literature. It is regrettable that the relevance of the analysis was further undermined by a delay of two-and-a-half years in publication of the report. During the intervening time between April 2002 and the end of 2004, a large number of additional articles reporting low-dose effects of BPA in experimental animals has been published. The result is that by the end of 2004, a PubMed search identified 115 published studies concerning effects of low doses of BPA in experimental animals.

The last US Environmental Protection Agency (US-EPA) risk assessment for BPA was conducted in the 1980s. The most recent risk assessment of BPA was based on a comprehensive review of the scientific literature conducted in 1998 by the European Union, at which time only 5 of the 115 low-dose BPA studies had been published (ECB 2003). We will describe below recent findings concerning mechanisms mediating effects of very low doses of BPA, the adverse effects being reported in animals, as well as recent findings from human studies. These published findings lead us to strongly recommend that a new risk assessment for BPA be initiated.

The Definition of "Low Dose"

We will begin with some comments about the issue of "low dose". The US-EPA considers low-dose effects of environmental endocrine disrupting chemicals to refer to effects being reported for chemicals at doses lower than those used in traditional toxicological studies conducted for risk assessment purposes. For BPA the lowest dose studied was 50 mg/kg/day, which is the currently accepted lowest adverse effect level (LOAEL) that was used to calculate a reference dose of 50 μg/kg/day based on experiments conducted in the 1980s (IRIS 1988).

Xenoestrogens at Picomolar to Nanomolar Concentrations Trigger Membrane Estrogen Receptor-α–Mediated Ca^{2+} Fluxes and Prolactin Release in GH3/B6 Pituitary Tumor Cells

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Xenoestrogens (XEs) are widespread in our environment and are known to have deleterious effects in animal (and perhaps human) populations. Acting as inappropriate estrogens, XEs are thought to interfere with endogenous estrogens such as estradiol (E_2) to disrupt normal estrogenic signaling. We investigated the effects of E_2 versus several XEs representing organochlorine pesticides (dieldrin, endosulfan, *o′,p′*-dichlorodiphenylethylene), plastics manufacturing by-products/detergents (nonylphenol, bisphenol A), a phytoestrogen (coumestrol), and a synthetic estrogen (diethylstilbestrol) on the pituitary tumor cell subline GH3/B6/F10, previously selected for expression of high levels of membrane estrogen receptor-α. Picomolar to nanomolar concentrations of both E_2 and XEs caused intracellular Ca^{2+} changes within 30 sec of administration. Each XE produced a unique temporal pattern of Ca^{2+} elevation. Removing Ca^{2+} from the extracellular solution abolished both spontaneous and XE-induced intracellular Ca^{2+} changes, as did 10 μM nifedipine. This suggests that XEs mediate their actions via voltage-dependent L-type Ca^{2+} channels in the plasma membrane. None of the Ca^{2+} fluxes came from intracellular Ca^{2+} stores. E_2 and each XE also caused unique time- and concentration-dependent patterns of prolactin (PRL) secretion that were largely complete within 3 min of administration. PRL secretion was also blocked by nifedipine, demonstrating a correlation between Ca^{2+} influx and PRL secretion. These data indicate that at very low concentrations, XEs mediate membrane-initiated intracellular Ca^{2+} increases resulting in PRL secretion via a mechanism similar to that for E_2, but with distinct patterns and potencies that could explain their abilities to disrupt endocrine functions. *Key words:* **bisphenol A, coumestrol, DDE, DES, diethylstilbestrol, dieldrin, endosulfan, estrogen receptor-α, exocytosis, L-type channels, membrane, nonylphenol, phytoestrogen, prolactin, xenoestrogen.** *Environ Health Perspect* **113:431–439 (2005).**

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Environmental chemicals with estrogenic activities [xenoestrogens (XEs)] have been implicated in harmful endocrine effects on animals and humans such as the feminization of male animal populations (Kloas et al. 1999; Sumpter 1995), reproductive tract malformations and endometriosis (Gotz et al. 2001; Lee 1998; Steinmetz et al. 1998), disorganization of the central nervous system (Laessig et al. 1999; Oka et al. 2003), and breast and ovarian cancer (Brown and Lamartiniere 1995; Mathur et al. 2002). By acting as estrogen mimetics and binding to estrogen receptors (ERs), XEs may disrupt normal endocrine function, leading to reproductive failure and the induction of tumors in estrogen-sensitive tissues. XEs can also cause alteration of hormone levels via changes in hormone production, metabolism, or transport (Sonnenschein and Soto 1998).

There are many potential endocrine-disrupting chemicals that are prevalent in the environment, or to which humans have been otherwise exposed (Singleton and Khan 2003); in this study we examined several representative compounds. Erroneously used to prevent miscarriages in the 1950s and 1960s, diethylstilbestrol (DES) acts developmentally as a potent estrogen agonist, causing adenocarcinomas, squamous neoplasia of the vagina and cervix (Hatch et al. 2001), oligospermia (vom Saal et al. 1997), and infertility (Palmer et al. 2001). The pesticide *o′,p′*-dichlorodiphenylethylene (DDE) and its metabolites can disorder prostate maturation (Gray et al. 1999). Endocrine disruptors are known to have great impact during fetal development when endogenous hormones regulate cell differentiation and growth, and thus slight alterations in hormonal activity due to endocrine disruption can lead to irreversible changes (Derfoul et al. 2003). However, the abilities of XEs to disrupt adult endocrine function and perhaps to exacerbate estrogen-dependent tumor growth (Soto et al. 1995) are also of concern. We also examined other XEs reported to have estrogen-like activities: detergents such as nonylphenol and bisphenol A (BPA), the organochlorine pesticides dieldrin and endosulfan, and the phytoestrogen coumestrol.

Estrogenic actions have been well studied with respect to genomic responses mediated by nuclear ERs. The nuclear ER-mediated gene transcription responses to XEs are very weak [effective only at 1,000- to 10,000-fold higher concentrations than estradiol (E_2; Massaad and Barouki 1999; Stevens et al. 1994; Witorsch 2002)], leading some to suggest that their presence in our environment is relatively harmless. However, in addition to classical genomic actions, estrogens can act through nongenomic or membrane-initiated signaling pathways via a membrane form of ER (mER). Examples of such actions are alterations in G-protein–coupled receptor responses, protein phosphorylation, lysosomal membrane destabilization, K^+ and Ca^{2+} channel activation, and nitric oxide secretion (reviewed by Watson and Gametchu 1999, 2003). XE actions via nongenomic pathways remain largely unstudied.

Ca^{2+} responses to extracellular stimuli can lead to changes in cell motility, intra- and extracellular signaling processes, and rapid hormone secretion [including prolactin (PRL)] through exocytosis (Campbell 1990; Pappas et al. 1994; Watson et al. 1999a). Changes in PRL secretion are associated with hormonal regulation of lactation, cell proliferation, the cellular immune response, and parental/maternal behavior (Freeman et al. 2000). We recently showed that picomolar to nanomolar concentrations of E_2 and XEs can initiate mitogen-activated protein kinase activation and that several signaling pathways, including Ca^{2+} elevation, may participate in this kinase activation (Bulayeva et al. 2004; Bulayeva and Watson 2004). We also demonstrated the ability of a physiological estrogen (E_2) to elicit cellular Ca^{2+} influx via a membrane version of ER-α (Bulayeva et al. 2005). Here we investigate in more detail the ability of several XEs (DES, coumestrol, *p*-nonylphenol, BPA, DDE, dieldrin, and endosulfan) to induce rapid intracellular Ca^{2+} changes leading to PRL secretion in mER-α–enriched or depleted sublines of GH3/B6 cells (Pappas et al. 1994). Misregulation of such cellular signaling events by XEs could lead to damaging endocrine disruptions such as tissue malformation, cancer, and reproductive system malfunctions.

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Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide

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The impairment of liver function by low environmentally relevant doses of glyphosate-based herbicides (GBH) is still a debatable and unresolved matter. Previously we have shown that rats administered for 2 years with 0.1 ppb (50 ng/L glyphosate equivalent dilution; 4 ng/kg body weight/day daily intake) of a Roundup GBH formulation showed signs of enhanced liver injury as indicated by anatomorphological, blood/urine biochemical changes and transcriptome profiling. Here we present a multiomic study combining metabolome and proteome liver analyses to obtain further insight into the Roundup-induced pathology. Proteins significantly disturbed (214 out of 1906 detected, $q < 0.05$) were involved in organonitrogen metabolism and fatty acid β-oxidation. Proteome disturbances reflected peroxisomal proliferation, steatosis and necrosis. The metabolome analysis (55 metabolites altered out of 673 detected, $p < 0.05$) confirmed lipotoxic conditions and oxidative stress by showing an activation of glutathione and ascorbate free radical scavenger systems. Additionally, we found metabolite alterations associated with hallmarks of hepatotoxicity such as γ-glutamyl dipeptides, acylcarnitines, and proline derivatives. Overall, metabolome and proteome disturbances showed a substantial overlap with biomarkers of non-alcoholic fatty liver disease and its progression to steatohepatosis and thus confirm liver functional dysfunction resulting from chronic ultra-low dose GBH exposure.

Glyphosate-based herbicides (GBH), such as Roundup, are the major pesticides used worldwide[1]. Residues of GBH are routinely detected in foodstuffs[2,3] and drinking water[4]. Epidemiological data on the human body burden of GBH residues is very limited but evidence suggests that glyphosate and its metabolites are widespread[5]. The active principle of GBH, glyphosate, is a competitive inhibitor of phosphoenolpyruvate[6]. Glyphosate acts as a herbicide by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate aromatic amino acid biosynthesis pathway present in plants and some bacteria[7].

A number of toxicity studies have shown that glyphosate and its commercial formulations have non-target effects on mammalian metabolism and provoke toxic effects, especially with respect to liver and kidney structure and function[8,9]. Potential adverse hepatic effects of glyphosate were first observed in the 1980s, including its ability to disrupt liver mitochondrial oxidative phosphorylation[10]. As glyphosate can act as a protonophore increasing mitochondrial membrane permeability to protons and Ca^{2+}[11], it can trigger the production of reactive oxygen species resulting in observed oxidative stress[12]. Elevation in oxidative stress markers is detected in rat liver and kidney after subchronic exposure to GBH at the United States permitted glyphosate concentration of 700 μg/L in drinking water[13]. Hepatic histological changes and alterations of clinical biochemistry are detected in rats consuming 4.87 mg/kg body weight (bw) glyphosate every 2 days over 75 days[14]. In farm animals, elevated glyphosate urinary levels are correlated with alterations in blood serum parameters indicative of liver and kidney oxidative stress and depletion in nutrient trace element levels[15].

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Nevertheless, it should be noted that most results from these GBH toxicity studies were obtained at doses far greater than general human population exposure. Doses tested were typically over the glyphosate acceptable daily intake (ADI), which is currently set at 0.3 mg/kg bw/day within the European Union (1.75 mg/kg bw/day in the USA) based on hepatorenal toxicity measurements after chronic exposure in rats[16,17]. However, no long-term studies investigating the toxicity of complete GBH commercial formulations, which contain a broad spectrum of largely undisclosed "adjuvants" as well as glyphosate, have been conducted (see ref. 9). In an effort to address this gap in commercial GBH toxicity evaluation, a 2-year study was conducted where rats were administered with a Roundup GBH via drinking water at a concentration of 0.1 ppb (0.05 μg/L glyphosate; daily intake 4 ng/kg bw/day), which is an admissible concentration within the European Union (0.1 μg/L) and USA (700 μg/L)[18]. The results showed that Roundup caused an increased incidence in signs of anatomical pathologies, as well as changes in urine and blood biochemical parameters suggestive of liver and kidney functional insufficiency[18].

Most pesticides exert their toxic effects by targeting proteins and modulating their activity. Herbicides act mostly by inhibiting plant enzymes responsible of photosynthesis, carotenoid synthesis, or amino acid synthesis[19]. Besides its well known interaction with EPSPS, it has been suggested that glyphosate could impact mitochondrial function by inhibiting succinate dehydrogenase[20], or on steroid biosynthesis by inhibiting aromatase enzyme activity[21]. Molecular profiling techniques can be used to identify specific signatures of chemical toxicity and thus provide greater insight into organ pathological status[22,23]. The proteome and metabolome are very sensitive to toxic chemical exposures and have been used to reveal non-targets effects of herbicides such as paraquat[24], atrazine[22] and organophosphate mixtures[25] in mammalian species. However, while transcriptome profiles reveal pathway disturbances that could be correlated to toxic effects, they do not always translate into alterations in protein levels and functional, metabolic disturbances. Overall, mRNA transcript abundance explains approximately one- to two-thirds of the variance in steady-state protein levels[26]. In yeast subjected to oxidative stress, a post-transcriptional regulation of a large fraction of the genes was observed independently of their up- or downregulation[27].

Given the insight molecular profiling methods can potentially provide into processes and mechanisms of toxicity, we have previously conducted a transcriptomics investigation of the same female cohort of animals subjected to ultra-low dose Roundup exposure, and which showed signs of liver and kidney damage at a anatomorphological and blood/urine biochemical level of function[28]. Our previous results showed alterations in the liver transcriptome reflective of fibrosis, necrosis, phospholipidosis, mitochondrial membrane dysfunction and ischemia[29]. However, as changes in the transcriptome may not fully translate into alterations in organ function, we hypothesized that a study of the proteome and metabolome of the same liver tissues will provide confirmation of, and possibly a mechanistic link to, the type of liver pathology developed by rats exposed to Roundup. Our results show that proteins whose levels were altered were reflective of oxidative stress and changes in fatty acid metabolism. Proteome alterations were typical of disturbances measured in cases of peroxisomal proliferation, steatosis and necrosis. Metabolome analysis confirmed the induction of oxidative stress, and revealed hallmarks of hepatotoxicity. Overall, metabolome and proteome disturbances showed a substantial overlap with biomarkers of non-alcoholic fatty liver disease (NAFLD) and thus confirm metabolic dysfunction resulting from chronic exposure to an ultra-low dose of Roundup.

Results

The female rat liver tissues, which formed the starting material for this investigation, were as previously described[29]. They were obtained from animals that formed part of a 2 year study of Roundup toxicity[18]. Harlan Sprague–Dawley rats were administered with Roundup via drinking water at a regulatory admissible dose (50 ng/L glyphosate). The average daily intake of Roundup was approximately 4 ng/kg bw/day glyphosate equivalent dose. Control and Roundup-treated animals were respectively euthanized at 701+/− 62 and 635+/− 131 days. Anatomopathological analysis of organs from these animals revealed that the liver was the most affected organ[18]. Roundup-treated female rats showed 3 times more anatomical signs of pathology (15 in 8 rats) than the control group (6 in 4 rats)[18]. Blood samples were collected from the tail vein of each rat under short isoflurane anesthesia after 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months of treatment. Serum biochemical analysis showed increased levels of serum triglycerides (Fig. 1). Thus, although no differences were observed during the first year of the experiment, the rats administered with Roundup started progressively to accumulate serum triglycerides as they aged.

The proteome discovery study consisted of a comparison between control (n = 10) and Roundup treated (n = 10) rat liver samples. Fractions for both non-enriched peptide and enriched phosphopeptides were analysed using Orbitrap Velos-Pro. A total of 1906 peptides were quantified across all liver samples. We began our analysis by looking at the variance structure in an unsupervised Principal Component Analysis (PCA). While percentages of explained variance on the 2 first components were low (22.3% and 14.3% respectively), a separation was observed between control and Roundup-treated rats (Fig. 2A). Of the 1906 quantifiable peptides taken forward for bioanalytical analysis, 214 were respectively found to be significantly regulated (Supplementary Table 3) at the cut off Benjamini-Hochberg adjusted p-value (or q-value) of 0.05 with a fold change (FC) of >1.2. Figure 2B shows the statistical significance of differential protein expression by volcano plot along with their respective FC.

We developed a high throughput Tandem Mass Tag - Selected Reaction Monitoring (TMT-SRM) method to verify the alterations observed in protein levels in liver of Roundup-treated rats. The raw discovery LC-MS/MS spectra from Orbitrap Velos-Pro were used to select transition ions for each peptide (Fig. 3). First, in order to determine a subset of peptides, which can be detected by SRM, individual liver samples marked by a heavy TMT were combined with an internal standard constituted by all 20 samples marked by a light TMT. This method was repeated 4 times and any peptides/transitions, which were not detected in all 4 repeats removed. A total of 9 proteins and 10 peptides have been analysed over a 35 minute gradient. Then, 20 combined (TMTLight and TMTHeavy) liver samples were analysed on the TSQ Vantage in triplicate, leading to the production of 60 raw

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Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats

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ABSTRACT

Glyphosate-based herbicides (GBHs) are widely used in agriculture. ***Recently, several animal and epidemiological studies have been conducted to understand the effects of these chemicals as an endocrine disruptor for the gonadal system.*** The aim of the present study was to determine whether GBHs could also disrupt the hypothalamic-pituitary-thyroid (HPT) axis. Female pregnant Wistar rats were exposed to a solution containing GBH Roundup® Transorb (Monsanto). The animals were divided into three groups (control, 5 mg/kg/day or 50 mg/kg/day) and exposed from gestation day 18 (GD18) to post-natal day 5 (PND5). Male offspring were euthanized at PND 90, and blood and tissues samples from the hypothalamus, pituitary, liver and heart were collected for hormonal evaluation (TSH—Thyroid stimulating hormone, T3—triiodothyronine and T4—thyroxine), metabolomic and mRNA analyses of genes related to thyroid hormone metabolism and function. The hormonal profiles showed decreased concentrations of TSH in the exposed groups, with no variation in the levels of the thyroid hormones (THs) T3 and T4 between the groups. Hypothalamus gene expression analysis of the exposed groups revealed a reduction in the expression of genes encoding deiodinases 2 (*Dio2*) and 3 (*Dio3*) and TH transporters *Slco1c1* (former *Oatp1c1*) and *Slc16a2* (former *Mct8*). In the pituitary, *Dio2*, thyroid hormone receptor genes (*Thra1* and *Thrb1*), and *Slc16a2* showed higher expression levels in the exposed groups than in the control group. Interestingly, *Tshb* gene expression did not show any difference in expression profile between the control and exposed groups. Liver *Thra1 and Thrb1* showed increased mRNA expression in both GBH-exposed groups, and in the heart, *Dio2*, *Mb*, *Myh6* (former *Mhca*) and *Slc2a4* (former *Glut4*) showed higher mRNA expression in the exposed groups. Additionally, correlation analysis between gene expression and metabolomic data showed similar alterations as detected in hypothyroid rats. Perinatal exposure to GBH in male rats modified the HPT set point, with lower levels of TSH likely reflecting post-translational events. Several genes regulated by TH or involved in TH metabolism and transport presented varying degrees of gene expression alteration that were probably programmed during intrauterine exposure to GBHs and reflects in peripheral metabolism. ***In conclusion, the role of GBH exposure in HPT axis disruption should be considered in populations exposed to this herbicide.***

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Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

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For decades, studies of endocrine-disrupting chemicals (EDCs) have challenged traditional concepts in toxicology, in particular the dogma of "the dose makes the poison," because EDCs can have effects at low doses that are not predicted by effects at higher doses. Here, we review two major concepts in EDC studies: low dose and nonmonotonicity. Low-dose effects were defined by the National Toxicology Program as those that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies. We review the mechanistic data for low-dose effects and use a weight-of-evidence approach to analyze five examples from the EDC literature. Additionally, we explore nonmonotonic dose-response curves, defined as a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined. We provide a detailed discussion of the mechanisms responsible for generating these phenomena, plus hundreds of examples from the cell culture, animal, and epidemiology literature. We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. *Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities.* We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. *Thus, fundamental changes in chemical testing and safety determination are needed to protect human health.* (*Endocrine Reviews* 33: 378–455, 2012)

Abbreviations: A4, Androstenedione; AhR, aryl hydrocarbon receptor; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; EDC, endocrine-disrupting chemical; EPA, Environmental Protection Agency; ER, estrogen receptor; FDA, Food and Drug Administration; GLP, good laboratory practices; LOAEL, lowest observed adverse effect level; mER, membrane-associated ER; NHANES, National Health and Nutrition Examination Survey; NIS, sodium/iodide symporter; NMDRC, nonmonotonic dose-response curve; NOEL, no observed effect level; NOAEL, no observed adverse effect level; NTP, National Toxicology Program; PIN, prostatic intraepithelial neoplasias; POP, persistent organic pollutants; ppb, parts per billion; SERM, selective ER modulator; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WoE, weight of evidence.

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Glyphosate

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Contents

Summary

Background

Glyphosate, commonly known by its original trade name Roundup™ (manufactured by Monsanto), is the world's most widely used herbicide. Glyphosate-based herbicides are manufactured by many companies in many countries.

The herbicidal action of glyphosate is primarily due to its capacity to block the production of essential amino acids in plants and some microorganisms through a pathway called "shikimate", which is present only in plants. Thus, it was sold as 'safe' for animals and humans.

Glyphosate is sprayed on numerous crops and plantations, including about 80% of genetically modified (GM) crops (canola, corn, cotton, soybean, sugar beet); with relatively high levels permitted as residues in food and animal feed. It is used as a pre-harvest desiccant, and because it is a systemic herbicide it cannot be completely removed from food by washing, peeling or processing. It is widely used in home gardens and public places including roadsides, and semi-

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natural and natural habitats. Human exposure is widespread and constantly recurring. Residues are widespread in foods, particularly those containing cereals (from pre-harvest use) or GM corn or soy-derived products. It has been detected in drinking water, wine and beer, and even in non-food products derived from GM cotton. The extent of human exposure is reflected in the widespread presence of glyphosate in human urine wherever it has been tested, principally in Europe. It has also been found in urine and breast milk in the USA.

Very aggressive public relations and marketing by its developer, Monsanto, has resulted in the widespread belief that glyphosate is 'safe'. For example, Monsanto claimed that glyphosate is 'biodegradable' and that it 'left the soil clean'. However, in 2009, France's Supreme Court upheld judgements by two previous courts that these claims were false (Anon 2009). Registration processes continue to allow the use of the herbicide without raising concerns about safety even as new data identifying adverse effects emerges.

However, the 2015 classification by the International Agency for Research on Cancer (IARC) of glyphosate as a probable human carcinogen is resulting in massive widespread concern about its continued use, especially preharvest and in public places. Additionally, independent scientific studies and widespread poisonings in Latin America (resulting from aerial application) have begun to reveal numerous acute and chronic effects of glyphosate-based herbicides.

As a result, national bans and restrictions, and voluntary action by local authorities and retailers to curb use are rising dramatically. Sri Lanka was the first country to ban it completely, although that ban may be partially relaxed. The European Union has extended approval for glyphosate for only 18 months instead of the usual 15 years, has banned the use of the surfactant POEA in formulations, and proposed minimised preharvest use and use in public places. The European Food Safety Authority stated that there are so many data gaps for POEA that establishing acceptable exposure limits is impossible. Italy has also banned the preharvest use of glyphosate, its use in public places and those frequented by children and the elderly, and non-agricultural use on soils with high sand content to reduce the potential for contamination of groundwater.

Huge production capacity for glyphosate in China has resulted in the world being oversupplied with the herbicide. Total global production capacity is more than twice the global demand, putting pressure on the industry to decrease prices and disperse GM Roundup Ready crops.

Highly Hazardous Pesticide

IARC's classification of glyphosate as a probable human carcinogen means that it now meets the criteria for a Highly Hazardous Pesticide as defined by PAN (PAN International 2016b) and by FAO/WHO Joint Meeting on Pesticide Management as implemented by FAO in Mozambique (Come et al 2013).

Poisonings

Glyphosate herbicides have been frequently used in self-poisonings and many deaths have occurred, especially in Asia, from as little as 3/4 of a cup of formulated product.

There have also been many cases of unintentional poisonings amongst users and bystanders, the former often experiencing severe chemical burns and respiratory problems.

Widespread poisonings have occurred in Latin America as a result of aerial spraying of GM soybean crops, and of coca crops in Colombia—effects being recorded as far as 10 km away from the supposed spray zone. The coca spraying (instigated by a US government-funded programme to eliminate cocaine production in Colombia) was reported to have also resulted in widespread animal deaths.

Doctors in Argentina report vomiting, diarrhoea, respiratory problems and skin rashes in association with aerial spraying of glyphosate on GM crops. Other acute symptoms of poisoning commonly reported from unintentional exposure include abdominal pain, gastrointestinal infections, itchy or burning skin, skin infections (particularly prevalent in children), blisters, burning or weeping eyes, blurred vision, conjunctivitis, headaches, fever, rapid heartbeat, palpitations, raised blood pressure, dizziness, chest pains, numbness, insomnia, depression, debilitation, difficulty in breathing, respiratory infections, dry cough, sore throat, and unpleasant taste in the mouth. Less common effects reported include balance disorder, reduced cognitive capacity, seizures, impaired vision, smell, hearing and taste, drop in blood pressure, twitches and tics, muscle paralysis, peripheral neuropathy, loss of gross and fine motor skills, excessive sweating, and severe fatigue.

Acute toxicity

Glyphosate has a low toxicity rating (WHO Table 5) despite the substantial evidence of adverse health effects. Surfactants added to formulated glyphosate products may be more toxic: the surfactant POEA present in many formulations is about 5 times more toxic than the glyphosate itself. There are a number of other chemicals added to glyphosate formulations or contaminating them; some are known to be harmful, but many are regarded as trade secrets and it is unknown which might be contributing to the health effects.

Long-term toxicity

Glyphosate-based herbicides can interfere with numerous mammalian organs and biochemical pathways, *including inhibition of numerous enzymes, metabolic disturbances and oxidative stress leading to excessive membrane lipid peroxidation, and cell and tissue damage.* Genotoxicity and endocrine disruption also lead to chronic health and developmental effects.

Glyphosate has long been known to have antimicrobial properties, and was patented by Monsanto as an antimicrobial in 2010, with claims to be active against a very wide range of organisms. Recent studies show it can cause imbalances in the normal gastrointestinal microbiome, increasing vulnerability to pathogenic bacteria and influencing the response to antibiotics and intestinal functioning, in humans and animals.

Scientists have also found harmful effects on human cells at levels of glyphosate too low to have a herbicidal effect, some at levels similar to those found in food. These effects are amplified by the adjuvants in the Roundup formulation, which assist penetration of the cells by glyphosate. Several researchers have reported that glyphosate appears to accumulate in human cells.

Glyphosate at low concentrations damages liver, kidney and skin cells; in the latter, it causes aging and potentially cancer. Its ability to penetrate skin increases 5-fold when skin is damaged.

Doctors in Argentina have reported a dramatic upsurge in long-term effects in areas where genetically modified soy crops are aerial-sprayed with glyphosate. They include cancer, infertility, pregnancy problems, birth defects, and respiratory diseases.

Kidney

Kidney and liver are the main target organs for glyphosate, and a wide range of adverse effects are reported from laboratory studies, including cell damage and death, DNA damage and tumours. Glyphosate is implicated in an epidemic of 'chronic kidney disease of unknown cause' (CKDu) amongst farmers in Sri Lanka, Andhra Pradesh (India), and Central America, in part because of the herbicide's ability to chelate nephrotoxic metals.

Cancer, genotoxicity

The IARC monograph on glyphosate, published in 2015, concludes that "there is limited evidence in humans for carcinogenicity of glyphosate" and "there is sufficient evidence in experimental animals for the carcinogenicity of glyphosate". Besides evidence from carcinogenicity studies in rats and mice, the IARC considered as a rationale "two key characteristics of known human carcinogens" and concluded that there is strong evidence that exposure to glyphosate or glyphosate-based formulations *is genotoxic and can induce oxidative stress. The latter mechanism was also ascribed to aminomethylphosphonic acid (AMPA), the major metabolite of glyphosate.* As a result the IARC classified glyphosate as probably carcinogenic to humans (Group 2A).

In the same year, the European Food Safety Authority (EFSA) insisted on its evaluation that glyphosate is neither carcinogenic nor genotoxic, thereby joining similar assessments made earlier by the International Programme on Chemical Safety (IPCS) and the United States Environmental Protection Agency (US EPA). This occurs in spite of substantial laboratory and some epidemiological evidence that continues to accumulate and points to the opposite conclusion.

The evaluation of glyphosate by the European Chemicals Agency (ECHA) is still ongoing. Final results are expected by end of 2017 when the extension of the current approval for glyphosate in the European union also expires.

Studies have demonstrated that glyphosate and/or Roundup cause genetic damage in human lymphocytes and liver cells; bovine lymphocytes; mouse bone marrow, liver, and kidney cells; fish gill cells and erythrocytes; caiman erythrocytes; tadpoles; sea urchin embryos; fruit flies; root-tip cells of onions; and in Salmonella bacteria. Other studies have shown that it causes oxidative

stress, cell-cycle dysfunction, and disruption to RNA transcription, all of which can contribute to carcinogenicity.

Several epidemiological studies have linked exposure to glyphosate with non-Hodgkin's lymphoma, hairy cell leukaemia, multiple myeloma, and DNA damage.

Glyphosate and Roundup caused DNA damage in human buccal cells, and was clastogenic in mouse bone marrow cells, adding to a number of previous studies showing it to be genotoxic.

Endocrine disruption

A number of studies have demonstrated that both glyphosate and the Roundup formulation disrupt oestrogen, androgen, and other steroidogenic pathways, and cause the growth of human breast cancer cells.

One study summarises these effects occurring at doses substantially lower than those used in agriculture, or permitted as residues: *at 0.5 mg/kg (40 times lower than levels permitted in soybeans in the US) they were anti-androgenic; at 2 mg/kg they were anti-oestrogenic; at 1 mg/kg they disrupted the enzyme aromatase; at 5 mg/kg they damaged DNA, and at 10 mg/kg they were cytotoxic.* These effects can result in adverse effects in sexual and other cell differentiation, bone metabolism, liver metabolism, reproduction, development and behaviour, and *hormone-dependent diseases such as breast and prostate cancer* (Gasnier et al 2009).

In vivo experiments in rats show that low levels of glyphosate-based herbicides disrupt the production of testosterone, oestradiol and other steroid hormones, down-regulate the expression of oestrogen progesterone receptors, induce the aromatase activity and protein levels in the testis and cause abnormal sperm morphology.

The implications of the endocrine-disrupting effects can be profound and far-reaching, involving a range of developmental impacts including sexual and other cell differentiation, bone metabolism, liver metabolism, lipid metabolism, reproduction, pregnancy, growth, brain and organ development, cognition, behaviour, and endocrine-related diseases such as breast, testicular and prostate cancer, neurodegenerative and metabolic disorders (diabetes, obesity).

Reproductive and developmental

Exposure to glyphosate-based herbicides, even at very low doses, may result in reproductive problems including miscarriages, pre-term deliveries, low birth weights, and birth defects. Laboratory studies have shown that very low levels of glyphosate, Roundup, POEA, and the metabolite AMPA all kill human umbilical, embryonic and placental cells. Roundup can kill testicular cells, reduce sperm numbers, increase abnormal sperm, retard skeletal development, and cause deformities in amphibian embryos.

Monsanto has known since the 1980s, and the German government since 1998, that glyphosate causes birth defects. After analysing the industry data reported in the German authorities 1998 draft assessment report, independent scientists concluded: "a substantial body of evidence demonstrates that glyphosate and Roundup cause teratogenic effects and other toxic effects on reproduction", including heart, kidney, skeletal, lung and cranial problems (Antoniou et al 2012).

More recent studies show malformation in the heads of frogs that are similar to birth defects amongst people exposed to aerial spraying of Roundup over GM soy crops in Latin America.

Neurological

Glyphosate is assumed by regulators to have no neurological effects—the US EPA did not require neurotoxicity studies to be carried out for the registration of Roundup. *However, a number of studies have shown that glyphosate can adversely affect nerve cells and affect neuronal development. There is emerging evidence that glyphosate can affect areas of the brain associated with Parkinson's disease, particularly the dopaminergic neurons. Epidemiological and case studies link glyphosate exposure with parkinsonian, Attention-Deficit/ Hyperactivity Disorder (ADHD) and autism.*

Immune

Several studies indicate that glyphosate formulations may interfere with the immune system resulting in adverse respiratory effects including asthma, rheumatoid arthritis, and autoimmune skin and mucous membrane effects.

Environmental effects

Glyphosate has direct eco-toxicological effects and indirect effects. The later result from the unprecedented elimination of flora termed weeds. Direct and indirect effects have cascading impacts on the food chain and on biodiversity. Ecosystem functions of insects, such as natural pest control and pollination services, are jeopardised by the almost complete elimination of *weeds because these plants are essential to most beneficial species.* This may lead to huge difficulties in returning to ecologically sound agricultural systems. In aquatic ecosystems, the direct eco-toxicological effects of glyphosate of greatest concern are those that occur at a subtle level, which can result in significant disruption of the ecosystem.

Aquatic effects

Glyphosate is water soluble, and is increasingly found in the environment at levels that have caused significant effects on species that underpin the entire aquatic food chain. Glyphosate and/or Roundup can alter the composition of natural aquatic communities, potentially tipping the ecological balance and giving rise to harmful algal blooms. It can have profound impacts on microorganisms, plankton, algae and amphibia at low concentrations: one study showed a 70% reduction in tadpole species and a 40% increase in algae. Insects, crustaceans, molluscs, reptiles, and fish can also be affected, with vulnerability within each group varying dramatically between species. Effects include reproductive abnormalities, developmental abnormalities and malformations, DNA damage, immune effects, oxidative stress, modified enzyme activity, decreased capacity to cope with stress and maintain homeostasis, altered behaviour, and impaired olfaction that can threaten their survival. Amphibians are particularly vulnerable. Roundup is generally more toxic than glyphosate, especially to fish.

Terrestrial effects

Soil & plant health

As with the aquatic environment, it is the subtle effects causing disruption of the ecosystem that are of greatest concern, particularly effects on the agroecosystem. Glyphosate is toxic to some but not all soil microorganisms, altering microbial community dynamics in ways that are harmful to plants and to ecological balance. It increases microorganisms capable of metabolising the chemical. It can reduce some beneficial organisms such as saprophytic fungi that decompose dead plant material and are important for soil fertility. *Numerous studies have shown that glyphosate stimulates the growth of a number of fungal pathogens that cause diseases in many crops.* The upsurge in use of glyphosate in no-till agriculture has brought about a resurgence of some diseases. *Glyphosate binds micronutrients in the soil and causes micronutrient deficiencies in plants that increase their susceptibility to disease, decrease their vigour, and produce micronutrient-deficient food crops. It can reduce the plant's production of lignin and phenolic compounds, which are also important for disease resistance. It can reduce nitrogen fixation in legumes such as soybean.*

Glyphosate can cause metabolic and compositional changes, including altering the nutritional composition of foods, for example the protein and fatty acid content of soybeans. It can cause iron deficiency in soybeans, which is a concern for human health as human iron deficiency is widespread.

Earthworms and beneficial insects

Glyphosate has adverse effects on some earthworms; and a number of beneficial insects useful in biological control, particularly predatory mites, carabid beetles, ladybugs, and green lacewings. It can also adversely affect other insects that play an important part in ecological balance such as springtails, wood louse, and field spiders. *Glyphosate, at levels commonly found in agricultural settings, impairs honeybees' cognitive capacities affecting their navigation with potential long-term negative consequences for colony foraging success*

Birds and other animals

Glyphosate use may result in significant population losses of a number of terrestrial species through habitat and food supply destruction. There have been reports of numerous deaths of livestock and domestic animals as a result of the aerial spraying of glyphosate in Colombia.

Environmental fate

Glyphosate is a widespread environmental contaminant found in soils and sediments, a wide range of surface water bodies, groundwater and the marine environment.

Soils

The European Food Safety Authority (EFSA) describes glyphosate persistence in soil as being low to very high, and that of AMPA as

being moderate to very high, with a half-life varying from less than a week to more than a year and a half, depending on the extent of soil binding and microbial breakdown (glyphosate is broken down by microbial degradation). Residues have been found up to 3 years after application in cold climates. It is less persistent in warmer climates, with a half-life between 4 and 180 days. It is bound onto soil particles, and this was once thought to mean that glyphosate is not biologically active within soil, nor will it leach to groundwater. However, it is now known that it can easily become unbound again, be taken up by plants or leach out, indicating a greater risk of groundwater contamination.

Phosphate fertilisers reduce binding of glyphosate to soil particles, and so increase the amount of unbound glyphosate remaining in the soil, which is available for root uptake, microbial metabolism, and leaching into groundwater. The risk of leaching is greater in fertilised soils. Conversely, the presence of glyphosate in some soils can reduce retention and availability of phosphate reducing soil fertility.

Water

Glyphosate is soluble in water, and slowly dissipates from water into sediment or suspended particles. Although it does break down by photolysis and microbial degradation, it can be persistent for some time in the aquatic environment, with a half-life of up to nearly 5 months, and still be present in the sediment of a pond after 1 year.

Residues of glyphosate have been found in a wide range of ditches, drains, streams, rivers, ponds, lakes and wetlands in many countries including Argentina, Canada, China, throughout Europe, Norway, USA, and the UK; in wastewater in France and Canada, landfill leachate in the UK. Urban use on road and rail sides is contributing significantly to this contamination, with residues being found in sewage sludge and wastewater treatment plants. Contamination of 'vernal pools'—pools that are shallow and disappear in dry weather—are a concern for amphibia, for which these water sources are critical.

Residues have also been found in groundwater in Canada, Austria, Belgium, Denmark, Germany, Ireland, Spain, Sweden, Switzerland, Netherlands, UK, Sri Lanka, and USA. They have been detected in the marine environment off the Atlantic Coast of France; and in marine sediment in New Zealand, believed to have come largely from the spraying of urban roadside vegetation.

Bioaccumulation

EFSA gives a bioconcentration factor (BCF) of 1.2 (+ 0.61). However, bioaccumulation of glyphosate may be greater than predicted). The BCF for glyphosate is increased in the presence of POEA in the aquatic environment. This may be because POEA, which is known to enhance glyphosate transport into plant cells, also facilitates increased permeability in animal cells. A BCF for glyphosate varying between 1.4 and 5.9 was found in freshwater blackworm. Bioaccumulation has also been demonstrated in land snails, fish, aquatic plants. There are also suggestions of bioaccumulation in some human cell studies.

Atmospheric transport and deposition

Glyphosate is of low volatility, and residues in the air have been found in particulate matter, suggesting that airborne transport is via particles with deposition being largely in dust rather than vapour. It has been found in the rain in Belgium, Canada, France, and USA.

Resistance

Weed resistance to glyphosate was first recorded in 1996, in Australia; it is now recorded in 35 species of weeds and in 27 countries, most notably the USA.

Most of this resistance has been caused by the repeated use of glyphosate in GM crops, no-till agriculture, and amenity use. Some has resulted from a gradual evolution of exposed weed species, and some from gene flow from GM crops to weed relatives. The latter has been observed with sugar beet in France, canola in Canada, creeping bentgrass in USA, and also with corn and soybean. Now even Monsanto is recommending the use of other herbicides in addition to glyphosate in Roundup Ready crops (crops genetically modified to be tolerant of Roundup), to slow the onset of resistance in weeds.

So widespread is the resistance now that Dow has developed a GM corn resistant to both 2, 4-D and glyphosate, and Monsanto to develop a soybean resistant to both dicamba and glyphosate.

Climate Change effects

A number of glyphosate's adverse effects can be expected to increase with climate change: higher

temperatures enhance glyphosate's reduction of chlorophyll and carotenoids in freshwater green algae, increase toxicity to fish, and increase susceptibility to *Fusarium* head scab in cereals.

One study has shown that increased levels of carbon dioxide can result in increased tolerance of some grasses to glyphosate, indicating that as climate change progresses, grasses may become less susceptible to the herbicide.

Alternatives

There are numerous design, mechanical and cultivational practices, as well as some non-chemical herbicides based on plant extracts that can be used instead of glyphosate herbicides, depending on the weed species and the situation. Care must first be taken to determine whether the plant regarded as a weed is in fact really a problem to production, or should be regarded as a non-crop plant with beneficial uses or ecosystem services.

Chemical Profile

Identification

Common name

Glyphosate

Common trade name

Roundup

Chemical names and form

N-(phosphonomethyl)glycine

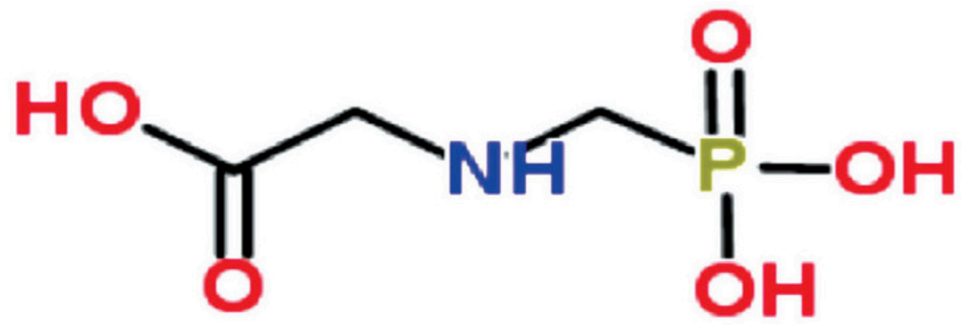
Glyphosate is a weak organic acid that consists of a glycine moiety (part of a molecule) and a phosphonomethyl moiety.

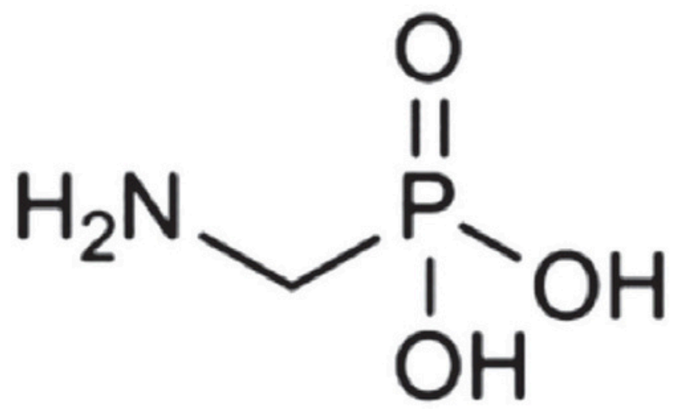
Technical grade glyphosate is a colourless, odourless crystalline powder, formulated as water-soluble concentrates and granules.

Most formulations contain the isopropylamine ammonium salt of glyphosate (glyphosate-isopropyl ammonium).

Molecular formula and structure

$C_3H_8NO_5P$





aminomethylphosphonic acid

Chemical group

Phosphinic acid

Other related chemicals

Glyphosate, diammonium salt
Glyphosate, dimethylammonium salt (glyphosate dimethylamine)
Glyphosate, ethanolamine salt
Glyphosate, monoammonium salt (glyphosate sel d'ammonium)
Glyphosate, potassium salt
Glyphosate, sesquisodium (or sodium) salt
Glyphosate, trimethylsulfonium salt (glyphosate-trimesium)

CAS numbers

Glyphosate	1071-83-6
Isopropylamine salt	38641-94-0
Monoamine salt	114370-14-8
Diammonium salt	69254-40-6
Sesquisodium salt	70393-85-0
Glyphosate-trimesium	81591-81-3
(Aminomethyl)phosphonic acid	1066-51-9

Trade names

Because glyphosate is so widely used and is off-patent, there are now many generic formulations and a very large number of trade names.

Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling

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The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosate-based herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. ***The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior−posterior (A-P) axis***. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. ***Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed,*** rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. ***The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.***

Introduction

The broad-spectrum glyphosate based herbicides (GBHs) are widely used in agricultural practice, particularly in association with genetically modified organisms (GMO) engineered to be glyphosate resistant such as soy crops. Considering the wide use of GBH/GMO agriculture, studies of the possible impacts of GBH on environmental and human health are timely and important. Given the intensive use of this technological package in South America, studies of the possible impacts on environment and human health are absolutely necessary, together with adequate epidemiological studies. The need for information about the developmental impact of GBH is reinforced by a variety of adverse health effects on people living in areas where GBH is extensively used, particularly since there is a paucity of data regarding chronic exposure to sublethal doses during embryonic development.

It is important to note that the bulk of the data provided during the evaluation stages of GBH/GMO safety were provided by the industry. Given the recent history of the endocrine disruptor field with low dose effects observed in numerous academic laboratories but not in industry-funded studies (*1, 2*), it is clear that a reasonable corpus of independent studies is necessary to fully evaluate the effects of agrochemicals on human health. This is particularly important when significant economic interests are concerned.

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There is growing evidence raising concerns about the effects of GBH on people living in areas where herbicides are intensively used. Women exposed during pregnancy to herbicides delivered offspring with congenital malformations, including microcephaly, anencephaly, and cranial malformations (*3*).

Relevant contributions to the subject were made by Seralini's group, among others (*4*). They showed that a GBH acts as an endocrine disruptor in cultures of JEG3 placental cells, decreasing the mRNA levels of the enzyme CYP19 (an essential component of cytochrome p450 aromatase) and inhibiting its activity. CYP19 is responsible for the irreversible conversion of androgens into estrogens. The GBH Roundup is able to disrupt aromatase activity. Importantly, the active principle glyphosate interacts with the active site of the purified enzyme and its effects in cell cultures, and microsomes are facilitated by other components in the Roundup formulation that presumably increase the bioavailability of glyphosate (*4*). Glyphosate penetration through the cell membrane and subsequent intracellular action is greatly facilitated by adjuvants such as surfactants (*5, 6*).

In addition, both glyphosate and the commercial herbicide severely affect embryonic and placental cells, producing mitochondrial damage, necrosis, and programmed cell death by the activation of caspases 3/7 in cell culture within 24 h with doses far below those used in agriculture. Other effects observed include cytotoxicity and genotoxicity, endocrine disruption of the androgen and estrogen receptors, and DNA damage in cell lines (*7, 8*).

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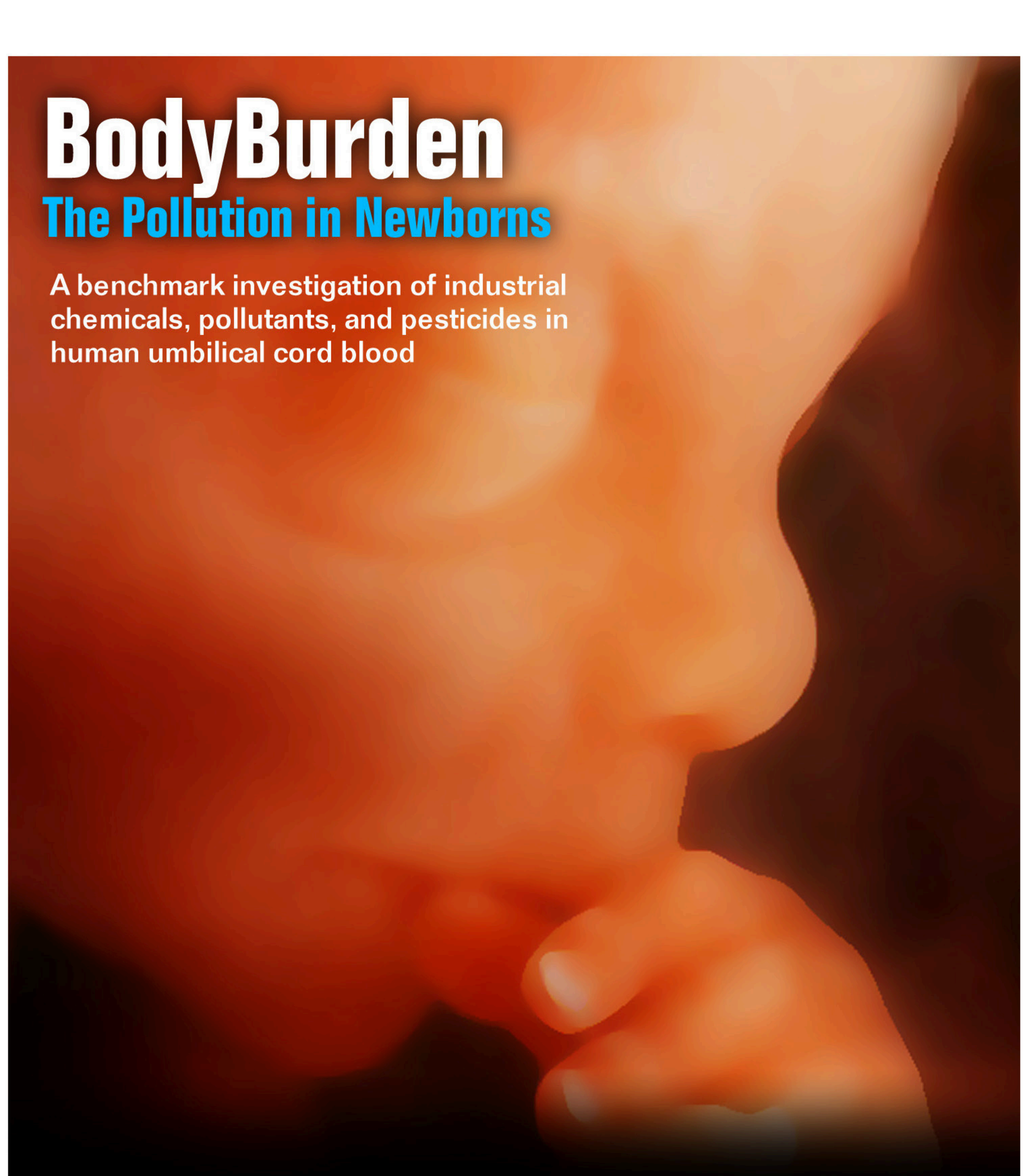
BodyBurden

The Pollution in Newborns

A benchmark investigation of industrial chemicals, pollutants, and pesticides in human umbilical cord blood



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JULY 14, 2005

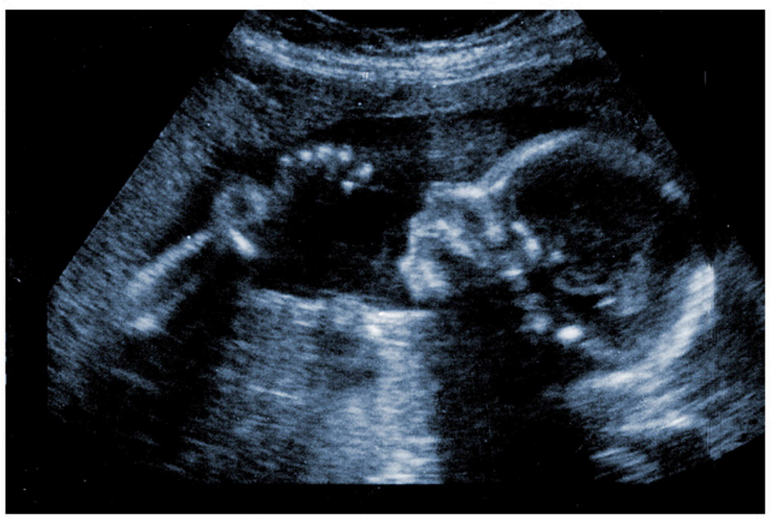
Part 1

Executive Summary

Summary. In the month leading up to a baby's birth, the umbilical cord pulses with the equivalent of 300 quarts of blood each day, pumped back and forth from the nutrient- and oxygen-rich placenta to the rapidly growing child cradled in a sac of amniotic fluid. This cord is a lifeline between mother and baby, bearing nutrients that sustain life and propel growth.

Not long ago scientists thought that the placenta shielded cord blood — and the developing baby — from most chemicals and pollutants in the environment. ***But now we know that at this critical time when organs, vessels, membranes and systems are knit together from single cells to finished form in a span of weeks, the umbilical cord carries not only the building blocks of life, but also a steady stream of industrial chemicals, pollutants and pesticides that cross the placenta*** as readily as residues from cigarettes and alcohol. This is the human "body burden" — the pollution in people that permeates everyone in the world, including babies in the womb.

In a study spearheaded by the Environmental Working Group (EWG) in collaboration with Commonweal, researchers at two major laboratories found an average of 200 industrial chemicals and pollutants in umbilical cord blood from 10 babies born in August and September of 2004 in U.S. hospitals. ***Tests revealed a total of 287 chemicals in the group. The umbilical cord blood of these 10 children, collected by Red Cross after the cord was cut, harbored pesticides, consumer product ingredients, and wastes from burning coal, gasoline, and garbage.***



A DEVELOPING CHILD'S CHEMICAL EXPOSURES ARE GREATER POUND-FOR-POUND THAN THOSE OF ADULTS.

This study represents the first reported cord blood tests for 261 of the targeted chemicals and the first reported detections in cord blood for 209 compounds. Among them are eight perfluorochemicals used as stain and oil repellants in fast food packaging, clothes and textiles — including the Teflon chemical PFOA, recently characterized as a likely human carcinogen by the EPA's Science Advisory Board — dozens of widely used brominated flame retardants and their toxic by-products; and numerous pesticides.

Of the 287 chemicals we detected in umbilical cord blood, we know that 180 cause cancer in humans or animals, 217 are toxic to the brain and nervous system, and 208 cause birth defects

or abnormal development in animal tests. The dangers of pre- or post-natal exposure to this complex mixture of carcinogens, developmental toxins and neurotoxins have never been studied.

Chemical exposures in the womb or during infancy can be dramatically more harmful than exposures later in life. Substantial scientific evidence demonstrates that children face amplified risks from their body burden of pollution; the findings are particularly

Chemicals and pollutants detected in human umbilical cord blood

Mercury (Hg) - tested for 1, found 1

Pollutant from coal-fired power plants, mercury-containing products, and certain industrial processes. Accumulates in seafood. Harms brain development and function.

Polyaromatic hydrocarbons (PAHs) - tested for 18, found 9

Pollutants from burning gasoline and garbage. Linked to cancer. Accumulates in food chain.

Polybrominated dibenzodioxins and furans (PBDD/F) - tested for 12, found 7

Contaminants in brominated flame retardants. Pollutants and byproducts from plastic production and incineration. Accumulate in food chain. Toxic to developing endocrine (hormone) system

Perfluorinated chemicals (PFCs) - tested for 12, found 9

Active ingredients or breakdown products of Teflon, Scotchgard, fabric and carpet protectors, food wrap coatings. Global contaminants. Accumulate in the environment and the food chain. Linked to cancer, birth defects, and more.

Polychlorinated dibenzodioxins and furans (PBCD/F) - tested for 17, found 11

Pollutants, by-products of PVC production, industrial bleaching, and incineration. Cause cancer in humans. Persist for decades in the environment. Very toxic to developing endocrine (hormone) system.

Organochlorine pesticides (OCs) - tested for 28, found 21

DDT, chlordane and other pesticides. Largely banned in the U.S. Persist for decades in the environment. Accumulate up the food chain, to man. Cause cancer and numerous reproductive effects.

Polybrominated diphenyl ethers (PBDEs) - tested for 46, found 32

Flame retardant in furniture foam, computers, and televisions. Accumulates in the food chain and human tissues. Adversely affects brain development and the thyroid.

Polychlorinated Naphthalenes (PCNs) - tested for 70, found 50

Wood preservatives, varnishes, machine lubricating oils, waste incineration. Common PCB contaminant. Contaminate the food chain. Cause liver and kidney damage.

Polychlorinated biphenyls (PCBs) - tested for 209, found 147

Industrial insulators and lubricants. Banned in the U.S. in 1976. Persist for decades in the environment. Accumulate up the food chain, to man. Cause cancer and nervous system problems.

Source: Chemical analyses of 10 umbilical cord blood samples were conducted by AXYS Analytical Services (Sydney, BC) and Flett Research Ltd. (Winnipeg, MB).

strong for many of the chemicals found in this study, including mercury, PCBs and dioxins. Children's vulnerability derives from both rapid development and incomplete defense systems:

- A developing child's chemical exposures are greater pound-for-pound than those of adults.
- An immature, porous blood-brain barrier allows greater chemical exposures to the developing brain.
- Children have lower levels of some chemical-binding proteins, allowing more of a chemical to reach "target organs."
- A baby's organs and systems are rapidly developing, and thus are often more vulnerable to damage from chemical exposure.
- Systems that detoxify and excrete industrial chemicals are not fully developed.
- The longer future life span of a child compared to an adult allows more time for adverse effects to arise.

The 10 children in this study were chosen randomly, from among 2004's summer season of live births from mothers in Red Cross' volunteer, national cord blood collection program. They were not chosen because their parents work in the chemical industry or because they were known to bear problems from chemical exposures in the womb. Nevertheless, each baby was born polluted with a broad array of contaminants.

U.S. industries manufacture and import approximately 75,000 chemicals, 3,000 of them at over a million pounds per year. Health officials do not know how many of these chemicals pollute fetal blood and what the health consequences of in utero exposures may be.

Had we tested for a broader array of chemicals, we would almost certainly have detected far more than 287. But testing umbilical cord blood for industrial chemicals is technically challenging. Chemical manufacturers are not required to divulge to the public or government health officials methods to detect their chemicals in humans. Few labs are equipped with the machines and expertise to run the tests or the funding to develop the methods. Laboratories have yet to develop methods to test human tissues for the vast majority of chemicals on the market, and the few tests that labs are able to conduct are expensive. Laboratory costs for the cord blood analyses reported here were $10,000 per sample.

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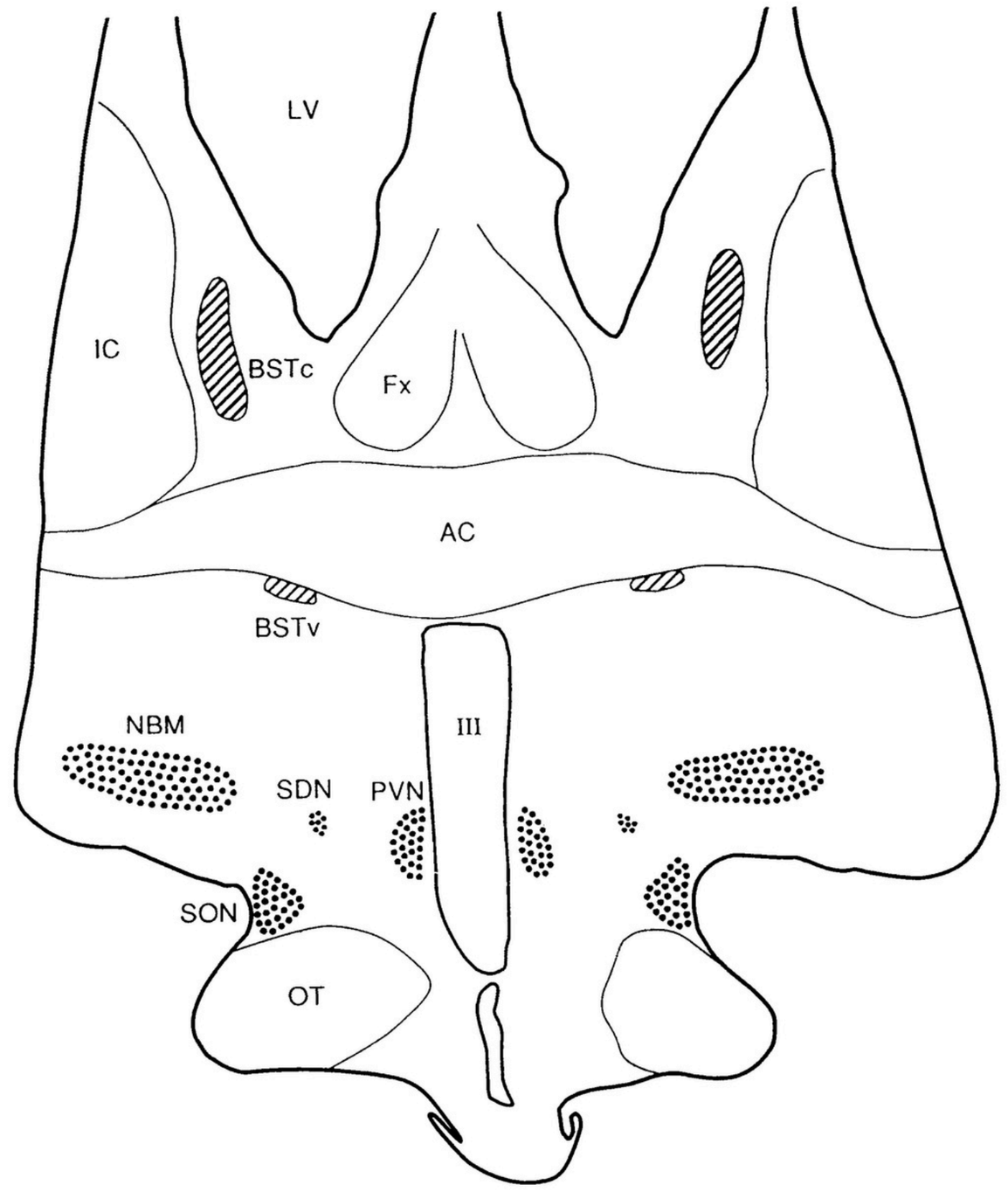

FIG. 1 Schematic frontal section through two subdivisions of the bed nucleus of the stria terminalis (BST). III, third ventricle; AC, anterior commissure; BSTc and BSTv, central and ventral subdivisions of the BST; FX, fornix; IC, internal capsule; LV, lateral ventricle; NBM, nucleus basalis of Meynert; OT, optic tract, PVN, paraventricular nucleus; SDN, sexually dimorphic nucleus; SON, supraoptic nucleus.

A sex difference in the human brain and its relation to transsexuality

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TRANSSEXUALS **have the strong feeling, often from childhood onwards, of having been born the wrong sex. The possible psychogenic or biological aetiology of transsexuality has been the subject of debate for many years[1,2]. Here we show that the volume of the central subdivision of the bed nucleus of the stria terminalis (BSTc), a brain area that is essential for sexual behaviour[3,4], is larger in men than in women. A female-sized BSTc was found in male-to-female transsexuals. The size of the BSTc was not influenced by sex hormones in adulthood and was independent of sexual orientation. Our study is the first to show a female brain structure in genetically male transsexuals and supports the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones[5,6].**

Investigation of the genetics, gonads, genitalia or hormone level of transsexuals has not, so far, produced any results that explain their status[1,2]. In experimental animals, however, the same gonadal hormones that prenatally determine the morphology of the genitalia also influence the morphology and function of the brain in a sexually dimorphic fashion[6,7]. This led to the hypothesis that sexual differentiation of the brain in transsexuals might not have followed the line of sexual differentiation of the body as a whole. In the past few years, several anatomical differences in relation to sex and sexual orientation have been observed in the human hypothalamus (see ref. 6 for a review), but so far no neuroanatomical investigations have been made in relation to the expression of cross-gender identity (transsexuality).

We have studied the hypothalamus of six male-to-female transsexuals (T1–T6); this material was collected over the past eleven years. We searched for a brain structure that was sexually dimorphic but that was not influenced by sexual orientation, as male-to-female transsexuals may be 'oriented' to either sex with respect to sexual behaviour. Our earlier observations showed that the paraventricular nucleus (PVN), sexually dimorphic nucleus (SDN) and suprachiasmatic nucleus (SCN) did not meet these criteria (ref. 6 and unpublished data). Although there is no accepted animal model for gender-identity alteration, the bed nucleus of the stria terminalis (BST) turned out to be an appropriate candidate to study for the following reasons. First, it is known that the BST plays an essential part in rodent sexual behaviour[3,4]. Not only have oestrogen and androgen receptors been found in the BST[8,9], it is also a major aromatization centre in the developing rat brain[10]. The BST in the rat receives projections mainly from the amygdala and provides a strong input in the preoptic–hypothalamic region[11,12]. Reciprocal connections between hypothalamus, BST and amygdala are also well documented in experimental animals[13–15]. In addition, sex differences in the size and cell number of the BST have been described in rodents which are influenced by gonadal steroids in development[16–18]. Also, in humans a particular caudal part of the BST (BNST–dspm) has been reported to be 2.5 times larger in men than in women[19].

The localization of the BST is shown in Fig. 1. The central part of the BST (BSTc) is characterized by its somatostatin cells and vasoactive intestinal polypeptide (VIP) innervation[20]. We measured the volume of the BSTc on the basis of its VIP innervation (Fig. 2). The BSTc volume in heterosexual men ($2.49 \pm 0.16\ mm^3$) was 44% larger than in heterosexual women ($1.73 \pm 0.13\ mm^3$) ($P<0.005$) (Fig. 3). The volume of the BSTc of heterosexual and homosexual men did not differ in any statist-

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Livebirth after uterus transplantation.

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Abstract

BACKGROUND: Uterus transplantation is the first available treatment for absolute uterine infertility, which is caused by absence of the uterus or the presence of a non-functional uterus. Eleven human uterus transplantation attempts have been done worldwide but no livebirth has yet been reported.

METHODS: In 2013, a 35-year-old woman with congenital absence of the uterus (Rokitansky syndrome) underwent transplantation of the uterus in Sahlgrenska University Hospital, Gothenburg, Sweden. The uterus was donated from a living, 61-year-old, two-parous woman. In-vitro fertilisation treatment of the recipient and her partner had been done before transplantation, from which 11 embryos were cryopreserved.

FINDINGS: The recipient and the donor had essentially uneventful postoperative recoveries. The recipient's first menstruation occurred 43 days after transplantation and she continued to menstruate at regular intervals of between 26 and 36 days (median 32 days). 1 year after transplantation, the recipient underwent her first single embryo transfer, which resulted in pregnancy. She was then given triple immunosuppression (tacrolimus, azathioprine, and corticosteroids), which was continued throughout pregnancy. She had three episodes of mild rejection, one of which occurred during pregnancy. These episodes were all reversed by corticosteroid treatment. Fetal growth parameters and blood flows of the uterine arteries and umbilical cord were normal throughout pregnancy. The patient was admitted with pre-eclampsia at 31 full weeks and 5 days, and 16 h later a caesarean section was done because of abnormal cardiotocography. A male baby with a normal birthweight for gestational age (1775 g) and with APGAR scores 9, 9, 10 was born.

INTERPRETATION: We describe the first livebirth after uterus transplantation. This report is a proof-of-concept for uterus transplantation as a treatment for uterine factor infertility. Furthermore, the results show the feasibility of live uterus donation, even from a postmenopausal donor.

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Comment in

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REVIEW ARTICLE

Aromatase in the Brain: Not Just for Reproduction Anymore

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Journal of Neuroendocrinology

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Aromatase, the enzyme that synthesises oestrogens from androgen precursors, is expressed in the brain, where it has been classically associated with the regulation of neuroendocrine events and behaviours linked with reproduction. ***Recent findings, however, have revealed new unex-pected roles for brain aromatase,*** indicating that the enzyme regulates synaptic activity, synaptic plasticity, neurogenesis and the response of neural tissue to injury, ***and may contribute to control non-reproductive behaviours, mood and cognition. Therefore, the function of brain aromatase is not restricted to the regulation of reproduction as previously thought.***

Key words: behaviour, cognition, neurogenesis, neuroprotection, oestrogens, synaptic plasticity.

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Aromatase in the brain: new unexpected roles for an old enzyme

Testosterone and other C19 steroids are converted to oestradiol by aromatase (Fig. 1), which is an enzyme that consists of two components: a cytochrome P450 (P450 aro), the product of the *cyp19* gene, and the ubiquitous flavoprotein NADPH (reduced nicotinamide adenine dinucleotide phosphate)-cytochrome P450 reductase (1, 2). Aromatase activity in the brain was first detected by Naftolin *et al.* (3–5) in the foetal human limbic system and in the rat hypothalamus. After these pioneering findings, numerous studies have shown the expression, activity and distribution of aromatase in the central nervous system of several species of vertebrates (6–12), including humans (13–18). Brain aromatase is thought to be involved in the regulatory effects of androgens, via conversion to oestrogens, on reproductive neuroendocrine development. Thus, by the regulation of local oestrogen levels, aromatase activity participates in the sexual differentiation of brain regions involved in the control of gonadotrophin secretion and sexual behaviour (19–22). During adult life, brain aromatase activity also controls local oestrogen levels within brain regions involved in the regulation of reproduction (23–25).

In addition to these classical reproductive roles of brain aromatase, its activity may also modulate mood and affective status (26). Thus, aromatase knockout (ArKO) female mice (27), but not ArKO male mice (28), show increased depressive-like behaviours and polymorphisms in the *cyp19* gene are associated with depressive symptoms in women (29). Furthermore, ArKO male mice develop compulsive behaviours, such as excessive barbering, grooming and wheel-running (30). Modifications in brain aromatase activity may also play an important role in the regulation of aggressive behaviour (31, 32) and in its modulation by social experience (32). Some clinical and experimental studies suggest that aromatase activity also impacts on cognitive function. Two randomised, placebo-controlled clinical trials have assessed the effect of aromatase inhibition on cognition. In one of these studies, conducted in postmenopausal women, the aromatase inhibitor letrozole (Fig. 1) did not affect the improvements in visual and verbal memory caused by testosterone administration (33). By contrast, another clinical trial demonstrated that aromatase inhibition in healthy older men prevents the improvement in verbal memory produced by testosterone (34). Other studies suggested that aromatase inhibitors, used as a treatment for breast cancer, may impair verbal and visual learning in women (35, 36). Studies in animals also suggested that aromatase activity may interfere with cognitive processing. Local aromatisation of testosterone to oestradiol within the brain of songbirds enhances hippocampal function, including spatial memory performance (37). By contrast, in male rats, inhibition of brain aromatase counteracts spatial learning impairment induced by the injection of testosterone into the hippocampus (38) and the systemic administration of an aromatase inhibitor facilitates working memory acquisition (39). Aromatase activity may therefore improve or impair specific cognitive modalities, probably by the fine regulation of oestradiol levels at precise moments and in specific brain regions, because oestradiol exerts dose, time and region-specific actions on cognition (40–42).

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Sexual Orientation after Prenatal Exposure to Exogenous Estrogen

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Thirty women aged 17 to 30 years with documented prenatal exposure to the nonsteroidal synthetic estrogen diethylstilbestrol (DES) were compared to thirty women of similar demographic characteristics from the same medical clinic who had a history of abnormal Pap smear findings. A subsample of the DES women were also compared to their DES-unexposed sisters. Sexual orientation in its multiple components was assessed by systematic semi-structured interviews. In comparison to both control groups, the DES women showed increased bisexuality and homosexuality. However, about 75% of the DES women were exclusively or nearly exclusively heterosexual. Nonhormonal and hormonal interpretations of these findings are discussed.

KEY WORDS: homosexuality; diethylstilbestrol; prenatal hormones; sexual orientation.

INTRODUCTION

Since the majority of heterosexual and homosexual individuals do not show consistent differences in peripheral hormone levels (Meyer-Bahlburg,

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BRIEF REPORT

Psychosexual Milestones in Women Prenatally Exposed to Diethylstilbestrol

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Thirty women aged 17 to 30 years with a record-confirmed history of prenatal exposure to diethylstilbestrol (DES) were compared to 30 women of similar age and demographic background with a history of abnormal Pap smear findings. Heterosocial and heterosexual histories were assessed by systematic semistructured interviews. The groups differed neither in the age at menarche nor in the age at attainment of various psychosexual milestones.

Before systemic estradiol can reach the developing brain of the fetus or neonate, it is assumed to be inactivated by binding to α-fetoprotein in the rat (Plapinger and McEwen, 1978) or by placental conversion to estrone in the rhesus monkey (Slikker, Hill, and Young, 1982) and probably other primates including human beings. The findings by Davidson, Stott, Longstaff, Abramovich, and Pearson (1983) suggested conjugation of steroidal estrogens in brain cytosols as an alternative or additional protective mechanism. The nonsteroidal estrogen, diethylstilbestrol (DES), however, bypasses all these mechanisms and remains in biologically active form to interact with the estrogen receptors of the fetal brain. In studies of behavioral effects, thus far only available for lower mammals, pre- or perinatal DES has been reported to show effects similar to those of androgen treatment (Hines, Döhler, and Gorski, 1982; Hines, Alsum,

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Sexual Activity Level and Sexual Functioning in Women Prenatally Exposed to Diethylstilbestrol

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Thirty women with a history of prenatal exposure to diethylstilbestrol (DES) underwent a detailed sexual history and were compared to a demographically similar sample of 30 women with a history of an abnormal Pap smear. The DES women were found to have less well-established sex-partner relationships and less experience with child-bearing, to be lower in sexual desire and enjoyment, sexual excitability, and orgasmic coital functioning, but to be comparable (and low) with regard to such sexual dysfunctions as vaginismus and dyspareunia. Both potential psychosocial and neuroendocrine explanations are discussed.

INTRODUCTION

The nonsteroidal synthetic estrogen, diethylstilbestrol (DES), was widely used for the treatment of at-risk pregnancies during the three decades preceding its ban for this indication by the Food and Drug Administration in 1971. Thus, at the present time, several million women and men are living in the United States who were exposed to DES during their fetal development (1). Herbst et al. (2) discovered an association of prenatal DES exposure with the development of clear-cell adenocarcinoma in women. Since then, a variety of medical abnormalities of the urogenital tract and of reproductive function have been described in both male and female offspring of DES-treated pregnancies (3). Although the occurrence of clear-cell adenocarcinoma is very rare, vaginal epithelial changes have been found in about a third of unselected DES daughters (4). In many DES daughters, structural abnormalities of both the cervix (e.g., cervical collar or cock's comb appearance) and the vagina (e.g., hoods, ridges, incomplete transverse septum) have been found (5, 6). Also increased are menstrual irregularities (7, 8; although not corroborated by 9), hirsutism with associated endocrine abnormalities (8), and pregnancy problems (10–12). Because of the medical side effects, DES daughters have been advised to regularly undergo specific gynecologic examinations, and a number of states in the United States have set up DES screening centers. It is, therefore, not surprising that the identification as a DES-exposed daughter constitutes an emotional stress that may lead to anxiety reactions (13) or to an initial grief reaction and anger (14).

It is not known, however, to what extent sexual functioning and behavior are affected. It appears plausible that medical

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Xenoestrogens at Picomolar to Nanomolar Concentrations Trigger Membrane Estrogen Receptor-α–Mediated Ca^{2+} Fluxes and Prolactin Release in GH3/B6 Pituitary Tumor Cells

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Xenoestrogens (XEs) are widespread in our environment and are known to have deleterious effects in animal (and perhaps human) populations. Acting as inappropriate estrogens, XEs are thought to interfere with endogenous estrogens such as estradiol (E_2) to disrupt normal estrogenic signaling. We investigated the effects of E_2 versus several XEs representing organochlorine pesticides (dieldrin, endosulfan, *o′,p′*-dichlorodiphenylethylene), plastics manufacturing by-products/detergents (nonylphenol, bisphenol A), a phytoestrogen (coumestrol), and a synthetic estrogen (diethylstilbestrol) on the pituitary tumor cell subline GH3/B6/F10, previously selected for expression of high levels of membrane estrogen receptor-α. Picomolar to nanomolar concentrations of both E_2 and XEs caused intracellular Ca^{2+} changes within 30 sec of administration. Each XE produced a unique temporal pattern of Ca^{2+} elevation. Removing Ca^{2+} from the extracellular solution abolished both spontaneous and XE-induced intracellular Ca^{2+} changes, as did 10 μM nifedipine. This suggests that XEs mediate their actions via voltage-dependent L-type Ca^{2+} channels in the plasma membrane. None of the Ca^{2+} fluxes came from intracellular Ca^{2+} stores. E_2 and each XE also caused unique time- and concentration-dependent patterns of prolactin (PRL) secretion that were largely complete within 3 min of administration. PRL secretion was also blocked by nifedipine, demonstrating a correlation between Ca^{2+} influx and PRL secretion. These data indicate that at very low concentrations, XEs mediate membrane-initiated intracellular Ca^{2+} increases resulting in PRL secretion via a mechanism similar to that for E_2, but with distinct patterns and potencies that could explain their abilities to disrupt endocrine functions. *Key words:* **bisphenol A, coumestrol, DDE, DES, diethylstilbestrol, dieldrin, endosulfan, estrogen receptor-α, exocytosis, L-type channels, membrane, nonylphenol, phytoestrogen, prolactin, xenoestrogen.** *Environ Health Perspect* **113:431–439 (2005).**

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Environmental chemicals with estrogenic activities [xenoestrogens (XEs)] have been implicated in harmful endocrine effects on animals and humans such as the feminization of male animal populations (Kloas et al. 1999; Sumpter 1995), reproductive tract malformations and endometriosis (Gotz et al. 2001; Lee 1998; Steinmetz et al. 1998), disorganization of the central nervous system (Laessig et al. 1999; Oka et al. 2003), and breast and ovarian cancer (Brown and Lamartiniere 1995; Mathur et al. 2002). By acting as estrogen mimetics and binding to estrogen receptors (ERs), XEs may disrupt normal endocrine function, leading to reproductive failure and the induction of tumors in estrogen-sensitive tissues. XEs can also cause alteration of hormone levels via changes in hormone production, metabolism, or transport (Sonnenschein and Soto 1998).

There are many potential endocrine-disrupting chemicals that are prevalent in the environment, or to which humans have been otherwise exposed (Singleton and Khan 2003); in this study we examined several representative compounds. Erroneously used to prevent miscarriages in the 1950s and 1960s, diethylstilbestrol (DES) acts developmentally as a potent estrogen agonist, causing adenocarcinomas, squamous neoplasia of the vagina and cervix (Hatch et al. 2001), oligospermia (vom Saal et al. 1997), and infertility (Palmer et al. 2001). The pesticide *o′,p′*-dichlorodiphenylethylene (DDE) and its metabolites can disorder prostate maturation (Gray et al. 1999). Endocrine disruptors are known to have great impact during fetal development when endogenous hormones regulate cell differentiation and growth, and thus slight alterations in hormonal activity due to endocrine disruption can lead to irreversible changes (Derfoul et al. 2003). However, the abilities of XEs to disrupt adult endocrine function and perhaps to exacerbate estrogen-dependent tumor growth (Soto et al. 1995) are also of concern. We also examined other XEs reported to have estrogen-like activities: detergents such as nonylphenol and bisphenol A (BPA), the organochlorine pesticides dieldrin and endosulfan, and the phytoestrogen coumestrol.

Estrogenic actions have been well studied with respect to genomic responses mediated by nuclear ERs. The nuclear ER-mediated gene transcription responses to XEs are very weak [effective only at 1,000- to 10,000-fold higher concentrations than estradiol (E_2; Massaad and Barouki 1999; Stevens et al. 1994; Witorsch 2002)], leading some to suggest that their presence in our environment is relatively harmless. However, in addition to classical genomic actions, estrogens can act through nongenomic or membrane-initiated signaling pathways via a membrane form of ER (mER). Examples of such actions are alterations in G-protein–coupled receptor responses, protein phosphorylation, lysosomal membrane destabilization, K^+ and Ca^{2+} channel activation, and nitric oxide secretion (reviewed by Watson and Gametchu 1999, 2003). XE actions via nongenomic pathways remain largely unstudied.

Ca^{2+} responses to extracellular stimuli can lead to changes in cell motility, intra- and extracellular signaling processes, and rapid hormone secretion [including prolactin (PRL)] through exocytosis (Campbell 1990; Pappas et al. 1994; Watson et al. 1999a). Changes in PRL secretion are associated with hormonal regulation of lactation, cell proliferation, the cellular immune response, and parental/maternal behavior (Freeman et al. 2000). We recently showed that picomolar to nanomolar concentrations of E_2 and XEs can initiate mitogen-activated protein kinase activation and that several signaling pathways, including Ca^{2+} elevation, may participate in this kinase activation (Bulayeva et al. 2004; Bulayeva and Watson 2004). We also demonstrated the ability of a physiological estrogen (E_2) to elicit cellular Ca^{2+} influx via a membrane version of ER-α (Bulayeva et al. 2005). Here we investigate in more detail the ability of several XEs (DES, coumestrol, *p*-nonylphenol, BPA, DDE, dieldrin, and endosulfan) to induce rapid intracellular Ca^{2+} changes leading to PRL secretion in mER-α–enriched or depleted sublines of GH3/B6 cells (Pappas et al. 1994). Misregulation of such cellular signaling events by XEs could lead to damaging endocrine disruptions such as tissue malformation, cancer, and reproductive system malfunctions.

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Variation in Length of the Estrous Cycle in Mice Due to Former Intrauterine Proximity to Male Fetuses[1]

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ABSTRACT

Female mouse fetuses occupying an intrauterine position between male fetuses exhibit longer estrous cycles in adulthood than do females formerly residing in utero next to other female fetuses. This difference in adult cycle length was observed consistently when the two types of females were maintained under a variety of social conditions. Thus, these data suggest a fundamental biasing of the intrinsic timing of the estrous cycle by former intrauterine proximity to males in this and possibly other species in which more than one fetus is present in the uterus.

INTRODUCTION

The mammalian estrous cycle encompasses a complex series of changes in the brain, pituitary and ovary, all synchronized by hormonal and environmental cues to promote ovulation and sexual receptivity. The length of this cycle varies considerably between species and, in most cases, variation among conspecifics is also observed (Alleva et al., 1971; Nequin et al., 1979; Schwartz, 1969). The present report documents one source of such individual variation in mice, namely, that there is a direct effect of intrauterine proximity to males on the length of a female's estrous cycle during later adult life.

In the present experiments, female mouse fetuses located in utero between two male fetuses (designated here as 2M females) and female fetuses adjacent to other female fetuses (0M females) were collected by cesarean delivery and fostered to dams that had just given birth naturally. The length of the estrous cycles of these two types of females was compared in adulthood while they were either 1) housed individually or grouped in the presence of a male or 2) housed individually or grouped in an environment free of males. This spectrum of housing conditions was deemed an experimental necessity because of the sensitivity of the mouse's estrous cycle to regulation by social (pheromonal and tactile) cues. It is well established now, for example, that female mice emit cues that prolong the estrous cycles of other females, while stimuli emanating from adult males accelerate cycling (Bronson, 1979; Vandenbergh, 1974; Whitten and Champlin, 1973). Thus, among females that are grouped together in the absence of males, prolonged and irregular cycles are usually observed, while adult females that are either grouped with males present or housed individually exhibit shorter and more regular cycles. The present experiments were designed to determine whether possible effects of intrauterine position on estrous cycle length would relate to differential sensitivity to extrinsic (social) cues or reflect a more fundamental influence on the intrinsic timing of the adult estrous cycle.

MATERIALS AND METHODS

One hundred and fifty adult CF-1 females were paired with males each morning between 0800–1000 h. Inseminated females bearing vaginal plugs (25–35 each day) were individually housed (Day 0 of pregnancy). On Day 19 of pregnancy at 0900 h, the females that had not yet begun to deliver (about 75% of the pregnant females) were killed by cervical dislocation and the fetuses were removed from the uterus. The sex of the pups was determined by observing the size of the anogenital space and the 0M and 2M females were saved. The pups were removed from the mothers that had just delivered naturally and 6 experimental pups from the same intrauterine position were

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Self-fertilization in human: having a male embryo without a father.

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Author information

Abstract

Chimeras are the result of fusion of two zygotes to form a single embryo, producing an individual with genetically different kinds of tissue. If the fused zygotes are of different sex, the individual develops both ovarian and testicular tissues. The majority of these people are best reared as females and many pregnancies with living offspring have been reported in persons reared as females, and several cases has fathered a child. During ovulation, a negative pressure occurs in the lumen of the oviduct and it produces a vacuum effect which has made several pregnancies possible in subjects lacking an ipsilateral ovary by allowing the transperitoneal migration of oocyte from the contralateral gonad. Self-fertilization was reported in many flowering plants, in a kind of fish and in a case of rabbit. They have both eggs and sperms in their body and at fertilization, one sperm cell fuses with oocyte to form an embryo. Self-fertilization may also occur in human. A scenario is presented here for a woman to have a son without a father: she is a chimera of 46,XX/46,XY type resulting from the fusion of two zygotes of different sex types and she develops both ovary and testis in her body. Since XX cells tend to gather on the left side while XY cells on the right, she develops an ovary on the left side with a oviduct and a testis on the right side located in an ovarian position with no duct. Müllerian duct regression on the right side is mediated by the antimüllerian hormone derived from the ipsilateral testis and testosterone secreted from Leydig cells does not prevent the regression of the Wolffian duct. Therefore, neither an oviduct nor an epididymis and vas deferens is present next to the testis on the right side, and lumens of a well-developed rete testis have an open access to the abdominal cavity allowing the sperms to be picked-up by the contralateral oviduct. Both gonads are functional and produce spermatozoa and oocyte respectively after puberty. At the time of ovulation, estrogens increase the motility of the oviduct on the left side which results in a negative pressure in the tube and oocyte and sperms are picked-up into the tube with the help of this vacuum effect, taking both gametes to the fertilization site in the oviduct. Since the sperm contains a Y chromosome, this fertilization gives rise to a XY male embryo.

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Research description

Research in my laboratory concerns the effects on fetal development of endogenous sex hormones, naturally occurring estrogenic chemicals in food, (such as phytoestrogens in soy), and estrogenic manmade chemicals in consumer products (such as bisphenol A in plastic). We collect fetal mouse reproductive tissues and use primary mouse and human cell culture to investigate cellular mechanisms that mediate responses to hormones and other chemicals. We have confirm observations from in vivo experiments using mice. An important aspect of this research is that we use a physiologically based approach to determine the doses of chemicals that we examine. This approach has led to findings that fetal tissues are extremely sensitive to much lower doses of endogenous estradiol and manmade estrogenic chemicals than had previously been appreciated.

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INFECTIONS, TOXIC CHEMICALS AND DIETARY PEPTIDES BINDING TO LYMPHOCYTE RECEPTORS AND TISSUE ENZYMES ARE MAJOR INSTIGATORS OF AUTOIMMUNITY IN AUTISM

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Similar to many complex autoimmune diseases, genetic and environmental factors including diet, infection and xenobiotics play a critical role in the development of autism. In this study, we postulated that infectious agent antigens such as streptokinase, dietary peptides (gliadin and casein) and ethyl mercury (xenobiotic) bind to different lymphocyte receptors and tissue enzyme (DPP IV or CD26). We assessed this hypothesis first by measuring IgG, IgM and IgA antibodies against CD26, CD69, streptokinase (SK), gliadin and casein peptides and against ethyl mercury bound to human serum albumin in patients with autism. A significant percentage of children with autism developed anti-SK, anti-gliadin and casein peptides and anti-ethyl mercury antibodies, concomitant with the appearance of anti-CD26 and anti-CD69 autoantibodies. These antibodies are synthesized as a result of SK, gliadin, casein and ethyl mercury binding to CD26 and CD69, indicating that they are specific. Immune absorption demonstrated that only specific antigens, like CD26, were capable of significantly reducing serum anti-CD26 levels. However, for direct demonstration of SK, gliadin, casein and ethyl mercury to CD26 or CD69, microtiter wells were coated with CD26 or CD69 alone or in combination with SK, gliadin, casein or ethyl mercury and then reacted with enzyme labeled rabbit anti-CD26 or anti-CD69. Adding these molecules to CD26 or CD69 resulted in 28-86% inhibition of CD26 or CD69 binding to anti-CD26 or anti-CD69 antibodies. The highest % binding of these antigens or peptides to CD26 or CD69 was attributed to SK and the lowest to casein peptides. We, therefore, propose that bacterial antigens (SK), dietary peptides (gliadin, casein) and Thimerosal (ethyl mercury) in individuals with pre-disposing HLA molecules, bind to CD26 or CD69 and induce antibodies against these molecules. In conclusion, this study is apparently the first to demonstrate that dietary peptides, bacterial toxins and xenobiotics bind to lymphocyte receptors and/or tissue enzymes, resulting in autoimmune reaction in children with autism.

As with many complex autoimmune diseases, genetic, immune and environmental factors, including diet, toxic chemicals and infections, play critical roles in the development of autism (1,2). Opioid peptides are considered to be part of the etiology of autism, and these peptides are available from a variety of food sources. These dietary proteins and peptides, including casein, casomorphins, gluten (GLU) and gluteomorphins, can stimulate T-cells, induce peptide-specific T-cell responses, and abnormal levels of cytokine production, which may result in inflammation, autoimmune reactions and disruption of neuroimmune communications (3). In celiac disease

Key words: chemotaxis, atherosclerosis, innate immunity, adaptive immunity, thrombin

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Disruption of aromatase homeostasis as the cause of a multiplicity of ailments: A comprehensive review.

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Abstract

Human health is beset with a legion of ailments, which is exacerbated by lifestyle errors. Out of the numerous enzymes in human body, aromatase, a cytochrome P450 enzyme is particularly very critical. Occurring at the crossroads of multiple signalling pathways, its homeostasis is vital for optimal health. Unfortunately, medications, hormone therapy, chemical additives in food, and **endocrine**-disrupting personal care products are oscillating the aromatase concentration beyond the permissible level. As this enzyme converts androgens (C19) into estrogens (C18), its agitation has different outcomes in different genders and age groups. Some common pathologies associated with aromatase **disruption** include breast cancer, prostate cancer, polycystic ovary syndrome (PCOS), endometriosis, osteoporosis, ovarian cancer, gastric cancer, pituitary cancer, Alzheimer's disease, schizophrenia, male hypogonadism, and transgender issues. Several drugs, cosmetics and pesticides act as the activators and suppressors of this enzyme. This carefully-compiled critical review is expected to increase public awareness regarding the threats resultant of the perturbations of this enzyme and to motivate researchers for further investigation of this field.

KEYWORDS: Aromatase; Aromatase inhibitor; Breast cancer; **Endocrine disruption**; Estrogen; Inflammation

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The Increasing Prevalence in Intersex Variation from Toxicological Dysregulation in Fetal Reproductive Tissue Differentiation and Development by Endocrine-Disrupting Chemicals.

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Abstract

An increasing number of children are born with intersex variation (IV; ambiguous genitalia/hermaphrodite, pseudohermaphroditism, etc.). Evidence shows that **endocrine**-disrupting chemicals (EDCs) in the environment can cause reproductive variation through dysregulation of normal reproductive tissue differentiation, growth, and maturation if the fetus is exposed to EDCs during critical developmental times in utero. Animal studies support fish and reptile embryos exhibited IV and sex reversal when exposed to EDCs. Occupational studies verified higher prevalence of offspring with IV in chemically exposed workers (male and female). Chemicals associated with **endocrine**-disrupting ability in humans include organochlorine pesticides, poly-chlorinated biphenyls, bisphenol A, phthalates, dioxins, and furans. Intersex individuals may have concurrent physical disorders requiring lifelong medical intervention and experience gender dysphoria. An urgent need exists to determine which chemicals possess the greatest risk for IV and the mechanisms by which these chemicals are capable of interfering with normal physiological development in children.

KEYWORDS: ambiguous genitalia; **endocrine** disrupting chemicals; fetal development; intersex variation; pesticides; reproductive birth defect

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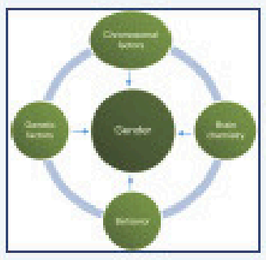

ORIGINAL ARTICLES

Scand J Work Environ Health 1990;16:232—8

Prevalence of adverse reproductive outcomes in a population occupationally exposed to pesticides in Colombia

by Mauricio Restrepo, MD,[1] Nubia Muñoz, MD,[2] Nicholas E Day, PhD,[3] José E Parra, MS,[1] Laura de Romero, MS,[1] Xuan Nguyen-Dinh, MS[2]

RESTREPO M, MUÑOZ N, DAY NE, PARRA JE, DE ROMERO L, NGUYEN-DINH X. Prevalence of adverse reproductive outcomes in a population occupationally exposed to pesticides in Columbia. *Scand J Work Environ Health* 1990;16:232—8. A prevalence survey of adverse reproductive outcomes was carried out in a population of 8867 persons (2951 men and 5916 women) who had been working in the floriculture industry in the Bogotá area of Colombia for at least six months. These workers were exposed to 127 different types of pesticides. The prevalence rates for abortion, prematurity, stillbirths, and malformations were estimated for pregnancies occurring among the female workers and the wives of the male workers before and after they started working in floriculture, and these rates were related to various degrees of exposure. A moderate increase in the prevalence of abortion, prematurity, and congenital malformations was detected for pregnancies occurring after the start of work in floriculture.

Key terms: abortion, birth defects, floriculture, pesticides, prematurity, stillbirths.

Experimental and epidemiologic evidence indicates that certain industrial and environmental chemicals, and also some pharmaceutical products, can cause reproductive toxicity (1, 2). Among these chemicals, pesticides pose special concern due to their ever increasing use, their large number, their wide environmental distribution with vast potential for human exposure, and their toxicologic characteristics.

Many epidemiologic studies have already been devoted to the adverse reproductive effects of human exposure to chemicals (1, 2), but only a few deal with exposure to phenoxy herbicides and related dioxins (3—13).

The present study was undertaken in an attempt to ascertain the occurrence of certain reproductive events among a population occupationally exposed to a heterogeneous group of pesticides and to assess the possible association between adverse events and such exposure. The occurrence of fetal loss, prematurity, congenital malformations, and cancer was studied among the offspring of workers in the floriculture industry in Bogotá, Colombia, by means of a questionnaire administered by an interviewer.

Subjects and methods

The flower-growing industry is located on the Bogotá plateau at 2653 m above sea level. The mean temperature is 13°C without seasonal variations in the weather, and there are two periods of rain, one in May and the other in November. The flowers are grown within semiclosed polyethylene greenhouses and then exported. The importance of this relatively new enterprise is illustrated by the fact that 90 % of the 350 million cut flowers imported every year into the United States comes from Colombia (14).

All persons who had been working for a period of at least six months in 58 companies affiliated with the Colombian Association of Flower Growers (Asocolflores) were defined as the study population. A list of 12 129 workers was obtained from the 58 companies, and 10 238 of the workers proved to be eligible for the study. After exclusions for various reasons (sickness, vacation, or retirement) 9328 were eligible for the interview, and 8867 (95 %) were actually interviewed.

A precoded questionnaire containing demographic information, detailed reproductive history of the female workers and all the partners of the male workers, and information about the length of time worked in floriculture and type of job was administered to each of the subjects by specially trained interviewers. The interview took place in 1981. It was usually conducted at the workplace and lasted approximately 10 min. It was supervised and assisted by the study statistician, who also checked each questionnaire for completeness and consistency before recording it onto a magnetic tape.

The reproductive history was oriented to establish the pregnancies of each woman and the outcome of each pregnancy. Such outcomes reported by each in-

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Abstract

A prevalence survey of adverse reproductive outcomes was carried out in a population of 8867 persons (2951 men and 5916 women) who had been working in the floriculture industry in the Bogotá area of Colombia for at least six months. These workers were exposed to 127 different types of pesticides. The prevalence rates for abortion, prematurity, stillbirths, and malformations were estimated for pregnancies occurring among the female workers and the wives of the male workers before and after they started working in floriculture, and these rates were related to various degrees of exposure. A moderate increase in the prevalence of abortion, prematurity, and congenital malformations was detected for pregnancies occurring after the start of work in floriculture.