2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) has been associated with many disease states in humans. A rising concern is that exposure early in life can lead to adult toxicity and toxicity in subsequent generations. Juvenile zebrafish exposed to TCDD (50 pg/ml in water; 1 h exposure) at 3 and 7 weeks post fertilization showed toxicity only later in adulthood. We have maintained the offspring of these exposed F0 fish to determine whether we could find adverse effects in the next two generations of F1 and F2 offspring. TCDD exposure produced a significantly higher female: male ratio in all three generations. Scoliosis-like axial skeleton abnormalities, not normally observed in controls, were present in the F1 and F2 generations descended from the treated F0 founders. Egg release and fertilization success were reduced in the TCDD lineage F1 and F2 generations. This reduction in fertility in the TCDD lineage F2 generation could be attributed to alterations in the F2 males. Using zebrafish as a model allowed the simultaneous maintenance of different generations with relatively small space and costs. The zebrafish showed clear signs of transgenerational responses persisting into generations never directly exposed to TCDD.

Key words: toxicity; transgenerational; TCDD; dioxin; zebrafish; endocrine disruption; skeletal; reproductive; sexual differentiation; ovary.

2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is a toxic global contaminant released into the environment as a byproduct of human activities, including industrial emissions and waste incineration. Dioxin exposure has been associated with diseases that include cancer, heart disease, skeletal malformations, and reproductive abnormalities (Eskenazi et al., 2004; Guo et al., 2000; Mocarelli et al., 2008; NAS-IOM, 2011; Ni et al., 2010). Among the most commonly reported toxic endpoints for TCDD exposure in humans, rodents, birds, or fish are alterations in the female/male sex ratio (Ikeda et al., 2005; Mocarelli et al., 2000), skeletal abnormalities (Bursian et al., 2013; Hormung et al., 1999; Peterson et al., 1993; Xiong et al., 2008), and reproductive defects (De Vito and Birnbaum, 1994; King Heiden et al., 2009; Yoshizawa et al., 2009).

TCDD exposure during development alters the characteristics of different tissues, leading to effects in adulthood. It has been recognized that chemicals that are able to reprogram developing tissues can in principle also alter the characteristics of germ cells, leading to effects that span generations after the exposure. Evidence for this sort of effect has been shown for several chemicals that alter development (Anway et al., 2006; Manikkam et al., 2012; Nilsson et al., 2012). Because these chemicals alter gene expression and phenotype in a heritable manner, yet do not alter the DNA sequence in the genome, these effects have been termed epigenetic.

Any chemical that can cause adverse health effects on the children or the grandchildren of the exposed individual, decades after an exposure, is the source of grave concern. This is especially so when the initial exposure produces little if any effects at the time of exposure.

Despite the importance of the problem, it is not easy to investigate. Epidemiology studies have great power, but are confounded by great diversity among the subjects, that can include differences in genetic background, factors such as diet, and great variety in the exposure events themselves. For chemicals with little initial impact, the exposure may never be noted at all, and can only be inferred. Furthermore, the human life span and generation time matches that of the investigators, making observation of multigenerational events difficult in humans.

Animal models fill many of these gaps, allowing controlled exposures, genetic background, and environment. Ideally, the model should be easy to examine for adverse effects, and have short generation times, allowing multigeneration studies in a reasonable period of time. A promising model species is the zebrafish. The zebrafish is well-established model for investigating development and disease, produces numerous offspring that are inexpensive to house, and have a relatively short generation time (3–4 months). Effects on development are easy to detect because the eggs are externally fertilized and the embryos are transparent during development.

In our previous work, sublethal TCDD exposures during development, that had no adverse effects at the time of exposure, caused reproductive and skeletal abnormalities in adulthood (Baker et al., 2013). From this work, it is clear that sublethal TCDD exposure alters the fate of cells or tissues that is...
manifested long after exposure. Similar effects in germ cells could produce epigenetic alterations affecting subsequent generations.

Our previous work included cohorts exposed to TCDD at 3 and 7 weeks post fertilization (wpf). This period of time was chosen because the exposures occur during gonad differentiation and development. Because this low-level exposure (1 h in water at 50 pg/ml) produced adult consequences, and coincided with germline development, we have followed the offspring of these exposed fish, looking for transgenerational effects. Here, we report that this exposure does indeed produce transgenerational toxicity, producing scoliosis and infertility.

During the initial dosing, progenitors that would become the gametes forming the F1 fish were directly exposed to TCDD. In principle then, it is possible to say that the F1 fish were exposed, even if the TCDD is gone by the time the embryo is formed (Fig. 1). Effects observed in the F2 generation can only be caused by epigenetic alterations in the germline. Here, we report that this exposure does indeed produce transgenerational phenotypic effects, producing scoliosis and infertility in both F1 and F2 zebrafish descended from exposed F0 founders.

**MATERIALS AND METHODS**

*Fish husbandry.* Zebrafish embryos (AB strain) were kept at 27–30°C in lightly buffered water (60 mg/l Instant Ocean Salts; Aquarium Systems, Mentor, OH) with a standard 14-h/10-h light/dark cycle as described by Westerfield (2000). Fish were fed twice per day. Fish were euthanized in Tricaine methanesulfonate (MS-222, 1.67 mg/ml). The protocol for zebrafish use and maintenance was approved by the Research Animal Resources Center of the University of Wisconsin-Madison, which follows the National Institutes of Health Guide to the Care and Use of Laboratory Animals (protocol no. M00489).

*TCDD exposure.* TCDD (>99% purity; Chemsyn) was used as a 0.4 ng/ml stock solution in dimethyl sulfoxide (DMSO). Zebrafish were exposed as previously described (Baker et al., 2013; Henry et al., 1997) at 3 wpf and again at 7 wpf to waterborne TCDD (50 pg/ml) or vehicle (0.1% DMSO) for 1 h each time in small glass beakers with gentle rocking. The number of fish per volume of dosing solution was 1 fish/ml at 3 wpf, and due to growth between 3 and 7 wpf, 1 fish/2 ml at 7 wpf. All results are derived from three independent TCDD exposure experiments done in successive blocks. Mean percent survival (± SEM) was recorded. These exposed fish are referred to as F0 fish. At maturity, F0 males and females were spawned to yield the F1 generation, which were kept as separate blocks, each derived from F0 fish from a single experiment. These were in turn crossed at adulthood to produce the F2 generation fish, again descended from discrete exposure experiments.

**FIG. 1.** Zebrafish generations. The schematic shows the F0, F1, and F2 generations relative to the initial exposure in the F0 as juveniles. The schematic shows dosing of juvenile at 3 and 7 wpf, and growth to adulthood. Progenitor cells of the gametes forming the F1 were present during exposure, and eventually produce offspring. The F1 were spawned, allowing examination of the unexposed F2 generation.
Scoring for sex ratios and abnormalities. At 16 wpf, fish were evaluated for skeletal abnormalities. Skeletal abnormalities were scored visually for cranial, jaw, or axial spine abnormalities. Fish with specific skeletal abnormalities were stained with alcian blue (0.015%) and alizarin red (0.01%) according to methods previously described (Baker et al., 2013; Walker and Kimmel, 2007). In order to score the ratio of females to males all fish were euthanized at 1 year of age and the gonads were identified and confirmed using a dissecting microscope.

Reproduction. Fish were spawned approximately once every two weeks from 20 to 36 wpf (n = 40 spawns for each generation) to assess reproductive capacity. For all spawning experiments, fish were placed together the night before the experiment in groups of three males and three females. Eggs were collected at 3 h after the beginning of the light cycle on the next day. Eggs were collected, counted, and examined for fertilization success 24 h after collection.

Histopathology. Fish from each generation, euthanized at 1 year, were fixed in 10% Zn formalin, decalcified with Cal-ExII, and bisected along the sagittal plane for fixing. The specimens were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, sectioned (5 mm), mounted onto slides, and stained with hematoxylin and eosin (H&E). Gonads were evaluated for lesions by examining four separate fields at 10× in each of two separate sections per fish (at least 10 males and 10 females). Sections were imaged using an Olympus DP72 digital camera on an Olympus S2×16 microscope.

Statistical analysis. Results are reported as mean ± SEM. Levene’s test was used as a preliminary screen for homogeneity of variances between or among groups with equal n values and the Brown–Forsythe test was used for groups with unequal n values. When necessary, natural log or square root data transformations were used to maintain homogeneity of variances between or among blocks. If, using these tests, variances were equal between or among the groups that were being compared (p > 0.05), Student’s t-test was used to identify differences between two groups or one-way analysis of variance (ANOVA) was used to detect differences among more than two groups. Fisher’s Least Significant Test was then used for multiple comparisons. A difference of p < 0.05 was considered significant.

RESULTS

Our previous work exposed cohorts of juvenile zebrafish to TCDD (1h at 50 pg/ml) at 3 and 7 wpf, a period coincident with gonad differentiation and development. Because of the simplicity and low cost of housing zebrafish in large numbers through several generations, we followed the offspring of these TCDD-exposed fish through two additional generations F1 and F2 (Fig. 1). Compared with parallel cohorts of control fish we observed several types of persistent, transgenerational toxicity.

Skeletal Abnormalities

Sublethal TCDD exposure at 3 and 7 wpf caused spinal kinks in the F0 generation (Baker et al., 2013), as well as in the subsequent TCDD lineage F1 and F2 generations. Examples of the deformations observed in the axial skeleton, scored at 16 wpf, are shown in Figure 2A. The axial skeleton was sharply angled or kinked in the lateral and dorsal-ventral planes, in the treated F0 fish and in many of their descendents. As shown in the figure, the spinal abnormalities in all three TCDD lineage generations were similar in overall appearance.

Using alizarin red staining, we looked more closely at the vertebrae at the sites of the axial kinks. The vertebrae in the control fish show a regular “hour glass”-like appearance with wide end plates and narrow vertebral bodies (Fig. 2B). The axial kinks in all three generations appear to correspond to a single malformed vertebra, surrounded by relatively normal vertebrae. Malformed vertebrae, indicated by the arrows in Figure 2B, appeared to have normal end plates, but the vertebral body was shortened, bent and/or collapsed in the center, contributing to the malformed trunk.

The incidence of axial skeleton malformations following F0 TCDD exposure was significantly increased in all three generations (Table 1). This deformation was not frequently observed in controls: across all experiments using 295 fish as controls, the incidence of spinal deformation was 1.7%.
TABLE 1
Effects on Skeletal Formation

<table>
<thead>
<tr>
<th></th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of any skeletal abnormality</td>
<td>1.1 ± 1.1%</td>
<td>1.8 ± 0.9%</td>
<td>1.9 ± 1.2%</td>
</tr>
<tr>
<td>Control</td>
<td>TCDD</td>
<td>TCDD</td>
<td>TCDD</td>
</tr>
<tr>
<td></td>
<td>82.4 ± 3.2%</td>
<td>34.9 ± 4.3%</td>
<td>22.1 ± 3.4%</td>
</tr>
<tr>
<td>Incidence of axial kink</td>
<td>1.1 ± 1.1%</td>
<td>1.8 ± 0.9%</td>
<td>1.9 ± 1.2%</td>
</tr>
<tr>
<td>Control</td>
<td>TCDD</td>
<td>TCDD</td>
<td>TCDD</td>
</tr>
<tr>
<td></td>
<td>54.5 ± 2.5%</td>
<td>28.1 ± 6.1%</td>
<td>17.3 ± 3.0%</td>
</tr>
<tr>
<td>Incidence of cranial malformation</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>TCDD-exposed</td>
<td>TCDD-exposed</td>
<td>TCDD-exposed</td>
</tr>
<tr>
<td></td>
<td>46.9 ± 9.9%</td>
<td>11.7 ± 4.3%</td>
<td>7.8 ± 2.8%</td>
</tr>
<tr>
<td>Incidence of jaw malformation</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>TCDD</td>
<td>TCDD</td>
<td>TCDD</td>
</tr>
<tr>
<td></td>
<td>34.5 ± 6.6%</td>
<td>3.7 ± 1.8%</td>
<td>0.9 ± 0.7%</td>
</tr>
</tbody>
</table>

Notes. Zebrafish were exposed as described in the Materials and Methods section. Incidences of the distinct skeletal malformations are reported as well as the incidence of fish having at least one skeletal abnormality (F0n = 50, F1n = 91, and F2n = 97). Values are expressed as mean ± SEM.

*a*Indicates significant difference (p < 0.05) from the control.

We also observed both cranial and jaw malformations in the TCDD-treated adult F0 fish as well as in their descendents. However, the incidences of all skeletal malformations decreased with each generation, such that by the F2 generation the incidences of jaw and cranial malformations were no longer statistically separable from those of the controls (Table 1). As the most prevalent skeletal abnormality, the axial skeletal kinks were the only skeletal abnormality that remained significantly increased in the TCDD lineage F2 generation.

Adult Sex Ratio

While fish husbandry conditions can affect the ratio of males to females, males typically outnumber females 2:1. We found that TCDD exposure at 3 and 7 wpf increased the ratio of females to males in the F0 generation (Baker et al., 2013; Table 2). This significant shift toward females, as verified by dissection, persisted in both the TCDD lineage F1 and F2 generations. We raised DMSO vehicle-control fish and their F1 and F2 offspring in parallel to the treated fish. For comparison, in all three generations of control fish (F0, F1, and F2), the percentages of females in the DMSO control groups were not significantly different from each other, and averaged at 29% female.

In the TCDD-exposed F0 generation, we observed a mismatch between the female secondary sex characteristics and the gonads (Baker et al., 2013). Of the 30 fish with typical rounded female bodies, 26.5% of them contained testes rather than ovaries. In the TCDD lineage F1 females, 6.8% (± 3.3%; P = 0.091; n = 43) fish with a female body plan and testes. This mismatch was not observed in the TCDD lineage F2 females. We have never observed this phenomenon in untreated fish.

Ovarian Development

As described above, fish were sacrificed at 1 year of age and dissected for gross gonad examination. Gonads were also collected for histological examination. Figure 3 shows an H&E stained section of an ovary from a control adult zebrafish. Examples of all three stages of ovarian maturation are present: small primary follicles, intermediate secondary follicles, and large tertiary (vitellogenic) follicles. Atretic follicles were not commonly observed in the controls, and are not visible in this field. Fish in the control group typically had zero atretic follicles per 10x field.

As previously reported, the sections of ovary from exposed F0 fish showed a loss of organized progression in follicle development (Baker et al., 2013; Fig. 3). We observed similar malformations in the ovaries of TCDD lineage F1 fish. In both examples, the sections show granulomatous inflammation, with numerous atretic follicles breaking down, and few if any mature vitellogenic follicles. While this lack of ovarian organization was observed commonly in the F0 and F1 fish, the defect was rarely seen in the TCDD lineage F2 generation. In these fish, the ovarian histology sections appeared similar to the DMSO controls, showing all three stages of ovarian development.

Table 2 shows the incidence of ovarian abnormalities observed in the H&E sections. There was a significant increase in atretic follicles in both the treated F0 fish (P < 0.001; n = 10) and their F1 descendents (P = 0.003; n = 12), but this did not extend into the F2 generation (n = 12). Ovarian abnormalities in structure and follicle progression were not observed in any sections among the control groups for all three generations (n = 32).

In contrast, histological examination of the testes revealed no observable effects. We found no lesions in the sections of testes taken from fish directly exposed to TCDD or from generations descended from the TCDD-exposed fish (not shown).

Reproductive Capacity

We previously reported that exposure at 3 and 7 wpf caused a reduction in egg release and fertilization success in the F0 generation (Baker et al., 2013). We examined the F1 and F2 descend-
Effects on Sex Ratio and Ovarian Development

<table>
<thead>
<tr>
<th></th>
<th>F₀</th>
<th>F₁</th>
<th>F₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (% female)</td>
<td>29.9 ± 1.9%</td>
<td>29.2 ± 4.3%</td>
<td>21.3 ± 3.6%</td>
</tr>
<tr>
<td>TCDD</td>
<td>44.5 ± 1.8%a</td>
<td>40.7 ± 3.4%a</td>
<td>38.6 ± 2.6%a</td>
</tr>
<tr>
<td>Incidence of ovarian atretic follicles</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>65.6 ± 6.9%a</td>
<td>46.1 ± 6.9%a</td>
<td>7.7 ± 6.9%</td>
</tr>
<tr>
<td>TCDD</td>
<td>1.8% 6.9% 40.7</td>
<td>4.3% 3.4% 38.6</td>
<td>3.4% 3.6% 21.3</td>
</tr>
</tbody>
</table>

Notes. Zebrafish were exposed, and sex ratio and histopathology examination of the ovaries were as described in the Materials and Methods section. The sex ratios for each generation (F₀n = 50, F₁n = 91, and F₂n = 97) as well as incidence of atretic ovarian follicles (n = 10–12) are reported as mean ± SEM. *Indicates significant difference (p < 0.05) from the control.

Discussions

Transgenerational and Epigenetic Effects

In our study, zebrafish were exposed as immature juveniles at 3 and 7 wpf as previously reported (Baker et al., 2013). They produced F₁ offspring, and the F₁ fish in turn produced an F₂ generation. TCDD caused effects and phenotypic changes in all three generations of fish. Both direct exposure toxic effects were observed in the F₀ fish, as well as transgenerational effects in the F₂. Some of the phenotypic changes were observed only in the F₀ fish directly exposed to TCDD, while others were observed in all generations, indicating that they were transgenerational.

From a mechanistic standpoint, it is important to distinguish effects caused directly when TCDD is present during exposure, from legacy effects of the initial exposure persisting when TCDD is no longer present. Clearly effects in the F₀ fish can be attributed to direct TCDD exposure, although there is reason to think that much of the TCDD had left the fish by the time these effects were manifested (Brambilla et al., 2007; Philips et al., 2006).

Any effects on F₁ and F₂ fish must have been carried along through the gametes. Because TCDD was present as the F₀ ovaries and testes were being formed, one can imagine nonspecific damage to the gametes yielding alterations in the offspring. In this sense, the offspring were directly affected by TCDD exposure, albeit before they came into existence. One would not expect this type of effect to be heritable. A perhaps more plausible explanation for effects passing into the F₁ generation is that exposure during gonad development caused epigenetic changes in the egg and sperm progenitors that carried effects into the F₁ fish. Perhaps the most convincing argument for this as the mechanism is the fact that many of the effects were observed in the unexposed F₂ generation.
Regardless of whether one considers the F1 fish to have been exposed to TCDD, an exposure that causes toxic effects in the F1, while producing no apparent effect at the time, is startling to consider. If we use zebrafish to model human events, teenagers exposed to a chemical could pass on toxic effects to their children. These effects might not be manifested until the children reached adulthood. From this perspective, the F1 effect is important.

As shown in Figure 1, the F2 had no opportunity for TCDD exposure by any route. Any mechanism describing F2 toxicity, must therefore account for the heritability of the effects. One such mechanism would be chromatin modifications that are replicated with the DNA, e.g. changes in DNA methylation, histone modification, non-coding DNA, or some other covalent or non-covalent effect that ultimately causing changes in the expression of genes that are critical for skeletal formation and reproduction.

Epigenetic changes are thought to play an important role in cell differentiation: all cell types carry the same genomic sequence; yet vary in gene expression. TCDD exposure can alter cell differentiation. Furthermore, TCDD produces multigenerational effects in rodents, including abnormal ovarian histology, increased premature births, perinatal mortality, and reduced pregnancy (Bruner-Tran and Osteen, 2011; Manikkam et al., 2012; Nilsson et al., 2012).

### TCDD and Reproductive Function

As previously reported, sublethal TCDD exposure at 3 and 7 wpf led to changes in sex ratios in the adults (Baker et al., 2013). This is consistent with the findings that human and rat exposures cause changes in the sex ratio of the offspring (Ikeda et al., 2005; Mocarelli et al., 2000). The significant increase in the proportion of females persisted in both the F1 and the F2 generations. The endpoints of toxicity that we observed were similar to effects documented in humans exposed to dioxin (Eskenazi et al., 2004; Mocarelli et al., 2008).

One striking effect that we reported for the F0 TCDD-exposed fish was a divergence between body shape and gonad, yielding fish that were outwardly female, yet inwardly carrying true testes (Baker et al., 2013). These fish were therefore neither male nor female, but carried characteristics of both sexes. We observed this in almost 7% of the outwardly female TCDD lineage F1 specimens examined, and not at all in the TCDD lineage F2 females. From this we conclude that if heritable, the effect is not as strongly inherited as some of the others we measured. It should be added that we have never encountered an example of this defect in untreated zebrafish.

Exposure to TCDD leads to infertility in many vertebrate species, including humans, and is associated with downregulation of enzymes in the estrogen synthesis pathway, decreased testosterone and sperm concentrations, decreased egg release, an increase in atretic ovarian follicles, and decreased fertilization success (De Vito and Birnbaum, 1994; Edeland et al., 1994; King-Heiden et al., 2012; Mayani et al., 1997; Mocarelli et al., 2008; Yoshizawa et al., 2009). The incidence of human infertility has increased steadily in many countries (Andersson et al., 2008), and environmental exposure may play a role in rising infertility (Fisher, 2004; Hauser and Sokol, 2008; Sadeu et al., 2010).

We found that TCDD exposure caused abnormal ovarian development in F0 and F1 zebrafish, but was not present in the third generation. However, decreased egg release and fertilization was observed all the way through the TCDD lineage F2 adults, leaving the population less fertile long after the initial exposure. Interestingly, this could best be attributed to changes in the males of the population. Previous work has shown that TCDD decreases sex organ weights, spermatogenesis, and litter size in rats (Mably et al., 1992; Theobald and Peterson, 1997), yet we found no apparent defects in the testes in the zebrafish. The decreased egg fertilization could be due to more subtle defects in the sperm cells or in the behavior of the fish.
The fact that TCDD exposure alters the ability of male zebrafish to elicit egg release from the females is remarkable, especially three generations after exposure. Yet other studies have reported altered sexual behavior in males exposed \textit{in utero} to low levels of TCDD (Colciago et al., 2009; Mably et al., 1992b). Although we do not know the mechanism, we speculate that TCDD has changed something in the male that is sensed by the female. Whether this is a behavioral cue or a chemical released into the water is a subject waiting for study.

**Skeletal Effects**

While not clearly understood, environmental factors play a role in causing birth defects and other skeletal abnormalities in humans (Giampietro et al., 2003; Sever, 1995). Spina bifida, an incomplete closing of the neural tube associated with malformed vertebrae, occurs in the offspring of humans exposed to TCDD via Agent Orange (NAS-IOM, 2011). In mink, skeletal abnormalities were observed in the F$_1$ offspring of TCDD-exposed adults (Bursian et al., 2013). TCDD exposure causes skeletal and bone abnormalities in several animal models (Bursian et al., 2013; Hornung et al., 1999; Peterson et al., 1993; Xiong et al., 2008).

TCDD produced a variety of skeletal defects, but the most common one was a pronounced kink in the axial skeleton caused by a vertebral defect. In these cases, the surrounding vertebrae were often unaffected, suggesting that TCDD exposure increased the probability of a stochastic defect in vertebral formation, leading to malformation or failure. The fact that TCDD exposure during early development interferes with chondrogenesis in zebrafish may be related to this effect on the spine. In acute toxicity, chondrogenesis of the jaw is altered by downregulation of the $sox9b$ gene, a transcriptional regulator of collagen genes (Xiong et al., 2008).

**Human Health Significance**

Human exposure to dioxin has been associated with diseases that are similar to the phenotypic effects that we have reported in this study. Epidemiological reports have documented that after environmental exposure to dioxin resulting from major industrial accidents there is a significant change in sex ratio (more female offspring), which is the same trend that we report in our exposed fish populations (Eskenazi et al., 2004; Mocarelli et al., 2008; NAS-IOM, 2011). Reports have also shown potential effects on fertility due to a decrease in sperm concentration and motility and there are implications that TCDD leads to endometriosis (Egeland et al., 1994; Mayani et al., 1997; Mocarelli et al., 2008). We show that there is a decrease in fertility in both the male and the female and speculate that it could be due to ovarian abnormalities and a sperm abnormality. Skeletal effects including spina bifida, an incomplete closing of the neural tube associated with malformed vertebrae, occurs in the offspring of humans exposed to TCDD (NAS-IOM, 2011). These results are comparable to the craniofacial malformations as well as axial skeletal abnormalities in response to dioxin exposure.
CONCLUSION

The zebrafish is a useful model for studying the transgenerational effects of toxic chemicals. Benefits include low expense, ease of scoring, simple exposure routes, and short generation times. In our example using TCDD, skeletal and reproductive effects were prominent. In light of the heritability of the effects, we suspect a chromosomal mechanism. Our data provide no clue regarding how the changes were inherited with chromatin, or which changes in gene expression led to toxicity, but we will need to know these things to understand the process. However, this is to our knowledge the first report of an environmentally induced transgenerational response observed in fish. This expands the known range of vertebrates using epigenetic inheritance as a response to environmental stimulus.

An important question is: How persistent are these changes? It seems possible that chromatin can exist at many loci in bistable states that remain heritable until switched. On the other hand, transgenerational responses would reduce fitness and be subject to the same type of Darwinian forces as DNA sequence. In our case, we consistently observed a diminution of effect across the generations, indicating that the system was being somehow reset. For humans, this ability to reset will be of consequence.

ACKNOWLEDGEMENTS

We would like to thank Dorothy Nesbit, Carrie Zellmer, Kelly Mularkey, and Helen Adu, and Darlene Forslyn for technical assistance, advice, zebrafish maintenance, and husbandry. We also thank Dr. Marie Pinkerton for expertise and advice. The contents are solely the responsibility of the authors and do not necessarily represent the official view of the National Institute of Environmental Health Sciences, National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES


