Early embryogenesis in zebrafish is affected by bisphenol A exposure

William K. F. Tse1,*, Bonnie H. Y. Yeung1,*, H. T. Wan1 and Chris K. C. Wong1,2,*
1Department of Biology and 2Croucher Institute for Environmental Sciences, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

*These authors contributed equally to this work
*Authors for correspondence (ckcwong@hkbu.edu.hk; kftse@hkbu.edu.hk)

Biology Open 2, 466–471
doi: 10.1242/bio.20134283
Received 30th January 2013
Accepted 25th February 2013

Summary
Exposure of a developing embryo or fetus to endocrine disrupting chemicals (EDCs) has been hypothesized to increase the propensity of an individual to develop a disease or dysfunction in his/her later life. Although it is important to understand the effects of EDCs on early development in animals, sufficient information about these effects is not available thus far. This is probably because of the technical difficulties in tracing the continuous developmental changes at different stages of mammalian embryos. The zebrafish, an excellent model currently used in developmental biology, provides new insights to the field of toxicological studies. We used the standard whole-mount in situ hybridization screening protocol to determine the early developmental defects in zebrafish embryos exposed to the ubiquitous pollutant, bisphenol A (BPA). Three stages (60–75% epiboly, 8–10 somite, and prim-5) were selected for in situ screening of different molecular markers, whereas BPA exposure altered early dorsoventral (DV) patterning, segmentation, and brain development in zebrafish embryos within 24 hours of exposure.

© 2013. Published by The Company of Biologists Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial Share Alike License (http://creativecommons.org/licenses/by-nc-sa/3.0).

Key words: Bisphenol A, Embryogenesis, Zebrafish

Introduction
The drastic advancement in industrialization and technology and the growth in human population in the past century have resulted in unprecedented environmental changes in the human history. The production of large amounts of synthetic industrial and biomedical chemicals as well as pollutants poses a risk to our ecosystem and induces negative effects on the health of wildlife and human beings. Some of the more damaging chemical contaminants are classified as endocrine disrupting chemicals (EDCs) because they can interfere with the synthesis, metabolism, and action of endogenous hormones (Phillips et al., 2008; Phillips and Foster, 2008). EDCs exert different biological effects via diverse mechanisms of actions (Judson et al., 2009; Rhind, 2008; Wigle et al., 2008). EDCs are believed to cause damages to human health and the ecological systems. With the emergence of the global problem of chemical contamination, the adverse biological effects of EDCs are gaining attention among the scientific communities, industry, governments, non-governmental organizations, and the public. There is an increasing need for the identification and quantification of all these ubiquitous chemical contaminants. The possible routes of exposure of humans to the EDCs are through the environments, consumer products, and foods (Feron et al., 2002; Mantovani et al., 2006; Poppenga, 2000; Wigle et al., 2008). To safeguard the public health, instrumental chemical analysis has been adopted globally for assessing the risk of human exposure to EDCs and their metabolites (Hotchkiss et al., 2008). Considering the severe long-term impact of EDCs on public health, a sensitive animal model is required to assess the risks of the EDCs for protecting human and ecological health.

Rapid structural and functional changes occur during the fetal life making it a vulnerable period of development. The process of development is not a simple process of unfolding the inherited genetic program, followed by the commitment of cells to specific lineages, and structural and functional differentiation in respective organs/tissues. Developmental plasticity in animals can be influenced by both genomic (epigenetic and genetic) and environmental factors, which leads to considerable changes in the developmental path for adaptive responses in the fetus (Bateson et al., 2004; Gluckman et al., 2009; Gluckman et al., 2008; Gluckman and Hanson, 2007; Gluckman et al., 2005a; Gluckman et al., 2005b; Hanson and Gluckman, 2008). To fill the information gap between exposures to EDCs and the outcomes of developmental failure, an experimental model that enables us to investigate the early developmental stages is essential. Zebrafish has been extensively used in developmental biology and has become an attractive model for chemical screening. This is a highly scalable model with a well-established genome database (Barros et al., 2008; Yeh et al., 2009; Zon and Peterson, 2005). This model has been used in general toxicology studies for decades. General toxicology studies such as identification of the median lethal concentration (LC50) and end-point phenotype have been performed in zebrafish after bisphenol A (BPA) exposure (Duan et al., 2008; McCormick et al., 2011; Saili et al., 2012). Recently, next-generation sequencing technology was used to identify potential...
Biology Open

al., 2009). Furthermore, BPA administration in rodents could
decrease in fertility and fecundity (Cabaton et al., 2011) and had
importantly, female mice prenatally exposed to BPA showed a
disruption in cardiac development (Krauss et al., 1991), and
pax2a development of reproductive and neuronal systems (Jas ˇarevic´e
et al., 2012). A considerable number of studies in rodents have
reported the negative effects of BPA on the function and
metabolic disorders in humans, such as cardiovascular diseases,
can interact with thyroid hormone receptors (Moriyama et al.,
selective estrogen receptor modulator (Richter et al., 2007) and
properties and toxicities of BPA have been reported. BPA is a
in situ characterization of exposure to effect. It is difficult to monitor
Effects of BPA exposure in zebrafish embryos (Lam et al., 2011).
Thus, the unique developmental features of zebrafish have not been used in many studies for
characterization of exposure to effect. It is difficult to monitor
the effects of EDCs on early development by using mammalian
embryos; therefore, zebrafish, which has the ability of external
fertilization, is used as an alternative model. Gibert and his
colleagues used different in situ molecular markers to examine
the developmental stage of otolith formation in zebrafish after
BPA exposure (Gibert et al., 2011). Here, we hypothesize that the
primary action of EDCs is to prevent normal development during
early embryogenesis and cell fate determination (i.e. cell
signaling and epigenetic modification) and thus affect normal
development (i.e. cell fate determination and organogenesis),
which leads to organ dysfunction. In this study, we used the
zebrafish model to show that exposure of zebrafish embryos to
low doses of BPA caused disturbance in dorsal/ventral patterning
and segmentation, which provides a new insight on
developmental toxicology of environmental pollutants.

Materials and Methods

Fish strains and maintenance
We used the AB wild-type line in this study. The zebrafish were raised and staged
as described previously (Kimmel et al., 1995). All experimental procedures on
zebrafish embryos were approved by the Hong Kong Baptist University, Hong
Kong Special Administrative Region.

BPA exposure in zebrafish embryos
BPA (Sigma–Aldrich, USA) was dissolved in DMSO and diluted in egg medium
(E3 medium). BPA was used at a final concentration of 50 μM in all experiments, which
is comparable to the concentrations used in other studies (Lam et al., 2011; Sun
et al., 2009). Embryos at 1–4 cell stage were directly exposed to BPA in 2 ml of
E3 medium in a 6-well plate. The embryos were grown at 28°C for the selected
time points (stages), 8 hours post-fertilization (hpf) (60–75% epiboly), 14 hpf (8–10 somite), 24 hpf (prim-5), and 72 hpf (protruding mouth). Control embryos were
treated with equal volume of DMSO as that in the BPA-exposed embryos.

Screening procedure and whole-mount in situ hybridization
We used the whole-mount in situ hybridization (WISH) procedure for screening on
the basis of our previous study (Tse and Jiang, 2012). Briefly, BPA-exposed
embryos were collected at 3 stages 60–75% epiboly, 8–10 somite (ss), and prim-5 and
were fixed in 4% paraformaldehyde (PFA). Standard WISH procedure was
applied using zebrafish embryos. Plasmids that were used to make antisense
mRNA probes have been published previously: chd (Miller-Bertoglio et al., 1997),
eve1 (Gajewski et al., 2001), eng2 (Schier et al., 1993), gata2 (Detrich et al., 1995), gsc
(Stachel et al., 1993), krox20 (Stahl et al., 1993), myod (Weinberg et al., 1996),
pan2a (Krauss et al., 1991), and otx2 (Heisenberg et al., 1996).

Results and Discussion

BPA is one of the most common EDCs, and the chemical
properties and toxicities of BPA have been reported. BPA is a
selective estrogen receptor modulator (Richter et al., 2007) and
can interact with thyroid hormone receptors (Moriyama et al.,
2002; Zoeller et al., 2005) and peroxisome proliferator-activated
receptors (Riu et al., 2011). At the physiological levels, BPA
is suggested to be a factor attributed to the development
of metabolic disorders in humans, such as cardiovascular diseases,
leptin, and insulin resistance (Polyzos et al., 2012; vom Saal et
al., 2012). A considerable number of studies in rodents have
reported the negative effects of BPA on the function and
development of reproductive and neuronal systems (Jašarević et
al., 2011; Wolstenholme et al., 2011; Xi et al., 2011). More
importantly, female mice prenatally exposed to BPA showed a
decrease in fertility and fecundity (Cabaton et al., 2011) and had
an adverse effect on the fertility of the male offspring (Salian et
al., 2009). Furthermore, BPA administration in rodents could
disturb neurons in the substantia nigra (Tando et al., 2007) and in
the hippocampus (Kunz et al., 2011). In previous studies,
zebrafish embryos have been exposed to BPA at concentrations
similar to those used in this study, and otolith malformations
(70 μM) and cardiac edema (65 μM) have been reported (Duan
et al., 2008; Gibert et al., 2011). Although the general effects and
the effects of BPA on development in rodents and zebrafish have
been reported, important information about the effects of BPA in
the initial stages of cell development remains to be addressed. To
understand the mechanism underlying these effects is important
because these data could reveal the fundamental cause of the
observed effects; further, the data can be utilized to predict and
evaluate the impact of in utero EDC exposure. In this study, we
performed screening in the early stage of embryogenesis; we
selected 3 critical stages, including dorsoventral (DV) patterning
(60–75% epiboly), segmentation (8–10 ss), and brain
development (prim-5), within 24 hpf (Tse et al., 2009; Tse et
al., 2011).

BPA exposure of embryos at 60–75% epiboly stage disturbs DV
classification
DV patterning is an important developmental process in zebrafish
(Schmitz and Campos-Ortega, 1994). Several zebrafish mutants
have been identified on the basis of their dorsal or ventral
phenotypes, which range from C5 (dorsal) to V4 (ventral)
(Mullins et al., 1996; Kishimoto et al., 1997). In this study, we
targeted on exposure to BPA at the early development period
(within 24 hours). Follow-up examination of the effects of the
exposure was performed up to 3 days after fertilization while
mild dorsalization (mainly C1–C3) was observed. Dorsalization
was characterized by their phenotype of shortened posterior parts
during the development (Fig. 1). The DV patterning

![Fig. 1. Morphology and phenotypic frequency of 3-day post-fertilized embryos exposed to bisphenol A. Bisphenol A (BPA)-exposed embryos in an AB wild-type zebrafish showed mild dorsalized phenotypes at 3 days post-fertilization. The control embryos were treated with DMSO (A). BPA exposed embryos showed C1 to C3 mild dorsalization phenotypes (B). Scale bar: 650 μm. Phenotypic frequency is indicated in panel C. C1–C3 phenotypes represent dorsalized phenotype as described (Tse et al., 2009; Mullins et al., 1996). n, number of scored embryos.](http://bio.biologists.org/Downloaded from http://bio.biologists.org/)}
occurs in the early stage of embryogenesis, the effects of the BPA action can be observed by using selected in situ molecular markers (ventral markers, eve1 and gata2; dorsal markers, chd and gsc) at stage of 60–75% epiboly (Tse et al., 2009). Among various validated markers, eve1 is a zebrafish homeobox gene similar to even-skipped in Drosophila (Joly et al., 1993). eve1 is strongly expressed in the ventrolateral marginal cells. The other gene marker gata2 is a hematopoietic transcription factor gene (Detrich et al., 1995) for ventral ectoderm and hematopoietic cells in the ventral mesoderm. To trace the dorsal patterning, we used 2 dorsally expressed markers chordin (chd) and goosecoid (gsc) (Sasai et al., 1995; Stachel et al., 1993). The expression patterns of eve1 and gata2 in embryos exposed to BPA were more restricted in the ventral half of the marginal and the animal zone than that in the controls (Fig. 2A–D). On the other hand, the expression of dorsal markers chd and gsc was greater in the embryos exposed to BPA (Fig. 2E–H). We measured the angles of expressions of the markers (Fig. 2I,J). Taken together, embryos at the 60–75% epiboly stage exposed to BPA showed reduced expression levels of the ventral markers but increased expression levels of the dorsal markers.

Exposure of embryos at 8–10 somite stage to BPA affects somatic muscle development
To monitor the trend of altered DV patterning, gata1 and pax2a were used as the markers at 8–10 somite stage. gata1 is ventrally expressed in presumptive hematopoietic cells in 2 lateral stripes (Detrich et al., 1995; Kimmel et al., 1990), while pax2a is used for marking the presumptive neural region (Krauss et al., 1991). The gata1 marker showed widening of the 2 lateral stripes of presumptive hematopoietic cells in BPA-exposed embryos (Fig. 3A,B). Additionally, pax2a staining showed a diffused expression pattern in the mid-hindbrain boundary (mhb). The otic vesicles were missing in the embryos exposed to BPA (Fig. 3C,D). On the other hand, the somite muscle widened in BPA-exposed embryos (Fig. 3E,F). The phenotype was further confirmed by using the myoD somite marker that is expressed in the dorsal mesoderm and somite muscles (Kimmel et al., 1990; Weinberg et al., 1996). Weak and abnormal myoD expression was detected in the BPA-exposed embryos (Fig. 3G,H). In addition to DV patterning, the follow-up in situ experiments illustrated the effects of BPA on somatic muscle formation in the segmentation period.

Fig. 2. Bisphenol A affects dorsal–ventral patterning at the 60–75% epiboly stage. Embryos exposed to bisphenol A (BPA) showed narrower expression pattern for the ventral markers eve1 and gata2 (A–D), but wider expression pattern for the dorsal markers chd and gsc (E–H). Red dotted lines indicate the normal expression margin of the ventral markers (ventricle) or dorsal (horizontal) in both BPA-exposed and control embryos. Images were captured in the lateral view (A–D) and animal pole view (E–H), dorsal towards the right in the 60–75% epiboly stage. Scale bar: 250 μm. Schematic diagrams indicate the expression angles of different markers. x marks the center of the embryos, angle of expression of different in situ markers in control and BPA-exposed embryos. θ indicates the angles of the ventral markers (eve1/gata2) with orange lines (I), while σ represents the angles of the dorsal markers (chd/gsc) with blue lines (J). The angles represent the mean of 20 embryos. The expression angles of the ventral markers were smaller (I) but those of the dorsal markers were larger (J), which represents the dorsalization phenotype (*P<0.05).
BPA exposure of embryos at the prim-5 stage alters brain development

On the basis of the diffused pax2a expression in the mhb region at the 8–10 somite stage, we suspected that BPA affected brain development during the developmental process. To prove this assumption, the genetic markers krox20, otx2, and eng2b were used to monitor the brain regionalization process at the prim-5 stage (Finkelstein and Boncinelli, 1994; Joyner and Guillemot, 1994). BRAIN regionalization is one of the fundamental processes in the early stages of vertebrate brain development, including the formation of the mhb and its adjacent brain regions (Joyner and Guillemot, 1994). The hindbrain develops into a series of rhombomeres along the anterior–posterior axis of the neural tube. Rhombomeres are believed to be involved in neuronal organization in development (Moens and Prince, 2002), while rhombomeres 3 (r3) and 5 (r5) could be involved in neuronal organization in brain development (Moens and Prince, 2002), while rhombomeres 3 and 5 (black asterisk) was found in BPA-exposed embryos (B), which indicates that regionalization was affected. The patterns of eng2b expression indicated that the mid-hindbrain boundary (mhb) was minimized in the BPA-exposed embryos (red asterisk). Compared to the controls (C), BPA-exposed embryos showed a smaller mhb (D), lateral view; magnified views of the dorsal region are shown in the inserts at the right bottom corner. otx2 expression pattern was restricted in the BPA-exposed embryos (blue asterisk). Compared to the controls (E), the BPA-exposed embryos (F) showed reduced size of the midbrain. Magnified views of the dorsal section are shown in the corner. mb, midbrain; mhb, mid-hindbrain boundary; r3/r5, rhombomere 3/5. All were head to the left. Scale bars: 100 µm (A,B); 125 µm (C–F). The number of embryos with the presented phenotype is shown in the top right corner of the panel.

Fig. 3. Bisphenol A alters somite formation at the 8–10 somite stage.

Lateral expansion of the presumptive hematopoietic cell marker (gata1) was indicated by an orange asterisk, which indicates the dorsalized phenotype in the bisphenol A (BPA)-exposed embryos (A,B). pax2a expression at the 8–10 somite stage, dorsal view (C,D). A blue asterisk indicates abnormal developmental pattern in the mid-hindbrain boundary (mhb) in BPA-exposed embryos. Additionally, an arrow marks the missing of the 2 otic vesicles in BPA-exposed embryos (D). Somite morphology of the control (E) and the BPA-exposed embryos (F) at the 8–10 somite stage. Lateral expansion of somite muscles was observed (red dotted lines). The somite marker, myoD, showed widened and diffused expression in the BPA-exposed embryos (red asterisk) as compared to the control (G,H). mhb, mid-hindbrain boundary; ot, otic vesicle; p, pronephric precursor expression domain. All were head to the left. Scale bars: 75 µm (A,B); 200 µm (C,D); 150 µm (E–H). The number of embryos with the presented phenotype is shown in the top right corner of the panel.

Fig. 4. Bisphenol A influences brain development in the prim-5 stage. The expression patterns of markers in different brain regions (krox20, eng2b, and otx2) at the prim-5 stage are shown. The expression of krox20, a marker of rhombomeres 3 and 5, dorsal view (A,B). Abnormal patterning of rhombomeres 3 and 5 (black asterisk) was found in BPA-exposed embryos (B), which indicates that regionalization was affected. The patterns of eng2b expression indicated that the mid-hindbrain boundary (mhb) was minimized in the BPA-exposed embryos (red asterisk). Compared to the controls (C), BPA-exposed embryos showed a smaller mhb (D), lateral view; magnified views of the dorsal region are shown in the inserts at the right bottom corner. otx2 expression pattern was restricted in the BPA-exposed embryos (blue asterisk). Compared to the controls (E), the BPA-exposed embryos (F) showed reduced size of the midbrain. Magnified views of the dorsal section are shown in the corner. mb, midbrain; mhb, mid-hindbrain boundary; r3/r5, rhombomere 3/5. All were head to the left. Scale bars: 100 µm (A,B); 125 µm (C–F). The number of embryos with the presented phenotype is shown in the top right corner of the panel.

Conclusions

In this study, the standard in situ hybridization method was used to examine the effects of EDCs on early embryogenesis. We found that BPA exposure influences DV patterning, somite formation, and brain development in zebrafish embryos. Our study showed the potential use of zebrafish for validating the effects of EDC at a particular developmental stage.
Kong. The work in the W.K.F.T. laboratory is supported by the Faculty start-up fund (BIOL3840101).

Competing Interests
The authors have no competing interests to declare.

References
Barros, T. P., Alderton, W. K., Reynolds, H. M., Roach, A. G. and Bergmann, S. (2008). Bisphenol A: an emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery. Br. J. Pharmacol. 154, 1400-1413.

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., U’Dine, B., Foley, R. A., Gluckman, P., Hetherington, K., Kirkwood, T., Labh, M. et al. (2004). Developmental plasticity and human health. Nature 430, 419-421.

Cabaton, N. J., Wadia, P. R., Rubin, B. S., Zalko, D., Schaeberle, C. M., Askenase, M. H., Gardbois, J. L., Tharp, A. P., Whitt, G. S., Sonnenschein, C. et al. (2011). Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environ. Health Perspect. 119, 547-552.

Detrich, H. W., 3rd, Kieran, M. W., Chan, F. Y., Barone, L. M., Yee, K., Kord, V. and Fjose, A. (1991). Expression of the zebrasfish paired box gene pax-2[pf] during early neurogenesis. Development 113, 1193-1206.

Kunz, N., Camm, E. J., Somm, E., Lodygensky, G., Darbre, S., Aubert, M. L., Hupp, P. S., Sizonenko, S. V. and Graeuter, R. (2011). Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation. J. Int. Dev. Neurosci. 29, 37-43.

Lam, S. H., Haing, M. M., Zhang, X., Yan, C., Duan, Z., Zhu, L., Ung, C. Y., Mathavans, S., Ong, C. N. and Gong, Z. (2011). Toxicogenomic and phenotypic alterations of bisphenol-A early-life exposure toxicity in zebrafish. PLoS ONE 6, e28273.

Mantovani, A., Maranghi, F., Purificato, I. and Macri, A. (2006). Assessment of feed additives and contaminants: an essential component of food safety. Ann. Ist. Super. Sanita 42, 427-432.

McCrormick, J. M., Van Es, T., Cooper, K. R., White, L. A. and Haggblom, M. M. (2011). Microbiologically mediated O-methylation of bisphenol A results in metabolites with increased toxicity to the developing zebrafish (Danio rerio) embryo. Environ. Sci. Technol. 45, 6567-6574.

Mercier, P., Simeone, A., Cotelli, F. and Bocconcelli, E. (1995). Expression pattern of two ots genes suggests a role in specifying anterior body structures in zebrafish. Int. J. Dev. Biol. 39, 559-573.

Mobilio, B., Millegre, V. E., Fisher, S., Sánchez, A., Mullins, M. C. and Halpern, M. E. (1997). Differential regulation of chordin expression domains in mutant zebrafish. Dev. Biol. 192, 537-550.

Morns, C. B. and Prince, V. E. (2002). Constructing the hindbrain: insights from the zebrafish. Dev. Dyn. 224, 1-17.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saito, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H. and Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. 87, 5185-5190.

Mullins, M. C., Hammerschmidt, M., Kano, D. A., Odenthal, J., Brand, M., van Eden, F. J., Furutani-Seiki, M., Granato, M., Haefner, H. P., Heisenberg, C. P. et al. (1996). Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral specifying genes. Development 123, 81-93.

Oxtoby, E. and Jowett, T. (1993). Cloning of the zebrafish krox-20 gene (kro20) and its expression during hindbrain development. Nucleic Acids Res. 21, 1087-1095.

Phillips, K. P. and Foster, W. G. (2008). Key developments in endocrine disruptor research and human health. J. Toxicol. Environ. Health B Crit. Rev. 11, 322-344.

Phillips, K. P., Foster, W. G., Leiss, W., Shahn, V., Karyakinina, N., Turner, M. C., Kacew, S. and Krewski, D. (2008). Assessing and managing risks arising from exposure to endocrine-active chemicals. J. Toxicol. Environ. Health B Crit. Rev. 11, 351-372.

Polizos, S. A., Kountouras, J., Deretzis, G., Zavos, C. and Mantzoros, C. S. (2012). The emerging role of endocrine disruptors in pathogenesis of insulin resistance: a concept implying nonalcoholic fatty liver disease. Curr. Med. Res. Opin. 26, 68-82.

Poppenga, R. H. (2000). Current environmental threats to animal health and productivity. Vet Clin North Am. Food Anim Pract. 16, 545-558, viii.

Rhind, S. M. (2008). Endocrine disruptors and other food-contaminating pollutants as risk factors for animal reproduction. Reprod. Dev. Anim. Is 43 Suppl. 2, 15-22.

Richter, A., Taylor, J. A., Ruhlen, R. L., Welshons, W. V. and vom Saal, F. S. (2007). Estradiol and bisphenol A stimulate androgen receptor and estrogen receptor gene expression in female mouse prostate mesenchymal cells. Environ. Health Perspect. 115, 902-908.

Ruin, A., Grimaldi, M., le Maire, A., Bey, G., Phillips, K., Boulahtouf, A., Boulergue, M., Bourgouer, W. and Balaguer, P. (2011) Peroxisome proliferator-activated receptor γ is a target for halogenated analogs of bisphenol A. Environ. Health Perspect. 119, 1227-1232.

Saill, K. S., Corvi, M. M., Weber, D. N., Patel, A. U., Das, S. R., Przybyla, J., Anderson, K. A. and Tangay, R. L. (2012). Neurodevelopmental low-dose bisphenol A exposure leads to early-life-stage hyperactivity and learning deficits in adult zebrafish. Toxicol. Sci. 980, 83-92.

Salian, S., Doshi, T. and Vanage, G. (2009). Perinatal exposure of rats to Bisphenol A alters the fertility of male rats. Life Sci. 85, 742-752.

Sasi, Y., Lu, B., Steinheisser, H. and De Robertis, E. M. (1995). Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. Nature 376, 333-336.

Schier, A. F., Neumann, S. C., Harvey, M., Malicki, J., Solnicka-Krezel, L., Stainier, D. Y. R., Zwartzkris, F., Abdelilah, S., Stemple, D. L., Rangini, Z. et al. (1996). Mutations affecting the development of the embryonic zebrafish brain. Development 123, 165-177.

Schier, A. F., Campos-Ortega, J. A. (1994). Dorso-ventral polarity of the zebrafish embryo is distinguishable prior to the onset of gastrulation. Roux’s Archives of Developmental Biology 203, 374-380.

Stachel, S. E., Grunwald, D. J. and Myers, P. Z. (1993). Lithium perturbation and glycosylated expression induce novel specification pathway in the pregastrula zebrafish embryo. Development 117, 1261-1274.

Strahle, U., Blader, P., Henrique, D. and Ingham, P. W. (1993). A axial, a zebrafish gene expressed along the developing body axis, shows altered expression in cyclops mutant embryos. Genes Dev. 7, 1436-1446.

Downloaded from http://bio.biologists.org/ by guest on March 7, 2021
Stuart, E. T., Kioussi, C. and Gruss, P. (1994). Mammalian Pax genes. *Annu. Rev. Genet.* 28, 219-238.

Sun, H., Shen, O.-X., Wang, X.-R., Zhou, L., Zhen, S.-Q. and Chen, X.-D. (2009). Anti-thyroid hormone activity of bisphenol A, tetrabromobisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. *Toxicol. In Vitro* 23, 950-954.

Tando, S., Itoh, K., Yasi, T., Ikeda, J., Fujivara, Y. and Fushiki, S. (2007). Effects of pre- and neonatal exposure to bisphenol A on murine brain development. *Brain Dev.* 29, 352-356.

Tse, W. K. F. and Jiang, Y. J. (2012). Functional screen of zebrafish deubiquitylating enzymes by morpholino knockdown and in situ hybridization. *Methods Mol. Biol.* 815, 321-331.

Tse, W. K. F., Eisenhaber, B., Ho, S. H. K., Ng, Q., Eisenhaber, F. and Jiang, Y.-J. (2009). Genome-wide loss-of-function analysis of deubiquitylating enzymes for zebrafish development. *BMC Genomics* 10, 637.

Tse, W. K. F., You, M.-S., Ho, S. H.-K. and Jiang, Y.-J. (2011). The deubiquitylating enzyme Cops6 regulates different developmental processes during early zebrafish embryogenesis. *Int. J Dev. Biol.* 55, 19-24.

Wigle, D. T., Arbuckle, T. E., Turner, M. C., Bérubé, A., Yang, Q., Liu, S. and Krewski, D. (2008). Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J. Toxicol. Environ. Health B Crit. Rev.* 11, 373-517.

Wolstenholme, J. T., Taylor, J. A., Shetty, S. R., Edwards, M., Connelly, J. J. and Rissman, E. F. (2011). Gestational exposure to low dose bisphenol A alters social behavior in juvenile mice. *PLoS ONE* 6, e25448.

Xi, W., Lee, C. K. F., Yeung, W. S. B., Giesy, J. P., Wong, M. H., Zhang, X., Hecker, M. and Wong, C. K. C. (2011). Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypotalamus-pituitary-gonadal axis of CD-1 mice. *Reprod. Toxicol.* 31, 409-417.

Yeh, J. R., Munson, K. M., Elagib, K. E., Goldfarb, A. N., Sweetser, D. A. and Peterson, R. T. (2009). Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. *Nat. Chem. Biol.* 5, 236-243.

Zoeller, R. T., Bansal, R. and Parris, C. (2005). Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist *in vitro*, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology* 146, 607-612.

Zon, L. I. and Peterson, R. T. (2005). *In vivo* drug discovery in the zebrafish. *Nat. Rev. Drug Discov.* 4, 35-44.