



ISSN: 2230-7850

IMPACT FACTOR : 5.1651 (UIF)

VOLUME - 7 | ISSUE - 1 | FEBRUARY - 2017

“THE TOXICOLOGY OF MERCURY AND INDUCED REPRODUCTIVE IMPAIRMENT IN FISH : A REVIEW”

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ABSTRACT:

Mercury is a potent neurotoxin, and increasing levels have led to concern for human and wildlife health in many regions of the world. During the past three decades, studies in fish have examined the effects of sub lethal mercury exposure on a range of endpoints within the reproductive axis. Mercury studies have varied from highly concentrated aqueous exposures to ecologically relevant dietary exposures using levels comparable to those currently found in the environment. This review summarizes data from both laboratory and field studies supporting the hypothesis that mercury in the aquatic environment impacts the reproductive health of fish. The evidence presented suggests that the inhibitory effects of mercury on reproduction occur at multiple sites within the reproductive axis, including the hypothalamus, pituitary, and gonads. Accumulation

of mercury in the fish brain has resulted in reduced neurosecretory material, hypothalamic neuron degeneration, and alterations in parameters of monoaminergic neurotransmission. At the level of the pituitary, mercury exposure has reduced and/or inactivated gonadotropin-secreting cells. Finally, studies have examined the effects of mercury on the reproductive organs and demonstrated a range of effects, including reductions in gonad size, circulating reproductive steroids, gamete production, and spawning success. Despite some variation between studies, there appears to be sufficient evidence from laboratory studies to link exposure to mercury with reproductive impairment in many fish species. Currently, the mechanisms underlying these effects are unknown; however, several physiological and cellular mechanisms are proposed within this review.

KEYWORDS: Toxicology, mercury and fish.

INTRODUCTION:

The presence of industrial contaminants in the aquatic environment has been of increasing concern for human and wildlife health. Mercury is of particular concern because of the bioaccumulation and potent neurotoxicity of some of its forms. Atmospheric emissions of mercury are the result of anthropogenic activities (metal production and coal-fired power generation) and natural geochemical processes (volcanic eruptions and forest fires). Based on lake sediment records from mid-continental North America, atmospheric deposition of mercury is estimated to have tripled over the past 140 years. Once in the atmosphere, mercury can exist for up to a year, allowing widespread distribution via long-range atmospheric transport. Mercury pollution is a ubiquitous problem, with



atmospheric deposition contaminating watersheds in areas far from anthropogenic or natural atmospheric point sources.

Several forms of mercury are present in the aquatic environment, including elemental, ionic, and organic. Methyl mercury, one of the most toxic forms, bio accumulates in fish primarily through dietary uptake. The level of bioaccumulation is a function of age, species, and trophic transfer. Preferential bioaccumulation of methyl mercury over inorganic mercury results from the greater assimilation efficiency and trophic transfer efficiency of methylmercury. Inorganic mercury is found in piscivorous birds and mammals, but its presence generally is attributed to the ability of these species to convert methylmercury to the less toxic inorganic form rather than to dietary uptake or bioaccumulation. Specific bioaccumulation of methyl mercury is evident, because the average proportion increases from approximately 5% methyl-mercury in the aqueous environment to 15% in phytoplankton, 30% in zooplankton, and more than 90% in fish. Total mercury levels in freshwaters of North America generally do not exceed the ng/L range. Methyl mercury in surface water normally comprises 0.1 to 5% of the total mercury content, but in anoxic waters, it can be one of the dominant mercury species. Concentrations of methyl mercury in aquatic organisms increase because of trophic transfer. Methyl mercury concentrations in phytoplankton range from 0.003 to 0.005 µg/g wet weight, whereas the range of methylmercury concentration increases to from 0.03 to 0.22 µg/g wet weight in muscle of lower-trophic-level fish, such as bluegill (*Lepomis macrochirus*) and black crappie (*Pomoxis nigromaculatus*). In higher-trophic-level fish, including largemouth bass (*Micropterus salmoides*), methylmercury concentrations range from 0.10 to 1.0 µg/g wet weight. Health Canada has established a human consumption guideline level of 0.5 µg/g wet weight of total mercury in commercial marine and freshwater fish. Even in remote regions, methylmercury in fish sometimes exceeds the concentration deemed to be safe for human consumption. A study of 775 water bodies in the province of Quebec, Canada, reported maximum muscle mercury levels of 2.30, 4.90, and 5.60 µg/g wet weight for northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), and lake trout (*Salvelinus namaycush*), respectively. The adverse effects of inorganic mercury and methylmercury on humans is well documented however, relatively little is known about the effects on the health or condition of wild-fish populations.

Methyl mercury exposure can affect behavior, biochemistry, growth, reproduction, development, and survival in fish. Long-term laboratory studies on fish exposed to dietary methylmercury have noted loss of coordination, diminished swimming activity, starvation, and mortality. Industrial activities have resulted in highly contaminated local fish populations, with muscle mercury concentrations ranging from 8.4 to 24 µg/g wet weight in Minamata Bay, Japan, and from 6.3 to 16 µg/g wet weight in Clay Lake, Ontario, Canada. At lower concentrations, mercury may affect fish populations indirectly, through impairment of physiological processes, rather than through the severe neurological impairment and death observed in extreme contamination incidents. During the past three decades, studies using sublethal concentrations of mercurials have demonstrated negative impacts on reproduction in numerous freshwater fish species. Weiner and Spry concluded that reduced reproductive success was the most plausible effect of mercury on wild-fish populations at current exposure levels in aquatic ecosystems. The mechanistic effects of methylmercury on reproduction, however, remain unclear.

DISRUPTION OF HYPOTHALAMIC AND PITUITARY FUNCTION:

The hypothalamus:

Multiple hypothalamic neuropeptides and neurotransmitters exert a dual stimulatory and inhibitory control over pituitary gland release of gonadotropic hormones that regulate gonadal function in fish. The inhibitory effects of pollutants on reproduction may be mediated via changes in regulation at the level of the hypothalamus and pituitary. Methylmercury is a known neurotoxin, the effects of which in fish after long-term dietary exposure are characterized by brain lesions, oxidative stress, and decreased activity. Various molecular targets within the nervous system have been implicated in methylmercury neurotoxicity, including the cytoskeleton, axonal transport, neurotransmission parameters, protein synthesis, and mitochondrial energy-

generating systems. Although methylmercury will readily react with any sulfhydryl group, it shows brain region specificity, with neuron loss only in certain areas. Methylmercury exposure in Atlantic salmon (*Salmo solar*; 0.68 µg Hg/g wet wt brain tissue) caused widespread vacuolation, with the most severe effects noted in the medulla and cerebellum and the cerebrum and other regions being less affected. Furthermore, mitochondria from cortical astrocytes are more sensitive to methylmercury toxicity than are those in cerebellar astrocytes. Thus, it is important to examine the effects of mercury exposure on isolated brain regions. In addition, the endocrine system amplifies signals such that minor changes in neuronal function may cause major disturbances in reproduction. Consequently, mercury may affect reproduction via the hypothalamus at levels lower than those that induce general neuronal damage.

In teleosts, gonadotropin-releasing hormone (GnRH) neurons, originating in the ventral telencephalic-preoptic-anterior region of the forebrain, project into the anterior pituitary gland. Gonadotropin-releasing hormone regulates the synthesis and release of the gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]), making it a crucial neuroendocrine mediator of reproductive function. Histological analysis of neurons in the hypothalamic preoptic nuclei of the murrel (*Channell punctatus*) has provided evidence for potential inhibitory effects of mercury on neurosecretion. The magnocellular isotocin and vasotocin-secreting neurons of the preoptic nuclei were smaller and inactive (characterized by reduced staining of neurosecretory material) in murrels treated for six months with 10 µg/L of inorganic mercury. This may be important, because isotocin and vasotocin control sexual behavior in teleosts. Many neurons also exhibited various degrees of degeneration, from apoptotic chromatin condensation to necrotic nuclear dropout. Some of the affected neurons were uncharacterized and may have included GnRH cells, although this was not proven. In rats, dietary methylmercury decreased GnRH content in the medial hypothalamus. The paucity of information on the effects of mercury on GnRH neurons in vertebrates may be a reflection of the difficulty in studying them, because they do not form a compact nucleus but, rather, a diffuse, interconnected network (i.e., hypothalamus and telencephalon) that is specifically involved in reproductive control. To our knowledge, a comprehensive assessment of gene expression changes in the hypothalamus in relation to methylmercury-induced reproductive impairment in any species remains to be done.

Neurotransmission:

Neurotransmitters are released by presynaptic nerve cells into the synapse, where they stimulate receptors on postsynaptic nerve cells. The release of hypothalamic and pituitary hormones is regulated by neurotransmitters, and in turn, neurotransmitter release is modulated by hormones. The effects of mercury on neurotransmission may be mediated by disruption of synthesis, storage, release, and/or reuptake by the presynaptic cell; by changes in activity of the deactivating enzyme; or by mimicking/blocking the neurotransmitter at the synapse. Individual neurotransmitters may be stimulatory and/or inhibitory in terms of reproduction and may modulate the effects of other neurotransmitter systems, resulting in a multitude of effects from alterations in one neurotransmitter. Furthermore, because each neurotransmitter may have more than one type of receptor, and because these various receptors may be on different types of cells, alterations in the level of a single neurotransmitter may affect multiple cells in different ways. In fish, mercury affects the levels of several neurotransmitters involved in regulating the synthesis and release of GnRH and the gonadotropins.

Mercury exposure decreased serotonin (5-HT) concentrations in the brains of several fish species. Serotonin is synthesized from the amino acid tryptophan and activates GnRH neurons to stimulate release of LH from the pituitary. In a laboratory study with Nile tilapia (*Oreochromis mossambicus*), 5-HT levels decreased significantly in the hypothalamus of males exposed for six months to 15 and 30 µg/L of inorganic mercury. No change in 5-HT levels was observed in the telencephalon or optic lobe. Levels of 5-HT also decreased in whole brain of walking catfish (*Clarias batrachus*) exposed for 90 to 180 d to 40 µg/L of methylmercury. A field study with mummichog (*Fundulus heteroclitus*) from an unpolluted site (0.029 µg Hg/g wet wt brain tissue) and a polluted site (0.066 µg Hg/g wet wt brain tissue) observed lower 5-HT levels in the medulla, but not in the

cerebellum, of fish from a mercury-contaminated site. Those authors also noted lower levels of the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), in contaminated fish, suggesting that the low 5-HT levels may be caused by reduced synthesis rather than by increased degradation. These results agree with those of Tsuzuki, who observed a decrease in 5-HT and 5-HIAA in the hypothalamus of rats exposed orally to 4.0 µg/g of methylmercury for 50 d. Further support in rats comes from the methylmercury-induced decrease in activity of tryptophan hydroxylase, the rate-limiting biosynthetic enzyme in the 5-HT pathway. The inhibitory effects of mercury on the 5-HT pathway may depend on the developmental stage of fish. For example, methylmercury exposure (10 µg/L) increased 5-HT in the heads of 7- and 14-d posthatch mummichog larvae with no concurrent change in 5-HIAA. Collectively, these studies indicate that in sexually mature fish, mercury exposure can decrease 5-HT levels, which in turn could be linked to decreased reproduction.

Levels of the catecholamines norepinephrine and dopamine also have been measured after mercury exposure in fish. Dopamine is the single most potent inhibitor of LH secretion in fish, and it acts by binding directly to D2 receptors on the gonadotrophs and by altering GnRH signalling. Dopamine was significantly increased in brains of walking catfish after a 90-d exposure to 40 µg/L of methylmercury. Levels of norepinephrine, a stimulatory monoamine synthesized from dopamine, also were increased significantly in whole brain of walking catfish after methylmercury exposure. Aqueous methylmercury (10 µg/L) elevated dopamine levels in heads of 7- and 14-d posthatch mummichog larvae without any change in its major metabolites, 3,4-dihydroxyphenylacetic acid or 3-methoxy-4-hydroxyphenylacetic acid. No significant differences in dopamine, norepinephrine, or 5-HT were observed in fathead minnows (*Pimephales promelas*) after a 10-d aqueous exposure to inorganic mercury (1.69–13.57 µg/L); however, whole-brain measurements were made that would have obscured any brain region-specific changes. In rats, methylmercury also has been shown to increase dopamine in the hypothalamus while decreasing the levels of 3,4-dihydroxyphenylacetic acid. Total mercury concentrations in the brain of wild mink correlated negatively with D2 receptor density and binding affinity, suggesting a potential adaptive mechanism to counter the mercury-induced increase in dopamine. Whether this adaptive mechanism exists in fish is not known. If mercury-exposed fish are unable to counter the increases in dopamine, then LH release may be reduced and reproduction impaired.

As discussed above, LH release in fish is affected by several monoamine neurotransmitters (dopamine, norepinephrine, and 5-HT), suggesting that alterations in their rate of synthesis or degradation could impact reproduction. The degradation of monoamines involves monoamine oxidase (MAO), which is an intraneuronal enzyme complex responsible for oxidative deamination. Inorganic mercury decreased MAO activity in murrel after a six-month aqueous exposure (10 µg/L) and in Atlantic salmon after a four-month dietary exposure (0.13 µg Hg/g wet wt brain tissue). Methylmercury induced a much more potent inhibitory effect compared with inorganic mercury on MAO activity in Atlantic salmon. Similarly, organic mercury (40 µg/L) was a stronger inhibitor of MAO compared with inorganic mercury (50 µg/L) in walking catfish after 45 d of aqueous exposure. Furthermore, methylmercury significantly inhibited the rat synaptosomal MAO activity in all brain regions tested, including the hypothalamus. These results indicate that mercury in both its inorganic and organic forms is capable of impairing the monoaminergic systems known to be key modulators of hypothalamic-pituitary control of gonadotropic function and, thus, reproduction. A direct, causal link between monoaminergic disruption and decreased reproduction is required for substantiation of this hypothesis; however, this link has yet to be clearly established.

The pituitary:

The gonadotropic hormones LH and FSH are released from the pituitary in fish and control the annual cycle of gonadal growth, ovulation in females, sperm release in males, and production of sex steroids in both sexes. Therefore, disruption of gonadotropin secretion can have a major impact on fertility. The effects of mercury on the pituitary have been limited to morphological analysis and measurements of pituitary-derived

hormone levels in plasma. Critically missing are studies regarding the direct effects of mercury on pituitary hormone release in vitro.

The morphological effects of mercurials on the pituitary gonadotroph in murrel and walking catfish have been studied by several groups. Histological analyses demonstrated that compared to controls, gonadotrophs were smaller, appeared to be inactive, and were fewer in number in both species of fish exposed to 10 to 50 µg/L of methylmercury, inorganic mercury, or a mercurial fungicide for six months. The mercury-induced inhibition of gonadotropic activity was correlated with impaired gonadal development. Whether the decrease in sex steroid levels and, thus, an alteration of gonadal feedback signals is causing the inhibition of gonadotropic function is not known. Conversely, if the action of mercurials is directed primarily at the hormone synthesis and release mechanisms of the pituitary, the resulting decrease in gonadotropin could lead to the inhibition of gonadal development. A thorough assessment of serum LH and FSH and associated pituitary mRNA levels would help to determine whether the pituitary is directly affected by methylmercury exposure. A recent study in ovariectomized rats observed a decrease in plasma LH after a 3-d exposure to dietary methylmercury. Moreover, basal LH and sex steroid levels are significantly reduced in postspawning female goldfish (*Carassius auratus*) after a 28-d dietary exposure to 0.88 µg/g of methylmercury (Crump and Trudeau, unpublished data). Further studies in fish are required to determine the mechanisms of mercury-induced inactivation of the gonadotrophs.

DISRUPTION OF MALE REPRODUCTIVE FUNCTION:

The cycle of spermatogenesis in which stem cells divide into mature spermatozoa is controlled by LH and FSH. Throughout this cycle, the testes increase in size, which can be measured by an increase in the gonadosomatic index (GSI; gonad wt/body wt 100) and usually is maximal in the breeding season. Disruption of testes function can be classified as either cytotoxicological or endocrine in origin. Cytotoxic effects are characterized by damage to cellular integrity or alterations in cell function, whereas endocrine effects involve the disruption of specific cells as a result of alterations in pituitary secretion or gonadal steroidogenesis.

Testicular morphology and spermatogenesis:

Compared to control fish, testicular growth and spermatogenesis in murrel were arrested following exposure to inorganic mercury (10 µg/L) or a mercurial fungicide (200 µg/L) for six months. Necrotic areas were visible within the testes of fish exposed to the mercurial fungicide, suggesting direct cytotoxic effects, however, no obvious signs of degeneration were observed after inorganic mercury exposure. Spermatogenesis also was inhibited and GSI decreased in male walking catfish after 180 d of exposure to organic and inorganic mercury at 30 to 50 µg/L. Similarly, guppies (*Poecilia reticulata*) exposed to 5.6 µg/L of methylmercury for three months and medaka (*Oryzias latipes*) exposed to 40 µg/L of methylmercury for 8 exhibited signs of testicular atrophy and arrested spermiation. Wester, as well as Wester and Canton concluded that necrosis of the sperm was occurring during the process of spermatogenesis, and those authors proposed a mechanism involving the disruption of the mitotic spindle. Mercury has been shown to result in depolymerization of microtubules that form the mitotic spindle, which is a possible mechanism underlying mercury-induced genotoxicity in mammalian cells. Disruption of testicular function also has been observed in exposures to environmentally relevant levels of methylmercury. Dietary exposure for six months to methylmercury concentrations (0.1–1.0 µg/g) similar to those encountered by piscivorous fish in North America resulted in a significant decrease of GSI in juvenile male walleye with mean mercury body burdens of 0.25 to 2.37 µg/g wet weight. The normal morphology of the testes was significantly altered, with the appearance of atrophy. Decreased spermatogenesis and atrophied seminiferous tubules were observed in male Nile tilapia (*Oreochromis niloticus*) after a seven-month exposure to methylmercury. Intraperitoneal implants in the male tilapia released methylmercury gradually, with final muscle concentrations ranging from 0.08 to 0.54 µg/g wet weight (assuming 80% water content), which are similar to levels reported for largemouth bass in the northeastern United States. From these experiments, it can be concluded that both inorganic and organic mercury exposure via a variety of exposure routes can arrest gonadal development

in male fish. This effect may be cytotoxic in nature, because effects at the level of the cell include structural alterations in Leydig cells and necrotic cell death. The inhibition of spermatogenesis in the murrel was correlated with small and inactive gonadotrophs of the pituitary.

The data are inconclusive regarding the impacts of mercury exposure on testicular growth. In addition to the studies mentioned previously, in several studies no change in GSI occurred. In wild-caught northern pike with muscle mercury content ranging from 0.12 to 0.62 $\mu\text{g/g}$ wet weight, no significant correlations were found between mercury content and GSI or gonadal sex steroid concentration. However, the small sample size ($n = 7$) and high variability may have masked any subtle effects. Dietary methylmercury decreased sex steroid levels but had no effect on gonadal development in male fathead minnows with mean total mercury carcass concentrations of 0.86 to 3.84 $\mu\text{g/g}$ wet weight after 250 d of exposure. Similarly, GSI was not affected in male tilapia exposed to methylmercury, despite changes in testicular morphology and spermatogenic arrest. This indicates that histology may provide a more sensitive indicator than GSI for assessing the effects of mercury on gonadal development.

In male fish, androgens control gonadal development, secondary sexual characteristics, and sexual behaviour. Testosterone and/or 11-ketotestosterone secretion is high during gonadal recrudescence and declines before spermiation. Plasma testosterone levels significantly decreased in male fathead minnows fed diets containing 0.87 to 3.93 $\mu\text{g/g}$ of methylmercury for 250 d. Decreases in plasma 11-ketotestosterone also were observed in male Nile tilapia (*O. niloticus*) after a six-month exposure to methylmercury and in large-mouth bass after an eight-week exposure to 3.12 to 6.25 $\mu\text{g/g}$ of dietary methylmercury, no concurrent decrease in 17 β -estradiol was observed in either exposure. These studies provide further evidence that mercury alters steroidogenesis in male fish. Further studies, however, are required to determine whether the effect is direct, at the gonad level, and/or indirect, via disruption of the hypothalamus or pituitary.

In wild fish, the effects of mercury accumulation on androgens, particularly 11-ketotestosterone, are unclear. Similar to the results of laboratory studies, both plasma testosterone and 11-ketotestosterone were negatively and significantly correlated with muscle mercury content (mean value, 0.17 $\mu\text{g/g}$ wet wt) in sexually immature white sturgeon (*Acipenser transmonatus*). From these results, a mercury concentration threshold associated with steroidogenic effects was estimated to be 0.2 $\mu\text{g/g}$ wet weight. This threshold was determined previously by Beckvar et al. to be protective for juvenile and adult fish based on a systematic analysis of studies assessing sublethal endpoints (growth, reproduction, development, and behavior) of mercury exposure. No significant alterations in 11-ketotestosterone levels were observed in sexually mature common carp (*Cyprinus carpio*), brown bullhead (*Ameiurus nebulosus*), smallmouth bass (*Micropterus dolomieu*), or largemouth bass containing mercury concentrations greater than this 0.2 $\mu\text{g/g}$ wet weight threshold. Nor were any alterations in testosterone levels correlated with mercury accumulation in recrudescing northern pike with muscle mercury concentrations ranging from 0.28 to 0.64 $\mu\text{g/g}$ wet weight. Although not statistically significant, testosterone levels in prespawning largemouth bass from a high-mercury lake (5.42 $\mu\text{g Hg/g}$ wet wt muscle tissue) were more than 50% lower than those of bass in a control lake (0.30 $\mu\text{g Hg/g}$ wet wt muscle tissue). Conversely, a positive correlation between plasma 11-ketotestosterone concentrations and mercury residues was observed in the male bass from the high-mercury lake. To our knowledge, this is the only report of increased steroidogenesis in male fish associated with methylmercury exposure. Increased steroidogenic enzyme activity and a concurrent increase in plasma steroid levels were observed in female fish exposed to inorganic mercury. Positive correlations also were observed between 11-ketotestosterone and muscle mercury concentrations in female smallmouth bass (0.21–0.51 $\mu\text{g/g}$ wet wt) and common carp (0.06–0.33 $\mu\text{g/g}$ wet wt). Field surveys are complicated by the presence of multiple contaminants in addition to methylmercury, which may impact steroidogenesis. Although correlations exist between mercury concentrations and sex steroid levels, the effect of additional contaminants cannot be ruled out. This is particularly true for the largemouth bass study showing increased 11-ketotestosterone, because the levels of other contaminants were not measured in these fish. In addition to the confounding factor of multiple contaminants, the differing results also may be attributed to species differences and the state of gonadal development during which the sampling took place. Cholesterol plays a key role in reproduction in teleosts,

servicing as the lipid precursor for gonadal steroids. Lipids mobilized from the liver gradually increase in the gonads from the preparatory-to-spawning phase of the reproductive cycle. Testicular cholesterol levels decreased in male catfish exposed for 180 d to both organic and inorganic mercury, whereas in freshwater murrel, hepatic cholesterol levels increased after a 180-d exposure to an organic mercury fungicide (Emisan-6; 200 µg/L). Both studies suggest that mercury exposure resulted in a decreased mobilization of lipids from the liver to gonads. Impaired lipid metabolism may be responsible, in part, for the inhibition of spermatogenic and steroidogenic activities in mercury-treated fish.

Sperm morphology and motility

The success of fertilization events likely are reduced or inhibited completely by alterations in sperm motility and morphology. Fertilization success was decreased after short-term (≤ 40 -min) exposure of rainbow trout (*Oncorhynchus mykiss*) sperm and mummichog sperm to inorganic mercury and methylmercury (1–1,000 µg/L). It is now known, however, that fertilization success is dependent on the ratio of sperm to egg used in each test, because high ratios will mask toxic effects by providing sufficient spermatozoa to overcome the spermotoxic effects. None of the above studies discuss the ratio used and, therefore, may have underestimated the toxicity of inorganic and organic mercury.

Sperm also can be exposed to mercury in seminal fluids (i.e., in vivo) of contaminated fish. A 24-h exposure in an extender buffer has been designed to mimic this type of exposure in vitro. Goldfish sperm, which exhibited no effects after instantaneous exposure, had decreased flagella length and motility after preincubation in an extender buffer with 100 µg/L of inorganic mercury. Attempts have been made to measure the effects of in vivo sperm exposure on fertilization success. Thus far, the results have been inconclusive because of the inherent difficulty in assessing the effects on sperm in isolation from other reproductive impairments.

DISRUPTION OF FEMALE REPRODUCTIVE FUNCTION

Mercury exposure also can affect reproductive endpoints in female fish. Mercury has been shown to affect ovarian morphology, delay oocyte development, and inhibit steroid hormone synthesis. As with the testes, it is difficult to determine whether mercury affects the ovaries directly or indirectly, via alterations at the level of hypothalamus or pituitary, and whether the effects are the cause or the result of changes in steroidogenesis. Unlike in males, female gamete production involves the synthesis and incorporation of a yolk protein, which may transfer some of the maternal mercury burden to the embryo. The effects of mercury on the female reproductive system discussed in the next section are separated into changes at the level of ovarian morphology and egg development, steroid hormone and vitellogenin synthesis, fecundity and spawning, and ultimately, egg and embryo survival.

Ovarian morphology and oogenesis:

Oogenesis in teleosts involves the maturation from immature or previtellogenic oocytes through the accumulation of yolk vesicles during vitellogenesis and, finally, the formation of mature oocytes. This cycle of oogenesis was significantly repressed in the freshwater murrel after exposure to inorganic mercury or mercurial fungicide (10–20 µg/L), with a concurrent decrease in GSI. After 180 d of exposure, only immature oocytes completely devoid of vitellogenin were present, whereas the control ovaries were fully developed, with a large number of mature and maturing oocytes. Degenerative changes—namely, nucleolar necrosis and atresia (follicular degeneration)—also were observed in some of the fungicide and inorganic mercury treatment groups. Ovarian recrudescence was arrested with some atretic changes and a decrease in GSI of walking catfish exposed over six months to inorganic and organic mercury (30–40 µg/L). Atretic follicles also were more common in female Nile tilapia (*O. niloticus*) after exposure to methylmercury implants; however, no change in GSI was observed. Common spiny loach (*Lepidocephalichthys thermalis*) exposed to inorganic mercury for 10 d at 100 µg/L had extensive vacuolation in the oocortex, necrosis of the oolemma, and hypertrophy of follicular cells, leading to atresia of the oocytes after 20 d. In sexually mature largemouth bass, the frequency of atretic oocytes

was positively correlated with sediment mercury concentrations in a Tennessee, USA, reservoir system. These degenerative changes are suggestive of cytotoxic actions on the ovary.

Apoptosis is the main trigger for follicle atresia in mammals, and is involved in normal teleost ovarian development and regression. A significant increase in the number of apoptotic cells was measured in ovaries of fathead minnows exposed to dietary methylmercury as juveniles through to sexual maturity (–250 d), with resultant mean total mercury carcass concentrations of 0.92 to 3.84 µg/g wet weight. Thus, the induction of apoptosis in steroidogenic gonadal cells is one possible mechanism by which sex steroid levels are reduced in fish exposed to methylmercury. Apoptosis may be a result of oxidative stress, which can be induced by methylmercury exposure. Apoptosis occurs spontaneously in ovarian follicles and is regulated by follicular survival factors, including FSH, LH, and 17β-estradiol. Therefore, an alternative mechanism may involve methylmercury-induced alterations in follicular survival factors resulting in apoptosis. Reduced serum levels of LH were observed in methylmercury-exposed goldfish (Crump and Trudeau, unpublished data), and decreased serum 17β-estradiol levels have been correlated with mercury concentrations in studies of wild-caught fish and laboratory fish. Furthermore, in the absence of degenerative changes, gonadal arrest has been related to endocrine alterations resulting from a decrease in activity of pituitary gonadotrophs.

Steroidogenesis:

In female fish, 17β-estradiol is the major reproductive hormone and is produced primarily in the follicle of the developing oocyte. Luteinizing hormone stimulates the production of testosterone, which is then converted into 17β-estradiol via the aromatase enzyme. One of the primary roles of 17β-estradiol is to stimulate liver production of the yolk protein, vitellogenin, which is then incorporated into the maturing oocyte to later serve as nourishment for the developing fish larva. Similar to testosterone, gonadal 17β-estradiol secretion and subsequent vitellogenin production both change with maturation of the oocytes; thus, plasma levels can be used to monitor the reproductive stage of fish.

In female Nile tilapia (*O. niloticus*), 17β-estradiol levels decreased over the summer months and remained low until November, when the hormone level surged to prepare for the new reproductive season. This hormone surge was repressed in fish exposed for seven months to methylmercury implants (0.3–1.3 µg Hg/g wet wt muscle tissue), which may suggest an interference with the production of stimulatory pituitary hormones or gonadal steroidogenesis. Methylmercury accumulation also was correlated with decreased 17β-estradiol in fathead minnows exposed for 250 d to diets containing 0.87 to 3.93 µg/g of methylmercury (mean carcass accumulation, 0.92–3.84 µg/g wet wt) and in previtellogenic largemouth bass after four to eight weeks of exposure to diets containing 3.12 and 6.25 µg/g of methylmercury. In addition to 17β-estradiol, 11-ketotestosterone levels were measured in laboratory exposures with female tilapia and largemouth bass; however, no significant changes were observed. In wild fish, sex steroid levels have been correlated both negatively and positively with total mercury concentrations. Similar to the results of laboratory studies, plasma 17β-estradiol concentrations in sexually mature female largemouth bass were negatively correlated with total mercury in muscle. A significant negative correlation between plasma 17β-estradiol and liver mercury (mean value, 0.14 µg/g wet wt) also was detected in sexually immature white sturgeon collected in the lower Columbia River, Oregon, USA. No significant correlations between muscle mercury and 17β-estradiol were measured in sexually mature female smallmouth bass and carp from the Hudson River, New York, USA; however, in the same fish, 11-ketotestosterone was significantly positively correlated with total muscle mercury (0.09–0.47 µg/g wet wt). Taken together, these laboratory and field studies suggest that methylmercury exposure, at concentrations currently found in the environment, can induce alterations in serum sex steroid levels in female fish.

Vitellogenesis:

Estradiol stimulates the liver to synthesize the phospholipoglycoprotein yolk precursor vitellogenin in oviparous vertebrates. Altered expression of vitellogenin mRNA and its protein is one of the most commonly used

biomarkers of aquatic contaminant exposure. In males, vitellogenin normally is not expressed, but it can be induced by exposure to estrogenic chemicals. In control female catfish, serum vitellogenin levels doubled after 17 β -estradiol injection. After injection of inorganic mercury, however, serum vitellogenin levels decreased and did not respond to subsequent 17 β -estradiol injection. Vitellogenin synthesis also was inhibited in catfish after a 90- to 180-d exposure to inorganic mercury (50 μ g/L) and organic mercury (40 μ g/L), as indicated by a decrease in ³²P-labeled phosphoprotein content in the liver and ovaries. In largemouth bass exposed to dietary methylmercury (3.12 and 6.25 μ g/g) for 56 d, females had significantly reduced levels of vitellogenin, whereas levels increased in males fed the high methylmercury diet. Female large-mouth bass with reduced vitellogenin levels also had significantly reduced levels of 17 β -estradiol. A survey of freshwater species from various sites along the Hudson River provided equivocal data; vitellogenin levels were negatively correlated with muscle mercury concentrations in female smallmouth bass (0.21–0.51 μ g/g wet wt) and male common carp (0.09–0.41 μ g/g wet wt) but were not related to muscle mercury concentrations in either sex of largemouth bass (0.12–0.61 μ g/g wet wt) or brown bullhead (0.055–0.52 μ g/g wet wt). This inhibition of vitellogenin synthesis on exposure to inorganic or organic mercury may be a result of impaired steroidogenesis or alterations in estrogen signaling pathways. Recent studies have demonstrated alterations in vitellogenin mRNA levels in livers from fish exposed to dietary methylmercury in laboratory-based and in situ exposures. Similar to the reduction in vitellogenin protein observed in catfish and bass, vitellogenin mRNA was down-regulated by two- to fourfold in female fathead minnows exposed for 600 d to dietary methylmercury (3.93 μ g/g). Conversely, some male fathead minnows had elevated vitellogenin levels, but the results were highly variable and not statistically significant. Cutthroat trout from a high-mercury lake (0.054 μ g Hg/g wet wt whole-fish tissues) had vitellogenin mRNA levels fivefold lower than those of fish sampled from a low-mercury lake (0.016 μ g Hg/g wet wt whole-fish tissues) based on cDNA microarray analysis of liver RNA. Unfortunately, the study with trout did not consider gene expression in males and females separately, thus making specific conclusions regarding the impact of methylmercury exposure on vitellogenin expression difficult. On the whole, significant evidence suggests that methylmercury alters vitellogenesis in females, with reductions measured in mRNA, protein, and lipid levels after exposure. Increased vitellogenin expression in males may be caused by an estrogen-like activity of methylmercury, because it has been shown to bind to and activate estrogen receptors.

Lipids:

During the gonadal cycle, a reciprocal relationship exists between hepatic and gonadal lipids, the balance of which may be altered by pollutants, such as mercury. In the peak of reproductive activity, hepatic lipid levels decrease, with a concomitant increase in gonadal levels. Phospholipids are an important component in the structure of proliferating and developing germ cells as reserve food in the form of yolk or as energy sources. Cholesterol, on the other hand, is the precursor for steroid hormones. Lipid content increased in the liver (and muscle) with a concurrent decrease in the ovaries of bronze featherback (*Notopterus notopterus*) exposed for 30 d to inorganic mercury (44–88 μ g/L). Lipid levels (total lipids, phospholipids, and cholesterol) also increased in the liver of walking catfish, with a simultaneous decrease in all forms of lipids in the ovaries after a 180-d exposure to inorganic mercury (50 μ g/L) and methylmercury (40 μ g/L). Murrel exposed to 200 μ g/L of inorganic mercury, however, had a significant reduction of lipid and cholesterol in the liver and ovary. In summary, these results suggest a mercury-induced change in lipid retention and/or inhibition of seasonal mobilization of lipids from liver to ovary during ovarian recrudescence. Disturbance of the lipid balance by mercury may result in other disruptions, including impairment of vitellogenesis (implicated in arrest of oogenesis) and steroidogenesis.

Effects on ovulation and spawning:

Mercury exposure can alter fecundity (number of eggs produced) and spawning of female fish. Zebrafish produced fewer eggs following exposure (19–25 d) to 1.0 μ g/L of a mercurial fungicide. A multigenerational study with brook trout (*Salvelinus fontinalis*) exposed to aqueous methylmercury observed no spawning in

second-generation trout exposed to 0.93 µg/L or more methylmercury for 108 weeks. Similarly, no spawning occurred in fathead minnows (whole-body concentrations, 4.47 µg Hg/g wet wt) after 41 weeks of inorganic mercury exposure at and above 1.02 µg/L. Fertilization success was reduced in offspring (whole-body mercury concentrations, 1.1–1.2 µg/g wet wt) of mummichog fed methylmercury-contaminated diets. Recently, two studies were performed with fathead minnows exposed to dietary methylmercury as juvenile fish through sexual maturation to determine the effects on reproduction. In these studies, an overall decrease in spawning success was observed as a consequence of reduced gonadal growth correlated to a decrease in 17β-estradiol levels, decreased fecundity, and increased number of days to spawning in fish, with carcass mercury concentrations ranging from 0.57 to 3.68 µg/g wet weight. Days to spawning appears to be influenced by the female, because contaminated females that mated with control males took 3 d longer to spawn, on average, than control females that mated with contaminated males. In male fathead minnows with carcass mercury concentrations of 0.71 to 4.23 µg/g wet weight, spawning behavior and spawning success were reduced after dietary methylmercury exposure as juveniles through to sexual maturity. Because androgens are critical in regulating reproductive behavior, methylmercury-induced impairment of reproduction may be caused by alterations in sex steroids that, in turn, result in altered behavior. A female-specific delay in spawning can be especially detrimental if sperm release is not similarly delayed. Delayed spawning can have significant effects on the offspring, because it may disrupt the timing of feeding relative to seasonal food resources. If young fish are unable to find food as an indirect effect of mercury exposure, their growth may be affected, leading to increased susceptibility to predation and reduced survival.

Eggs and embryos:

Mercury in eggs originates primarily from maternal transfer and is predominantly (>90%) in the methylated form. Originally, it was believed that maternal transfer occurred by mobilization and transfer of stored mercury to the gonads during oogenesis. The actual amount transferred, however, is only 0.2 to 2.3% of the maternal burden. In contrast, organochlorines are transferred to the eggs at 5.5 to 25.5% of the whole-body burden. The diet of the maternal adult during oogenesis, rather than the body burden, is now accepted as the principal source of methylmercury in eggs. Therefore, the exposure of embryos to maternally transferred methylmercury depends on the levels of mercury in the prey of the adult during oogenesis, which can vary intra- and interannually.

Mercury exposure may diminish the reproductive potential of maternal fish by reducing the hatching success of embryos. Hatching success of zebrafish embryos in unpolluted water was decreased after maternal exposure (19–25 d) to 0.2 µg/L of a mercurial fungicide. Maternal exposure also may alter the sensitivity of eggs to mercury in the environment. Mummichog eggs from a polluted site and a control site were exposed to methylmercury (0.5–5.0 mg/L) for 20 min before fertilization with untreated sperm. Eggs from the polluted creek demonstrated a higher tolerance to methylmercury. Scanning-electron microscopy revealed that the control eggs exposed to 1.0 mg/L of methylmercury for 20 min had an increased incidence of spontaneous micropyle blockage, resulting in reduced fertilization success. These studies indicate that pre-exposure of eggs to mercury in maternal fish can decrease hatching success in the laboratory, whereas multigenerational exposure in wild populations may convey increased resistance to mercury pollution.

CONCLUSION:

The present review has collated data from both laboratory and field studies supporting the hypothesis that mercury in the aquatic environment negatively impacts the reproductive health of fish. The evidence presented suggests that the inhibitory effects of mercurials on reproduction may occur at multiple sites within the reproductive axis. A variety of endpoints have been measured, but the majority of studies have focused on the level of the gonads, measuring alterations in GSI, gametogenesis, steroidogenesis, and vitellogenesis. Some notable variations in response to mercury exposure may be attributed to one or more of the following factors: Species, life stage (larval, juvenile, or adult), state of gonadal development (recrudescent, spawning, or

regressed), route of exposure, length of exposure, mercurial species, and mercury concentration. For example, spawning success was reduced in fathead minnows exposed as juveniles but not as sexually mature adults, suggesting that some life or gonadal development stages may be more sensitive to methylmercury exposure. Species differences likely are important, because negative correlations were significant between vitellogenin levels and mercury content in smallmouth bass and carp but not in bullhead or largemouth bass collected from the same locations and with comparable mercury burdens. The heterogeneity of species sensitivity to mercury also was demonstrated by Birge et al., who studied lethal concentrations of inorganic mercury in embryo- larval stages of a variety of freshwater species. Nevertheless, across all studies, there appears to be a trend for reduced gonadal development, impaired gametogenesis, and decreased sex steroid levels. A consensus list of the effects of mercury exposure on the reproductive axis in fish was developed through extensive surveying of the literature. Some evidence suggests that fish and other wildlife species may adapt to mercury in their environment. Mercury exposure can affect reproduction by reducing gamete quantity and/or quality, and surviving offspring could be more resistant to mercury toxicity. This natural selection scenario could result in populations made up of individuals who have greater resistance to the effects of mercury. Studies with mummichog from both a clean reference site and a mercury-contaminated site have provided evidence for this occurring in wild-fish populations. Gametes and embryos of contaminated mummichogs exhibited tolerance to methylmercury and, as a result, were relatively unaffected by concentrations that had severe effects on gametes and embryos from the reference site. The effects in mummichog are stage-specific, with contaminated adults exhibiting reduced growth and condition. Receptor-binding studies in wild mink have shown correlations between neurotransmitter receptor densities and mercury concentrations in the brain, which those authors suggest may represent adaptive mechanisms to maintain normal neurotransmission. Adaptation and/or natural selection for mercury resistance may occur to some extent in wild populations; however, the long-term effects and ecological significance remain unknown.

Despite the large number of studies indicating the harmful effects of mercurials on reproduction, there remains a paucity of studies examining the specific mechanism and site of action. Methylmercury is both an endocrine-disrupting chemical and a neurotoxicant. In mammalian models, research has focused primarily on the underlying mechanisms of methylmercury-induced neurotoxicity while research into potential endocrine-disrupting effects has been limited to developmental alterations in the fetus. Conversely, the majority of fish studies have followed the classical endocrine-disruptor approach of measuring reproductive impairment through fecundity, spawning success, and alterations in the hypothalamic-pituitary-gonadal axis. Studies in fish have identified impacts of methylmercury on reproduction. To understand the underlying mechanisms of these effects, however, it is critical to look at alterations in neuroendocrine control, because the central nervous system is the primary target for methylmercury action.

Several physiological and cellular mechanisms have been proposed in the present review. Reproductive impairment may be caused by alterations in any of the factors involved in regulating the hypothalamic-pituitary-gonadal axis. First, changes in neurotransmitter levels or other parameters of neurotransmission could affect the release of GnRH and/or LH from the hypothalamus and pituitary. Second, inhibition of GnRH or LH synthesis and release would cause a cascade effect, resulting in decreased gonadal hormone production and subsequent decreases in vitellogenesis, gonadal growth, and GSI. Third, alterations in gonadal steroidogenesis would affect gamete production and pituitary hormone synthesis and release via positive-feedback mechanisms. Last, altered lipid balance may lead to disruption of normal processes, including steroidogenesis and vitellogenesis. Cellular mechanisms also have been proposed that may result in general toxicity and/or specific impairment of endocrine processes. The cytoskeleton is a target for methylmercury, and disruption of cytoskeletal components could impair the normal mitotic division that occurs during gamete development. Apoptosis also has been proposed as a mechanism by which methylmercury impairs the steroidogenic capacity of gonads. The reproductive axis is an integrated system; however, many of the studies reviewed here examined only a single parameter of the axis. Furthermore, data implicating the direct involvement of any of these mechanisms in reproductive impairment are still lacking. These studies provide evidence of reproductive inhibition, but they do not clarify whether the

primary site of action is the gonad itself or whether it is caused by inhibition of pituitary secretion or altered neurotransmission in the hypothalamus or other brain regions. Additionally, changes at the level of the transcriptome or proteome associated with reproductive dysfunction remain to be determined.

In summary, there appears to be sufficient evidence from laboratory studies to link exposure to mercury with reproductive impairment in many fish species. It is now necessary to determine the primary site of action and the mechanisms involved in endocrine disruption at environmentally relevant exposures to methylmercury. Linking reproductive impairment with an ecologically relevant impact on real fish populations is a further challenge. Fish are a large food source for both humans and wildlife, and the effects of mercury exposure on fish, as described in the present review, may similarly occur in these piscivorous vertebrates. Therefore, mercury pollution is a concern for both fish populations and their consumers, because mercury can act both indirectly, to decrease the food supply, and directly, by concentrating in the food chain itself to affect top predators, such as humans.

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