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The pesticide methoxychlor given orally during the perinatal/juvenile period, reduced the spermatogenic potential of males as adults by reducing their Sertoli cell number

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Abstract — Perinatal and juvenile oral treatment of rats with the insecticide, methoxychlor (MXC), reduced testicular size and other reproductive indices including the number of epididymal spermatozoa in those animals as adults [6]. The objective was to determine if these males exposed during development had fewer Sertoli cells which might explain these testicular effects. Rat dams were gavaged with MXC at 0, 5, 50, or 150 mg·kg⁻¹·day⁻¹ for the week before and after they gave birth. Resulting male pups (15/group) then were dosed directly from postnatal day 7 to 42. Testes were fixed in Bouin's and in OsO₄, embedded in Epon and sectioned at 0.5 μm, stained with toluidine blue, and evaluated stereologically or cut at 20 μm to measure Sertoli cell nuclei with Nomarski optics. Sertoli cell number was calculated as the volume density of the nucleus times the parenchymal weight (90% of testicular weight) divided by the volume of a single Sertoli cell nucleus. Across dose groups, there were no changes in the nuclear volume density, the volume of a single nucleus, or the number of Sertoli cells per g parenchyma. There were highly significant dose-related changes in the volume of Sertoli cell nuclei per testis and the number of Sertoli cells per testis. Reduced testicular weight ($r = 0.94$) and reduced numbers of epididymal spermatozoa ($r = 0.43$) were significantly ($p < 0.01$) correlated to reduced number of Sertoli cells per testis. Hence, perinatal and juvenile oral exposure to MXC can reduce spermatogenic potential of males as adults by reducing their number of Sertoli cells.

methoxychlor / estrogenic / oral exposure / Sertoli cell number

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1. INTRODUCTION

Upon request of the United States Congress, the National Academy of Sciences [36] evaluated the likely risk posed to infants and children by pesticide residue in the food supply. They recommended a change in surveillance of the food supply and expanding the knowledge of the effects of pesticides on the developing reproductive system. The last trimester of pregnancy to 18 years of age was identified as the exposure period of concern for humans [36].

Compounds of several major groups of chemicals have been categorized as being weakly estrogenic when evaluated by *in vitro* and *in vivo* screening methods [50]. These include organochlorine pesticides, polychlorinated biphenyls, phenolic compounds, and phthalate esters. Since these compounds are widespread and persist in the environment, they are likely to be present in the food supply [50]. However, little is known about the risk that weakly estrogenic compounds in the food supply pose to human health. The route of exposure of these estrogenic compounds is important. Injected doses as low as 0.1 μg diethylstilbestrol (DES) six times over 12 days reduced testicular weight and Sertoli cell number [1], but young rats fed 17 β -estradiol in their diets at 2.5 parts per million did not have reduced testicular weight or Sertoli cell number [8].

Methoxychlor (MXC), first synthesized more than 100 years ago [30] and used for 50 years for insect and larval control, is one of the four remaining chlorinated pesticides approved for use in the U.S. It is more readily metabolized and excreted by mammals and has less potential for bioconcentration than DDT. Younger animals appear to be more affected than older animals, possibly due to hormonal imprinting, resulting in permanent changes in hormonal status when these animals become adults [6]. Since the endocrine system plays a critical role in the development of the male reproductive sys-

tem and in the initiation and maintenance of normal function, this developing male system is an especially vulnerable target of potential endocrine disruptions [23].

Because the exposure period of concern for humans corresponded to the perinatal/juvenile period in rats and because the concern raised by the National Academy of Sciences was on direct consumption of pesticide residue, Chapin et al. [6] conducted a study in which they orally dosed dams with the pesticide MXC from gestation day 14 until postnatal day (pnd) 7, and then directly orally dosed the pups from pnd 7 to pnd 42. They found that the body weight was not altered by their MXC treatment, but testicular weight, epididymal weight, and number of spermatozoa in the tail of the epididymis were significantly reduced in a dose-dependent fashion [6].

The objective of this present study was to determine if these males had reduced number of Sertoli cells. It was found that prenatal/neonatal oral exposure of rats to MXC reduced spermatogenic potential of males as adults by reducing their Sertoli cell number.

2. METHODS

2.1. Animals

Tissues analyzed in this study came from animals treated and specimens prepared as described in the original study [6]. Groups of timed-mated, pregnant rats [from Taconic Farms (Tac:N(SD)fBR)] were gavaged daily from gestation day 14 to pnd 17, with Methoxychlor (Sigma Chemical Co., St. Louis, MO) at dosages of 0, 5, 50, or 150 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$. The pups were individually gavaged daily from pnd 7 until pnd 42, and sacrificed at \sim pnd 152. This exposure period for rats corresponds to the exposure period of concern for humans [36]. The number of animals was 15 per treatment group.

2.2. Stereology

Testes were fixed in Bouin's fixative. Five pieces of testicular tissue from each rat were further fixed in 1% osmium in sodium cacodylate buffer, embedded in Epon, and sectioned at 0.5 or 20- μm . The 0.5- μm Epon sections were stained with toluidine blue and used for stereologic determination of the volume density (percentage) of Sertoli cell nuclei [20, 24, 27]. The average width and height based on measurement of 35–50 nuclei per sample were used to calculate a rough estimate of the volume of a single nucleus, assuming the nucleus to be a sphere. Since Sertoli cell nuclei are not spherical, a correction factor (calculated at 0.663 ± 0.025 for intact rats [27]) was used (volume based on sphere $\times 0.663$) to obtain a corrected final volume for an individual nucleus.

Volume density of Sertoli cell nuclei was based on the number of points over Sertoli cell nuclei divided by total points applied using a point-counting method and a 50-point ocular grid at $1000 \times$ magnification [11, 22]. Sections averaging over 10 mm^2 each were analyzed for each tissue block by two observers for a total of 10000 points. Precision of Sertoli cell nuclei volume density has been estimated at 14% coefficient of variation for our laboratory [20]. Sertoli cell number per testis was calculated by dividing the product of the volume density of Sertoli cell nuclei, parenchymal volume, and the approximated histological correction factor for section thickness and nuclear diameter assuming the most closely related spherical model [52] by the corrected volume of a single Sertoli cell nucleus. The approximated histological correction factor was 0.96 for volume density of Sertoli cell nuclei. The relative section thickness (average maximum nuclear diameter divided by section thickness of 0.5 μm) was much less than the < 0.1 cutoff point (at which correction has no significant value) needed to correct for spherical structures [5]. While the correction factor for section thickness and nuclear

diameter lowered the estimate by 4%, no correction would result in an overestimation of only a few percentage points of the absolute value [5].

In addition to the Sertoli cell number per testis, the relationship between the testicular weight or epididymal sperm number (reported previously, [6]) and Sertoli cell number was determined.

2.3. Statistical analyses

One-way analysis of variance and Student-Newman-Keuls procedure were used to identify treatment differences in parameters evaluated [42, 46]. Correlation coefficients among various parameters of spermatogenesis were determined [42].

3. RESULTS

There was no effect of oral MXC treatment during the perinatal/juvenile period on body weight of adult rats (508 ± 10 , 503 ± 10 , 506 ± 12 , and $486 \pm 15 \text{ g}$ at 0, 5, 50, or $150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, respectively). However, there was a negative, dose-dependent effect of MXC on testicular weight (Fig. 1a) and number of caudal epididymal spermatozoa (29.1 ± 1.7 , 26.8 ± 1.5 , 23.3 ± 1.6 , and $16.8 \pm 1.4 \times 10^6$). The volume density of Sertoli cell nuclei (1.47 ± 0.04 , 1.48 ± 0.04 , 1.40 ± 0.04 , and $1.41 \pm 0.03\%$), and the volume of individual Sertoli cell nuclei (474 ± 16 , 480 ± 12 , 472 ± 12 , and $501 \pm 14 \text{ fl}$) were not apparently affected by MXC.

The volume of Sertoli cell nuclei per testis (Fig. 1b) and the number of Sertoli cells per testis (Fig. 1c) in adult rats were significantly reduced with increasing doses of MXC. Hence, there was a dose-dependent reduction in Sertoli cell number per testis in adults. The number of Sertoli cells per g parenchyma (Fig. 1d) was not significantly affected even though there was a trend for a dose-dependent effect.

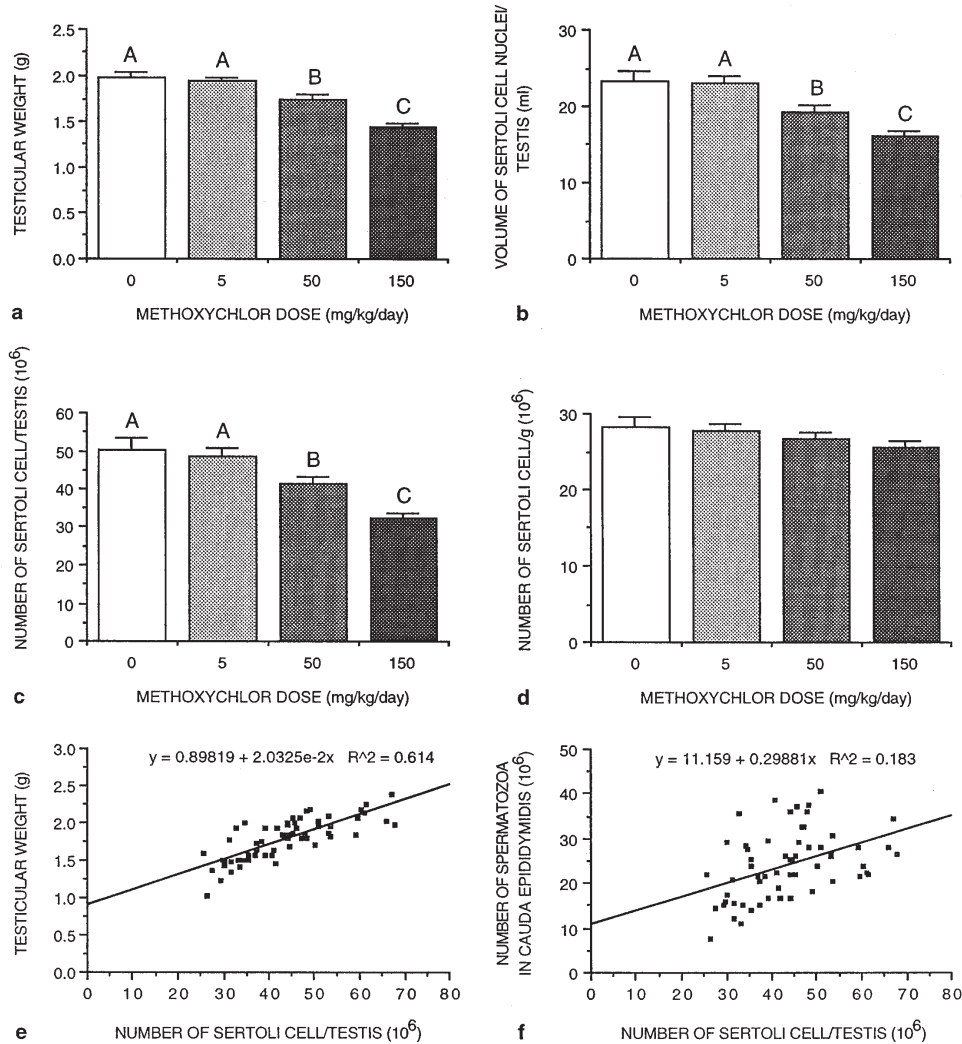


Figure 1. Effect of methoxychlor (MXC) given orally during the perinatal/juvenile period on testicular characteristics. **(a)** Testicular weight, **(b)** volume of Sertoli cell nuclei per testis, and **(c)** number of Sertoli cells/testis are reduced in a dose-dependent fashion. **(d)** Sertoli cell number per g parenchyma is not affected by MXC treatment. **(e)** The number of Sertoli cells per testis is significantly correlated with testicular weight and **(f)** number of spermatozoa in the cauda epididymidis. ABC means with different superscript are different $p < 0.05$.

Testicular weight (Fig. 1e) and number of epididymal spermatozoa (Fig. 1f) in adult rats were significantly ($p < 0.01$) correlated with the number of Sertoli cells. The correlation was high ($r = 0.94$), such that 61% of the variation in testicular weight was

explained by variation in Sertoli cell number. Due to the inherent variation in estimating number of epididymal sperm (whose values change with ejaculation frequency), only 18% of the variation in epididymal spermatozoan number could be explained by

variation in number of Sertoli cells of the attached testes ($r = 0.43$).

4. DISCUSSION

Oral exposure to MXC during the perinatal and juvenile period reduced spermatogenic potential of males as adult (Fig. 1a) by reducing their number of Sertoli cells per testis (Fig. 1c). Indeed, there were significant correlations between testicular weight and Sertoli cell number or between epididymal spermatozoan number and Sertoli cell number/testis (Figs. 1e and 1f). This finding that juvenile exposure to an estrogenic and anti-androgenic compound reduces Sertoli cell number in adults is consistent with the finding of abnormalities in functional development of Sertoli cells in rats injected neonatally with DES [45] and a reduction of Sertoli cell number in 18 day old rats following injection of DES or estradiol on days 2–12 [1]. However, dietary 17β -estradiol given to pregnant rats did not alter the number of Sertoli cells in F_1 males as adults [8]. Likewise, an estrogenic compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at $1.0 \text{ mg}\cdot\text{kg}^{-1}$, did not reduce Sertoli cell number, but it did reduce epididymal weight and caused epididymal abnormalities at a dose of $2.0 \mu\text{g}\cdot\text{kg}^{-1}$ [54]. A similar in utero and juvenile exposure to an aryl hydrocarbon receptor agonist, indole-3-carbinol (I3C) at $100 \text{ mg}\cdot\text{kg}^{-1}$ resulted in an increased Sertoli cell number above the control in 62 day old male rats [54]. Differences in route of exposure (e.g., oral vs. injection), different test substances, different modes of action, and the fact that MXC has both estrogenic and antiandrogenic effects make it difficult to compare these studies. Given that the study requested by the U.S. Congress and recommendations made by the National Academy of Sciences to change surveillance of human food and expand knowledge of pesticides on the developing reproduction system, it appears that effects detected from oral/dietary exposure (Figs. 1a, 1b and 1c)

would be more important and would more likely indicate the potential effects of human exposures to pesticides on foods, if exposure was of sufficient magnitude.

Release of endocrine-disrupting industrial compounds into the environment has resulted in developmental effects in exposed wildlife populations [7, 10]. A large number of these environmental contaminants such as *o-p*-DDT [1,1,1-trichloro-2-(*p*-chlorophenyl) 2-(*o*-chlorophenyl)ethane], kepone, hydroxypolychlorinated biphenyls (PCBs), dieldrin, and several other organochlorine compounds exhibit estrogen receptor agonist activities [3, 4, 15, 31, 41, 47, 49, 53]. Sharpe and Skakkebaek [44] have hypothesized that in utero exposure to environmental estrogens and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds may be responsible for decreased spermatozoan counts (concentration in human ejaculates) and other male reproductive tract disorders [12, 32–34, 40, 43, 53].

In contrast to perinatal and juvenile exposure to estrogenic effects on Sertoli cell number, antiandrogenic compounds like cyproterone acetate given to adult humans for cancer therapy caused damage of the Leydig cells and a reduction in DNA synthesis in spermatogonia and preleptotene primary spermatocytes [35]. Markewitz et al. [35] concluded that the primary and direct effect of this antiandrogenic compound was on primitive (stem cell) spermatogonia. We have recently confirmed this result in a study about the effects of MXC on male germ cells. We showed that the MXC-treated rats had fewer spermatogonia per Sertoli cell than did the control group [48]. When the different types of spermatogonia were identified, it appeared that MXC particularly affected early spermatogonia [48]. To put these results in the context of the current MXC study, the effect of a reduced number of spermatogonia could drive a reduced need for Sertoli cells and the resulting reduction in their number in adults. This is similar to what happens during the equine breeding season, when the need to support an increasing

number of spermatogonia may lead to the seasonal increased number of supporting Sertoli cells in the horse [21].

Reduction in Sertoli cell number in adults by perinatal/juvenile exposure to the pesticide MXC (Fig. 1) is important because the number of Sertoli cells has been directly related to daily spermatozoan production in several species [19, 23, 29]. Sertoli cell number and testicular size are important in determining spermatozoan production rates, as they are directly related to spermatogonial number, spermatid number, or daily spermatozoan production in rams [17], bulls [2], boars [37], horses [18, 25, 28], rats [39], and humans [26]. Age-related reduction in human Sertoli cell number is associated with reduced length of seminiferous tubules and reduced daily spermatozoan production [18, 26]. FSH and other hormones are known to influence Sertoli cell proliferation and DNA synthesis [9, 13, 14, 16, 22, 38, 51]. Although not measured in the males of this study, FSH concentrations were reduced in estrus females in all MXC-treated rats [6].

The results of this study indicate that perinatal/juvenile oral exposure to the pesticide methoxychlor, a compound with estrogenic/antiandrogenic effects, reduced the number of Sertoli cells in animals as adults (Fig. 1). Consistent with the importance of Sertoli cell number on numbers of germ cells and daily spermatozoan production on various species [23], the number of epididymal spermatozoa and testicular weight in adults were significantly reduced following juvenile exposure to the pesticide MXC by the dose-dependent reduction of Sertoli cell number.

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REFERENCES

- [1] Atanassova N., McKinnell C., Walker M., Turner K.J., Fisher J.S., Morley M., Millar M.R., Groome N.P., Sharpe R.M., Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood, *Endocrinology* 140 (1999) 5364–5373.
- [2] Berndtson W.E., Igboeli G., Numbers of Sertoli cells, quantitative rates of sperm production, and the efficiency of spermatogenesis in relation to the daily sperm output and seminal quality of young beef bulls, *Am. J. Vet. Res.* 50 (1989) 1193–1197.
- [3] Bitman J., Cecil H.C., Estrogenic activity of DDT analogs and polychlorinated biphenyls, *J. Agric. Food Chem.* 18 (1970) 1108–1112.
- [4] Bitman J., Cecil H.C., Harris S.J., Fries G.F., Estrogenic activity of o,p'-DDT in the mammalian uterus and avian oviduct, *Science* 162 (1968) 371–372.
- [5] Bolender R.P., Correlation of morphometry and stereology with biochemical analysis of cell fractions, *Int. Rev. Cytol.* 55 (1978) 247–289.
- [6] Chapin R.E., Harris M.W., Davis B.J., Ward S.M., Wilson R.E., Mauney M.A., Lockhart A.C., Smialowicz R.J., Moser V.C., Burka L.T., Collins B.J., The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function, *Fundam. Appl. Toxicol.* 40 (1997) 138–157.
- [7] Colborn T., vom Saal F.S., Soto A.M., Developmental effects of endocrine-disrupting chemicals in wildlife and humans, *Environ. Health Perspect.* 101 (1993) 378–384. Comment in: *Environ. Health Perspect.* 102 (1994) 256–257.
- [8] Cook J.C., Johnson L., O'Connor J.C., Biegel L.B., Krams C.H., Frame S.R., Hurtt M.E., Effects of dietary 17beta-estradiol exposure on serum hormone concentrations and testicular parameters in male Crl:CD BR rats, *Toxicol. Sci.* 44 (1998) 155–168.
- [9] Cooke P.S., Kirby J.D., Porcelli J., Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole, *J. Reprod. Fertil.* 97 (1993) 493–499.
- [10] Davis D.L., Bradlow H.L., Wolff M., Woodruff T., Hoel D.G., Anton-Culver H., Medical hypothesis: xenoestrogens as preventable causes of breast cancer, *Environ. Health Perspect.* 101 (1993) 372–377.
- [11] Elias H., Pauly J.E., Burns E.R., Quantitative microscopy (stereology and morphometry), in: *Histology and Human Microanatomy*, 1978, pp. 547–576.

- [12] Gray L.E. Jr., Kelce W.R., Monosson E., Ostby J.S., Birnbaum L.S., Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: Reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status, *Toxicol. Appl. Pharmacol.* 131 (1995) 108–118.
- [13] Griswold M.D., Mably E.R., Fritz I.B., FSH stimulation of DNA synthesis in Sertoli cells in culture, *Mol. Cell. Endocrinol.* 4 (1976) 139–149.
- [14] Griswold M.D., Solari A., Tung P.S., Fritz I.B., Stimulation by FSH of DNA synthesis and of mitosis in cultured Sertoli cells prepared from the testes of immature rats, *Mol. Cell. Endocrinol.* 151 (1977) 151–165.
- [15] Hammond B., Katzenellenbogen B.S., Krauthammer N., McConnell J., Estrogenic activity of the insecticide chlordane (Kepone) and interaction with uterine estrogen receptors, *Proc. Natl. Acad. Sci. USA* 76 (1979) 6641–6645.
- [16] Hess R.A., Cooke P.S., Bunick D., Kirby J.D., Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers, *Endocrinology* 132 (1993) 2607–2613.
- [17] Hochereau-de Reviere M.T., Courot M., Sertoli cells and development of seminiferous epithelium, *Ann. Biol. Anim. Bioch. Biophys.* 18 (1978) 573–583.
- [18] Johnson L., Spermatogenesis and aging in the human, *J. Androl.* 7 (1986) 331–354.
- [19] Johnson L., Efficiency of spermatogenesis, *Microsc. Res. Tech.* 32 (1995) 385–422.
- [20] Johnson L., Nguyen H.B., Annual cycle of the Sertoli cell population in adult stallions, *J. Reprod. Fertil.* 76 (1986) 311–316.
- [21] Johnson L., Tatum M.E., Temporal appearance of seasonal changes in numbers of Sertoli cells, Leydig cells, and germ cells in stallions, *Biol. Reprod.* 40 (1989) 994–999.
- [22] Johnson L., Petty C.S., Neaves W.B., A comparative study of daily sperm production and testicular composition in humans and rats, *Biol. Reprod.* 22 (1980) 1233–1243.
- [23] Johnson L., Welsh T.J., Wilker C., Anatomy and Physiology of the Male Reproductive System and Potential Targets of Toxicants, in: Boekleheide K., Chapin R.E., Hoyer P.B., Harris C. (Eds.), *Comprehensive Toxicology*, First ed., Vol. 10, Pergamon, New York, 1997, pp. 5–61.
- [24] Johnson L., Suggs L.C., Norton Y.M., Zeh W.C., Effect of developmental age or time after transplantation on Sertoli cell number and testicular size in inbred Fischer rats, *Biol. Reprod.* 54 (1996) 948–959.
- [25] Johnson L., Varner D.D., Tatum M.E., Scrutchfield W.L., Season but not age affects Sertoli cell number in adult stallions, *Biol. Reprod.* 45 (1991) 404–410.
- [26] Johnson L., Zane R.S., Petty C.S., Neaves W.B., Quantification of the human Sertoli cell population: its distribution, relation to germ cell numbers, and age-related decline, *Biol. Reprod.* 31 (1984) 785–795.
- [27] Johnson L., Suggs L.C., Norton Y.M., Welsh T.J., Wilker C.E., Effect of hypophysectomy, sex of host, and/or number of transplanted testes on Sertoli cell number and testicular size of syngeneic testicular grafts in Fischer rats, *Biol. Reprod.* 54 (1996) 960–969.
- [28] Johnson L., Carter G.K., Varner D.D., Taylor T.S., Blanchard T.L., Rembert M.S., The relationship of daily sperm production with number of Sertoli cells and testicular size in adult horses: role of primitive spermatogonia, *J. Reprod. Fertil.* 100 (1994) 315–321.
- [29] Johnson L., Varner D.D., Roberts M.E., Smith T.L., Keillor G.E., Scrutchfield W.L., Efficiency of spermatogenesis: a comparative approach, *Anim. Reprod. Sci.* 60–61 (2000) 471–480.
- [30] Kapoor I.P., Metcalf R.L., Nystrom R.F., Sangha G.K., Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem, *J. Agric. Food Chem.* 18 (1970) 1145–1152.
- [31] Korach K.S., Sarver P., Chae K., McLachlan J.A., McKinney J.D., Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes, *Mol. Pharmacol.* 33 (1988) 120–126.
- [32] Mably T.A., Bjerke D.L., Moore R.W., Gendron-Fitzpatrick A., Peterson R.E., In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability, *Toxicol. Appl. Pharmacol.* 114 (1992) 118–126.
- [33] Mably T.A., Moore R.W., Goy R.W., Peterson R.E., In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood, *Toxicol. Appl. Pharmacol.* 114 (1992) 108–117.
- [34] Mably T.A., Moore R.W., Peterson R.E., In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Effects on androgenic status, *Toxicol. Appl. Pharmacol.* 114 (1992) 97–107.
- [35] Markewitz M., Veenema R.J., Fingerhut B., Nehme-Haily D., Sommers S.C., Cyproterone acetate (SH714) effect on histology and nucleic acid synthesis in the testes of patients with prostatic carcinoma, *Invest. Urol.* 6 (1969) 638–649.
- [36] National Academy of Sciences, Pesticides in the diets of infants and children, National Academy Press, Washington, DC, 1993.

- [37] Okwun O.E., Igboeli G., Ford J.J., Lunstra D.D., Johnson L., Number and function of Sertoli cells, number and yield of spermatogonia, and daily sperm production in three breeds of boar, *J. Reprod. Fertil.* 107 (1996) 137–149.
- [38] Orth J.M., The role of follicle stimulating hormone in controlling Sertoli cell proliferation in testes of fetal rats, *Endocrinology* 115 (1984) 1248–1255.
- [39] Orth J.M., Gunsalus G.L., Lamperti A.A., Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development, *Endocrinology* 122 (1988) 787–794.
- [40] Peterson R.E., Theobald H.M., Kimmel G.L., Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons, *CRC Crit. Rev. Toxicol.* 23 (1993) 283–335.
- [41] Robinson A.K., Mukku V.T., Spalding D.M., Stancel G.M., The estrogenic activity of DDT: The in vitro induction of an estrogen-inducible protein by o,p-DDT, *Toxicol. Appl. Pharmacol.* 76 (1984) 537–543.
- [42] SAS Institute. Statistical Analysis System User's Guide. Statistics, SAS Institute, Inc., Cary, NC, 1985, pp. 113–709.
- [43] Sharpe R.M., The roles of oestrogen in the male, *Trends Endocrinol. Metab.* 9 (1998) 371–377.
- [44] Sharpe R.M., Skakkebaek N.E., Are oestrogens involved in falling sperm counts and disorders of the reproductive tract?, *Lancet* 341 (1993) 1392–1395.
- [45] Sharpe R.M., Atanassova N., McKinnell C., Parte P., Turner K.J., Fisher J.S., Kerr J.B., Groome N.P., Macpherson S., Millar M.R., Saunders P.T.K., Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development, *Biol. Reprod.* 59 (1998) 1084–1094.
- [46] Sokal R.R., Rohlf F.J. (Eds.), *Biometry*, W.H. Freeman and Co., San Francisco, CA, 1969.
- [47] Soto A.M., Chung K.L., Sonnenschein C., The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells, *Environ. Health Perspect.* 102 (1994) 380–383.
- [48] Staub C., Hardy V.B., Chapin R.E., Harris M.W., Johnson L., The hidden effect of estrogenic/antiandrogenic methoxychlor on spermatogenesis, *Toxicol. Appl. Pharmacol.* 180 (2002) 129–135.
- [49] Tullner W.W., Uterotropic action of the insecticide methoxychlor, *Science* 133 (1961) 647–648.
- [50] Turner K.J., Sharpe R.M., Environmental oestrogens—present understanding, *Rev. Reprod.* 2 (1997) 69–73.
- [51] Van Haaster L.H., De Jong F.H., Docter R., De Rooij D.G., High neonatal triiodothyronine levels reduce the period of Sertoli cell proliferation and accelerate tubular lumen formation in the rat testis, and increase serum inhibin levels, *Endocrinology* 133 (1993) 755–760.
- [52] Weibel E.R., Paumgartner D., Integrated stereological and biochemical studies on hepatocytic membranes. II. Correction of section thickness effect on volume and surface density estimates, *J. Cell Biol.* 77 (1978) 584–597.
- [53] Welch R.M., Levin W., Conney A.H., Estrogenic action of DDT and its analogs, *Toxicol. Appl. Pharmacol.* 14 (1969) 358–367.
- [54] Wilker C.E., Safe S.H., Johnson L., Indole-3-carbinol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) reduces daily sperm production and alters epididymal function, *Fundam. Appl. Toxicol.* 30 (1996) 142.