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THE EFFECT OF TCDD ON CONCENTRATION OF STEROID HORMONES IN OVIDUCT OF THE DOMESTIC HEN (*GALLUS DOMESTICUS*)*

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The aim of the study was to determine the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the concentration of steroid hormones, progesterone (P4), testosterone (T) and estradiol (E2) in individual parts of the chicken oviduct. The experiment was carried out on 12 Hy-Line laying chickens, which were randomly divided into two equal groups. The birds of the control group received a single intramuscular injection of saline, while the experimental group was injected with TCDD at a dose of 1 μg/kg body weight. The birds were decapitated 24 h after TCDD injection, and subsequently individual parts of the oviduct were isolated. P4, T and E2 concentrations in the oviductal parts were determined by radioimmunoassay. In the control group, the highest concentrations of these steroids were detected in the magnum and shell gland. TCDD injection resulted in a statistically significant increase in P4 and E2 concentrations in all examined parts of the oviduct and T in the magnum and shell gland. The obtained results suggest that dioxins – by inhibiting the uptake and/or synthesis of sex steroids in the oviduct tissues, as well as by blocking the steroid hormone binding to the appropriate receptor – may significantly affect the function of this organ.

Key words: hen, TCDD, oviduct, steroid hormones

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Polychlorinated dibenzo-p-dioxins (PCDDs) are a group of aromatic organochlorine compounds characterized by high toxicity, ability to accumulate in tissues (especially in adipose tissue), and resistance to environmental degradation. Because of their low susceptibility to microbial breakdown, PCDDs accumulate in the soil and water, from which they can biotransferred into living organisms. The high resistance of these compounds to biochemical processes and hydrolysis makes their biodegradation very slow (Wan et al., 2014). Among the 75 PCDD congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most

hazardous and toxic to humans and animals (Yonemoto, 2000; Makles et al., 2001). The majority of TCDD in the environment originate from uncontrolled burning of: (i) waste in old-style incinerators, (ii) loose waste in the open air, in boiler houses (e.g. hospital boiler plants), and (iii) plastic in household heating stoves. Substantial sources of dioxin emissions also include iron works and non-ferrous metal smelters, secondary material processing facilities, and car engines (in particular diesel engines). However, inappropriate thermal conditions during waste treatment (i.e. too low combustion temperature) are the main reason why this dioxin finds its way into the environment (Grochowalski, 2002).

It is widely accepted that the toxic effect of TCDD results from activation of the aryl hydrocarbon receptor (AhR) (Mimura and Fujii-Kuriyama, 2003; Walisser et al., 2004; Yasui et al., 2004; Beischlag et al., 2008). AhR is a transcription factor activated by a ligand, which, after forming the heterodimer AHR/ARNT complex with ARNT protein (AHR nuclear translocator) and binding to the dioxin response element (DRE) DNA sequence, regulates the transcription of many genes, including genes encoding xenobiotic metabolism enzymes (Gu et al., 2000; Lee et al., 2011; Yang et al., 2013). Planar hydrophobic compounds have the highest affinity for the AhR receptor. It is now thought that the AhR mediates many biochemical, biological and toxicological reactions, which are observed following exposure to TCDD (Yang et al., 2013; Sechman et al., 2014). The lack of expression of the AhR gene as well as activation of the AhR pathway by a ligand lead to reproductive dysfunction in females, which may be indicative of the potential role of this receptor in reproductive function (Baba et al., 2005; Barnett et al., 2007).

The harmful effects of TCDD are associated primarily with the endocrine system; this dioxin is classified as an endocrine disruptor (Yonemoto, 2000). Due to high homology in the structure of TCDD molecule and steroid hormones, TCDD may bind to the receptors of these hormones and thus interfere with reproductive function and hormonal balance (Giesy et al., 2003; Mimura and Fujii-Kuriyama, 2003). Many data from the literature suggest that in both mammals (Gregoraszczuk et al., 2000; Petroff et al., 2002; Jabłońska et al., 2011) and birds, TCDD adversely affects reproductive function (Giesy et al., 2003; Hrabia et al., 2013; Sechman et al., 2014). Our earlier research demonstrated that TCDD inhibits the expression of genes coding for the key enzymes of steroidogenesis in the follicular wall of the hen's ovary and reduces secretion of steroid hormones from these follicles (Sechman et al., 2014). TCDD were also shown to act through AhR receptors, which were found to be expressed in different structures of the ovary (Antos et al., 2015).

The direct effect of TCDD on the avian oviduct, the development and function of which is regulated by ovarian steroid hormones, remains unknown. Estrogens have an effect on differentiation of cells in the oviduct wall and on synthesis of egg albumen proteins (Yu and Marquardt, 1973; Schimke et al., 1975; Dougherty and Sanders, 2005; Socha and Hrabia, 2018). Together with androgens, estrogens work synergistically in stimulating hypertrophy and hyperplasia of smooth muscle cells of the oviduct (Yu and Marquardt, 1973; Dougherty and Sanders, 2005). Along with gestagens, estrogens control the synthesis and secretion of egg

albumens such as ovalbumin, conalbumin (Palmiter, 1971) and avidin (Joensuu et al., 1991; Socha and Hrabia, 2018). Many studies confirmed that all these three hormones are required for the full secretory potential of the oviduct to develop (Mika et al., 1987; Rząsa, 2007).

There are no studies in the literature concerning the effect of dioxins on the level of sex steroids in the avian oviduct. Therefore, the objective of the study was to determine the effect of TCDD injection on the concentration of progesterone (P4), testosterone (T) and estradiol (E2) in different segments of the oviduct in the domestic hen.

Material and methods

The study was carried out with 36-week-old Hy-Line hens, which were kept in individual cages with constant access to feed and water. Birds received a DJ2 diet and were exposed to a 14L:10D light programme. During the month preceding the experiment, oviposition time of each egg was recorded at 30-minute intervals from 7:00 to 15:30 hours, and ovulation time was determined on the assumption that it occurred within 15 min after oviposition of the previous egg in the sequence. Birds that laid eggs between 7:00 and 9:00 hours (n=12) were randomly divided into two equal groups. Control chickens received a single injection of 0.9% NaCl (with 0.1% dimethyl sulfoxide as a solvent for TCDD), while the experimental birds were intramuscularly injected with TCDD at a dose of 1 μg/kg body weight. 24 h after TCDD administration, pullets were slaughtered and their oviducts were dissected into four segments: infundibulum, isthmus, magnum, and shell gland (uterus). Sections were sampled from each segment and homogenized in liquid nitrogen and then in phosphate buffer at pH 7.2. The so prepared homogenate was analysed for the concentrations of P4, T and E2 by radioimmunoassay using commercial diagnostic kits (E2-RIA-CT, P4-RIA-CT and TESTO-RIA-CT, DIAsource ImmunoAssays S.A., Belgium).

The results were statistically analysed with two-way analysis of variance (ANOVA). Significant differences between the means were tested with Tukey's post-hoc test at $P<0.05$. The results were shown as means ±S.E.M.

Results

Under control conditions, the highest concentration of P4 (427.7±21.8 pg/g tissue) was found in the magnum; it was 2.2-fold higher than in the isthmus ($P<0.01$; Fig. 1). Compared to the control birds, TCDD injection caused a significant increase in P4 concentration in the studied parts of the oviduct (Fig. 1; $P<0.05$–0.01). The greatest difference was observed in the infundibulum, where the concentration of this steroid in the experimental group was 49% higher than in the control group ($P<0.01$).

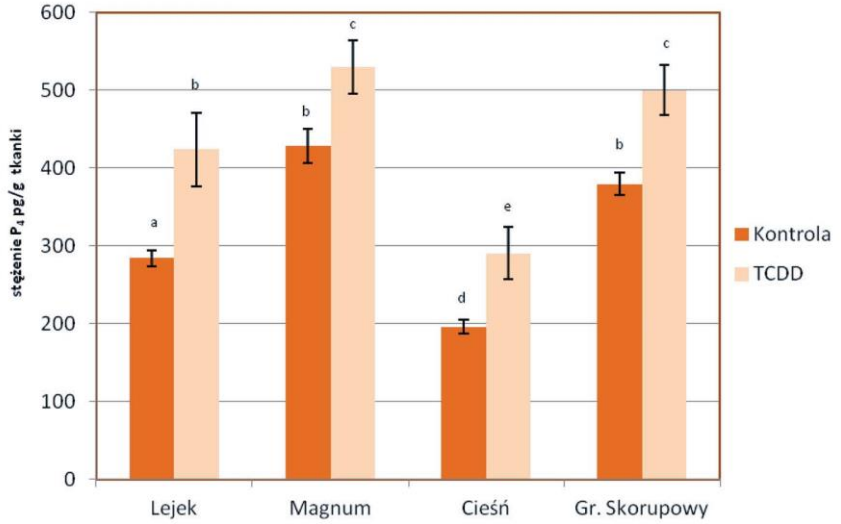

Fig. 1. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on progesterone (P4) concentration in hen oviduct (means ± S.E.M.; n=6); a, b, c, d – means with different letters are significantly different at P<0.01–0.05

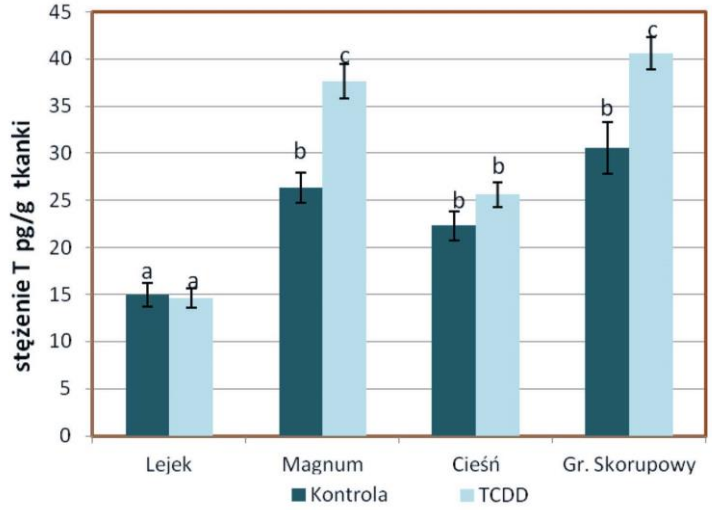

Fig. 2. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on testosterone (T) concentration in hen oviduct (means ± S.E.M.; n=6); a, b, c – means with different letters are significantly different at P<0.01–0.05

T concentration in the oviduct segments of control hens and those exposed to TCDD is presented in Figure 2. In the control group, T concentration was highest (30.5±2.7 pg/g tissue) in the shell gland; it was 2-fold higher than the lowest level noted in the infundibulum (P<0.01). TCDD injection caused T concentration to increase in the magnum and shell gland by 42% and 33%, respectively, when compared to the control group values (P<0.05). No significant changes in T concentration in the infundibulum and isthmus in response to TCDD were observed (Fig. 2).

In the control group, the highest concentration of E2 (42.6±2.9 pg/g tissue) was noted in the magnum. It was 2.5-fold higher than in the isthmus (P<0.01; Fig. 3). TCDD significantly increased the level of the analysed steroid in all the oviduct segments under study. The greatest effect was noted in the shell gland, in which TCDD injection increased E2 concentration by 56% in comparison with the control group (P<0.01; Fig.3).

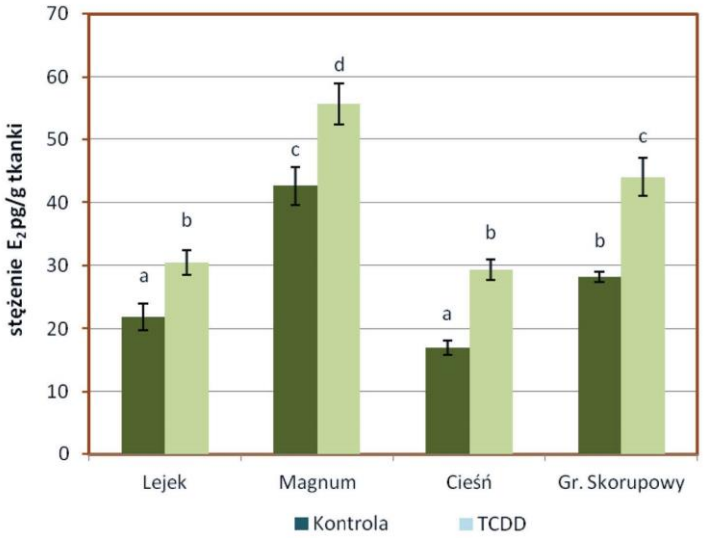

Fig. 3. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on estradiol (E2) concentration in hen oviduct (means ± S.E.M.; n=6); a, b, c, d – means with different letters are significantly different at P<0.01–0.05

Discussion

Under control conditions, the highest concentration of the examined steroids was found in the magnum and shell gland. These results are consistent with earlier data for P4, T and E2 concentrations in oviductal segments of laying hens (Socha et al., 2017). The high level of steroids (in particular P4 and E2) in the magnum and shell gland is associated with their effect on mRNA expression, and on synthesis of egg albumen proteins (including ovalbumin and avidin) and proteins of the eggshell matrix (including ovocalyxin 36 and ovocleidin 116) (Hincke et al., 1999; Nys et al., 2001; Gautron et al., 2011; Socha et al., 2017; Socha and Hrabia, 2018).

TCDD injection had no effect on the total oviduct weight and the weight of oviductal segments, which was probably due to the too short exposure to this dioxin. On the other hand, it caused a significant increase in P4 and E2 concentrations in all the oviductal segments compared to the control group values. There were no statistically significant changes in T concentration in the infundibulum and isthmus of the hens exposed to TCDD, but the concentration of this hormone showed a significant increase in the magnum and shell gland. The present authors suggest that the observed changes in P4, T and E2 concentrations in hens injected with TCDD are associated with stimulation of the uptake of the examined steroids from the blood and/or their synthesis *in situ* in the oviduct tissues. The current literature contains no data concerning steroidogenesis in the avian oviduct. However, given that mRNA expression of AhR takes place in different segments of the hen's oviduct and TCDD injection reduces the concentration of sex steroids in hen's blood (unpublished data), it cannot be excluded that steroid hormones are synthesized and secreted in the segments of the hen's oviduct. This hypothesis should be rechecked in further research.

The observed increase in P4 and E2 concentrations in the examined oviduct segments and of T in the magnum (the longest part of the oviduct characterized by high metabolic activity) and in the shell gland (in which the eggshell is formed), following TCDD injection, suggests that dioxins may

indirectly affect the function of these oviductal segments. Because TCDD molecule is similar in structure to steroid hormones, the main activity sites of this dioxin are the gonads, uterus and other organs in which steroids are synthesized (Całkosiński et al., 2005). Mika et al. (1992) demonstrated the presence of estrogen receptor (ER) and progesterone receptor (PR) proteins in four segments of the oviduct: the infundibulum, magnum, isthmus and shell gland, while Hrabia et al. (2013) and Socha et al. (2017) revealed mRNA expression of ER, PR and androgen receptors (AR) in the magnum and shell gland. Research results published to date show that molecular interactions may occur between AhR and steroid hormone receptors. (Pocar et al., 2005; Ohtake et al., 2008). Accordingly, it cannot be ruled out that TCDD may affect the function of different oviductal segments (including protein synthesis) by blocking the binding of steroid hormones with corresponding receptor. This may result in the observed increase in the level of steroid hormones in different segments of the oviduct following injection of the studied dioxin.

References

Antos P.A., Błachuta M., Hrabia A., Grzegorzewska A.K., Sechman A. (2015). Expression of aryl hydrocarbon receptor 1 (AHR1), AHR1 nuclear translocator 1 (ARNT1) and CYP1 family monooxygenase mRNAs and their activity in chicken ovarian follicles following *in vitro* exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Lett., 237 (2): 100–111.

Baba T.,Mimura J.,Nakamura N.,Harada N.,Yamamoto M.,Morohasahi K.,Fu-j i i -K u r i y a m a Y. (2005). Intrinsic function of aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. Mol. Cell Biol., 25: 10040 –10051.

Barnett K.R.,Tomic D.,Gupta R.K.,Babus J.K.,Roby K.F.,Terranova P.F.,Flaws J.A. (2007). The aryl hydrocarbon receptor is required for normal gonadotropin responsiveness in the mouse ovary. Toxicol. Appl. Pharmacol., 223: 66–72.

Beischlag T.V.,Luis Morales J.,Hollingshead B.D.,Perdew G.H.(2008).Thearylhy-drocarbon receptor complex and the control of gene expression. Crit. Rev. Eukaryot. Gene Express., 18: 207–250.

Całkosiński I.,Stańda M.,Borodulin-Nadzieja L.,Wasilewska U.,Majda J.,Cegielski M., Dzięgiel P., Woźniak W. (2005). Wpływ 2,3,7,8-tetrachlorodibenzo-p-dioksyny (TCDD) na strukturę narządów miąższowych oraz na stężenie cholesterolu i estradiolu u szczurów. Adv. Clin. Exp., 4, 2: 211–215.

Dougherty D.C., Sanders M.M. (2005). Estrogen action: revitalization of the chick oviduct model. Trends Endocrinol. Metab., 16: 414–419.

Gautron J.,Rehault-Godbert S.,Pascal G.,Nys Y.,Hincke M.T.(2011).Ovocalyxin-36 and other LBP/BPI/PLUNC-like proteins as molecular actors of the mechanisms of the avian egg natural defences. Biochem. Soc. Trans., 39: 971–976

Giesy J.P., Feyk L.A., Jones P.D., Kannan K., Sanderson T. (2003). Review

of the effects of endocrine-disrupting chemicals in birds. Pure Appl. Chem., 75: 2287–2303.

Gregoraszczuk E.L.,Wójtowicz A.K.Zabielany E.,Grochowalski A.(2000).Dose and time dependent effect of 2,3,7,8-tetrachlorodibenzeno-p-dioxin (TCDD) on progesterone secre-tion by porcine luteal cells cultured *in vitro*. J. Physiol. Pharmacol., 51: 127–135.

Grochowalski A. (2002). Badania nad oznaczaniem dioksyn w środowisku. Normalizacja, 4: 3–9.

Gu Y.Z., Hogenesch J.B., Bradfield C.A. (2000). The PAS superfamily: sensors of environmental and developmental signals. Annu. Rev. Pharamacol. Toxicol., 40: 519–561.

Hincke M.T., Gautron J., Tsang C.P., McKee M.D., Nys Y. (1999). Molecular cloning and ultrastructural localization of the core protein of an eggshell matrix proteoglycan, ovocleidin-116. J. Biol. Chem., 274 (46): 32915–32923.

Hrabia A., Leśniak A., Sechman A. (2013). In vitro effects of TCDD, PCB126, PCB153 on estrogen receptors, caspases, and metalloproteinase-2 mRNA expression in the chicken shell gland. Folia Biol. (Krakow), 61: 277–282.

Jabłońska O., Piasecka J., Peroff B.K., Nynca A., Siawrys G., Wąsowska B., Żmijewska A.,Lewczuk B.,Ciereszko R.E.(2011). *In vitro* effects of 2,3,7,8-tetrachlo-rodibenzeno-p-dioxin (TCDD) on ovarian pituitary and pineal function in pigs. Theriogenology, 76: 921–932.

Joensuu T.K., Tuohimaa P., Vilja P. (1991). Avidin and ovalbumin induction by progesterone in chicken oviduct detected by sensitive immunoenzymometric assays. J. Endocrinol., 130: 191–197.

Lee J.S., Eun-Young K., Iwabuchi K., Iwata H. (2011). Molecular and functional characterization of aryl hydrocarbon receptor nuclear translocator 1 (ARNT1) and ARNT2 in chicken (*Gallus gallus*). Comp. Biochem. Physiol. C Toxicol. Pharmacol., 153: 269–279.

Makles Z., Świątkowski A., Grybowska S. (2001). Niebezpieczne dioksyny. Wyd. Arkady, Warszawa, 9: 31–35.

Mika M., Rząsa J., Ewy Z. (1987). Interaction of progesterone, estradiol and testosterone in the regulation of growth and development of the chick oviduct. Folia Biologica, 35: 85–94.

Mika M., Rząsa J., Wolińska-Witort E. (1992). Presence of estrogen and progesterone receptors in the oviduct of the domestic hens. VIIth International Symposium of Young Poultry Science, Warszawa, 1: 36–38.

Mimura J., Fujii -Kuriyama Y. (2003). Functional role of AhR in the expression of toxic effects by TCDD. Biochem. Biphys. Acta, 1619: 263–268.

Nys Y., Gautron J., McKee M.D., Garcia-Ruiz J.M., Hincke M.T. (2001). Biochemical and functional characterisation of eggshell matrix proteins in hens. World Poult. Sci. J., 57: 401–413.

Ohtake F., Baba A., Fujii-Kuriyama Y., Kato S. (2008). Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. Biochem. Biophys. Res. Commun., 370: 541–546.

Palmiter R.D. (1971). Interaction of estrogen, progesterone and testosterone in the regulation of protein synthesis in chick oviduct.

Biochemistry, 10: 4399–4403.
Petroff B.K., Roby K.F., Gao X., Son D., Williams S., Johnson D., Rozman K.K., T e r r a n o v a P.F. (2002). A review of mechanism controlling ovulation with implications for the anovulatory effects of polychlorinated dibenzo-p-dioxins in rodents. Toxicology, 158: 91–107.
Pocar P.,Fischer B.,Klonisch T.,Hombach-Klonischm S.(2005).Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. Reproduction, 129: 379–389.
Rząsa J. (2007). Regulacja rozrodu ptaków. W: Biologia rozrodu zwierząt. Fizjologiczna regulacja procesów rozrodczych samicy. Krzymowski T. (red.). Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego w Olsztynie, ss. 555–577.
Schimke R.T., McKnight G.S., Shapiro D.J., Sullivan D., Palacios R. (1975). Hormonal regulation of ovoalbumin synthesis in the oviduct. Recent Prog. Horm. Res., 31: 175–211.
Sechman A.,Antos P.,Katarzyńska D.,Grzegorzewska A.,Wojtysiak D.,Hra-b i a A. (2014). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on secretion of steroids and STAR, HS-D3B and CYP19A1 mRNA expression in chicken ovarian follicles. Toxicol. Lett. 225 (2): 264–274.
Socha J.K., Hrabia A. (2018). Alterations in apoptotic markers and egg-specific protein gene expression in the chicken oviduct during pause in laying induced by tamoxifen. Theriogenology, 105: 126–134.
Socha J.K., Sechman A., Mika M., Hrabia A. (2017). Effect of growth hormone on steroid concentrations and mRNA expression of their receptor, and selected egg-specific protein genes in the chicken oviduct during pause in laying induced by fasting. Domest. Anim. Endocrinol., 61: 1–10.
Walisser J.A., Bunger M.K.,Glover F.,Harstand E,B.,Bradfield C.A.(2004).Patent ductus venosus and dioxin resistance in mice harboring a hypomorphic Arnt allele. J. Biol. Chem., 279: 16326–16331.
Wan C., Liu J., Nie X., Zhao J., Zhou S., Duan Z., Tang C., Liang L., Xu G. (2014). 2,3,7,8-Tetrachlorodibenzo-P-dioxin (TCDD) induces premature senescence in human and rodent neuronal cells via ROS-dependent mechanisms. US National Library of Medicine National Institutes of Health, 25: 327–341.
Yang J., An J., Li M., Hou X., Qiu X. (2013). Characterization of chicken cytochrome P450 1A4 and 1A5: inter-paralog comparisons of substrate preference and inhibitor selectivity. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 157: 337–343.
Yasui T., Kim E.Y., Iwata H., Tanabe S. (2004). Identification of aryl hydrocarbonreceptor 2 in aquatic birds: cDNA cloning of AHR1 and AHR2 and characteristics of their amino acid sequences. Mar. Environ. Res., 58: 113–118.
Yonemoto Y. (2000). The effects of dioxin on reproduction and development. Industrial Health, 38: 259–268.
Yu J.Y.-L., Marquardt R.R. (1973). Development, cellular growth and function of the avian oviduct. Biol. Reprod., 8: 283–298.

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SUMMARY

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