THE EFFECTS OF ENDOCRINE DISRUPTING COMPOUNDS ON THE DEVELOPMENT OF THE NERVOUS SYSTEM: USE OF THE FROG, *XENOPUS LAEVIS*, AS A MODEL SYSTEM

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INTRODUCTION

Due to the alarming numbers of animals from many species that have been found with gross morphological abnormalities, the topic of endocrine disruption, or "hormone-mimicking" chemicals in the environment, has attracted great attention in recent years. Animals as diverse as mammalian species like the Florida panther,[1] avian species,[2] and even reptilian species such as alligators[3] have all been reported with defects, particularly in reproductive organs. Frog populations across the country and here in Vermont have been rapidly declining, and the numbers of deformities being

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1. C. Facemire, T. Gross, & L. Guillette, *Reproductive impairment in the Florida panther: nature or nurture?*, 103 ENVTL. HEALTH PERSP. 79–86 (1995).

2. J. Giesy, J. Ludwig, & D. Tillitt, *Deformities in birds of the great lakes region: assigning causality*, 28 ENVTL. SCI. & TECH. 128–35 (1994).

3. L.J. Guillette Jr, T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival, & A.R. Woodward, *Developmental abnormalities of the gonadal and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida*, 102 ENVTL. HEALTH PERSP. 680–88 (1994).

reported are also on the rise.[4] Because exposure to these chemicals has such serious implication for both wildlife populations and for human health, research directed at identifying endocrine disrupting chemicals and their biological effects is at the foreground of active research efforts.

I. ENDOCRINE DISRUPTING COMPOUNDS (EDCS)

Endocrine disrupting compounds (EDCs) are synthetic compounds found in pesticides, herbicides, nonionic surfactants, environmental pollutants, and common plastics, as well as natural compounds derived from plants[5] that have deleterious effects on the development of a wide range of species by disrupting hormone-sensitive processes. Many studies have shown that exposure to EDCs during early development induces abnormalities in peripheral reproductive organs and in reproductive behaviors,[6] as well as disruption of limb development.[7] In addition to causing infertility and fetal malformations,[8] EDCs have also been shown to act as carcinogens in mammalian populations.[9]

Studies demonstrating that early EDC exposure leads to aberrant reproductive *behaviors* in adult life suggest that these compounds affect not only the formation of peripheral reproductive structures, but also the developing central nervous system (CNS). Endogenous hormones (i.e.,

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4. A. Blaustein, D. Wake, & W. Sousa, *Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions*, 8 CONSERVATION BIOLOGY 60–71 (1994).

5. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–182 (1994); A.M. Soto, H. Justicia, J.W. Wray, & C. Sonnenschein, *p-Nonylphenol: an estrogenic xenobiotic released from "modified" polystyrene*, 92 ENVTL. HEALTH PERSP. 167–73 (1991); T. Zacharewski, *Identification and assessment of endocrine disruptors: limitations of* in vivo *and* in vitro *assays*, 106 ENVTL. HEALTH PERSP. 577–82 (1998).

6. L.E. Gray, J.S. Ostby, & W.R. Kelce, *Developmental effects of an environmental antiandrogen: The fungicide vinclozolin alters sex differentiation of the male rat*, 129 TOXICOLOGY & APPLIED PHARMACOLOGY 46–52 (1994); R.L. Cooper & R.J. Kavlock, *Endocrine disruptors and reproductive development: a weight-of-evidence overview*, 152 J. ENDOCRINOLOGY 159–66 (1997); L.W. Reiter, C. DeRosa, R.J. Kavlock, G. Lucier, M.J. Mac, , J. Melillo, R.L. Melnick, T. Sinks, & B.T. Walton, *The U.S. federal framework for research on endocrine disruptors and an analysis of research programs supported during fiscal year 1996*, 106 ENVTL. HEALTH PERSP. 105–13 (1998).

7. http://www.npwrc.usgs.gov/narcam.

8. E. Giavini, M. Prati, & C. Vismara, *Embryonic effects of 2,3,7,8 tetrachlorodibenzo-p-dioxin administered to female rats before mating*, 31 ENVTL. RES. 105–10 (1983); L.E. Gray, J.M. Ferrell, & J.S. Ostby, *Alteration of behavioral sex differentiation by exposure to estrogenic compounds during a critical neonatal period: effects of zearalenone, methoxychlor, and estradiol in hamsters*, 80 TOXICOLOGY & APPLIED PHARMACOLOGY 127–36 (1985).

9. B. Weiss, *A risk assessment perspective on the neurobehavioral toxicity of endocrine disruptors*, 14 TOXICOLOGY & INDUS. HEALTH 341–59 (1998); C.W. Schmidt, *Answering the endocrine test questions*, 107 ENVTL. HEALTH PERSP. 458–60 (1999).

gonadal steroids: androgens, estrogens and progestins) are known to have significant and widespread effects on the development of the nervous system,[10] providing a myriad of potential targets for disruption by EDCs. Determining how EDCs alter nervous system development, however, is a complicated affair since the endpoints of assessment for nervous system abnormalities are often less easily defined than with assessments for limb malformation or tumor formation.[11] Moreover, the EPA has identified over 87,000 chemicals that need to be screened for potential EDC effects.[12] This overwhelming number of chemicals, coupled with the fact that effects on nervous system development may be both hard to categorize (changes in cognitive function or affect) and variable (different in individuals with different genetic backgrounds), makes for a daunting task. Finally, assessments of which EDCs pose a health danger and at what level are controversial and, at this time, unresolved. For example, it has been estimated that ~60% of the greater than 300,000 tons of alkylphenol polyethoxylates end up in the water supply each year. At the source (e.g., sewage treatment plants, mills, and factories), these compounds are detected at ~0.1 to 1 mg/litre or 10^{-6} M.[13] However, the metabolites of alkylphenol polyethoxylates are highly stable and accumulate in sediment and sludge at concentrations that exceed those of the parent EDC.[14] Compounding this physical accumulation, EDCs bioaccumulate in fatty animal tissues. TCDD (dioxin), a contaminant that derives from the

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10. K.D. D'hler, *The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus*, 131INT'L REV. CYTOLOGY 1–57 (1991).

11. B. Weiss, *A risk assessment perspective on the neurobehavioral toxicity of endocrine disruptors*, 14 TOXICOLOGY & INDUS. HEALTH 341–59 (1998); H.A. Tilson, *Developmental neurotoxicity of endocrine disruptors and pesticides: identification of information gaps and research needs*, 106 ENVTL. HEALTH PERSP. 807–11 (1998).

12. U.S. EPA, Endocrine Disruptor Screening Program, Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) 63 FED. REG. §§ 42852–55 (1998).

13. L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.L. Lippincott, & J.D. Rosen, *Determination of alkylphenol ethoxylates and their acetic acid derivatives in drinking water by particle beam liquid chromatography/mass spectrometry*, 47 INT'L J. ENVTL. ANALYTICAL CHEMISTRY 167–80 (1992); R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, Environmentally persistent alkylphenolic compounds are estrogenic, 135 J. ENDOCRINOLOGY 175–82 (1994).

14. W. Giger, M. Ahel, M. Koch, H.U. Laubscher, C. Schaffner, & J. Schneider, *Behaviour of alkylphenol-polyethoxylate surfactants and of nitriloacetate in sewage treatment*, 19 WATER SCI. & TECH. 449–60 (1987); L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.L. Lippincott, & J.D. Rosen, *Determination of alkylphenol ethoxylates and their acetic acid derivatives in drinking water by particle beam liquid chromatography/mass spectrometry*, 47 INT'L J. ENVTL. ANALYTICAL CHEMISTRY 167–80 (1992); R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994).

commercial preparation of certain herbicides,[15] has been measured at up to 6 ppt per mL serum in human adults.[16] The EDC metabolites that are consumed by bottom feeding fish become increasingly concentrated as fish become eaten by birds and so on up the food chain.[17]

What are the concentrations of EDCs required to elicit significant biological effects? Studies of how EDCs can activate estrogen-sensitive ("reporter") genes in isolated cells in culture indicate that concentrations from 10^{-8} to 10^{-5} M induce significant effects.[18] Hypothalamic neurons maintained in dissociated cell culture are highly sensitive to EDCs, and significant effects in neurotransmitter uptake can be elicited by concentrations of alkylphenol polyethoxylates as low as 10^{-11} M.[19] In addition, it should be noted that assays of cultured cells or reporter gene constructs do not take into account a number of critical parameters including metabolism of EDCs, bioaccumulation, or bioavailability (that is whether they are free or bound to proteins in serum that preclude them from having a biological effect at intracellular steroid receptors). Moreover, these simple assays do not take into account steroid-receptor independent mechanisms of action,[20] or cell-cell interactions that may induce effects in an intact animal that would not be evident in cultured cells. Finally, differences in genetic background[21] and developmental age (see below) will impose significant differences in the ability of EDCs to elicit biological effects.

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15. C.H. WALKER, S.P. HOPKIN, R.M. SIBLY, & D.B. PEAKALL, PRINCIPLES OF ECOTOXICOLOGY (1997).

16. K. Krishnan & J. Broduer, *Toxic interactions among environmental pollutants: corroborating laboratory observations with human experience*, 102 ENVTL. HEALTH PERSP. 11–17 (1994).

17. T. COLBORN, D. DUMANOSKI, & J.P. MYERS, OUR STOLEN FUTURE (1997).

18. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994); K.G. Osteen & R. Sierra-Rivera, *Does disruption of immune and endocrine systems by environmental toxins contribute to development of endometriosis?*, 15 SEMINARS IN REPRODUCTIVE ENDOCRINOLOGY 301–08 (1997).

19. M. Christian & G. Gillies, *Developing hypothalamic dopaminergic neurones as potential targets for environmental estrogens*, 160 J. ENDOCRINOLOGY R1–R6 (1999).

20. *See* T. Zacharewski, *Identification and assessment of endocrine disruptors: limitations of* in vivo *and* in vitro *assays*, 106 ENVTL. HEALTH PERSP. 577–82.

21. J.L. Spearow, P. Doemeny, R. Sera, R. Leffler, & M. Barkley, *Genetic variation in susceptibility to endocrine disruption by estrogen in mice*, 285 SCI. 1259–61 (1999).

II. CELLULAR AND MOLECULAR MECHANISM UNDERLYING EDC EFFECTS

EDCs cause adverse effects by interfering with endogenous hormonal signaling mechanisms.[22] Endogenous steroid hormones, such as testosterone or the estrogen 17fl-estradiol, are small hydrophobic molecules that easily diffuse through the plasma membrane of a cell and into the cytoplasm where they then bind to a specific target receptor (androgen or estrogen receptors). This steroid/receptor complex then travels to the nucleus where its actions ultimately alter the biological response of the targeted cells and the organism. The overall mechanism of these steroid effects is relatively well understood. Once in the nucleus, the steroid/receptor complex directly regulates the expression, or "transcription," of specific genes by binding to discrete regulatory sequences of these genes called hormone response elements. These steroid-dependent changes in gene expression result in changes in the synthesis, or "translation," of specific proteins. It is the actions of these proteins which determine the biological responses of the targeted cells, and therefore of the organism.[23]

EDCs could have potential deleterious actions in either of two ways: (1) if they interfere with the normal activation of a specific receptor by the natural hormone (i.e., act as an antagonist); or (2) if they act in the same way as the endogenous hormone (i.e., act as a hormone mimic or agonist), but at an inappropriate developmental time, or if they are present at the wrong concentration. Recent studies indicate that both mechanisms come into play. Many of the EDCs are known to exert their effects by acting as weak estrogens.[24] For example, alkylphenolic polyethoxylates were shown to bind to estrogen receptors over twenty years ago,[25] and more recent studies have demonstrated that putative EDCs can mimic the molecular effects of estrogen. Specifically, EDCs produce transcriptional activation of reporter gene constructs containing consensus estrogen response elements

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22. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994); W.R. Kelce, L.E. Gray, & E.M. Wilson, *Antiandrogens as environmental endocrine disruptors*, 10 REPRODUCTION FERTILITY & DEV. 105–11 (1998).

23. *See* B. ALBERTS, D. BRAY, J. LEWIS, M. RAFF, K. ROBERTS, & J.D. WATSON, MOLECULAR BIOLOGY OF THE CELL (3rd ed. 1994).

24. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994).

25. G. Mueller & U.H. Kim, *Displacement of estradiol from estrogen receptors by simple alkylphenols*, 102 ENDOCRINOL. 1429–35 (1978); P.D. NIEUWKOOP & J. FABER, NORMAL TABLE OF *XENOPUS LAEVIS* (DAUDIN) (1967).

in cell lines.[26] In the past few years, however, it has also become clear that a number of EDCs exert their effects *not* as weak estrogens, but rather by acting as anti-androgens. Specifically, the fungicide, vinclozolin,[27] the ubiquitous pesticides 1,1,1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane (DDT) and its major metabolite, *p, p'*-dichlorodiphenyldichloro-ethylene (*p, p'*-DDE),[28] and bisphenol A and butyl benzyl phthalate[29] all interfere with androgen-dependent activation of reporter constructs and alter sexual differentiation in male rodents in a manner consistent with anti-androgenic activity.[30]

III. CRITICAL PERIODS IN DEVELOPMENT

For most biological processes, but especially those related to hormone effects on the nervous system, there is incontrovertible data indicating that neural processes are significantly more susceptible to steroid effects during embryonic and early postnatal development than in adulthood.[31] In particular, it is known that naturally-occurring hormones can induce significant changes in neurogenesis (the birth of nerve cells or *neurons*), neuronal survival, neuronal migration, the connections neurons make with one another, as well in the expression of specific proteins that determine neuronal function during these early developmental "critical periods."

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26. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994).

27. L.E. Gray, J.S. Ostby, & W.R. Kelce, *Developmental effects of an environmental antiandrogen: The fungicide vinclozolin alters sex differentiation of the male rat*, 129 TOXICOLOGY & APPLIED PHARMACOLOGY 46–52 (1994); W.R. Kelce, E. Monosson, M.P. Gamcsik, S.C. Laws, & L.E. Gray, *Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites*, 126 TOXICOLOGY & APPLIED PHARMACOLOGY 276–85 (1994).

28. W.R. Kelce, C.R. Stone, S.C. Laws, L.E. Gray, J.A. Kemppainen, & E.M. Wilson, *Persistent DDT metabolite* p,p'-*DDE is a potent androgen receptor antagonist*, 375 NATURE 581–85 (1995); W.R. Kelce, & E.M. Wilson, *Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications*, 75 J. MOLECULAR MED. 198–07 (1997); P. Sohoni & J.P. Sumpter, *Several environmental oestrogens are also anti-androgens*, 158 J. ENDOCRINOLOGY 327–39 (1998).

29. P. Sohoni & J.P. Sumpter, *Several environmental oestrogens are also anti-androgens*, 158 J. ENDOCRINOLOGY 327–39 (1998).

30. L.E. Gray, J.S. Ostby, & W.R. Kelce, *Developmental effects of an environmental antiandrogen: The fungicide vinclozolin alters sex differentiation of the male rat*, 129 TOXICOLOGY & APPLIED PHARMACOLOGY 46–52 (1994); W.R. Kelce, E. Monosson, M.P. Gamcsik, S.C. Laws, & L.E. Gray, *Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites*, 126 TOXICOLOGY & APPLIED PHARMACOLOGY 276–85 (1994); W.R. Kelce, L.E. Gray, & E.M. Wilson, *Antiandrogens as environmental endocrine disruptors*, 10 REPRODUCTION & FERTILITY DEV. 105–11 (1998).

31. K.D. D'hler, *The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus*, 131 INT'L REV. CYTOLOGY 1–57 (1991).

Moreover, these changes are permanent and do not require continued presence of high levels of hormones. As development proceeds, however, many facets of this hormone-sensitivity are lost, and the adult brain is far less malleable with respect to these "organizational" actions of steroid hormones.[32] While there is far less known about the organizational actions of EDCs, several studies suggest that they, too, induce more deleterious effects in early development than in adulthood. For example, abnormalities in the reproductive system are induced by EDCs when animals are exposed embryonically or as neonates, but not when they are exposed as adults.[33] Epidemiological studies have also shown that children exposed to EDCs early in life, even for a highly restricted period of time, may suffer significant and long-term consequences that arise later in life.[34] Thus caution must be taken in assessing if particular EDCs (or EDCs at particular levels) are harmful if data is taken from adult populations (whether human or animal). Exposure to these compounds may be relatively benign in adults. However, even if present only transiently during a critical period of development, EDCs may induce significant detrimental effects that may not emerge until later in life.

IV. EXPERIMENTAL MODELS FOR TESTING EDC EFFECTS

Given the expansive number of chemicals that need to be screened for EDC activity, what is the best experimental system to use? Several laboratories have utilized rapid screens in cell lines or in yeast to assess estrogen or androgen binding activity.[35] These tests are fast, but they do not adequately address how EDCs will alter development of complex tissues, such as those comprising the central nervous system.

Numerous studies designed to investigate the effects of EDCs on development of peripheral reproductive structures have been carried out in

32. S.A. Laessig, M.M. McCarthy, & E.K. Silbergeld, *Neurotoxic effects of endocrine disruptors*, 12 CURRENT OPINION NEUROLOGY 4745–51 (1999).

33. W.R. Kelce, L.E. Gray, & E.M. Wilson, *Antiandrogens as environmental endocrine disruptors*, 10 REPRODUCTION FERTILITY DEV. 105–11 (1998); S.A. Laessig, M.M. McCarthy, & E.K. Silbergeld, *Neurotoxic effects of endocrine disruptors*, 12 CURRENT OPINION NEUROLOGY 4745–51 (1999).

34. E. Dewailly, P. Ayotte, S. Bruneau, C. Laiberte, D.C.G. Muir, R.J. Norstrom, *Inuit exposure to organochlorides through the aquatic food chain in arctic Quebec*, 101 ENVTL. HEALTH PERSP. 618–20 (1993); L.S. Birnbaum, Developmental effects of dioxins, 103 ENVTL. HEALTH PERSP. 89–99 (1995); B. Eskenazi & G. Kimmel, *Workshop on perinatal exposure to dioxin-like compounds, II Reproductive effects*, 103 ENVTL. HEALTH PERSP. 143–45 (1995).

35. P. Sohoni & J.P. Sumpter, *Several environmental oestrogens are also anti-androgens*, 158 J. ENDOCRINOLOGY 327–39 (1998); WR Kelce & LE Gray, *Environmental antiandrogens: in vitro and in vivo screening mechanisms*, 28 LAB ANIMAL 26–32 (1999).

rodents,[36] and mammals provide an excellent system in which to assess how EDC exposure may interfere with human development. However, rodents and other mammals have limitations as an experimental system. Specifically, the number of pups obtained with each mating is small, the EDCs have to be administered by methods that do not mirror how organisms are exposed under natural conditions, and rodent development is relatively slow. Therefore, using rats and mice to screen the thousands of chemicals that are on the list of potential EDCs would necessitate a very long period of study.

Finally, while rodents provide arguably the best system in which to model EDC effects in humans, they may not provide the best system in which to address EDC effects on wildlife populations, in particular those that are aquatic. For example, it has been estimated that greater than 60% of the alkylphenol polyethoxylates that are ingredients of nonionic detergents, paints, herbicides, and pesticides (which are produced at a rate greater than 300,000 tons per year) end up in aquatic environments where they may accumulate in both sediment and biological material.[37] Moreover, environmental studies indicate that aquatic species are particularly sensitive indicators of the deleterious effects of EDCs.[38]

An excellent model system, which provides both the ability to rapidly screen a large number of chemicals and to assess the effects of EDCs on complex vertebrate development, is the African clawed frog, *Xenopus laevis* ("*Xenopus*"). There are many advantages to using *Xenopus* as the experimental model. First, these frogs are totally aquatic, so the EDCs under study can be added directly to the water that the frogs live in, a situation that simulates how many wildlife populations are exposed to EDCs in the environment. Second, mating on a daily basis can be induced year round, thus a large number of embryos can be obtained: on the order of 100 -1000 with each mating. Third, early development in these frogs is rapid with respect to other vertebrates: animals develop from a single-celled fertilized egg to a freely swimming tadpole in only 2.5 days. Finally, *Xenopus* is arguably the best understood preparation for studying molecular

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36. W.R. Kelce & L.E. Gray, *Environmental antiandrogens: in vitro and in vivo screening mechanisms*, 28 LAB ANIMAL 26–32 (1999).

37. *See* R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, & M.G. Parker, *Environmentally persistent alkylphenolic compounds are estrogenic*, 135 J. ENDOCRINOLOGY 175–82 (1994).

38. L.J. Guillette Jr, T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival, & A.R. Woodward, *Developmental abnormalities of the gonadal and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida*, 102 ENVTL. HEALTH PERSP. 680–88 (1994); A. Blaustein, D. Wake, & W. Sousa, *Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions*, 8 CONSERVATION BIOLOGY 60–71 (1994).

mechanisms underlying vertebrate development[39] and is particularly amenable to studies of neurogenesis and neuronal differentiation.[40]

V. BIOLOGY OF THE DEVELOPING NERVOUS SYSTEM IN XENOPUS LAEVIS

The developmental events that underlie formation of the nervous system are extraordinarily well characterized in *Xenopus*. Neurogenesis begins approximately thirteen hours after fertilization.[41] As development proceeds, more new neurons are born, and they undergo a complex and highly regulated set of developmental changes that include: migration to appropriate places within the nascent nervous system; elongation of the processes called axons and dendrites that transmit and receive the electrical signals that are the coinage of information transfer in the nervous system; formation of chemical contacts called synapses between individual nerve cells and; expression of selective sets of neural-specific genes that allow specific subclasses to perform their appropriate functions (e.g., sensory neurons that receive information from the environment versus motoneurons that control muscle cells and movement).[42] In particular, the generation and differentiation of primary sensory neurons that innervate the skin,[43] primary motoneurons that provide efferent control of axial (trunk) musculature,[44] the formation of neuromuscular synapses,[45] and the relationship of

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39. M.J. Slack, *Embryonic Induction*, 41 MECHANISMS DEV. 91–107 (1993); J.R. Tata, *Amphibian metamorphosis: an exquisite model for hormonal regulation of postembryonic development in vertebrates*, 38 DEV. & GROWTH DIFFERENTIATION 223–31 (1996); A.M. Zorn, *Cell-cell signaling: frog frizbees*, 7 CURRENT BIOLOGY 501–04 (1997).

40. D.C. Weinstein & A. Hemmati-Brivanlou, *Neural induction*, 15 ANN. REV. CELL & DEVELOPMENTAL BIOLOGY 411–33 (1999); A Chitnis & C. Kintner, *Neural induction and neurogenesis in amphibian embryos*, 31 PERSP. DEVELOPMENTAL NEUROBIOLOGY 3–15 (1995); N.C. Spitzer, *Development of voltage-dependent and ligand-gated channels in excitable membranes*, 102 PROGRESS BRAIN RES. 169–79 (1994).

41. P.D. NIEUWKOOP & J. FABER, NORMAL TABLE OF *XENOPUS LAEVIS* (DAUDIN) (1967).

42. M. JACOBSON, DEVELOPMENTAL NEUROBIOLOGY 401–51 (3rd ed. 1991); S.F. GILBERT, DEVELOPMENTAL BIOLOGY 257, 284–87 (5th ed. 1997); D.C. Weinstein & A. Hemmati-Brivanlou, *Neural induction*, 15 ANN. REV. CELL & DEVELOPMENTAL BIOLOGY 411–33 (1999).

43. A. Hughes, *The development of the primary sensory system in* Xenopus laevis *(Daudin)*, 91 J. ANATOMY 323–38 (1957); A. Roberts & J.D.W. Clarke, The neuroanatomy of an amphibian embryo spinal cord, 296 PHIL. TRANSACTIONS ROYAL SOC'Y B 195–12 (1982); J.D.W. Clarke, B.P. Hayes, S.P. Hunt, & A. Roberts, *Sensory physiology, anatomy and immunohistrochemistry of Rohon-Beard neurones in embryos of* Xenopus laevis, 348 J. PHYSIOLOGY 51125. (1984).

44. A. Hughes, *Studies in embryonic and larval development in amphibia. II. The spinal motor-root*, 7 J. EMBRYOLOGY EXPERIMENTAL MORPHOLOGY 128–45 (1959); A. Roberts & J.D.W. Clarke, *The neuroanatomy of an amphibian embryo spinal cord*, 296 PHIL. TRANSACTIONS ROYAL SOC'Y B 195–12 (1982).

45. F. Moody-Corbett, *Formation of the vertebrate neuromuscular junction*, DEVELOPMENTAL BIOLOGY, VOL 2, 605–35 (L.W. Browder ed., 1986); P. Brehm & L.P. Henderson, *Regulation of*

neuromuscular development to swimming behavior[46] are all highly stereotypic developmental programs that have been thoroughly characterized at the level of the whole embryo. Moreover, the concomitant cellular and molecular changes that occur within single identified populations of neurons and muscle cells (myocytes) which underlie these developmental processes are just as reproducible and well-documented. This extensive understanding of normal development in *Xenopus* is of great advantage when trying to determine precisely which developmental processes are altered, deterred or aborted when animals are exposed to EDCs.

In addition to the wealth of literature describing development of the nervous system of intact *Xenopus* embryos, numerous studies have now shown that cells destined to become neurons or myocytes (but ones that have not yet adopted the defining characteristics of these specialized cells), can be isolated from the developing embryo and maintained in a dish as a dissociated cell culture (*in vitro*). Under these conditions, these cells will not only survive, but will go on to differentiate as neurons and faithfully reproduce many aspects of normal neural development, including elongation of processes, appropriate expression of ion channels that generate both electrical signals and transduce chemical signals at synapses, and the formation and maturation of synaptic contacts with appropriate targets (e.g., motoneurons will form synapses with muscle cells *in vitro*).[47]

Thus, the *Xenopus* embryo provides the advantage of being able to observe effects of putative EDCs not only in the intact embryo, but also under *in vitro* conditions where the environment can be directly manipulated and controlled, and where the molecular actions of specific factors can be determined. For example, the conservation of developmental programs extends to understanding how specific trophic signals (compounds released by developing cells including other neurons, as well as nonneuronal target cells) promote neuronal survival, neuronal differentiation, guide axon outgrowth and govern synaptogenesis.[48]

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acetylcholine receptor channel function during development of skeletal muscle, 129 DEVELOPMENTAL BIOLOGY 1–11 (1988).

46. P. van Mier, J. Armstrong, & A. Roberts, *Development of early swimming in* Xenopus laevis *embryos: myotomal musculature, its innervation and activation*, 32 *Neurosci.* 113–26 (1989).

47. F. Moody-Corbett, *Formation of the vertebrate neuromuscular junction*, DEVELOPMENTAL BIOLOGY, VOL. 2, 605–35 (L.W. Browder ed., 1986); P. Brehm & L.P. Henderson, *Regulation of acetylcholine receptor channel function during development of skeletal muscle*, 129 DEVELOPMENTAL BIOLOGY 1–11 (1988).

48. A.M. Lohof, N.Y. Yip, & M-M Poo, *Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF*, 363 NATURE 350–53 (1993); Ti Wang, K. Xie, & B. Lu, *Neurotrophins promote maturation of developing neuromuscular synapses*, 15 J. NEUROSCIENCE 4796–05 (1995); J.C. Liou, R.S. Yang, & W.M. Fu, *Regulation of quantal secretion by neurotrophic factors at*

Because steroid hormones are known to regulate the expression of neurotrophic factors and neurotrophin receptors,[49] interference with neurotrophin signaling pathways may be a likely mechanism by which EDCs could disrupt neuronal differentiation and synaptogenesis. In particular, preliminary data from our laboratory indicates that early exposure (prior to formation of the nervous system) to both the endogenous estrogen 17fl-estradiol, and to the EDCs, methoxychlor and nonylphenol, induces significant deficits in neural development, with the most notable changes observed in cells derived from part of the developing nervous system termed the neural crest.[50] These neural crest cells require specific trophic factors for both survival and differentiation,[51] and consistent with this requirement, we have also shown that the ability of these trophic factors to induce differentiation of neurons developing *in vitro* is inhibited by these EDCs.

CONCLUSION

These data suggest that *Xenopus* embryos can be used to rapidly and reliably screen for detrimental effects on vertebrate neural development, and that the ability to study neuronal differentiation both in whole embryos and in dissociated cell cultures makes *Xenopus* an excellent model system not only for screening potential EDCs for estrogenic and anti-androgenic activity, but for delineating the molecular mechanism of EDC action. With advances in this field, it is hoped that the dangers posed by EDCs to wildlife and to human populations will be fully realized so that further action can be taken to decrease environmental contamination.

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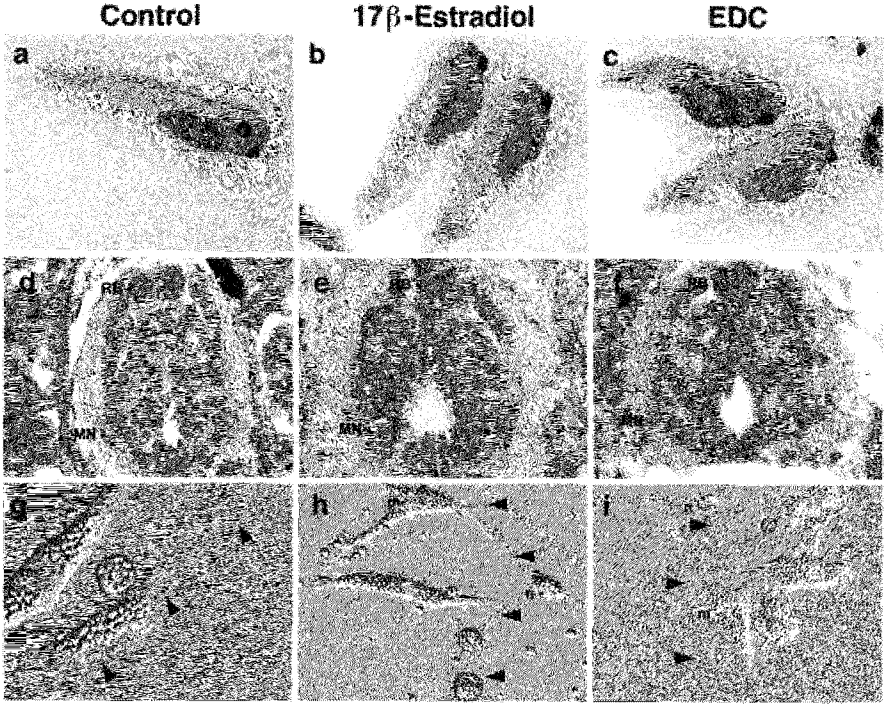

Figure 1.

Photographs in panels a-c show representative examples of tadpoles exposed to normal saline environment alone (control) or those exposed to the naturally occurring estrogen, 17fl-estradiol, or the EDC, nonylphenol. Animals were exposed at ~10 hrs after fertilization (a time before nervous system tissue begins to form) and maintained in the hormone treatment for ~2 days. Both 17fl-estradiol and nonylphenol had significant deleterious effects on the development of these tadpoles. Panels d-f show representative cross-sections through the spinal cords of control animals and those exposed to steroids or EDCs. Large sensory neurons (RB) and motoneurons (MN) can be identified in all animals, suggesting that the gross development of the central nervous system is not disrupted, but some of the motoneurons in the 17fl-estradiol- and the EDC-treated animals seem pale and not healthy. Panels g-i show representative examples of muscle cells (m) and neurons (n and arrowheads) obtained from dissociating the part of the embryo that contains the developing spinal cord and some of the tail musculature at an early stage. These dissociated cell cultures provide a convenient system in which to test directly the effects of EDCs on the ability of specific cell types to survive and differentiate.