Dicamba Affects Sex Steroid Hormone Level and mRNA Expression of Related Genes in Adult Rare Minnow (*Gobiocypris rarus*) at Environmentally Relevant Concentrations

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ABSTRACT: Dicamba is a benzoic acid herbicide that has been detected in surface and ground water. The herbicide has been shown to have cytogenic and DNA damaging effects and to cause organ damage in mammals; however, little is known about the endocrine disrupting effects of dicamba in fish. In this study, histological changes, plasma vitellogenin (VTG) and sex hormone levels, and mRNA expression of sex steroid hormone-related genes were determined in adult rare minnow exposed to environmentally relevant concentrations of dicamba (0, 0.05, 0.5, 5, and 50 $\mu\text{g/L}$) for 40 days. The results showed inhibition of spermatogenesis in male testes and ovarian degeneration in females. Plasma 17 β -estradiol (E2) levels were significantly increased in both genders, and plasma VTG levels were significantly increased in males ($p < 0.05$). These results indicate that sex hormone homeostasis and normal reproduction of fish could be affected by dicamba. Moreover, the mRNA levels of *vtg* were significantly upregulated in the livers and gonads of both male and female rare minnows ($p < 0.05$). The downregulation of cytochrome P450c19a (*cyp19a*) and steroidogenic acute regulatory protein (*star*) mRNA levels, and the upregulation of cytochrome P450c17 (*cyp17*) mRNA levels were observed in the livers and ovaries ($p < 0.05$). The results of the mRNA analysis suggest that changes in steroid hormone-related gene expression could serve as a regulatory mechanism to maintain sex hormone homeostasis. Overall, dicamba exposure could result in histological lesions, plasma VTG increases, changes in sex hormone levels, and alterations of hormone-related gene expression. Therefore, dicamba should be considered to be a potential endocrine disruptor.

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INTRODUCTION

Herbicides provide an abundance of economic benefits to agriculture, but the potential agronomic, environmental, ecological, and human health problems resulting from herbicide use cannot be ignored. Pesticides contain a significant amount of endocrine disrupting chemicals (EDCs), which interfere with hormone synthesis, metabolism, or action resulting in a deviation from normal homeostatic

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control and reproduction. Many of the herbicides, such as atrazine, acetochlor, and glyphosate, are EDCs (Crump et al., 2002; Le et al., 2010; Yang et al., 2010). Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a chlorinated benzoic acid-derivate herbicide that is widely used to control annual and perennial broadleaf weeds (Gonzalez et al., 2009). Dicamba poses a potential risk to aquatic organisms because of its relatively high water solubility, and it has been frequently detected in aqueous environment. For example, the maximum concentration of dicamba was 13 µg/L in surface water samples and up to 517 µg/L in groundwater sample in Canada (Caux et al., 1993). In the northern Great Plains of North America, dicamba has been detected in 15 drinking water reservoirs at a maximum concentration of 1040 ng/L with a mean annual calculated concentration of 4 ng/L (Donald et al., 2007). Furthermore, dicamba was one of most frequently detected herbicides in water and sediment samples obtained from three sites in California that were unaffected by agricultural inputs from 2008 to 2011 (Ensminger et al., 2012).

Dicamba has been proved to promote activity in two-stage hepatocarcinogenesis (Espandiar et al., 1999). Additionally, dicamba has been shown *in vitro* to have cytogenetic effects on whole blood human lymphocyte cultures and to be a DNA damaging agent, making it a potentially hazardous compound to humans (Gonzalez et al., 2006). Moreover, sister chromatid exchanges, micronucleus induction, decreased mitotic activity, and increased genomic instability were observed in Chinese hamster ovary cells after dicamba exposure (Gonzalez et al., 2009, 2011). However, little is known about the endocrine disrupting effects of dicamba on aquatic organisms.

Sex steroid hormones play important roles in sex differentiation, sexual development, and normal reproduction in fish (Rougeot et al., 2007; Lubzens et al., 2010). The sex steroids testosterone (T) and 17β-estradiol (E2) are produced from cholesterol through a series of reactions. Steroidogenic acute regulatory protein (StAR) regulates the transfer of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroid hormone synthesis, and has been detected in many organs, such as head kidney, testis, ovary, and brain (Nunez and Evans, 2007). The steroid-metabolizing enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD) is essential for formation of sex steroids (Wang et al., 2011). Cytochrome P450c17 (CYP17) is the key enzyme that catalyzes androgen biosynthesis, and the cytochrome P450c19 (CYP19) transforms these androgens to estrogens (Uno et al., 2012). In addition, estrogens induce vitellogenin (VTG) production in the liver of fish by binding to estrogen receptors (ERs) (Marlatt et al., 2008). Measurements of plasma VTG levels or gene transcription in fish are commonly used biomarkers for exposure to estrogenic EDCs in the aqueous environment (Zha et al., 2007; Zhang et al., 2008).

Fish are appropriate models for studying EDCs in aqueous environment due to its sensitivity to sex steroids or xenobiotics. As an ideal appropriate species for toxicological studies, Chinese rare minnow (*Gobiocypris rarus*) has been widely used to assess EDCs (Zha et al., 2007; Li et al., 2009). The objectives of this study were to analyze the histopathology, plasma VTG and sex steroid hormone levels, and sex steroid hormone-related gene expression of adult rare minnows after exposure to environmentally relevant concentrations of dicamba to investigate the possible molecular mechanisms underlying the response to this toxicant.

MATERIALS AND METHODS

Chemicals

Dicamba (3,6-dichloro-2-methoxybenzoic acid) was purchased from Sigma-Aldrich Chemical Co. (USA). Stock solutions of dicamba were dissolved in deionized water. Working solutions were freshly diluted by the stock solutions with dechlorinated tap water.

Culture Conditions and Experimental Design

The brood stock of rare minnows was raised in a flow-through system with dechlorinated tap water (pH 7.2–7.6; hardness 44.0–61.0 mg CaCO₃/L) at a constant temperature (25°C ± 1°C) with a photoperiod of 16:8 hr (light:dark), and has been used for testing chemicals in our laboratory for more than 9 years. The fish were fed with a commercial pellet food (Tetra, Germany) at a rate of 0.1% body weight per day and newly hatched brine shrimp (*Artemia*) nauplii twice daily. Appropriate doses of the stock solutions were added to glass mixing vessels by means of a peristaltic pump at a rate of 1 mL/min. The solutions were then diluted by dechlorinated tap water to the mixing vessels at a rate of 4 L/hr. A dilution apparatus and mixing chambers supplied a constant flow equivalent to five times the aquaria volume (18 L) per day. The flow rate of dilution water was checked daily by using a measuring cylinder. Waste and residue were removed daily while the test equipment and chambers were cleaned once a week.

Healthy, 5-month-old, adult rare minnows that were the offspring of the same pair of brood stock were randomly divided into five groups (15 male and female fish in each group). Fish were exposed under flow-through conditions to various concentrations (0, 0.05, 0.5, 5, and 50 µg/L; nominal concentrations) of dicamba; each treatment was conducted in triplicates. The fish in each group were euthanized after 40 days exposure. Individual fish were captured sequentially from each exposure group to avoid the confounding influence of sampling time. Blood was collected from each fish using a heparinized microcapillary tube and centrifuged immediately at 8000 × g for 10 min at 4°C. The plasma was stored at –80°C until analysis. The gonads and liver of each fish were removed and weighed after the blood was

TABLE I. Primers used for PCR and the quantification of the mRNA expression by RT-PCR

Gene	Genbank Accession No.	Sequence (5'→3')	Product Size (bp)
<i>β-actin</i>	DQ539421	F: CAGGGCGTGATGGTGGGGAT R: GGTTGGCTTTGGGGTTGAG	226
<i>star</i>	JQ219671	F: TGAAACACCTGGAAATGTGG R: TCTCAGCCCTTACAAACCCTT	143
<i>3β-hsd</i>	JN858104	F: TGGACAGAAACATCCGCTC R: TGAGAGACGCAGTGTGGAA	153
<i>cyp17</i>	GU290037	F: TTTCTCCGCTCCTCATCCCT R: TGCACAAACCATCACCTCC	183
<i>cyp19a</i>	GU290038	F: GTCGTTTCTTCCAGCCGTTTC R: GTTGTGGTTTGCGGGATGT	158
<i>vtg</i>	EU623080	F: ACAAGCCAACCGCAAGAGT R: TCAAGTCTAAAGCCCCGCT	280
<i>era</i>	HM045496	F: CTCACGACAGAAACACAGCC R: GCTCTCCAACAACCTGAACCTG	159

collected. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as follows: GSI = gonad weight (g)/body weight (g) × 100; HSI = liver weight (g)/bodyweight (g) × 100. In addition, the livers and gonads were flash-frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Histological Analysis

Liver and gonad tissue were fixed in Bouin's solution, processed in graded alcohol and xylene, and then embedded in paraffin. Histologic sections 3–4 μm in thickness were deparaffinized, hydrated, and stained with hematoxylin and eosin (H&E) for morphological studies. Five randomly selected separate nonoverlapping microscopic fields were examined under light microscope. Digital images were collected and viewed using Axiovision LE Version software. The spermatozoan and spermatocyte volume in the testis area relative to the total area of the field was analyzed and calculated as a percentage. The average score from 10 non-overlapping fields per section was determined.

Plasma VTG and Sex Hormone Measurement

Plasma VTG, E2, and 11-ketotestosterone (11-KT) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Hufeng Chemical Industry Co., China), following manufacturer's instructions. All samples were analyzed in duplicates, and a separate standard curve was run for each ELISA plate. The assay detection limits were 30 μg/L for VTG, 1.5 pg/mL for E2, and 7.0 pg/mL for 11-KT, respectively. All samples were measured at 450 nm using TECAN Infinite M200 plate reader.

RNA-isolation and Reverse Transcription

The total RNA was isolated from the livers and gonads (five male and female) using the Trizol reagent (Life technology) and the manufacturer's protocol. Traces of DNA were removed by incubation with DNase-I (Promega, Madison, WI). Then, RNA samples were dissolved in ribonuclease-free water and stored at −80°C.

The reverse transcription reaction mixtures containing 10 μL of total RNA, 2 μL (0.05 μg/μL) of Oligo(dT)₁₅ primers

were heated to 70°C for 5 min and quickly chilled on ice. After cooling, 40 units (U) of RNasin (RNase inhibitor; Promega), 200 U of Moloney Murine Leukemia Virus Reverse Transcriptase (Promega), and buffer (Promega) were added to a total volume of 25 μL and incubated for 1 hr at 37°C. Thereafter, the reaction mixture was heated to 70°C for 10 min to inactivate the reverse transcription.

Real-time Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed in a MX3005P RT-PCR system (Stratagene) in a total volume of 25 μL, consisting of the Brilliant II SYBR Green QPCR master mix, 300 nM forward primer and 300 nM reverse primer. RT-PCR primers were designed with the DNAMAN software programs (Lynnon BioSoft, Vaudreuil, Quebec, Canada), and the details of forward and reverse primers used and PCR products are listed in Table I. The thermal cycle parameters used were: 10 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 57°C, and 30 s at 72°C. All the samples were analyzed in triplicates and the mean values of these triplicate measurements were used for calculations of the mRNA expression. The results were analyzed according to the delta–delta Ct method (Livak and Schmittgen, 2001). The *star*, *3β-hsd*, *cyp17*, *cyp19a*, *era*, and *vtg* mRNA expression were normalized for *β-actin* mRNA expression. Dissociation curve analysis was performed for each gene to check the amplification of non-target fragments. Only one peak was observed for each amplification reaction, which was indicative of only the amplification of the target gene. Gene expression data are presented as the fold change relative to control fish within the same treatment period.

Statistics

All statistical analyses were performed with the SPSS (version 16.0). All quantitative data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis of the data was performed using analysis of variance, followed by Bonferroni's multiple comparison test. A probability of $p < 0.05$ was considered statistically significant, and $p < 0.01$ was considered highly statistically significant.

TABLE II. Growth, GSI, and HSI of adult rare minnow after 40 days exposure of dicamba

Concentration	Body Length (mm)		GSI (%)		HSI (%)	
	Females	Males	Females	Males	Females	Males
Control	48.32 ± 3.16	45.97 ± 1.98	17.95 ± 3.52	5.04 ± 0.66	1.95 ± 0.37	2.21 ± 0.46
0.05 µg/L	48.50 ± 3.11	46.78 ± 2.71	19.55 ± 1.98	5.37 ± 0.45	2.49 ± 0.75	2.14 ± 0.62
0.5 µg/L	46.80 ± 2.67	47.85 ± 2.68	19.92 ± 3.33	5.15 ± 0.71	2.31 ± 0.53	1.69 ± 0.54
5 µg/L	46.91 ± 2.78	45.23 ± 4.08	20.14 ± 4.77	6.65 ± 0.70	2.07 ± 0.52	1.70 ± 0.56
50 µg/L	46.99 ± 3.56	46.33 ± 3.00	21.81 ± 5.80	5.33 ± 0.87	2.15 ± 0.87	2.04 ± 0.50

Data expressed as mean ± SEM of each treatment (males or females, $n = 15$).

RESULTS

Mortality and Growth

No mortality was observed in treatment and control groups during the exposure period. Malformations were not observed during the exposure. There were no significant differences in body weight and body length between treatment and control fish (Table II). The HSI and GSI of fish in all

treatments did not show statistically significant differences compared with the control.

Histological Analysis

Several pathological changes were observed in the livers and gonads of fish after dicamba exposure. The most relevant histological alterations in livers of male fish were cytoplasmic degeneration and bile stagnation observed in the 50 µg/

Fig. 1. Light micrographs of hepatic tissue histology in adult rare minnow (*Gobiocypris rarus*) after 40 days of exposure to dicamba, stained with hematoxylin and eosin (H&E): (A) normal male hepatic tissue; (B) male hepatic tissue from 50 µg/L dicamba-treated group, it showed biliary stagnation (black arrow) and cytoplasmic degeneration (square); (C) normal female hepatic tissue; (D) female hepatic tissue from 50 µg/L dicamba-treated group, it showed enlargement of the cell nuclei (white arrow) and bile stagnation (black arrow). Scale bar corresponds to 10 µm.

Fig. 2. Light micrographs of gonads histology in adult rare minnow (*Gobiocypris rarus*) after 40 days of exposure to dicamba, stained with H&E: (A) normal male testis tissue; (B) male testis tissue from 50 µg/L dicamba-treated group, it showed an inhibition of spermatogenesis (asterisk); (C) normal female ovary tissue; (D) female ovary tissue from 50 µg/L dicamba-treated group, it showed ovarian degeneration (black arrow).

L treatment group [Fig. 1(B)]. In females, enlargement of the cell nuclei and bile stagnation in the livers were observed in the 50 µg/L treatment group [Fig. 1(D)].

The testes of the male control fish was found with numerous cysts containing germ cells in all stages of spermatogenesis [Fig. 2(A)]. The seminiferous tubules of the testes were filled with large numbers of fertile sperm. The spermatozoa and spermatocytes in male control fish totaled $45.90\% \pm 4.52\%$ and $39.63\% \pm 5.89\%$ of testis volume, respectively. However, an inhibition of spermatogenesis was observed in the 50 µg/L treatment group [Fig. 2(B)]. At the end of the exposure, spermatozoan and spermatocyte totaled $37.25\% \pm 3.62\%$ and $25.30\% \pm 4.23\%$ in the 50 µg/L treatment group, respectively. Ovaries of the control females contained oocytes at various stages of development [Fig. 2(C)]. In comparison, ovarian degeneration and few of primary and secondary follicles were observed in the 50 µg/L treatment group [Fig. 2(D)].

Plasma VTG and Sex Hormone Levels

At the end of the exposures, plasma VTG levels were significantly increased in the males in all treatment groups com-

pared with the control males [$p < 0.05$; Fig. 3(A)]. With respect to the females, a slight decrease in plasma VTG was observed in the treatment groups, but there were no significant differences compared with the control females.

The plasma E2 levels were significantly increased in both gender in all treatment groups compared with the corresponding controls [$p < 0.05$; Fig. 3(B)]. Additionally, 11-KT was measured because it is thought to be the major active androgen in male teleosts (Desjardins et al., 2005). There was no significant difference in plasma 11-KT levels between treatment and control groups in either gender [Fig. 3(C)]. However, the hormone ratios (E2/11-KT) were significantly increased for both genders in all treatment groups after the 40-day exposure ($p < 0.05$).

Quantitation of Gene Expression by RT-PCR

The expression of *star*, *3β-hsd*, *cyp17*, *cyp19a*, *era*, and *vtg* mRNA levels in the livers of both male and female rare minnows was determined after the 40-day exposure to dicamba (Fig. 4). The mRNA levels of *star* gene were significantly downregulated in all female treatment groups and in male

Fig. 3. Plasma concentrations of vitellogenin (VTG), estradiol-17 β (E2), and 11-ketotestosterone (11-KT) after 40 days of exposure to dicamba in adult rare minnow (*Gobio cypris rarus*). * $p < 0.05$, relative to control.

treatment groups above 5 µg/L ($p < 0.05$). There was no significant difference in the expression of 3β -*hsd* mRNA levels in either male or female fish compared with the gender-matched control. The expression of *cyp17* and *vtg* mRNA was significantly upregulated in both genders ($p < 0.05$). The upregulation of *er α* was observed in the livers of females above 5 µg/L ($p < 0.01$), whereas no significant changes in *er α* were observed in the livers of males. The *cyp19a* mRNA levels were significantly downregulated in livers of male as well as females in the 0.05 and 0.5 µg/L treatment groups ($p < 0.05$).

The expression of *star*, 3β -*hsd*, *cyp17*, *cyp19a*, *er α* , and *vtg* mRNA in gonads was determined after dicamba exposure (Fig. 5). The result showed that ovarian *star* mRNA levels were significantly downregulated at 5 and 50 µg/L ($p < 0.05$). Moreover, 3β -*hsd* mRNA levels were significantly upregulated in the ovaries and testis in the 5 µg/L groups. The *cyp17* mRNA levels were significantly upregulated in the ovaries, but were only upregulated in the testes of the 0.05 µg/L group. The expression of *cyp19a* mRNA was significantly downregulated at 0.5, 5, and 50 µg/L in the ovaries, whereas no significant changes were observed in the testes. The expression of the *vtg* mRNA levels was significantly upregulated in the ovaries and testes ($p < 0.01$). However, the expression of *er α* was not significantly different after dicamba exposure.

DISCUSSION

As a result of increased levels of chemicals detected in aqueous environments, the accumulation and effects of these chemicals on fish are mainly a result of direct exposure in the water, bioaccumulation through the food chain, or even through indirect parental transfer (Yu et al., 2011; Tetreault et al., 2012). In the present study, the histological changes, plasma VTG, and hormone levels of adult Chinese rare minnows were determined after exposure to environmentally relevant concentrations of dicamba. Moreover, the expression of sex steroid hormone-related genes were evaluated simultaneously to reveal possible endocrine disrupting effects and molecular mechanisms underlying toxic response.

The liver is an essential organ for the metabolism and excretion of xenobiotics in vertebrates. Sex steroids have crucial roles in normal reproduction in the gonads, and small changes in the levels of sex steroids could cause endocrine disrupting effects (Jensen et al., 2001). The evaluation of histological changes in the livers and gonads of fish have been used as tools for monitoring EDCs (Zha et al., 2007). In this study, enlargement of cell nuclei and bile stagnation were observed in livers, and inhibition of spermatogenesis and ovarian degeneration were observed in testes and ovaries, of dicamba-exposed fish. These results confirm that dicamba could induce hepatic tissue damage and cause adverse effects on spermatogenesis and potentially the fertility of the fish. Furthermore, our observed increases in plasma E2 levels are consistent with previous studies showing that estrogen could induce bile stagnation in mammalian livers (Zucchetti et al., 2011). Our studies found that the hormone ratios (E2/11-KT) were significantly increased in both genders in all treatment groups. Previous research has indicated that the ratio of E2/11-KT is a very sensitive indicator of the balance of these two hormones in fish (Mitchellmore and Rice, 2006). After a 21-day exposure to ethynylestradiol (EE2), alterations in reproductive behavior, decreased hormone levels and secondary sex characteristics were observed in male fathead minnow

Fig. 4. Relative expression levels of *star*, *3β-hsd*, *cyp17*, *cyp19a*, *era*, and *vtg* in livers of adult rare minnow (*Gobiocypris rarus*) after 40 days of exposure to dicamba. The results were evaluated as the relative ratio of the expression level of each mRNA to that of β -actin. Data expressed as mean $\pm$ SEM of each treatment ($n = 5$). * $p < 0.05$, ** $p < 0.01$, relative to control.

(*Pimephales promelas*) (Salierno and Kane 2009). In adult zebrafish, the increase in plasma E2 was accompanied by decreased fecundity and hatching rates after exposure to fluoretolomer alcohol (8:2 FTOH) (Liu et al., 2010). These results suggest that hormonal changes may be associated with reproductive effects in fish. Therefore, sex hormone homeostasis and normal reproduction in fish could be affected by dicamba at environmentally relevant concentrations.

Our data demonstrate that plasma VTG levels were significantly increased in male, and *vtg* mRNA levels were sig-

nificantly upregulated in the livers and gonads of both genders. Previous studies have shown that plasma VTG levels in Chinese rare minnows are likely to be responsive to treatment with environmental estrogens (Zha et al., 2007), and upregulation of *vtg* gene transcription was observed after exposure to E2 and 2,4-dichlorophenol (Zhang et al., 2008). Treatment with estrogenic wastewater treatment works effluents significantly increased plasma VTG levels in male of fathead minnows (Thorpe et al., 2009). Moreover, chronic exposure to low concentrations of EE2 can increase *vtg*

Fig. 5. Relative expression levels of *star*, *3β-hsd*, *cyp17*, *cyp19a*, *era*, and *vtg* in gonads of adult rare minnow (*Gobiocypris rarus*) after 40 days of exposure to dicamba. The results were evaluated as the relative ratio of the expression level of each mRNA to that of β -actin. Data expressed as mean $\pm$ SEM of each treatment ($n = 5$). * $p < 0.05$, ** $p < 0.01$, relative to control.

mRNA and protein levels in male fathead minnows in lake experiments and adversely impact the sustainability of wild populations (Kidd et al., 2007). These results may support the hypothesis that chronic exposure to dicamba may affect the reproductive health of fish. In this study, increased E2 levels were accompanied by increased hepatic *era* mRNA expression in females at higher-dose groups, whereas significant effects in gonads were not observed. Previous research showed that the *era* mRNA levels were upregulated in the livers of male and female largemouth bass (*Micropterus salmoides*), but were downregulated in the ovaries of females

after exposure to *p,p'*-DDE (Garcia-Reyero et al., 2006). Additionally, after exposure of common goldfish (*Carassius auratus*) to 1 nM E2, the expression profiles of *era* differed by sex and exposure time (Marlatt et al., 2010). Taken together, these evidences suggest that changes in *era* transcription levels induced by exogenous estrogen might be tissue-specific, sex-specific, and time-dependent in fish. The present data suggested that dicamba should be considered a potential estrogenic endocrine disruptor.

The transcription of *star* could be a sensitive biomarker for EDCs in fish (Arukwe, 2008). Our studies found *star*

mRNA levels were downregulated in the livers of both genders and in the ovaries of females after a 40-day exposure. Previous studies have indicated that the *star* and *3 β -hsd* were predominantly expressed in the gonads of rare minnows (Liu et al., 2012). Consistent with our present study, it has been reported that the mRNA levels of *star* were downregulated in the ovaries but unchanged in the testes after 5 months E2-treated goldfish (Sharpe et al., 2007). Moreover, the downregulation of *star* expression was observed in male largemouth bass livers exposed to organochlorine pesticide methoxychlor and E2 (Blum et al., 2008). However, the time- and concentration-specific effects on the expression of the *star* genes were observed in gonads of EE2-treated female Atlantic salmon (*Salmo salar*) (Vang et al., 2007). In the present study, the downregulation of *star* expression might be a compensatory response of steroidogenesis for rare minnows exposed to higher E2 levels. Our data demonstrate that the transcription of *3 β -hsd* was upregulated in gonads, but was not significantly changed in the liver after dicamba exposure. The 3 β -HSD protein plays an important role in biosynthesis of both androgens and estrogens during steroidogenesis (Raghuveer and Senthilkumaran, 2012). Previous research has observed that *3 β -hsd* type 1 mRNAs were increased in human trophoblast cells treated with E2 (Beaudoin et al., 1997). The upregulation of *3 β -hsd* expression has been observed in the testes of fathead minnows after exposure to the 3 β -HSD inhibitor trilostane (Ankley et al., 2011). Taken together, the changes in *star* and *3 β -hsd* expression might be compensatory responses to circulating sex steroid hormone levels in rare minnows after dicamba exposure.

In the present study, *cyp17* mRNA expression was upregulated in the livers and gonads of both male and female fish after dicamba exposure, but *cyp19a* mRNA levels were significantly downregulated in the livers and ovaries of females, and in the livers of males. The *cyp17* and *cyp19a* genes are mainly expressed in gonads of fish (Uno et al., 2012). Previous research has indicated that upregulation of *cyp17* and downregulation of *cyp19a* was observed in ovaries of fathead minnows after E2 exposure (Filby et al., 2007). Taken together, the changes of *cyp17* and *cyp19a* mRNA levels may increase T and decrease conversion of T to E2 in fish, which responded well to the changes in sex hormone levels. It is possible that the increased E2 might depress the expression of *cyp19a* as a feedback regulation, whereas the upregulation of *cyp17* mRNA levels might serve as a compensatory mechanism to increase T in rare minnows. Overall, these results indicate that the changes in mRNA expression of sex steroid production related genes reflect a regulatory mechanism to maintain sex hormone homeostasis in fish.

CONCLUSION

In summary, our study showed histological lesions and changes in plasma VTG, sex hormone levels, and sex steroid

hormone-related genes after 40 days dicamba exposure. Notably, the exposure concentrations were closely to those measured in surface water. The plasma E2 increase, spermatogenesis inhibition, and ovarian degeneration suggest that sex hormone homeostasis and the normal reproduction of fish could be affected by dicamba at environmentally relevant concentrations. Sex steroid hormone-related genes served as a regulatory mechanism to maintain sex hormone homeostasis in rare minnow. The gene expression patterns indicated that dicamba caused disruption of the sex steroid synthesis system. Therefore, dicamba should be considered a potential endocrine disruptor in fish.

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