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# Dichlorodiphenyldichloroethylene (DDE) residue limit exceeded in pig tissues after feed-borne exposure to maximum allowed concentration

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## ABSTRACT

Monitoring of undesirable substances by the European Union indicates a presence of natural and anthropogenic pollutants in animal feed that may be of concern for the producers, as well as the veterinary services. Although the literature concerning toxicity of DDT (an insecticide widely used in the past) is extensive, less attention has been focused on the biological properties of DDE and its interactions with other contaminants. This study reports on the concentration profile of *p,p′*-DDE and two other ogranochlorines (*p,p′*-DDT, *p,p′*-DDD) in different tissues of immature gilts after 14, 28, and 42 days of oral exposure to *p,p′*-DDE alone (0.5mg·kg$^{-1}$feed·day$^{-1}$) and in mixture with naturally occurring mycotoxin zearalenone, ZEN (0.5+0.1mg·kg$^{-1}$feed·day$^{-1}$). The treatment resulted in a time-dependent accumulation of *p,p′*-DDE in fat-rich tissues. Although the pesticide's concentration found in the adipose tissue exceeded the FAO/WHO maximum residue limit (5mg·kg$^{-1}$ of fat), human dietary risk is little, as it requires a substantial consumption of such contaminated pork fat. Importantly, the high concentration of *p,p′*-DDE found in the adrenal glands suggests a threat to the animals' health. Finally, a difference in the accumulation of *p,p′*-DDE was observed between the groups treated with this compound alone or in mixture with ZEN. This is most likely related to growth performance of the animals, altered by the endocrine disrupting activity of both compounds. Further research should evaluate the effects of *p,p′*-DDE at the observed concentrations on the pigs' health status and enable the studies of possible interactions with other environmental pollutants.

## INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) is an organochlorine pesticide, used worldwide in the past to control insect vectors of infectious diseases (Rogan and Chen 2005). DDT was also used as an insecticide to protect crops, including Poland, before it was banned in most countries in the 1970's for its negative effects on humans, wildlife, and the environment. Despite the ban, this insecticide is still used as a cost-effective method to prevent malaria in some tropical regions (Bettinetti et al. 2011). DDT is a highly persistent pollutant. In the environment, it may last for many years, as it is slowly biodegraded to DDE (dichlorodiphenyltrichloroethylene) and DDD (dichlorodiphenyldichloroethane) in processes generally driven by the action of microorganisms in the soil (ATSDR 2002). Common and intensive use of DDT has resulted in worldwide pollution with this compound. It has been found in organisms living in deserts as well as in the depths of oceans (Turusov et al. 2002). Since the times of DDT extensive use, its residual levels in the environment have greatly declined.

However, due to its persistence, the pesticide will be present at low concentrations for decades (Glynn et al. 2009; NTP 2011). Current human exposure to DDT and its metabolites is known to occur mainly through dietary intake, particularly consumption of contaminated fish or meat (ATSDR 2002; NTP 2011).

Technical grade DDT is a mixture of different isomers: approximately 85% of the *p,p*-DDT isomer with *o,p*'-DDT and *o,o*'-DDT present in lesser amounts (ATSDR 2002). In mammals, the *p,p*'-DDT isomer is metabolized mainly to *p,p*'-DDE and *p,p*'-DDD by the microsomal cytochrome P450 system (Kitamura et al. 2002). Due to their high lipophilic properties (*p,p*'-DDD<*p,p*'-DDT<*p,p*'-DDE), these compounds are readily distributed to the body once absorbed and stored in the tissues in proportion to the tissues' lipid content; and they leave the body very slowly. This bioaccumulation leads to increasing concentrations of the compounds at higher trophic levels (ASTDR 2002).

The literature concerning the biological properties of DDT and its metabolites is extensive, and considerable attention has focused on their adverse effects on the development and function of the reproductive system of animals. It has been shown that these compounds have the ability to modulate endocrine function and influence gene expression after binding to nuclear receptors. For example, unlike *p,p*'-DDT, an environmental estrogen which binds the estrogen receptor and induces estrogenic effects, *p,p*'-DDE has been shown to be a weak estrogen receptor agonist but a potent androgen receptor antagonist (Kelce et al. 1995). In a study on fetal rats, exposure to *p,p*'-DDE led to reduced anogenital distance at birth and retained thoracic nipples on postnatal day, which are indicative of antiandrogen activity (Kelce et al. 1995).

Poland is an important producer of pork in the European Union. Aside from cattle, the domesticated pig (*Sus scrofa*) is the most popular species in Polish production of farm livestock (CSO 2011). Health problems related to reproductive system dysfunction of unknown etiology are generally considered an important factor leading to increased costs of animal production. It is believed that the alimentary route of exposure to various endocrine disrupting compounds (EDCs), both natural (e.g. mycotoxins) and/or of anthropogenic origin (e.g. pesticides), may be responsible for occurrence of the disorders in the reproductive organs. Moreover, gilts are particularly susceptible to the hormone mimicking action of EDCs (Jakimiuk et al. 2009). In legislation, these animals are often considered as sentinel species with the lowest limits for undesirable substances in feed (e.g. PMARD 2012).

Monitoring of undesirable substances in animal feed materials indicates a common presence of pollutants, including DDT and its metabolites (Nag and Raikwar 2011). As a result of the ban on meat and bone meal in feed production, fish meal has been introduced as a source of protein and fat for animals (Weiner et al. 2012). However, fish meal may be a potential source of persistent, lipophilic contaminants. In this context, control of the pesticides' residues at safe levels may constitute an issue for feed producers as well as veterinary services. Since contaminants rarely occur as single compounds, there is a need to investigate possible interactions of different compounds and understand their combined effects.

Although *p,p*'-DDE is among the most frequently studied DDT metabolites and commonly detected isomers (ASTDR 2002), no research by now has been published describing the toxicokinetics of this compound in porcine tissues. Zearalenone (ZEN) is a mycotoxin commonly found in animal feed or feed material of plant origin. This natural contaminant is recognized as an environmental estrogen with associated hormone mimicking effects that disrupts the reproductive system of livestock, especially gilts (Jakimiuk et al. 2009). Current knowledge on the effects of the two pollutants in pigs and their possible interactions is poor.

The present study reports on the concentration profile of *p,p*'-DDE and two other organochlorine compounds (*p,p*'-DDT, *p,p*'-DDD) in different tissues of immature gilts after 14, 28, and 42 days of oral exposure to *p,p*'-DDE alone and combined with ZEN at the maximum doses allowed by current legislation. The measurements were taken to examine the distribution of organochlorine pesticides between different porcine tissues and to evaluate DDE's accumulation potential and its related human and animal health risks.

## MATERIALS AND METHODS

The study was performed on female pigs (Polish Large White breed) aged 8 weeks (mean body mass 19±2kg) which were obtained from a commercial fattening farm in Bałdy (Poland). The animals were housed and handled in accordance with resolution No. 24/2009 of the Local Ethics Committee. The gilts were housed in pens for 1 week to allow them to adapt to their new environment. During the acclimation and the further exposure period, all pigs were fed with "blank feed" that was tested for the presence of background contamination, and found to be free of mycotoxins (aflatoxin, ochratoxin, ZEN, α-zearalenol, and deoxynivalenol) and organochlorine pesticides (*p,p*'-DDT, *p,p*'-DDE, and *p,p*'-DDD). Throughout the study, the animals were maintained indoors and had constant access to water.

Doses of DDE and ZEN were chosen based on the current legislation limits. According to the Polish regulation (PMARD 2012), the maximum content of any combination of DDT, DDD, and DDE (expressed as ΣDDT) in all feed materials should not exceed 0.05mg·kg$^{-1}$, except fats and oils for which the limit is 0.5mg·kg$^{-1}$. Since fats and oils are also used in feed production, the dose of *p,p*'-DDE at 0.5mg·kg$^{-1}$ was chosen as the theoretically maximum allowable concentration of this compound in animal feeding. As for the guidance value for ZEN, its concentration in complementary

and complete feeding stuffs intended for piglets and gilts should not exceed 0.1mg·kg$^{-1}$ (EC 2006), thus this value was selected for the exposure.

Prior to oral exposure, the gilts were divided into 3 groups: an untreated (control) group (n=9) and 2 treatment groups (n=9 each) that were exposed to i) *p,p*'-DDE at a dose of 0.5mg·kg$^{-1}$feed·day$^{-1}$ (group DDE) or ii) exposed to *p,p*'-DDE together with ZEN at a dose of 0.5+0.1mg·kg$^{-1}$feed·day$^{-1}$ (group DDE+ZEN). Analytical samples of *p,p*'-DDE (#123897; Sigma-Aldrich) and ZEN (#Z2125; Sigma-Aldrich) were administered to the treated groups daily per os with their first (morning) feeding. After 14, 28, and 42 days of the experiment, the gilts (n=3 from each group at respective time-point) were randomly selected from their pens, weighted, then anesthetized with sodium pentobarbital (Biowet, Poland) and exsanguinated. Immediately after cardiac arrest, the animals were decapitated and the hypothalamus with the pituitary gland was excised; then fragments of the adrenal gland, the uterus (from the uterine horns), the ovary, the duodenum, the liver, and adipose tissue (the fatback) were collected. All samples were stored at -20°C for further analysis. Sample collection was always performed before the pigs' first morning feeding (after fasting for 12h).

Organochlorine pesticides (*p,p*'-DDE, *p,p*'-DDD, and *p,p*'-DDT) were extracted from the tissue samples using a hexane and acetone mixture with tissues' fat. The extracts were cleaned with sulphuric acid. The purified extracts were analysed by capillary gas chromatography with electron capture detection (GLC-ECD), according to the procedure described below. The concentrations of the organochlorine pesticides were expressed in $\mu$g·kg$^{-1}$.

The analyses were performed with an Agilent Technologies chromatograph, model 6890 Plus equipped with 7683B series autosampler, a split-splitless injector in pulsed splitless mode, and a 63Ni-EC detector. Chromatographic separation was performed in an HP-5MS capillary column (60m×0.25mm ID×0.25$\mu$m film thickness, J&W Scientific, USA). The following oven temperature programme was used: initial temperature of 60°C for 2min, increased to 180°C at 40°C·min$^{-1}$ and held for 2min, increased to 245°C at 2°C·min$^{-1}$ and held 2min, increased to 265°C at 4°C·min$^{-1}$ and held for 15min, and then increased to 290°C at 8°C·min$^{-1}$ and held for 20min. A secondary capillary column DB 1701 (60m×0.25mm ID×0.25$\mu$m film thickness, J&W Scientific, USA) was used for confirmation. The injector and the detector were operated at 285°C and 325°C, respectively. The GC was controlled by ChemStation software (Agilent Technologies).

The analyses were carried out using a validated method in an accredited laboratory. The validation was performed in accordance with SANCO/12495/2011 (Method validation and quality control procedures for pesticide residues analysis in food and feed). Validation tests demonstrated that the analytical method fulfilled the method performance acceptability criteria (recoveries from fortified samples ranging from 88% to 102%, coefficients of variation (CV) were <12% for repeatability and <15% for reproducibility, limit of quantification (LOQ) for each analyte was 0.5$\mu$g·kg$^{-1}$). The laboratory has regularly and successfully participated in international proficiency testing organized by FAPAS (Food Analysis Performance Assessment Schemes, UK) and the European Union Reference Laboratory (CVUA Freiburg). In addition, the certified reference material was analysed (CRM 430 - Organochlorine pesticides in pork fat).

The analytical method used had technical limitations related to the minimal mass of a sample required for the analysis. Due to a number of other analyses, we were also limited by the size of smaller organs. We were therefore unable to obtain data in multiple replicates. Organ or tissue samples of the hypothalamus with pituitary gland, the uterus, the duodenum, the liver, and the adipose tissue obtained from the 3 individuals of each experimental group were pooled together (homogenized) and analyzed singly according to the treatment (DDE, DDE+ZEN) and its respective exposure period (n=3 → n=1; Table 1). In the case of samples with smaller sizes, i.e. the adrenal gland and the ovary, the tissues collected from all exposure periods (14+28+42d) were pooled together within each experimental group and analyzed in single (n=9 → n=1; Figure 1). The same pooling method was used for tissues of control pigs (Table 1), as we did not expect high concentrations of the residues in these samples.

In order to deal with the small population sizes and the resulting heterogeneous data distribution, non-parametric tests were used for statistical analysis. To test the significance of differences between the pooled concentrations of *p,p*'-DDE in the control (*Placebo*) and treated groups of pigs (DDE or DDE+ZEN), the Mann-Whitney U test was used. The same test was used to assess differences in mass of pigs between experimental groups in each exposure period. In addition, the Wilcoxon test was used for paired comparisons of data from different exposure periods to assess the treatment- (i.e. DDE vs. DDE+ZEN) and time-dependent differences (e.g. 14d vs. 42d). Results of all statistical tests were considered to be significant at a level of $P<0.05$, except the comparisons of pigs' body masses. In this case, $P=0.081$ was considered to be significant as it is the minimum value that can be obtained with the Mann-Whitney U test with groups of n=3.

## RESULTS AND DISCUSSION

Despite the constantly declining concentration of DDT in different areas of the natural environment, organochlorine pesticides are still gaining considerable attention, mainly due to their harmful effects on reproduction and possible carcinogenic activity (ATSDR 2002). In this paper, we report on the concentration profile of *p,p*'-DDE in different organs and tissues of immature gilts after 14, 28, and 42 days of oral exposure to this compound, alone or in mixture with ZEN. To our knowledge, this is the first study documenting the treatment of pigs with *p,p*'-DDE in a time course experiment and showing possible interaction between the two compounds.

**Table 1. Concentrations ($\mu g \cdot kg^{-1}$) of organochlorine pesticides residues in different tissues of immature gilts after 14, 28, and 42 days of oral exposure to *p,p'*-DDE ($0.5 mg \cdot kg^{-1} \cdot day^{-1}$) and ZEN+*p,p'*-DDE ($0.1+0.5 mg \cdot kg^{-1} \cdot day^{-1}$), compared to control group (*Placebo*).**

| Organ or tissue | Residue | Experimental groups and exposure periods | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control *(Placebo)* | *p,p'*-DDE ($0.5 mg \cdot kg^{-1} feed \cdot day^{-1}$) | | | ZEN+*p,p'*-DDE ($0.1+0.5 mg \cdot kg^{-1} feed \cdot day^{-1}$) | | |
| | | Pool* (14+28+42 d) | 14 d | 28 d | 42 d | 14 d | 28 d | 42 d |
| **Hypothalamus, pituitary gland** | *p,p'*-DDT | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | ***p,p'*-DDE** | **0.7** | **38.6** | **49.1** | **59.0** | **46.1** | **72.0** | **51.2** |
| | *p,p'*-DDD | <LOQ | <LOQ | <LOQ | <LOQ | < LOQ | <LOQ | <LOQ |
| **Uterus** | *p,p'*-DDT | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | ***p,p'*-DDE** | **6.4** | **6.6** | **9.5** | **10.0** | **5.5** | **9.6** | **9.0** |
| | *p,p'*-DDD | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| **Duodenum** | *p,p'*-DDT | 1.4 | 2.1 | 1.4 | 1.3 | 1.8 | 1.7 | 2.0 |
| | ***p,p'*-DDE** | **1.2** | **94.4** | **65.7** | **131.6** | **69.7** | **93.6** | **162.5** |
| | *p,p'*-DDD | <LOQ | 0.8 | <LOQ | 0.8 | <LOQ | <LOQ | <LOQ |
| **Liver** | *p,p'*-DDT | 1.0 | 1.4 | 0.9 | 0.9 | 1.2 | 1.1 | 1.0 |
| | ***p,p'*-DDE** | **3.3** | **68.5** | **83.1** | **92.2** | **70.0** | **95.2** | **87.8** |
| | *p,p'*-DDD | <LOQ | 1.1 | <LOQ | 1.0 | <LOQ | 1.0 | <LOQ |
| **Adipose tissue** | *p,p'*-DDT | 82.3 | 101.1 | 50.5 | 46.2 | 62.0 | 43.0 | 43.6 |
| | ***p,p'*-DDE** | **116.0** | **3510.0** | **4954.0** | **5480.0** | **3294.0** | **5187.0** | **5382.0** |
| | *p,p'*-DDD | 18.4 | 23.6 | 23.9 | 23.3 | 20.7 | 27.5 | 22.7 |

* – value obtained from tissues collected after 14, 28, 42 days of exposure and pooled together for the concentration analysis (see: Material and Methods for details); LOQ – level of quantification ($0.5 \mu g \cdot kg^{-1}$).

## Highest concentration of DDE found in adipose tissue

In the present study, *p,p'*-DDE was found in all of the analyzed organs, with the concentrations in adipose tissue at least an order of magnitude higher than in the rest of organs (Table 1). The concentration of *p,p'*-DDE residue in tissues pooled from the three sampling times was significantly higher in the DDE- or DDE+ZEN-treated groups of pigs than in the control ($P=0.015$; Figure 1). However, no difference was observed between the two treatment groups ($P>0.05$; Figure 1). In the case of *p,p'*-DDT and *p,p'*-DDD, the concentrations of these compounds were much lower (<LOQ for most tissues; data not shown) with no significant differences between control and treated groups of pigs ($P>0.05$). The obtained results confirm the tendency of DDE to accumulate in adipose tissue, which is related to the compound's high solubility in lipids (ATSDR 2002).

Organochlorine pesticides are known to be distributed in fatty tissues of wild animals worldwide (Turusov et al. 2002). Likewise, it has been shown that the concentration of these compounds in human adipose tissue may be a hundred times greater than in breast milk or serum (Arrebola et al. 2012). Since it is known that highly lipophilic, persistent chemicals are almost entirely sequestered in the lipid fraction of a tissue, adipose tissue is often used as material-of-choice for assessment of DDT or DDE contamination.

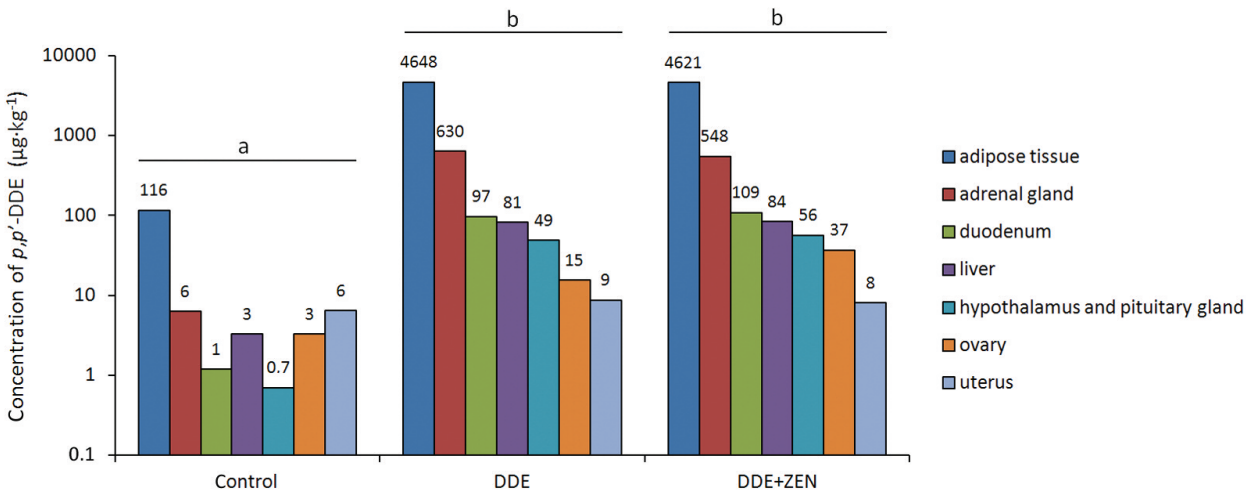


**Figure 1. Concentrations of *p,p'*-DDE ($\mu g \cdot kg^{-1}$) in different tissues of immature gilts after oral exposure to *p,p'*-DDE alone ($0.5 mg \cdot kg^{-1} \cdot day^{-1}$) and *p,p'*-DDE together with ZEN ($0.5+0.1 mg \cdot kg^{-1} \cdot day^{-1}$), compared to control group (*Placebo*; uncontaminated feed). The bars indicate values obtained from samples collected after 14, 28, 42 days of exposure and pooled together for the concentration analysis. The values for tissues were averaged from the data of Table 1, except for the adrenal gland and the ovary which were analyzed in pooled homogenates by GLC-ECD (see Materials and Methods section). Different letters denote significant differences between experimental groups ($P<0.05$; Mann-Whitney U test).**

## Human health risks of food products from DDE-exposed pigs

Raw pork fatback and lard are commonly used in traditional Polish cuisine, but these products are also popular in other countries. Information on Polish consumption of these products is limited, with only general estimates available for production of pork or animal fat (CSO 2011). According to FAO/WHO food standards, the maximum residue limit (MRL) for any combination of DDT, DDD, and DDE (ΣDDT) in (non-marine) mammalian meat is $5 mg \cdot kg^{-1}$ of fat (FAO/WHO 2001).

In the present study, *p,p'*-DDE concentrations in the adipose tissue (the fatback) of pigs treated with feed contaminated with this compound (at a dose of $0.5 mg \cdot kg^{-1} feed \cdot day^{-1}$) were found to range from 3.5 to $5.4 mg \cdot kg^{-1}$ of fat, depending on the exposure period (Table 1). This finding indicates that feeding gilts for 42 days with feed containing the maximum legal concentration of DDE results in an accumulation of this compound at a level exceeding the MRL. However, with regards to the provisional tolerable daily intake (PTDI), established at $0.01 mg \cdot kg^{-1} \cdot day^{-1}$ (FAO/WHO 2001), even without information on the pork fat consumption in Poland, we believe that the highest concentration of the pesticides found in our study would contribute only marginally to human exposure. Pork fat contaminated at the level found in our study is unlikely to pose a risk unless it was consumed continuously in substantial amounts. For example, to achieve a peak of acute dietary intake above the PTDI level, a person of 70kg body mass should eat daily at least 130g of pork lard that has been contaminated with DDE within the range that has been found in our study.

## Potential effects of DDE presence in endocrine organs and tissues

In addition, *p,p'*-DDE was also found in the gilts adrenal gland at high concentrations (Figure 1). The high level of DDE accumulation may be easily explained by the organ's high blood supply and its lipid-rich cortical tissue. However, since the adrenal glands are engaged in the synthesis and release of hormones (such as corticoids and sex steroids), our results suggest that this organ, as a major constituent of the endocrine system, may be a possible target for organochlorine pesticides. Indeed, studies have been previously published on DDT-induced disruption of adrenal function. For example, it was determined that methylsulfonyl-DDE ($MeSO_2$-DDE, a DDT metabolite originally discovered in Baltic seals) is a potent and tissue-specific toxicant that induces vacuolation and necrosis in the glucocorticoid-forming *zona fasciculata* of mice adrenals (Lund et al. 1988). Other metabolites of DDT have long been known for their cytotoxic effect on the adrenal cortex. For example, *o,p'*-DDD has been used as an anticancer drug for treatment of adrenocortical carcinoma as well as the overproduction of glucocorticoids due to a pituitary tumour in Cushing's syndrome (Bergenstal et al. 1960).

Although the adrenocorticolytic activity of DDT's metabolites (i.e. $MeSO_2$-DDE, *o,p'*-DDD) is evident, little information is available concerning similar properties of *p,p'*-DDE. For example, Mayne et al. (2004) observed a significant negative correlation between *p,p'*-DDE and the capacity of bluebird chicks to elevate blood

corticosterone levels in response to injection of exogenous adrenocorticotropic hormone (ACTH) which suggested a disruption of corticosterone synthesis and secretion by adrenal cortical steroidogenic cells. In another study by Adamsson et al. (2009), *p,p*'-DDE-treatment caused clear histological and ultrastructural abnormalities in the adrenals of rats exposed in utero (at dose of 100mg·kg$^{-1}$ from 13.5 to 17.5 embryonic days). However, adrenal protein expression of key steroidogenic enzymes (i.e. steroid acute regulatory protein, cholesterol desmolase, and 3-beta-hydroxysteroid dehydrogenase) in DDE-treated rats remained unchanged (Adamsson et al. 2009).

The observed concentration of *p,p*'-DDE in the hypothalamus with pituitary gland in both treated groups of pigs (Figure 1) was in agreement with the literature concerning the properties of this compound to cross the blood-brain barrier (Covaci et al. 2004). The lipophilic properties and small molecular weight of these organochlorine pesticides enable them to accumulate in different structures of the nervous system (Keifer and Firestone 2007). Acute toxicity related to the neurotoxic effects of *p,p*'-DDE has been reported after exposure to doses (ATSDR 2002) much higher than in this study. However, taking into consideration the presence of *p,p*'-DDE in the hypothalamus with pituitary gland (Figure 1), one may suspect that even at a low concentration, this compound may exert endocrine disruptive effects as a result of indirect action through endocrine system feedback loops (i.e. the hypothalamic-pituitary-thyroid, hypothalamic-pituitary-adrenal, and/or hypothalamic-pituitary-gonadal axis) (Gore 2010). Whether the concentrations of *p,p*'-DDE found in the pigs' hypothalamus with pituitary gland would affect the animals' health needs to be elucidated in further studies.

Interestingly, the concentration of *p,p*'-DDE that accumulated in the pigs' ovaries differed, depending on whether they were treated with DDE alone or in a mixture with ZEN (15 vs. 37$\mu$g·kg$^{-1}$; Figure 1). This may reflect the amounts of lipophilic substrates used for production of endogenous steroids by the ovaries. However, due to the very small number of the analyzed samples and the limited results of our study, it is not possible to discuss in detail about whether the different accumulation of *p,p*'-DDE in the porcine ovary was a result of increased hormonal activity (hormonal activation) caused by the estrogenic properties of ZEN.

**Table 2. Statistical significance for relationship between the concentration of organochlorine pesticides residue and the treatment factor (i.e. treatment-dependent accumulation) in respective exposure periods.**

| Residue | Matched pairs of the same timepoints but different treatment groups (DDE vs. DDE+ZEN) | | |
|---|---|---|---|
| | 14 d vs. 14 d | 28 d vs. 28 d | 42 d vs. 42 d |
| ***p,p'*-DDT** | 0.109 | 1.000 | 1.000 |
| ***p,p'*-DDE** | 0.500 | 0.043 | 0.345 |
| ***p,p'*-DDD** | 0.109 | 0.180 | 0.109 |

The probability (*P*-values) were obtained with Wilcoxon paired comparison test based on values from Table 1.

### Plausible effects of DDE and ZEN on body mass

To assess treatment-dependent differences in the concentrations of pesticide residues, pair-wise comparisons were made with the Wilcoxon test (Table 2). No significant differences in *p,p*'-DDT, and *p,p*'-DDD concentrations were found between DDE- and DDE+ZEN-treated groups of animals ($P>0.05$). However, the body burden of *p,p*'-DDE found in the gilts' tissues after 28 days of exposure (Table 1) was significantly higher in the group exposed to a mixture of DDE and ZEN in comparison to the group treated with DDE alone ($P=0.043$; Table 2). This suggests that ZEN could affect the accumulation of DDE in the co-treated group; however, we believe that this result may be simply explained by the differences in the gilts' body masses in our experiment (Figure 2). The animals treated with DDE alone (40.6kg) were bigger than those from the control group (34.9kg; $P=0.081$), whereas the DDE+ZEN-treated group (32.7kg) were smaller than those treated with DDE alone ($P=0.081$) but not the control group ($P>0.05$; Figure 2). Taking into consideration that the same dose of DDE was taken by both treatment groups (0.5mg·kg$^{-1}$feed·day$^{-1}$), it is most likely that the lower concentration of *p,p*'-DDE residue in the DDE-treated group was just because the animals from this group were larger. In the literature this phenomenon is often referred to as growth dilution (Herendeen and Hill 2004). On the other hand, it is possible that co-treatment of pigs with ZEN could have diminished their growth and therefore caused the higher ("concentrated") *p,p*'-DDE body burden found in the tissues of the DDE+ZEN group. We cannot fully exclude other, currently unknown possibilities, i.e. involving direct influence of ZEN on DDE uptake and storage in fat deposits, or detoxification mechanisms; however, we believe that the growth dilution hypothesis is the simplest and the most elegant way to explain the differences observed after 28 days of exposure.

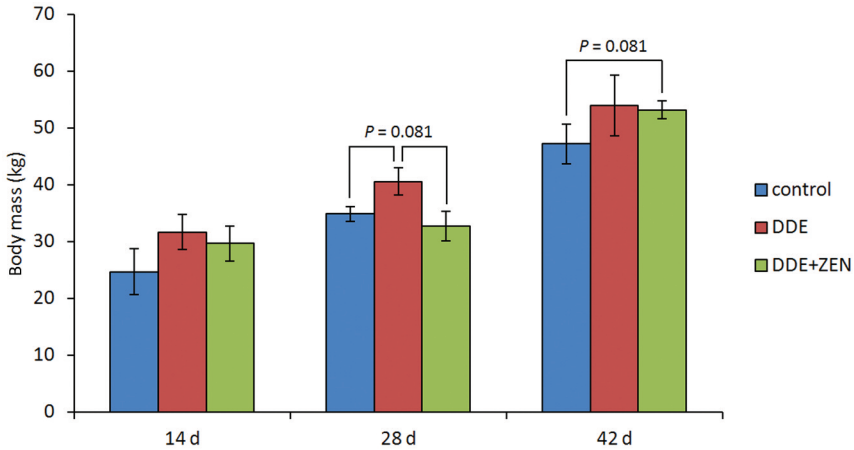


**Figure 2. Body mass of experimental individuals used in this study. Bars represent mean (n=3) total mass of the gilts after 14, 28, or 42 days of oral exposure to *p,p'*-DDE alone (0.5mg·kg$^{-1}$·day$^{-1}$) and *p,p'*-DDE together with ZEN (0.5+0.1mg·kg$^{-1}$·day$^{-1}$), compared to control group (*Placebo*; uncontaminated feed). Bars connected with lines indicate a minimal value for statistical significance of a difference (expressed as the *P*-value) that can be assessed with Mann-Whitney U test by comparison of two independent groups of data with n=3 count.**

It is known that sex steroids are involved in metabolism, accumulation and distribution of adipose tissues, however this regulation remains yet to be explained in detail (Mayes and Watson 2004). For example, men with a decreased level of circulating testosterone experience an increase in body mass (Gould et al. 2007) and women with complete androgen insensitivity syndrome have an increase of body fat (Dati et al. 2009). Likewise, lack of estrogen in ovarioctemized rats leads to obesity, and estrogen replacement in these animals abrogates the changes triggered by ovariectomy (reviewed in Mayes and Watson 2004). *p,p'*-DDE has been shown to be a weak estrogen receptor agonist but a potent androgen receptor antagonist, as it inhibited androgen binding to the androgen receptor and androgen-induced transcriptional activity (Kelce et al. 1995). In the same study, treatment of weanling male rats with *p,p'*-DDE resulted in inhibition of androgen action (observed as delayed onset of puberty) and concomitant increase of animal growth (Kelce et al. 1995). Therefore, it is plausible to speculate that the anti-androgenic properties of *p,p'*-DDE could be responsible for the higher growth performance of the pigs treated with DDE alone in our study. In addition, in terms of the well-known estrogenic properties of ZEN, it is also reasonable to think that this mycotoxin diminished the influence of DDE on the pigs' body mass (i.e. prevented DDE-induced lipid accumulation) in a similar way, as natural estrogens affect lipid deposition (Mayes and Watson 2004). Although our results do not warrant lengthy discussion in this manner, studies on the influence of both compounds on body mass are worthy of consideration in further experiments, involving larger experimental groups (i.e. n>3).

**Table 3. Statistical significance for relationship between the concentration of organochlorine pesticides residue and the exposure period (i.e. timepoint-dependent accumulation) for respective treatment group(s)*.**

| Residue | Matched pairs of timepoints (for respective treatment groups) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 14 d vs. 28 d | | | 14 d vs. 42 d | | | 28 d vs. 42 d | | |
| | DDE | DDE+ZEN | Pool | DDE | DDE+ZEN | Pool | DDE | DDE+ZEN | Pool |
| ***p,p'*-DDT** | - | - | **0.028** | - | - | 0.059 | - | - | 0.893 |
| ***p,p'*-DDE** | 0.345 | **0.043** | - | **0.043** | **0.043** | - | **0.043** | 0.686 | - |
| ***p,p'*-DDD** | - | - | 0.686 | - | - | 1.000 | - | - | 0.281 |

* due to significant differences in the concentration of *p,p'*-DDE between both treatment groups (after 28 days of exposure; Table 2), the calculations for timepoint-dependent accumulation of this pesticide were performed separately for each treatment group (DDE or DDE+ZEN); for *p,p'*-DDT and *p,p'*-DDE, the values were calculated without any division to separate groups (Pool) as no significant treatment-dependent accumulation was found ($P>0.05$; Table 2). The probability (*P*-values) were obtained with Wilcoxon paired comparison test based on values from Table 1.
- not appropriate.

The lipophilic character and high persistence of DDT and its metabolites enable these compounds to be stored easily in fatty tissue, but they are eliminated from the body very slowly (ATSDR 2002). Table 3 presents the relationships between the concentrations of pesticide residues and the exposure periods in our study. The results show a time-dependent increase of *p,p*'-DDE concentration in the tested tissues (Table 1) as pair-wise comparisons revealed a significant difference between 14 and 42 days of exposure ($P=0.043$; Table 3) in both treatment groups. The concentration of *p,p*'-DDD remained unchanged over time ($P>0.05$; Table 3). However, an interesting decrease over time in *p,p*'-DDT concentration was observed in the analyzed tissues (Table 1) with significant differences between the 14th and 28th day of exposure ($P=0.028$; Table 3). Although the increasing organ concentrations of *p,p*'-DDE confirm the constant dietary uptake of this contaminant throughout the experiment, the opposite effect (i.e. time-dependent decrease) observed for *p,p*'-DDT may be once again explained by the pigs' mass (Figure 2). Since the animal feed used in this study was DDT-free (see the Materials and Methods section), the notable drop of DDT was most likely due to the growth of the animals over time and resulting dilution of the DDT body burden that was probably received earlier (e.g. with the mother's milk or in the feed from the fattening farm).

## SUMMARY

In summary, our study indicated that oral treatment of immature gilts for 42 days with feed contaminated with the maximum allowable dose of *p,p*'-DDE results in a time-dependent accumulation of this compound in fat-rich tissues. Although the pesticide's concentration found in the adipose tissue exceeded the FAO/WHO maximum residue limit, human dietary risk is little, as it would require a substantial consumption of such contaminated pork fat to exceed the provisional tolerable daily intake (FAO/WHO 2001). However, the high concentration of *p,p*'-DDE found in the adrenal glands suggests a threat to the exposed animals' health. Finally, the difference in accumulation of *p,p*'-DDE between the groups treated with this compound alone or in mixture with zearalenone is most likely related to the growth performance of the animals. Further research should evaluate the effects of *p,p*'-DDE at the observed concentrations on pigs' health and focus on its possible interactions with other environmental pollutants, such as mycotoxins.

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