Effects of chronic sublethal progestogen exposure on development, reproduction, and detoxification system of water flea, *Daphnia magna*

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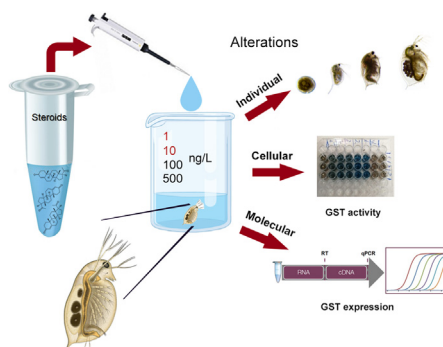[c] *Ecophysiology and Environmental Toxicology, Balaton Limnological Research Institute, Eötvös Loránd Research Network, 8237 Tihany, Hungary*
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HIGHLIGHTS

- Effects of progestogens on *Daphnia magna* were investigated.
- Long-term progestogen exposure influenced the egg production.
- Short-term exposure affected the expression and activity of GST enzyme.
- Results suggest that animals living under aquatic conditions are sensitive to progestogens.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

The presence of sex steroid hormones in aquatic ecosystems is of rapidly growing concern worldwide since they can affect the different non-target species including cladocerans. Although data are available on the effects of estrogens on the well-established ecotoxicological model organism *Daphnia magna*, the molecular or behavioural alterations induced by environmentally relevant concentrations (from a few ng L^{-1} to a few hundred ng L^{-1} in average) of progestogens have not been investigated on this species. In the present study, we exposed neonates of *D. magna* to relevant equi-concentrations (1, 10, 100, 500 ng L^{-1}) of mixtures of four progestogens (progesterone, drospirenone, gestodene, levonorgestrel) in short-term (6 days) and long-term (21 days) experiments. Significant alterations were observed at the molecular, cellular, and individual levels. During the short-term exposure, all of the mixtures increased the gene expression of glutathione S-transferase (GST) detoxification enzyme, moreover, the activity of GST was also significantly increased at the concentrations of 10, 100, and 500 ng L^{-1}. In long-term exposure, the number of days until production of the first eggs was reduced at the 10 ng L^{-1} concentration compared to control, furthermore, the maximum egg number per individual increased at the concentrations of 1 and 10 ng L^{-1}. Based on the authors' best knowledge, this is the first study to investigate the effects of progestogens in mixtures and at environmentally relevant concentrations on *D. magna*. Our findings contribute to the understanding of the possible physiological effects of human progestogens. Future research should be aimed at understanding the potential mechanisms (e.g., perception) underlying the changes induced by progestogens.

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1. Introduction

Nowadays, when large quantities of different chemicals, including pharmaceutically active compounds (PhACs), are released into the environment, the widespread presence of oral contraceptives (basically applying estrogens and/or progestogens) as endocrine disrupting chemicals in the global ecosystem has generated a rapidly growing concern worldwide (Can et al., 2014; Guzel et al., 2019; Liu et al., 2011; Maasz et al., 2019; Molnar et al., 2020; Postigo et al., 2010; Stefano et al., 2003). Understanding how animals are capable of coping successfully with these external factors and how they can develop appropriate adaptive responses from the molecular to the behavioural level is a complex problem. For a long time, the estrogens were the most extensively studied contraceptive compounds, for example, the molecular mechanism of the effects of 17β-estradiol (E2) was studied on various organisms and these studies revealed that E2 can induce biphasic changes in aquatic models (Zheng et al., 2020). However, more limited data are available for the relevant effects of mixtures of progestogens (progesterone and its synthetic analogue progestins) on non-target organisms, including fish and cladocerans, especially as the ecotoxicological studies usually only apply a single compound in concentrations (μg L^{-1}, mg L^{-1}) that are not environmentally relevant.

The water flea (Daphnia magna) is a well-established model organism in ecotoxicology (Stollewerk, 2010). Various advantages such as high fecundity, high growth rate (reaches sexual maturity within 6–8 day), ease of rearing and manipulation in the laboratory, short and well-characterized life cycle (7–8 weeks at 20 °C), and high sensitivity to a wide range of chemicals including steroid hormones (Luna et al., 2015; Ten Berge, 1978; Torres et al., 2015; Zheng et al., 2020) make this species ideal for evaluating aquatic toxicity in freshwater ecosystems (Martins et al., 2007; OECD, 2012). Furthermore, its genome has been published enabling investigations at the molecular level as well (Orsini et al., 2011). In the laboratory, specimens can be easily maintained through female parthenogenesis (Hebert, 1987).

In a previous study, the global environmental concentration range of the four main contraceptive progestogen compounds in ng L^{-1} was summarized as follows: 0.06–9330.00 for progesterone (PRG), 0.26–4.30 for drospirenone (DRO), 0.61–8.30 for gestodene (GES), and 0.20–170.00 for levonorgestrel (LNG) (Svigruha et al., 2020). Although data are available on the effects of estrogens on D. magna, the ability to tolerate the concentrations of progestogens and changes in molecular and cellular mechanisms following short- or long-term exposure to progestogens have not yet been investigated.

Keeping this in mind, the present study was conducted to investigate the possible effects of progestogens on D. magna during short-term (6 days) and long-term (21 days) exposures, in order to obtain further information regarding the mode of action of progestogens. To achieve this, considering the global progestogen concentrations presented above, neonates were exposed to environmentally relevant (1 and 10 ng L^{-1}) and higher (100 and 500 ng L^{-1}) equi-concentrations of mixtures of progestogens (PRG, DRO, GES, LNG). In this way, our experimental approach considers both the average environmental concentrations and rarer, considerably higher values. At the whole organism level, life cycle parameters, such as survival, growth, and reproduction, were examined. Enzymatic biomarkers from different metabolic pathways (e.g., oxidative stress) have been shown to be early indicators of environmental pollution (Coelho et al., 2011) and are widely used to evaluate the early responses to different PhACs (e.g., Liu et al., 2017; Nunes et al., 2006; Oliveira et al., 2015; Wang et al., 2016). Since sex steroid exposure has been shown to cause antioxidant enzyme responses in different aquatic non-target species (e.g., Cardoso et al., 2019; Linlan et al., 2016; Woo et al., 2012), the changes in the expression and activity of detoxification-related glutathione S-transferase (GST) were also investigated.

2. Materials and methods

2.1. Chemicals

HPLC grade PRG (CAS No.: 57-83-0), DRO (CAS No.: 67392-87-4), GES (CAS No.: 60282-87-3), and LNG (CAS No.: 797-63-7) were used for the treatments as progestogen agents (Sigma-Aldrich, Hungary). From these, 1 mg mL^{-1} stock solutions were prepared in acetone (ACS reagent, ≥99.5%; CAS No.: 67-64-1; VWR, Hungary). From these stock solutions, 1 μg mL^{-1} working solutions were prepared in artificial water (OECD, 2012) (solvent at ≤0.01%) and then added to the experimental glass beakers to reach the desired nominal equi-concentrations of 1, 10, 100, and 500 ng L^{-1} (mixtures of progestogens).

2.2. Daphnia magna culture and chronic treatments

D. magna specimens, originated from Daphtoxkit F magna (DM247, MicroBio Tests), have been maintained at a constant temperature of 23 ± 1 °C on a light:dark regime of 16:8 h at the Department of Experimental Zoology (Balaton Limnological Institute, Tihany, Hungary) for over 4 years. They were cultured in 500 mL glass beakers containing 450 mL artificial water (OECD, 2012) and were fed daily on Scenedesmus obliquus (0.5×10^{-6} cells mL^{-1}) and ground juvenile tropical fish food (0.9–1 mg L^{-1}, FIX1, Aqua-Life Ltd., Hungary) and the medium was renewed two times a week.

From our healthy parent stock, neonates (<24 h) were selected for chronic progestogen exposure. In order to investigate the adaptive developmental and fecundity responses, parthenogenetic neonates ($n = 6$/experimental group) were individually placed into 100 mL media in 150 mL glass beakers and hence individually exposed to the mixtures (1, 10, 100, and 500 ng L^{-1}) of PRG, LNG, GES, and DRO for 21 days. Animals in the control group were kept in 100 mL media originally containing the solvent (≤0.00001%). Hence, the 5 experimental groups contained $n = 30$ total animals per replicates. No effects of the solvent were observed. As we utilized a static exposure system and at least one of the test chemicals, progesterone, is known to be unstable in aqueous (Kumar et al., 2015; Ojoghoro et al., 2017), water was totally refreshed three times a week and progestogens were re-added to continuously maintain the nominal concentrations. The recovery results of our preliminary experiment (analytical methodology and results are presented in the Supplementary information), indicated that total water renewal is required in every second day. During the 21-day exposure, specimens were fed three times a week. In order to examine the changes in the GST enzyme expression and activity, parthenogenetic neonates ($n = 20$/experimental group; $n = 100$ total animals per replicates) were kept in 500 mL glass beakers containing 450 mL media and exposed to the mixtures of progestogens for 6 days. Similar to the long-term exposure, the water was totally refreshed three times and progestogens were re-added to continuously maintain the nominal concentrations. Three replicates were set up for experimental groups in both of the short- and long-term exposure. Before treatment, neonates were always acclimatized for 2 h in glass beakers in artificial water.

2.3. Growth and reproduction

Observations of D. magna growth and reproduction were obtained using LEICA M205C stereomicroscope (BioMarker Ltd., Hungary) and evaluated with LAS software (version: 4.12). Body size was calculated using the length and width parameter of the specimens (Eq. (1)).

$$K \approx 4\frac{\pi ab + (a-b)^2}{(a+b)} \tag{1}$$

2.4. qRT-PCR measurement of GST expression

For RNA sample collection, twenty adult specimens were collected from each group and then homogenized using a TissueLyser LT (QIAGEN) in TRI reagent (#93289, Sigma-Aldrich). RNA was isolated with Direct-zol™ RNA MiniPrep (#R2050, Zymo Research) following the instructions of the manufacturer. The RNA samples were quantified using a Qubit 4.0 device (#Q33238, Thermo Fisher Scientific) with Qubit BR RNA Kit (#Q10211, Thermo Fisher Scientific). The RevertAid H Minus First Strand cDNA Synthesis Kit (#K1631, Thermo Fisher Scientific) was used for reverse transcription, applying random hexamer primers and 200 ng RNA for each sample.

To quantify the change of mRNA expression level of GST, qRT-PCR was performed. Each 10 μL reaction volume contained 5 μL 2× PowerUp™ SYBR™ Green Master Mix with ROX as a passive reference dye (#A25741, Thermo Fisher Scientific), 3.0 μL distilled water, 1 μL cDNA sample, and 0.5 μL for each primer (10 μM) selectively amplifying GST or β-actin. The applied primer set, adapted from Liu et al. (2017), was as follows: the forward primer for GST: GGG AGT CTT TTA TCA CCG TTT C; the reverse primer for GST: TCG CCA GCA GCA TAC TTG TT; the forward primer for β-actin: GCC CTC TTC CAG CCC TCA TTC T; the reverse primer for β-actin: TGG GGC AAG GGC GGT GAT TT. The amplification efficiency of both primer pairs calculated from the standard curves was within the desired range of 95%–105% (GST: 98%, β-actin: 100%). Each preparation of the GST and β-actin was added to a 96-well plate in triplicate. The reaction was performed at 50 °C for 2 min, 95 °C for 2 min (holding stage), followed by 40 cycles of 95 °C for 3 s, 60 °C for 30 s (cycling stage) using StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies) and StepOneTM (version 2.3) software. At the end of the measurement, a melting curve analysis was performed (plate read every 0.3 °C from 60 to 95 °C) to determine the formation of the specific products. The relative mRNA levels of GST were calculated by comparative C_T ($\Delta\Delta C_T$) method using the mRNA of β-actin as an endogenous control.

2.5. Total protein content and GST enzyme activity

Twenty adult animals from each group were homogenized using a TissueLyser LT (QIAGEN) device in 150 μL phosphate buffer saline (PBS, pH = 7.4). After centrifugation (8000g for 10 min at 4 °C), 20 μL supernatant was used for each biochemical measurements. The total protein content was measured using Bradford assay (#B6916, Sigma-Aldrich) and the GST activity with GST Assay Kit (#CS0410, Sigma-Aldrich) using a Victor 3 plate-reader (Perkin Elmer). GSTs catalyze the conjugation of the substrate 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione, forming a thioether that can be followed by the increment of absorbance at 340 nm. Extinction coefficient (ε) of CDNB conjugate used was 9.6 mM^{-1} cm^{-1} and length of well (l) was 0.8 cm. GST activity was calculated using (Eq. (2)). Results were expressed as nanomoles of thioether produced per minute, per milligram of protein.

$$GST\ activity = \frac{\Delta Abs_{340}\ X\ V_{total}}{\Delta t\ X\ l\ X\ \varepsilon\ X\ V_{sample}\ X\ [protein]} \tag{2}$$

2.6. Statistical analysis

Statistical analysis was performed using the OriginPro_2018 software (OriginLab Corp., Northampton, Massachusetts, USA). The normality of the dataset was investigated using the Shapiro-Wilk test, homogeneity of variances between groups investigated using Levene's statistic. For the analysis of average body size, two-way repeated-measures ANOVA was used to assess main the effects of time, treatment, and time x treatment interaction. This analysis was followed by one-way ANOVA and Scheffe's post hoc test to identify significant differences between control and treatment groups within a given time point. The

Table 1
The reproductive performance of D. magna during a 21-days progestogen exposure. The reproduction was monitored every day. Note: values are mean $\pm$ SD; significant results (bold), in comparison with the control, were indicated by asterisks (ANOVA, *$P < 0.05$); $n = 6$ animals/experimental group/replicates.

Treatments (ng L^{-1})	First eggs production (days)	Eggs number in the first production	Maximum eggs number per individual
Control	8.7 ± 0.47	4.2 ± 0.48	10.5 ± 0.37
1	7.2 ± 0.25	4.8 ± 0.51	**16.0 ± 2.02***
10	**6.5 ± 0.28***	5.2 ± 0.67	**16.0 ± 1.59***
100	8.0 ± 0.40	5.0 ± 0.85	14.3 ± 1.13
500	7.2 ± 0.62	5.7 ± 0.42	12.0 ± 0.57

expression ($\log_2(x + 1)$ transformed) and enzyme activity data were analysed using one-way ANOVA with Scheffe's post hoc test. Differences were considered statistically significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

3. Results

3.1. Growth and reproduction of D. magna during long-term progestogen exposure

No lethality was observed for D. magna at any of the concentrations tested during the entire period of 21 days. The results of the exposure on reproduction are shown in Table 1. The number of days until production of the first eggs was significantly shorter at the 10 ng L^{-1} concentration (6.5 ± 0.28; $P < 0.05$) than the control (8.7 ± 0.47). However, there was no significant difference in the number of eggs in the first production. The maximum egg number per individual significantly increased at concentrations of 1 ng L^{-1} (16.0 ± 2.02; $P < 0.05$) and 10 ng L^{-1} (16.0 ± 1.59; $P < 0.05$) compared to the control (10.5 ± 0.37).

The average body size of the animals during progestogen exposure is presented in Fig. 1. Two-way repeated-measures ANOVA revealed significant effects of time (observation days) [F(9, 263) = 282.32, $P < 0.001$] and treatment [F(4, 263) = 10.41, $P < 0.001$], but not significant time × treatment interaction [F(36, 214) = 0.59, $P > 0.05$]. Further
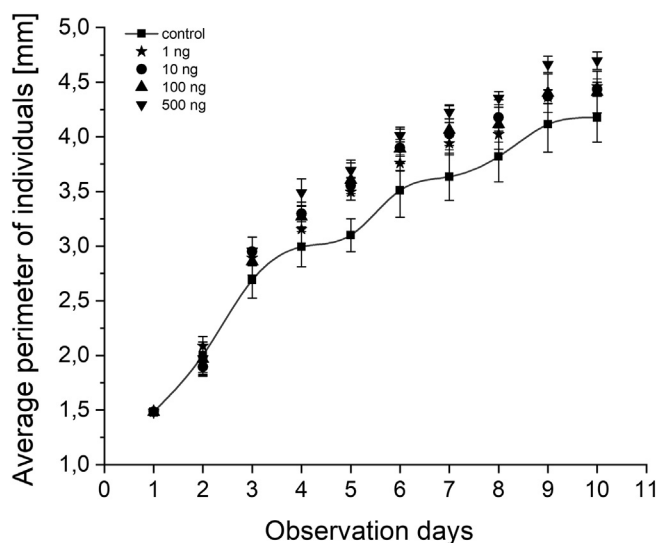

Fig. 1. Average body size of individuals in different experimental groups. The continuous line indicates control. Body size was measured every second day during the 21-day exposure. No significant differences were observed between the control and the treated groups during the whole observation period. Each data point represents mean ± SEM ($n = 6$ animals/experimental group/replicates).

analysis with one-way ANOVA and post hoc test indicated that there was no significant difference between the control and the treated groups during the whole observation period.

3.2. Effects of progestogens on the GST gene expression and enzymatic activity after short-term exposure

The expression profile of GST enzyme is shown in Fig. 2A. Compared to the control, gene expression significantly increased at all the concentrations: by 4.56 ± 0.17 ($P < 0.01$), 4.84 ± 0.20 ($P < 0.01$), 5.35 ± 0.39 ($P < 0.001$), and 8.00 ± 0.07 ($P < 0.001$) fold in the 1, 10, 100, and 500 ng L^{-1} groups, respectively. There was a gradual increase in expression as the equi-concentrations of the mixtures increased, demonstrating a concentration-response relationship.

The alterations of GST enzymatic activity are presented in Fig. 2B. There was no significant difference between the control (1.39 ± 0.02) and 1 ng L^{-1} (1.56 ± 0.06) progestogen treated group. However, GST activity was significantly higher in the 10 (1.69 ± 0.06; $P < 0.05$), 100 (1.81 ± 0.03; $P < 0.01$), and 500 (1.66 ± 0.01; $P < 0.01$) ng L^{-1} progestogen treated groups with showing a hormesis response (Calabrese and Baldwin, 2003).
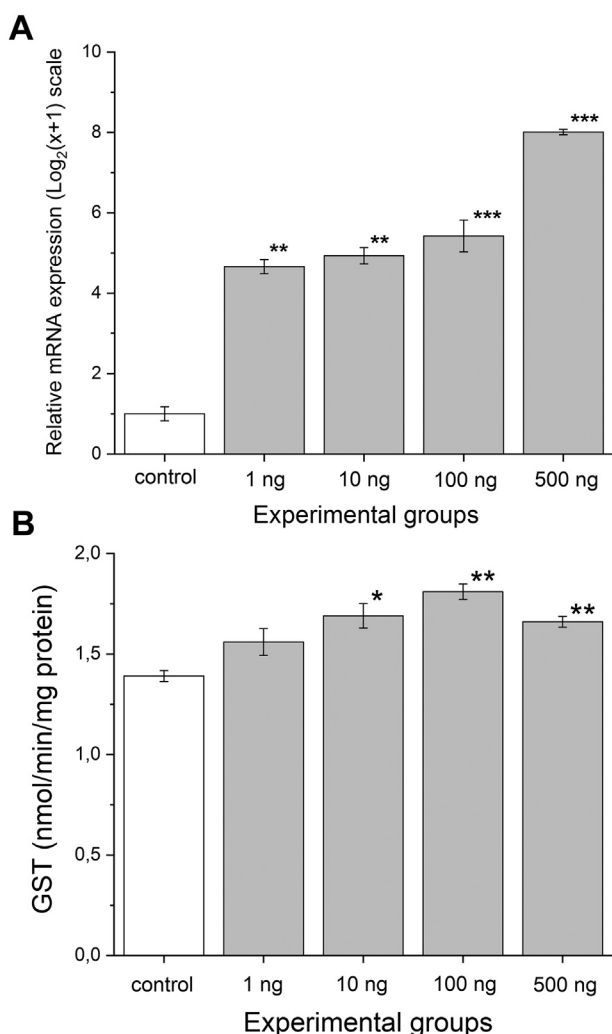

Fig. 2. Effects of different concentrations of progestogens on the GST gene expression (A) and activity (B) in *D. magna* after short-term exposure (6 days). Each bar represents mean $\pm$ SEM ($n = 20$ animals/experimental group/replicates). Fold change mRNA expression of GST (on $\log_2(x + 1)$ scale) is relative to the control. The white column represents the control while the grey columns represent the treated groups. Significance of differences to the control is indicated by asterisks (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

4. Discussion & conclusions

The global presence of vertebrate-type sex steroids, such as progestogens and estrogens, in ecosystems raises considerable environmental issues since these active compounds can cause toxic effects to freshwater organisms (Fent, 2015; Liu et al., 2012). Previous studies have already investigated their effects on fish (Maasz et al., 2017; Miracle et al., 2006; Runnalls et al., 2013; Zeilinger et al., 2009), molluscan (Contardo-Jara et al., 2011; Giusti et al., 2014; Svigruha et al., 2020; Tillmann et al., 2001; Zrinyi et al., 2017), and planktonic crustacean freshwater species (Barbosa et al., 2008; Brennan et al., 2006; LeBlanc, 2000; Luna et al., 2015; Rodríguez et al., 2007). Although the single effect of PRG at μg L^{-1} and mg L^{-1} concentrations has previously been investigated on the development of *D. magna* (Kashian and Dodson, 2004; Torres et al., 2015), this is the first study to examine the effects of progestogens applied in mixtures and at environmentally relevant concentrations on this classic ecotoxicological model species.

Similar to the previous two studies (Kashian and Dodson, 2004; Torres et al., 2015), no lethality was observed at any concentrations during the entire period of 21 days. Also, no significant changes were detected in the average body size during development at concentrations tested. This observation is consistent with a previous study where 100 μg L^{-1} PRG in long-term (25 days) exposure also failed to change growth-rate (Kashian and Dodson, 2004). In contrast to these findings, 17α-ethinylestradiol (EE2) reduced body size of *D. magna* at 0.1 ng L^{-1} concentration (Dietrich et al., 2010).

Many PhACs (e.g., diclofenac, ibuprofen) are known to cause a significant delay in terms of days to the first egg production (Heckmann et al., 2007; Liu et al., 2017). We found that the time to the first eggs produced was significantly shorter at the 10 ng L^{-1} progestogen exposure compared to the control. Similarly to growth-rate, the number of eggs in the first production was not affected by any of the progestogen concentrations in our laboratory experiment. In contrast, Dietrich et al. (2010) observed that daphnids exposed to (0.1 ng L^{-1}) EE2 produced a reduced number of offspring. In this study, the maximum eggs number per individual was significantly higher at the concentrations of 1 and 10 ng L^{-1} compared to the control. The significant increase in the maximum egg number caused an increase in fecundity. A similar effect was shown when the molluscan species, the great pond snail (*Lymnaea stagnalis*), was exposed to 10 ng L^{-1} of mixture of these four progestogens for 21 days (Zrinyi et al., 2017). This effect of progestogens in invertebrates remains to be resolved, because the environmental relevant concentrations of these compounds are known to consequently inhibit egg production in fish (Orlando and Ellestad, 2014; Thrupp et al., 2018). Unlike progestogens, long-term testosterone exposure at mg L^{-1} concentrations resulted in a reduced fecundity in *D. magna* (Barbosa et al., 2008; Clubbs and Brooks, 2007). Brennan et al. (2006) published that E2 had no significant impact on the fecundity of *D. magna* following exposure to mg L^{-1} concentrations. Moreover, long-term (40 days) environmentally relevant (100 ng L^{-1}) EE2 exposure decreased the number of neonates produced per female (Luna et al., 2015). Previously, specimens exposed for 25 days to 100 μg L^{-1} progesterone in long-term assays produced more male dominated broods during the second clutch of young (Kashian and Dodson, 2004). In contrast, no male specimens were observed in any of our experiments.

Many environmental pollutants can interfere with detoxification- and antioxidant-related enzyme activity and gene expression (Liu et al., 2017; Wang et al., 2016). Previous studies have shown that the molecular and cellular effects of sex steroid exposure can be investigated by enzymatic biomarkers from different metabolic pathways such as antioxidant enzyme responses in different aquatic non-target species (Cardoso et al., 2019; Costa et al., 2010; Lee and Choi, 2007; Linlan et al., 2016; Teles et al., 2005; Woo et al., 2012). One such multi-component enzyme is the GST that is known to be involved in the detoxification of many anthropogenic toxicants (e.g., active drug

residues, pesticides) and to play an important role in protecting tissues from oxidative stress (Yu et al., 2009; Mörtl et al., 2020; Yoo et al., 2019). With regard to sex steroids and GST, ecotoxicological studies have mainly investigated the effect of E2 and revealed an increase in the expression and activity of GST in fish (Teles et al., 2005; Woo et al., 2012) and worm species (Linlan et al., 2016). However, to the best of our knowledge, there has been no information on the effects of progestogens on GST expression and activity in aquatic species. In the present study, after short-term progestogen exposure, we found that gene expression was significantly higher at all applied progestogen concentrations compared to the control. Besides, the activity of GST was also increased at the concentrations of 10, 100, and 500 ng L^{-1}. Since GST is involved in removing reactive oxygen species from cells (Kim et al., 2009), our results indicate that, similar to estrogens, progestogens may also interact with pathways related to oxidative stress responses.

There is a continuing debate about whether natural vertebrate and synthetic sex steroid residues, occurring in the environment, can affect the neuroendocrine system and physiological processes of invertebrate species (Dang et al., 2012; Fodor et al., 2020; Luna et al., 2015; Rodríguez et al., 2007; Scott, 2013). Importantly, at least two homologues of the enzymes that catalyze the classical vertebrate steroid biosynthetic pathway, as well as the functional nuclear sex steroid receptors, have so far not been found in arthropod genomes (Markov et al., 2017). However, focusing on progesterone perception, homolog sequences to membrane progesterone receptor gamma (mPRγ) and progesterone membrane receptor component 1 (PGMRC1) are found in crustaceans (Ren et al., 2019). Although the progesterone-binding ability of these molecules has not been studied, they can be a potential target in the pathway of progestogen-induced changes and thus the effects could be mediated through a non-genomic pathway. This would include the activation of second messengers and various protein-kinase cascades that can eventually lead to indirect changes in gene expression due to phosphorylation of transcription factors. A similar mode of action has been proposed in the case of estrogen-induced alterations in molluscs (Tran et al., 2019).

Based on molecular, cellular, and behavioural responses (Barbosa et al., 2008; Brennan et al., 2006; Clubbs and Brooks, 2007; Dietrich et al., 2010; Kashian and Dodson, 2004; Luna et al., 2015; Torres et al., 2015; Zheng et al., 2020), *D. magna* specimens seem to be sensitive to sex steroid contaminations that occur in their natural habitat. Our experiments confirm this finding since 10 ng L^{-1} progestogen concentration influenced the time to the first egg production as well as 1 and 10 ng L^{-1} concentrations affected the maximum egg number per individual. Both the average environmental and higher concentrations affected the expression (1, 10, 100, and 500 ng L^{-1}) and activity (10, 100, and 500 ng L^{-1}) of GST enzyme and so potentially the related detoxification and antioxidant processes. Without functional nuclear sex steroid receptors, the molecular mechanisms underlying the induced effects of these synthetic PhACs remains to be determined and future research should be aimed at understanding their mode of action. The possible site of action could, for example, take place via the identified mPRγ or non-endocrine-mediated responses/non-specific interactions (e.g., with ancient multifunctional receptors or receptors for other compounds). However, we stress that this study does not focus on identifying the fundamental molecular background of the current findings. We just readily accept that progestogens are present in the natural habitats of *D. magna* and investigated whether their presence can cause any detectable molecular, cellular, and behavioural alterations.

CRediT authorship contribution statement

Réka Svigruha: Conceptualization, Investigation, Writing – original draft, Data curation, Visualization, Funding acquisition. **István Fodor:** Investigation, Writing – original draft. **János Győri:** Investigation, Writing – review & editing. **János Schmidt:** Investigation. **Judit Padisák:** Supervision, Writing – review & editing. **Zsolt Pirger:** Conceptualization, Methodology, Writing – review & editing, Data curation, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147113.

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