

# DDE

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of DDE. DDE (CAS# 72-55-9) is the initial and predominant environmental breakdown product of DDT, and is also a biological metabolite of DDT.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. This information was used in a screen to select appropriate chemicals for presentation to the Developmental and Reproductive Toxicant Identification Committee as possible candidates for Committee consideration. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to environmental exposures to DDE. Since blood levels of DDE will result from environmental exposures to DDE, all study abstracts that reported serum levels of DDE are considered to be relevant to environmental exposures to DDE, even if in some cases the environmental exposure may have been to DDT or a mixture of DDT and DDE. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Thirty-eight epidemiologic studies of DDE reporting increased risk of adverse developmental or reproductive outcomes were identified, seventeen of which were analytical studies of adequate quality. Two meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes were also identified. Thirty-three epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified, as well as two meeting abstract reporting no increased risk of adverse developmental or reproductive outcomes. Four studies were considered unclear, while there were six related studies and one study without an abstract.
- Four animal studies of DDE reporting reproductive or developmental toxicity were identified, as well as eleven studies reporting no reproductive or developmental toxicity. Twenty-two related articles were also identified.

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## I. Epidemiologic DART Studies

### A. Studies reporting increased risk of adverse developmental or reproductive outcomes

#### **Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa.**

Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C.  
J Androl. 2007 May-Jun;28(3):423-434.

The pesticide DDT [1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane] is 1 of the 12 persistent organic pollutants (POPs) under negotiation at the Stockholm Convention to restrict or ban their production and use because of their toxicity, resistance to breakdown, bioaccumulation, and potential for being transported over long distances. DDT has estrogenic potential, and the main metabolite, p,p'-dichlorodiphenyl-dichloroethylene (p,p'-DDE), is a potent antiandrogen. In response to mounting evidence on the endocrine-disrupting influence of environmental chemicals on human health, this epidemiological study was initiated to test the hypothesis that nonoccupational exposure to DDT affects male reproductive parameters. In a cross-sectional study, healthy male subjects (n=311) between 18 and 40 years (23+/-5) of age were recruited from 3 communities in an endemic malaria area in which DDT is sprayed annually. A semen analysis according to World Health Organization (WHO) standards was performed. The Hamilton Thorne Computer Assisted Sperm Analysis (CASA) system was simultaneously used to determine additional sperm motility parameters. Blood plasma samples were assayed for p,p'-DDT and metabolites as a measure of exposure. The exposure levels were expressed as lipid-adjusted p,p'-DDT and p,p'-DDE values. The mean p,p'-DDT and p,p'-DDE concentrations were 90.23 microg/g(+/-102.4) and 215.47 microg/g(+/-210.6), respectively. The multivariate linear regression analyses indicated that mean CASA motility was lower with a higher p,p'-DDE concentration (beta=-0.02, P=.001) and the CASA parameter beat cross-frequency (BCF) was higher with a higher p,p'-DDT concentration (beta=0.01, P=.000). There was also a statistically significant positive association between percent sperm with cytoplasmic droplets and p,p'-DDT concentration (beta=0.0014, P=.014). The ejaculate volume (mean 1.9+/-1.33 mL) was lower than the normal range (>or=2.0 mL) according to WHO, and a significant decrease with increasing p,p'-DDE values was seen for both square root-transformed volume (beta=-0.0003; P=.024) and count (beta=-0.003; P=.04). Although there were no associations between either p,p'-DDT or p,p'-DDE concentrations and the rest of the seminal parameters, the incidence of teratozoospermia (99%; normal sperm<15%) was high. Twenty-eight percent of the study group presented with oligozoospermia (<20x10(6) sperm/mL), which had a significant positive association with p,p'-DDE (odds ratio [OR]=1.001, P=.03). There was a significant positive association between participants with asthenozoospermia (32%) and p,p'-DDT (OR 1.003, P=.006) and p,p'-DDE (OR 1.001, P=.02). The results imply that nonoccupational exposure to DDT is associated with impaired seminal parameters in men. The high exposure levels of p,p'-DDT and p,p'-

DDE are of concern because these levels could have far-reaching implications for reproductive and general health.

**Modifying effect of the AR gene trinucleotide repeats and SNPs in the AHR and AHRR genes on the association between persistent organohalogen pollutant exposure and human sperm Y:X ratio.**

Tiido T, Rignell-Hydbom A, Jönsson BA, Rylander L, Giwercman A, Giwercman YL  
Mol Hum Reprod. 2007 Apr;13(4):223-9. Epub 2007 Jan 23.

Persistent organohalogen pollutants (POPs) have been suggested to be involved in changing the proportion of ejaculated Y-bearing sperm. The androgen receptor (AR), aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor repressor (AHRR) may modulate the effect of POPs with regard to previously observed sperm Y:X ratio changes. The objective of this study was to investigate whether sperm Y:X ratio changes in subjects exposed to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and dichlorodiphenyl dichloroethene (p,p'-DDE) were modified by polymorphisms in the AR, AHR and AHRR genes. Semen for analysis of Y- and X-bearing sperm by two-colour fluorescence in situ hybridization and blood for leukocyte DNA genotyping and analysis of CB-153 and p,p'-DDE concentrations were obtained from 195 Swedish fishermen. The polymorphic CAG and GGN repeats in the AR and the R554K and P185A single-nucleotide polymorphisms in the AHR and AHRR genes, respectively, were determined by direct sequencing and allele-specific PCR. The effect of p,p'-DDE was modified by CAG or GGN repeat category in relation to the proportion of Y-bearing sperm ( $P = 0.005$  and  $0.02$  for CAG and GGN, respectively). Moreover, p,p'-DDE, but not CB-153, levels were associated with Y-sperm proportion in men with  $CAG < 22$  ( $P < 0.001$ ), but not in those carrying  $CAG \geq 22$  ( $P = 0.73$ ). This association was even more pronounced in subjects carrying a short CAG repeat in combination with an AHRR G-allele. The association in regard to p,p'-DDE was found for  $GGN = 23$  but not for the  $GGN < 23$  or  $GGN > 23$  subgroups ( $P = 0.01, 0.44$  and  $0.99$ , respectively). In conclusion The endocrine-disrupting action of POPs, in relation to the observed changes in sperm Y:X ratio, may be modulated by the genes involved in sex steroid and dioxin-mediated pathways.

\* **In utero p,p'-DDE exposure and infant neurodevelopment: a perinatal cohort in Mexico.**

Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen Hernandez M, Garcia-Hernandez RM, Del Rio-Garcia C, Wolff MS, Lopez-Carrillo L.  
Environmental health perspectives. 2007 Mar;115(3):435-439.

BACKGROUND: Evidence suggests that p,p'-dichlorodiphenyldichloroethene (DDE) affects neurodevelopment in infants, although a critical exposure window has not yet

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been identified. **OBJECTIVES:** Our goal was to assess the prenatal DDE exposure window and its effect on the psychomotor development index (PDI) and mental development index (MDI) during the first year of life. **METHODS:** We recruited 244 children whose pregnancies and deliveries were uncomplicated, and whose mothers were monitored throughout the pregnancy. Participating mothers were not occupationally exposed to DDT (dichlorodiphenyltrichloroethane) but were residents of a zone in Mexico with endemic malaria. We measured serum levels of DDE before pregnancy and during each trimester of the pregnancy. We evaluated PDI and MDI of the Bayley Scales for Infant Development (BSID-II), at 1, 3, 6, and 12 months of age. We adjusted for quality of the home environment and maternal intellectual coefficient (IQ). We used generalized mixed-effects models for statistical analysis. **RESULTS:** Third-trimester DDE level (7.8 +/- 2.8 ppb) was significantly higher than the level at baseline, first, and second trimesters, but the differences never exceeded 20%. Only DDE levels during the first trimester of pregnancy were associated with a significant reduction in PDI (every doubled increase of DDE level reduced the PDI 0.5 points). DDE was not associated with MDI. **CONCLUSIONS:** A critical window of exposure to DDE in utero may be the first trimester of the pregnancy, and psychomotor development is a target of this compound. Residues of DDT metabolites may present a risk of developmental delay for years after termination of DDT use.

**\* Prenatal pesticide and PCB exposures and birth outcomes.**

Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, Wetmur J. *Pediatr Res.* 2007 Feb;61(2):243-50.

Evidence is inconsistent or poorly understood for links between polychlorinated biphenyls (PCBs), 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene (DDE), and organophosphate (OP) pesticides and adverse pregnancy outcomes, although they are known developmental toxicants. We measured biomarkers of maternal exposure to DDE, PCB, and OP metabolites in the third trimester of pregnancy among 404 mothers in a multiethnic cohort in New York City. We also determined maternal paraoxonase (PON1), butyrylcholinesterase (BuChe), and PON1Q192R gene variant. Higher multivariate-adjusted DDE levels (but not PCB) were associated with lower birth weight (-98 g/log<sub>10</sub> DDE,  $p = 0.096$ ) and head circumference (-0.54 cm/log<sub>10</sub> DDE,  $p = 0.030$ ). DDE and PCB levels were not related to birth length, Ponderal index, or gestational age. Birth length was shorter for mothers with PON192RR slow genotype compared with PON192QQ ( $p = 0.026$ ), and head circumference was inversely associated with maternal PON1 activity ( $p = 0.004$ ). With slow-activity PON1 or PON192, urinary diethylphosphates (SigmaDEPs) were associated with lower birth weight and dimethylphosphates (SigmaDMPs) with shorter birth length. No associations were found between birth outcomes and BuChe. In summary, we found suggestive relationships between prenatal environmental biomarkers and birth outcomes in this population.

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Maternal susceptibility factors including PON1 and maternal weight contributed to the observed effects.

**\* A prospective study of serum DDT and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age.**

Perry MJ, Ouyang F, Korrick SA, Venners SA, Chen C, Xu X, Lasley BL, Wang X.  
Am J Epidemiol. 2006 Dec 1;164(11):1056-1064.

The authors explored whether exposure to 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and its isomers and metabolites affects female reproductive hormones characterized by urinary pregnanediol-3-glucuronide (PdG) and estrone conjugate (E(1)C) levels. During 1996-1998, 287 newly married Chinese women nonsmokers intending to conceive were prospectively studied. Serum for DDT measurement was collected at enrollment, and daily menstrual diaries and urine specimens were collected for 1 year or until a clinical pregnancy was achieved. More than 500 menstrual cycles were studied totaling over 8,000 days. Day of ovulation was determined for each cycle, and the association of serum DDT levels with daily PdG and E(1)C levels in a +/-10-day window around ovulation was analyzed. After adjustment for covariates including age, body mass index, and occupational exposures, consistent inverse associations of most DDT forms occurred with urine E(1)C during the periovulation phase and with urine PdG during the luteal phase of the menstrual cycle. For example, a 10-ng/g increase in serum p,p'-DDE was associated with a 0.05-log(E(1)C) decrease ( $p = 0.03$ ) in the periovulation phase and a 0.06-log(PdG) decrease ( $p = 0.03$ ) in the luteal phase. These results support the potential for DDT to be associated with decrements in estrogen and progesterone levels at times during the menstrual cycle that are critical for ovulation and early pregnancy maintenance.

*The population in the following study includes subjects from Sunyer et al., 2005 cited later in this document.*

**\* Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six.**

Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO.  
Clin Exp Allergy. 2006 Oct;36(10):1236-1241.

Our aims were to assess association of dichlorodiphenyldichloroethylene (DDE) with childhood asthma measured up to age 6 and the effect of DDE on the protective effect of breastfeeding on asthma. In addition, we attempted to assess the relevant time-window of

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DDE exposure (i.e. at birth or at 4 years). All women presenting for antenatal care in Menorca, Spain over a 12-month period beginning in mid-1997 were invited to take part in a longitudinal study that included a yearly visit. Four hundred eighty-two children were enrolled and 462 provided complete outcome data after 6.5 years of follow-up. Organochlorine compounds were measured in cord serum of 402 (83%) infants and in blood samples of 285 children aged 4. We defined asthma as the presence of wheezing at age 6 and during any preceding year or doctor-diagnosed asthma, and used skin prick test at age 6 to determine atopic status. Results At birth and 4 years of age, all children had detectable levels of DDE (median 1 ng/mL and 0.8 ng/mL, respectively). From birth to age 4, the mean DDE level among children with artificial feeding decreased by 72%, while among breastfed children it increased by 53%. Diagnosed asthma and persistent wheezing were associated with DDE at birth [odds ratio (OR) for an increase in 1 ng/mL, OR=1.18, 95% confidence interval (95% CI)=1.01-1.39 and OR=1.13, 95% CI=0.98-1.30, respectively], but not with DDE at 4 years. Neither breastfeeding nor atopy modified these associations ( $P>0.3$ ). Breastfeeding protected against diagnosed asthma (OR=0.33, 95% CI=0.08-0.87) and wheezing (OR=0.53, 95% CI=0.34-0.82) in children with low and high DDE levels at birth. Conclusion: In a community without known dichlorodiphenyltrichloroethane environmental releases, this study strengthens the evidence for an effect of DDE on asthma by measuring the disease at age 6 and does not support the hypothesis that DDE modifies the protective effect of breastfeeding on asthma.

#### **Distribution of persistent organochlorine contaminants in infertile patients from Tanzania and Germany.**

Weiss JM, Bauer O, Bluthgen A, Ludwig AK, Vollersen E, Kaisi M, Al-Hasani S, Diedrich K, Ludwig M.

J Assist Reprod Genet. 2006 Sep-Oct;23(9-10):393-399.

**PURPOSE:** To test whether environmental pollutants could affect fertility in humans. **METHODS:** 31 women and 16 men from Tanzania and 21 couples from Germany were included (n = 89). Pesticides and polychlorinated biphenyls were measured in serum, follicular fluid or seminal plasma by gas chromatography and related to sperm quality and pregnancy rates. **RESULTS:** Higher concentrations of DDT+DDE and dieldrin in Tanzania and higher concentrations of PCBs in Germany and in men were detected. All compounds showed higher concentrations in serum and lowest concentrations in seminal plasma. A lower pregnancy rate in German women with high serum concentrations of DDT+DDE was observed. The toxins had no impact on sperm quality. **CONCLUSIONS:** The distribution of toxins between agricultural and industrial countries is different. Seminal plasma seems to be inert against chemicals. In patients with high serum concentrations of DDT and DDE pregnancy rates were impaired.

**Reproductive hormone levels in men exposed to persistent organohalogen pollutants: a study of inuit and three European cohorts.**

Giwercman AH, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, Lindh C, Pedersen HS, Ludwicki JK, Lesovoy V, Shvets M, Spano M, Manicardi GC, Bizzaro D, Bonfeld-Jorgensen EC, Bonde JP.

Environ Health Perspect. 2006 Sep;114(9):1348-53.

**OBJECTIVE:** Persistent organohalogen pollutant (POP) exposure may have a negative impact on reproductive function. The objective of this study was to assess the impact of POP exposure on the male hypothalamo-pituitary-gonadal axis. **PARTICIPANTS:** Participants included 184 Swedish fishermen and spouses of pregnant women from Greenland (n = 258), Warsaw, Poland (n = 113) , and Kharkiv, Ukraine (n = 194). **EVALUATIONS/MEASUREMENTS:** Serum levels of 2,2,4,4,5,5-hexachlorobiphenyl (CB-153) and dichlorodiphenyl dichloroethene (p,p -DDE) were determined in the four populations, showing different exposure patterns: Swedish fishermen, high CB-153/low p,p -DDE; Greenland, high CB-153/high p,p -DDE; Warsaw, low CB-153/moderate p,p -DDE; Kharkiv, low CB-153/high p,p -DDE. Serum was also analyzed for testosterone, estradiol, sex hormone-binding globulin (SHBG) , inhibin B, luteinizing hormone (LH) , and follicle-stimulating hormone (FSH) . Free testosterone levels were calculated based on testosterone and SHBG. **RESULTS:** We found significant center-to-center variations in the associations between exposure and the outcomes. The most pronounced effects were observed in Kharkiv, where statistically significant positive associations were found between the levels of both CB-153 and p,p -DDE and SHBG, as well as LH. In Greenland, there was a positive association between CB-153 exposure and LH. In the pooled data set from all four centers, there was positive association between p,p -DDE and FSH levels [ $\beta = 1.1$  IU/L; 95% confidence interval (CI), 1.0-1.1 IU/L]. The association between CB-153 levels and SHBG was of borderline statistical significance ( $\beta = 0.90$  nmol/L; 95% CI, -0.04 to 1.9 nmol/L). **CONCLUSIONS:** Gonadotropin levels and SHBG seem to be affected by POP exposure, but the pattern of endocrine response is the subject of considerable geographic variation.

**\* Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-DDE) in relation to child growth.**

Ribas-Fito N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP.

International Journal of Epidemiology. 2006 Aug;35(4):853-858.

**OBJECTIVE:** To examine the relation between prenatal 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) exposure (a metabolite of the insecticide DDT) and child growth during the first 7 years of life. Design Prospective cohort study. Participants 1,712 children born between 1959 and 1966 with measured p,p'-DDE concentrations in their mother's serum samples from pregnancy. Setting Multicenter US Collaborative

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Perinatal Project (CPP). RESULTS: The highest prenatal concentrations of p,p'-DDE ( $\geq 60$  microg/l), as compared with the lowest ( $< 15$  microg/l), were associated with decreased height at age 1 year [adjusted coefficient (SE) = -0.72 cm (0.37), n = 1,540], 4 years [-1.14 cm (0.56), n = 1,289], and 7 years [-2.19 (0.46), n = 1,371]. Among subjects in lower categories of exposure no association was observed. CONCLUSIONS: The findings suggest that high prenatal exposure to p,p'-DDE decreases height in children. Impaired growth may be a general indicator of toxicity and suggests that specific organ systems (e.g. endocrine) could be affected.

**\* In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children.**

Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, Jewell NP. Pediatrics. 2006 Jul;118(1):233-241.

OBJECTIVE: We investigated the relationship between prenatal exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment of Mexican farm-workers' children in California. METHODS: Participants from the Center for the Health Assessment of Mothers and Children of Salinas study, a birth cohort study, included 360 singletons with maternal serum measures of p,p'-DDT, o,p'-DDT, and p,p'-DDE. Psychomotor development and mental development were assessed with the Bayley Scales of Infant Development at 6, 12, and 24 months. RESULTS: We found a approximately 2-point decrease in Psychomotor Developmental Index scores with each 10-fold increase in p,p'-DDT levels at 6 and 12 months (but not 24 months) and p,p'-DDE levels at 6 months only. We found no association with mental development at 6 months but a 2- to 3-point decrease in Mental Developmental Index scores for p,p'-DDT and o,p'-DDT at 12 and 24 months, corresponding to 7- to 10-point decreases across the exposure range. Even when mothers had substantial exposure, breastfeeding was usually associated positively with Bayley scale scores. CONCLUSIONS: Prenatal exposure to DDT, and to a lesser extent DDE, was associated with neurodevelopmental delays during early childhood, although breastfeeding was found to be beneficial even among women with high levels of exposure. Countries considering the use of DDT should weigh its benefit in eradicating malaria against the negative associations found in this first report on DDT and human neurodevelopment.

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**Semen quality and exposure to persistent organochlorine pollutants.**

Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, Lindh CH, Pedersen HS, Ludwicki JK, Lesovoy V, Hagmar L, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Thulstrup AM, Bonde JP.  
Epidemiology. 2006 Jul;17(4):450-458.

**BACKGROUND:** Inconsistent results have been found in previous human studies on male reproductive toxicity of persistent organochlorine pollutants. The majority of studies have been conducted among selected populations of infertility clients or among occupational cohorts including a limited number of participants. **METHODS:** We conducted a cross-sectional study of semen quality and serum concentration of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE) among 763 men. We included men from all regions in Greenland (n = 194), fishermen from Sweden (n = 185), inhabitants of the city of Kharkiv, Ukraine (n = 195), and inhabitants of the city of Warsaw, Poland (n = 189). Blood samples were analyzed for CB-153 and p,p'-DDE using gas chromatography-mass spectrometry and adjusted for serum lipids. **RESULTS:** Sperm concentration was not impaired with increasing serum CB-153 or p,p'-DDE levels in any of the separate groups or overall. Similarly, the proportion of morphologically normal sperm was not associated with either CB-153 or p,p'-DDE blood concentration. However, sperm motility was inversely related to CB-153 concentration in Greenland and the Swedish fishermen population. Across all 4 regions, the sperm motility decreased on average by 3.6% (95% confidence interval = 1.7% to 5.6%) per one-unit increase in the log of blood CB-153 (ng/g lipid). The concentration of p,p'-DDE was negatively associated with sperm motility in the Greenlandic population and in the compiled dataset. **CONCLUSION:** Adult exposure to persistent organochlorine pollutants within the ranges observed in the present study is not likely to cause reduction in sperm concentration or morphology. However, higher exposure may be associated with impaired sperm motility.

**\* The association between organochlorine and thyroid hormone levels in cord serum: a study from northern Thailand.**

Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B.  
Environment International. 2006 May;32(4):554-559.

It is now known that many organochlorines (OCs) act as endocrine disruptors, causing harmful effects on wildlife and humans. Several field and laboratory animal studies have reported that OCs cause adverse effects on thyroid hormone status. However, data regarding their effects on thyroid hormone status in humans are inconclusive. Because a developing fetus is especially sensitive to hormonal disruption by exposure to OCs, the adverse health effects on infants are of concern. The present study aimed to investigate

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the association between OC levels in maternal and cord serum, and the association between OC and thyroid hormone levels in cord serum. The study was performed with 39 mother-infant pairs from Mae Rim District of Chiang Mai Province, northern Thailand, who had normal delivery and full term gestation. Maternal blood was collected for measuring OCs and total lipids. Umbilical cord blood was collected for measuring OCs, total lipids, and thyroid hormones, including total thyroxine (TT(4)), free thyroxine (FT(4)), and thyroid stimulating hormone (TSH). 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (p,p'-DDE) had the highest level in all serum samples with a geometric mean of 1,191 ng/g lipids in maternal serum and 742 ng/g lipids in cord serum. The second highest level was that for 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (p,p'-DDT), followed by 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (p,p'-DDD). Levels of p,p'-DDE, p,p'-DDT, p,p'-DDD, and dieldrin in maternal serum were positively associated with levels in cord serum ( $r = 0.86, 0.77, 0.66,$  and  $0.60,$  respectively;  $P < 0.001$ ). The important findings were that cord serum TT(4) levels were negatively associated with cord serum levels of p,p'-DDE ( $r = -0.37, P = 0.024$ ), p,p'-DDT.3 ( $r = -0.33, P = 0.048$ ), and 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene (o,p'-DDE) ( $r = -0.76, P = 0.019$ ). These results therefore suggest that exposure to DDT and its metabolites during fetal development may cause some effects on thyroid hormonal status in infants.

*The population in the following study includes subjects from Tiidor et al., 2005 cited later in this document.*

**Impact of PCB and p,p'-DDE contaminants on human sperm Y:X chromosome ratio: studies in three European populations and the Inuit population in Greenland.**

Tiido T, Rignell-Hydbom A, Jönsson BA, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvyezday V, Spano M, Manicardi GC, Bizzaro D, Bonefeld-Jørgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A; INUENDO. *Environ Health Perspect.* 2006 May;114(5):718-24.

**OBJECTIVE:** Recent studies indicate that persistent organohalogen pollutants (POPs) may contribute to sex ratio changes in offspring of exposed populations. Our aim in the present study was to investigate whether exposure to 2,2,4,4,5,5-hexachlorobiphenyl (PCB-153) and dichlorodiphenyldichloroethene (p,p-DDE) affects sperm Y:X chromosome distribution. **SUBJECTS AND METHODS:** We obtained semen and blood for analysis of PCB-153 and p,p-DDE levels from 547 men from Sweden, Greenland, Poland (Warsaw), and Ukraine (Kharkiv), with regionally different levels of POP exposure. The proportion of Y- and X-chromosome-bearing sperm in the semen samples was determined by two-color fluorescence in situ hybridization analysis. **RESULTS:** Swedish and Greenlandic men had on average significantly higher proportions of Y sperm (in both cohorts, 51.2%) and correspondingly higher lipid-adjusted concentrations of PCB-153 (260 ng/g and 350 ng/g, respectively) compared with men from Warsaw (50.3% and 22 ng/g) and Kharkiv (50.7% and 54 ng/g). In the Swedish cohort, log-transformed PCB-153 and log-transformed p,p-DDE variables were significantly

positively associated with Y-chromosome fractions (p-values 0.04 and <0.001, respectively). On the contrary, in the Polish cohort PCB-153 correlated negatively with the proportion of Y-bearing fraction of spermatozoa (p=0.008). CONCLUSIONS: The present study indicates that POP exposure might be involved in changing the proportion of ejaculated Y-bearing spermatozoa in human populations. Intercountry differences, with different exposure situations and doses, may contribute to varying Y:X chromosome ratios.

**Reduced seminal parameters associated with environmental DDT exposure and p,p'-DDE concentrations in men in Chiapas, Mexico: a cross-sectional study.**

De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, Dombrowski C, Rousseau F, Sanchez VD, Bailey JL.  
J Androl. 2006 Jan-Feb;27(1):16-27.

In response to mounting concerns about the endocrine-disrupting influence of environmental chemicals on human health, this epidemiological study was initiated to test the hypothesis that nonoccupational exposure to the estrogenic pesticide 1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (DDT) affects male reproductive parameters. One hundred and sixteen men aged 27 years (SD = 8.2) living in malaria endemic-areas in Chiapas (Mexico), where DDT was sprayed until 2000, participated in a cross-sectional study. Semen analyses were conducted according to World Health Organization methods and a quality control program was followed. DDT exposure was defined as the level of blood plasma p,p'-dichlorodiphenyl dichloroethylene (DDE), the major metabolite of DDT. The p,p'-DDE concentration adjusted for total lipids was 100 times higher than that reported for nonexposed populations at 45 plus or minus 32 mug/g (mean +/- SD). Crude regression analysis showed that several sperm motion parameters, including the percentage of motile sperm, decreased with higher p,p'-DDE concentrations (beta = -8.38; P = .05 for squared motility), and the percentage of sperm with morphological tail defects increased with higher plasma p,p'-DDE concentration (beta = 0.003; P = .017). Insufficient sperm chromatin condensation was observed in 46.6% of participants, and the most severe category of incomplete DNA condensation was also positively correlated with p,p'-DDE concentration (r = .223; P = .044). Therefore, nonoccupational exposure to DDT, as assessed by plasma p,p'-DDE concentrations, is associated with poorer semen parameters in men, indicating adverse effects on testicular function and/or the regulation of reproductive hormones. Previously, a causal role of environmental toxicants in human male infertility has been lacking because observed effects have been the result of unusually high exposures, either occupationally or as a result of industrial accidents, resulting in unprecedented controversy (reviewed by Cheek & McLachlan, Environmental hormones and the male reproductive system. J Androl. 1998;19:5). This is the first epidemiological study demonstrating effects after nonoccupational exposures to DDT. Based on these findings, the effect of DDT on male reproductive health should not be ignored.

**Serum DDT, age at menarche, and abnormal menstrual cycle length.**

Ouyang F, Perry MJ, Venners SA, Chen C, Wang B, Yang F, Fang Z, Zang T, Wang L, Xu X, Wang X.

Occupational and environmental medicine. 2005 Dec;62(12):878-884.

**BACKGROUND:** Although dichlorodiphenyl trichloroethane (DDT) exposure is known to affect human endocrine function, few previous studies have investigated the effects of DDT exposure on age at menarche or menstrual cycle length. **METHODS:** A cross sectional study was conducted to study the effects of DDT exposure on age at menarche and menstrual cycle length among 466 newly married, nulliparous female Chinese textile workers aged 20-34 years enrolled between 1996 and 1998. Serum was analysed for DDT and its major metabolites. Multivariate linear regression was used to estimate DDT exposure effects on age at menarche and multivariate logistic regression was used to estimate DDT exposure effects on odds of experiencing short or long cycles. **RESULTS:** Relative to those in the lowest DDT quartile, the adjusted mean age at menarche was younger in those in the fourth quartile (-1.11 years). Modeled as a continuous variable, a 10 ng/g increase in serum DDT concentration was associated with an adjusted reduction in age at menarche of 0.20 years. Relative to those in the lowest DDT quartile, odds of any short cycle (<21 days) in the previous year were higher for those in the fourth quartile (odds ratio = 2.78; 95% CI 1.07 to 7.14). There were no associations between serum DDT concentrations and odds of experiencing a long cycle (>40 days). **CONCLUSION:** Results suggest that DDT exposure was associated with earlier age at menarche and increased risk of experiencing a shortened menstrual cycle.

**\* Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children.**

Sunyer J, Torrent M, Munoz-Ortiz L, Ribas-Fito N, Carrizo D, Grimalt J, Anto JM, Cullinan P.

Environmental Health Perspectives. 2005 Dec;113(12):1787-1790.

Prevalence of asthma increases with increasing dichlorodiphenyldichloroethylene (DDE) levels. However, the effect of early-life exposure, the fundamental window of exposure, is unknown. We assessed the association between prenatal DDE and other organochlorine compounds, and atopy and asthma during infancy. All women presenting for antenatal care in Menorca (Spain) over 12 months starting in mid-1997 were invited to take part in a longitudinal study; 482 children were subsequently enrolled, and 468 (97.1%) provided complete outcome data up to the fourth year of study. Prenatal exposure of organochlorine compounds was measured in cord serum in 405 (83%) children. Asthma was defined on the basis of wheezing at 4 years of age, persistent wheezing, or doctor-diagnosed asthma. We measured specific immunoglobulin-E (IgE) against house dust mite, cat, and grass in sera extracted at 4 years of age. DDE (median = 1.03 ng/mL) was detected in all children, as well as hexachlorobenzene (0.68 ng/mL) and

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polychlorobiphenyls (0.69 ng/mL). Wheezing at 4 years of age increased with DDE concentration, particularly at the highest quartile [9% in the lowest quartile (< 0.57 ng/mL) vs. 19% in the highest quartile (1.90 ng/mL); relative risk = 2.63 (95% confidence interval 1.19-4.69), adjusting for maternal asthma, breast-feeding, education, social class, or other organochlorines]. The association was not modified by IgE sensitization and occurred with the same strength among nonatopic subjects and among those with persistent wheezing or diagnosed asthma. DDE was not associated with atopy alone. Prenatal exposure to DDE residues may contribute to development of asthma.

**Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross-sectional study.**

Toft G, Axmon A, Giwercman A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Ludwicki JK, Zvyezday V, Zinchuk A, Spano M, Manicardi GC, Bonfeld-Jorgensen EC, Hagmar L, Bonde JP.

Environ Health. 2005 Nov;4:26.

**BACKGROUND:** Persistent organochlorine pollutants (POPs) may interfere with reproductive function but direct evidence in humans is very limited. **METHODS:** Fertility was examined in four regions with contrasting blood levels of POPs. Pregnant women and their partners in Warsaw (Poland), Kharkiv (Ukraine) and Greenland were consecutively enrolled during antenatal visits. Swedish fishermen and their spouses were recruited separately and independently of current pregnancy. Lipid adjusted serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (DDE) were available for both partners. Time to pregnancy interviews were obtained among 2269 women and 798 men provided a semen sample. **RESULTS:** Inuits had high levels of both POP markers, Swedish fishermen were high in CB-153 but low in DDE, men from Kharkiv were high in DDE and low in CB-153 while men from Warsaw were low in CB-153 and had intermediate DDE levels. Compared to Warsaw couples, fecundability was reduced among couples from Kharkiv [adjusted fecundability ratio (FR) 0.64 (95% CI 0.5-0.8)] and elevated in Swedish fishermen families [FR 1.26 (95% CI 1.0-1.6)]. Adjusted geometric means of sperm counts and morphology did not differ between regions while sperm motility was higher in men living in Warsaw. **CONCLUSION:** We observed regional differences in time to pregnancy and sperm motility that may be related to regional differences in POP blood levels, but other interpretations are also plausible. In particular, differences in access to safe contraception and in the prevalence of contraceptive failures are most likely to bias comparisons of time to pregnancy.

**\* Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy.**

Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A, Altshul L, Perry M, Fu L, Wang X.

Am J Epidemiol. 2005 Oct 15;162(8):709-716.

Previous studies of pregnancy losses and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) were limited because they did not include losses prior to clinical detection of pregnancy and because exposures were measured after the pregnancies of interest. The authors examined the association of preconception serum total DDT (sum of DDT isomers and metabolites) concentration and subsequent pregnancy losses in 388 newly married, nonsmoking, female textile workers in China between 1996 and 1998. Upon stopping contraception, subjects provided daily urine specimens and records of vaginal bleeding for up to 1 year or until clinical pregnancy. Daily urinary human chorionic gonadotropin was assayed to detect conception and early pregnancy losses, and pregnancies were followed to detect clinical spontaneous abortions. There were 128 (26%) early pregnancy losses in 500 conceptions and 36 (10%) spontaneous abortions in 372 clinical pregnancies. Subjects were grouped in tertiles by preconception serum total DDT concentration (group 1: 5.5-22.9 ng/g; group 2: 23.0-36.5 ng/g; group 3: 36.6-113.3 ng/g). Compared with group 1, group 2 had adjusted relative odds of early pregnancy losses of 1.23 (95% confidence interval (CI): 0.72, 2.10), and group 3 had adjusted odds of 2.12 (95% CI: 1.26, 3.57). The relative odds of early pregnancy losses associated with a 10-ng/g increase in serum total DDT were 1.17 (95% CI: 1.05, 1.29). The small number of spontaneous abortions following clinical detection of pregnancy were not associated with serum total DDT. In this population, there was a positive, monotonic, exposure-response association between preconception serum total DDT and the risk of subsequent early pregnancy losses.

**\* Maternal concentration of dichlorodiphenyl dichloroethylene (DDE) and initiation and duration of breast feeding.**

Karmaus W, Davis S, Fussman C, Brooks K.

Paediatr Perinat Epidemiol. 2005 Sep;19(5):388-398.

Dichlorodiphenyl dichloroethylene (DDE) has been shown to reduce the duration of breast feeding in two studies. In addition to duration, we examined whether DDE lowers the initiation of breast feeding. Between 1973 and 1991, the Michigan Department of Community Health conducted three surveys to assess polychlorinated biphenyls (PCBs) and DDE serum concentrations in Michigan anglers. Through telephone interviews with parents, we retrospectively ascertained information on breast feeding. Based on repeated maternal serum measurements between 1973 and 1991, we arrived at the level of

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exposure at the time of delivery by extrapolating PCB and DDE serum levels. One mother may have contributed more than one child; however, serum concentrations varied between children from the same mother. The maternal DDE and PCB serum concentrations were categorised as follows: 0 to <5 microg/L, 5 to <10 microg/L, >or=10 microg/L. Repeated measurement models and survival analyses were used to determine the relationship between DDE and PCBs and characteristics of breast feeding while controlling for cohort effects, maternal age at delivery, education, and smoking during pregnancy. We focused on 176 pregnancies of 91 mothers who had maternal exposure information and gave birth between 1969 and 1995. Initiation of breast feeding was lowered by 39.5% and duration shortened by 66.4% in children of mothers who smoked during pregnancy. In children of non-smoking mothers, the incidence ratio for breast-feeding initiation was 0.45 [95% CI 0.15, 0.94] and 0.42 [95% CI 0.10, 1.03] when maternal DDE concentrations were 5 to <10 microg/L and >or=10 microg/L respectively, compared with the lowest DDE exposure group. In these offspring (of non-smoking mothers), breast-feeding duration was shorter when DDE concentrations were higher: 13 weeks for >or=10 microg/L DDE, compared with 21.7 weeks for lower DDE. We did not detect any association between PCBs and breast feeding. In the absence of the distorting effects of maternal smoking, DDE exposure may decrease initiation and duration of breast feeding.

### **Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury.**

Takser L, Mergler D, Baldwin M, de Grosbois S, Smargiassi A, Lafond J. Environmental Health Perspectives. 2005 Aug;113(8):1039-1045.

Polychlorinated biphenyls (PCBs), chlorinated pesticides, and mercury are global environmental contaminants that can disrupt the endocrine system in animals and humans. However, there is little evidence that they can interfere with endocrine status in pregnant women and neonates at low levels of exposure. The aim of this study was to examine thyroid hormone levels during pregnancy and in cord blood in relation to blood concentrations of organochlorine compounds (OCs) and Hg in healthy women recruited during pregnancy. We found a significant negative correlation between maternal total triiodothyronine levels and three non-coplanar congeners (PCB-138, PCB-153, and PCB-180), three pesticides (p,p -DDE, cis-nanochlor, and hexachlorobenzene), and inorganic Hg independently, without any other changes in thyroid status. No significant relationships were observed between OCs and cord serum thyroid hormones. Cord serum free thyroxin was negatively correlated with inorganic Hg. These results suggest that at even low levels of exposure, persistent environmental contaminants can interfere with thyroid status during pregnancy.



**Exposure to persistent organochlorine pollutants associates with human sperm Y:X chromosome ratio.**

Tiido T, Rignell-Hydbom A, Jönsson B, Giwercman YL, Rylander L, Hagmar L, Giwercman A.

Hum Reprod. 2005 Jul;20(7):1903-9.

**BACKGROUND:** During the last decades, there has been concern that exposure to endocrine disruptors, such as persistent organochlorine pollutants (POPs), may contribute to sex ratio changes in offspring of exposed populations. **METHODS:** To investigate whether exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and dichlorodiphenyl dichloroethene (p,p'-DDE) affect Y:X chromosome proportion, semen of 149 Swedish fishermen, aged 27-67 years, was investigated. The men provided semen and blood for analysis of hormone, CB-153 and p,p'-DDE levels. The proportion of Y- and X-chromosome bearing sperm in semen samples was determined by two-colour fluorescence in situ hybridization (FISH) analysis. **RESULTS:** Log transformed CB-153 as well as log transformed p,p'-DDE variables were both significantly positively associated with Y chromosome fractions (P-values = 0.05 and <0.001, respectively). Neither age, smoking nor hormone levels showed any association with Y-chromosome fractions. **CONCLUSIONS:** This is the first study to indicate that exposure to POPs may increase the proportion of ejaculated Y-bearing spermatozoa. These data add to the growing body of evidence that exposure to POPs may alter the offspring sex ratio.

**Polychlorinated biphenyls and menstrual cycle characteristics.**

Cooper GS, Klebanoff MA, Promislow J, Brock JW, Longnecker MP.

Epidemiology. 2005 Mar;16(2):191-200.

**BACKGROUND:** Experimental studies in nonhuman primates provide evidence that low-level exposure to persistent organochlorine pollutants such as polychlorinated biphenyls (PCBs) may affect aspects of their menstrual cycle, including cycle length, regularity, and bleeding duration. Few studies have examined these associations in humans. **METHODS:** We used data from 2314 pregnant women who participated in the Collaborative Perinatal Project, a cohort study that enrolled pregnant women in the 1960s in 12 centers in the United States. Information about usual (prepregnancy) menstrual cycle length, regularity, bleeding duration, and dysmenorrhea was collected at enrollment, and 11 PCBs and p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) were measured in stored blood samples collected during the third trimester of pregnancy. We used multivariate linear and logistic regression to examine the association between organochlorine levels and menstrual cycles, adjusting for demographic factors, cholesterol, and triglycerides. **RESULTS:** Total PCBs were positively associated with increasing menstrual cycle length (adjusted difference across 5 categories of PCB exposure = 0.7 days, trend-test P value = 0.02). Irregular cycles were slightly more frequent among those in the 2 highest categories of PCB exposure (odds ratio for highest category = 1.5; 95% confidence interval = 0.70-3.3), and there also was some evidence of an association with DDE. The strengths of these associations increased with various

exclusions made to decrease potential misclassification of the outcome and the exposures. There was little evidence for associations between DDE or PCBs and bleeding duration, heavy bleeding, or dysmenorrhea. **CONCLUSIONS:** This study supports experimental studies in monkeys showing an effect of low-dose PCB exposure on menstrual function.

**\* Exposure to organochlorine compounds and effects on ovarian function.**

Windham GC, Lee D, Mitchell P, Anderson M, Petreas M, Lasley B.  
Epidemiology. 2005 Mar;16(2):182-190.

**BACKGROUND:** Some chemicals appear to have hormonally active properties in animals, but data in humans are sparse. Therefore, we examined ovarian function in relation to organochlorine compound levels. **METHODS:** During 1997-1999, 50 Southeast Asian immigrant women of reproductive age collected urine samples daily. These samples were assayed for metabolites of estrogen and progesterone, and the women's menstrual cycle parameters were assessed. Organochlorine compounds (including DDT, its metabolite DDE, and 10 polychlorinated biphenyl [PCB] congeners) were measured in serum. **RESULTS:** All samples had detectable DDT and DDE, with mean levels higher than typical U.S. populations. Mean cycle length was approximately 4 days shorter at the highest quartile concentration of DDT or DDE compared with the lowest. After adjustment for lipid levels, age, parity, and tubal ligation, and exclusion of a particularly long cycle, the decrements were attenuated to less than 1 day, with wide confidence intervals (CIs). The adjusted mean luteal phase length was shorter by approximately 1.5 days at the highest quartile of DDT (95% CI = -2.6 to -0.30) or DDE (-2.6 to -0.20). With each doubling of the DDE level, cycle length decreased 1.1 day (-2.4 to 0.23) and luteal phase length decreased 0.6 days (-1.1 to -0.2). Progesterone metabolite levels during the luteal phase were consistently decreased with higher DDE concentration. PCB levels were not generally associated with cycle length or hormone parameters after adjustment, and they did not alter the DDE associations when included in the same models. **CONCLUSIONS:** This study indicates a potential effect of DDE on ovarian function, which may influence other end points such as fertility, pregnancy, and reproductive cancers.

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**\* Maternal serum level of the DDT metabolite DDE in relation to fetal loss in previous pregnancies.**

Longnecker MP, Klebanoff MA, Dunson DB, Guo X, Chen Z, Zhou H, Brock JW. Environ Res. 2005 Feb;97(2):127-33.

Use of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) continues in about 25 countries. This use has been justified partly by the belief that it has no adverse consequences on human health. Evidence has been increasing, however, for adverse reproductive effects of DDT, but additional data are needed. Pregnant women who enrolled in the Collaborative Perinatal Project (United States, 1959-1965) were asked about their previous pregnancy history; blood samples were drawn and the serum frozen. In 1997-1999, the sera of 1717 of these women who had previous pregnancies were analyzed for 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), the major breakdown product of DDT. The odds of previous fetal loss was examined in relation to DDE level in logistic regression models. Compared with women whose DDE level was <15 microg/L, the adjusted odds ratios of fetal loss according to category of DDE were as follows: 15-29 microg/L, 1.1; 30-44 microg/L, 1.4; 45-59 microg/L, 1.6; and 60+ microg/L, 1.2. The adjusted odds ratio per 60 microg/L increase was 1.4 (95% confidence interval 1.1-1.6). The results were consistent with an adverse effect of DDE on fetal loss, but were inconclusive owing to the possibility that previous pregnancies ending in fetal loss decreased serum DDE levels less than did those carried to term.

**\* Maternal exposure to Great Lakes sport-caught fish and dichlorodiphenyl dichloroethylene, but not polychlorinated biphenyls, is associated with reduced birth weight.**

Weisskopf MG, Anderson HA, Hanrahan LP, Kanarek MS, Falk CM, Steenport DM, Draheim LA. Environ Res. 2005 Feb;97(2):149-62.

Fish consumption may be beneficial for a developing human fetus, but fish may also contain contaminants that could be detrimental. Great Lakes sport-caught fish (GLSCF) are contaminated with polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethylene (DDE), but the effects of these contaminants on birth outcome are not clear. To distinguish potential contaminant effects, we examined (1) whether the decrease over time in contaminant levels in GLSCF is paralleled by an increase in birth weight of children of GLSCF-consuming mothers and (2) the relation between maternal serum concentrations of these contaminants and birth weight. Mothers (n=511) were interviewed from 1993 to 1995, and maternal serum was collected from 1994 to 1995 (n=143). Potential confounders considered were child gender, maternal age at delivery, maternal prepregnancy body mass index, maternal cigarette and alcohol use during pregnancy, maternal education level, maternal parity, and maternal breastfeeding.

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Children born during 1970-1977, 1978-1984, and 1985-1993 to mothers who ate more than 116 meals of GLSCF before pregnancy were, on average, 164 g lighter, 46 g heavier, and 134 g heavier, respectively, than children of mothers who ate no GLSCF before pregnancy (P trend=0.05). GLSCF-consuming mothers had higher serum PCB and DDE concentrations, but only increased DDE was associated with lower birth weight. The data suggest that fetal DDE exposure (as indicated by maternal serum DDE concentration) may decrease birth weight and that decreased birth weight effects associated with GLSCF consumption have decreased over time.

### **Age at natural menopause and exposure to organochlorine pesticides in Hispanic women.**

Akkina J, Reif J, Keefe T, Bachand A.

Journal of Toxicology and Environmental Health. 2004 Sep 24;67(18):1407-1422.

A cross-sectional study was conducted to evaluate the relationship between exposure to selected organochlorine pesticides (OCP) (p,p'-DDT, p',p'-DDE, dieldrin, hexachlorobenzene, beta-hexachlorocyclohexane [beta-HCH], oxychlordan, trans-nonachlor) and age at natural menopause in a sample of 219 menopausal women participating in the Hispanic Health and Nutrition Examination Survey in 1982-1984. Information on age at menopause, reproductive history, demographic variables, and potential confounding variables was collected via interview. Analysis of variance was employed to compare adjusted mean age at natural menopause among women by category of serum OCP level. Serum levels of p,p'-DDT, p,p'-DDE, beta-HCH, and trans-nonachlor were associated with a younger age at menopause. In particular, women with exposure levels in the highest exposure categories (serum p,p'-DDT > or = 6ppb, beta-HCH > or = 4ppb, or trans-nonachlor > or = 2ppb) had an adjusted mean age at menopause on average 5.7, 3.4, and 5.2 yr earlier, respectively, than women with serum levels of these pesticides below the detection limit. Women with serum p,p'-DDE levels greater than 23.6 ppb (highest quintile) had an adjusted mean age at menopause 1.7 yr earlier than women with serum p,p'-DDE levels less than 5.5 ppb (lowest quintile). However, no consistent dose-response effect was apparent across low, medium, and high exposure categories. Interactions were detected for p,p'-DDT in combination with beta-HCH, trans-nonachlor, or oxychlordan, as well as beta-HCH in combination with oxychlordan.

### **In utero exposure to organochlorines and age at menarche.**

Vasiliu O, Muttineni J, Karmaus W.

Hum Reprod. 2004 Jul;19(7):1506-12. Epub 2004 May 6.

**BACKGROUND:** To examine the effect of in utero exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) on age at menarche in offspring, we conducted a cohort study over two generations. **METHODS:** Female participants (and their offspring) in a Michigan angler cohort in which organochlorine

levels had been determined previously were studied. Of their 213 female offspring aged 20-50 years, 151 participated in the study (71%). We retrospectively determined age at first menstrual bleeding. Based on repeated maternal serum measurements between 1973 and 1991, we extrapolated PCB and DDE serum levels at the time of pregnancy. To estimate the association between in utero PCB and DDE exposure and age at menarche, we used linear regression analyses controlling for birth date period, maternal age at delivery, birth weight, breastfeeding, education status and maternal height. RESULTS: An increase in the in utero DDE exposure of 15 micro g/l reduced age at menarche by 1 year (P = 0.04). There was no association with maternal PCB exposure. When controlling for estimated body size at menarche, the DDE association was no longer significant, based on a subsample of 102 women. CONCLUSION: The DDE effect on age at menarche encourages further research about in utero exposures. Prospective studies including the offspring's DDE level before menarche are of particular interest.

### **Reproductive effects of occupational DDT exposure among male malaria control workers.**

Salazar-Garcia F, Gallardo-Diaz E, Ceron-Mireles P, Loomis D, Borja-Aburto VH. Environ Health Perspect. 2004 Apr;112(5):542-7.

To assess potential effects of human DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] exposure, we evaluated the reproductive history of 2,033 workers in the antimalaria campaign of Mexico. Data on occupational exposure to DDT and reproductive outcomes were gathered through a questionnaire, and workers provided information about 9,187 pregnancies. We estimated paternal exposure to DDT before each pregnancy using three approaches: a) a dichotomous indicator for pregnancies before and after exposure began, b) a qualitative index of four exposure categories, and c) an estimation of the DDT metabolite DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] accumulated in fat. To assess associations, we used logistic regression models that accounted for correlated observations and adjusted for parents' age at each child's birth, exposure to other pesticides, exposure to chemical substances in other employment, smoking, and alcohol consumption. The odds ratio for birth defects comparing pregnancies after and before the first exposure was 3.77 [95% confidence interval (95% CI), 1.19-9.52]. Compared with the lowest quartile of estimated DDE in fat, the ORs were 2.48 (95% CI, 0.75-8.11), 4.15 (95% CI, 1.38-12.46), and 3.76 (95% CI, 1.23-11.44) for quartiles 2, 3, and 4, equivalent to p,p -DDE in fat of 50, 82, and 298 microg/g fat, respectively. No significant association was found for spontaneous abortion or sex ratio. We found an increased risk of birth defects associated with high occupational exposure to DDT in this group of workers. The significance of this association at lower exposure levels found in the general population remains uncertain.

**\* Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants.**

Ribas-Fito N, Cardo E, Sala M, Eulalia de Muga M, Mazon C, Verdu A, Kogevinas M, Grimalt JO, Sunyer J.  
Pediatrics. 2003 May;111(5 Pt 1):e580-585.

**OBJECTIVE:** Exposure to organochlorine compounds (OCs) occurs both in utero and through breastfeeding. Levels of hexachlorobenzene (HCB) found in the cord serum of newborns from a population located in the vicinity of an electrochemical factory in Spain were among the highest ever reported. We studied the association between exposure to OCs and breastfeeding on neurodevelopment in the 1-year-old infants of this population. **METHODS:** A birth cohort including 92 mother-infant pairs was recruited between 1997 and 1999 in 5 neighboring villages (84% of possible recruits). The mental and psychomotor development of each infant was assessed at 13 months using the Bayley and the Griffiths Scales of Infant Development. OCs were measured in cord serum. **RESULTS:** Dichlorodiphenyl dichloroethylene (p,p'DDE) cord serum levels were negatively associated with both mental and psychomotor development. For each doubling of a dose of p,p'DDE, we found a resultant decrease of 3.50 points (standard error: 1.39) on the mental scale and 4.01 points (standard error: 1.37) on the psychomotor scale. Exposure to polychlorinated biphenyls was only marginally associated with psychomotor development. Prenatal exposure to HCB had no effect on child neurodevelopment. Long-term breastfeeding was associated with better performance on both the mental and motor scales. Short-term breastfed infants with higher p,p'DDE levels in cord serum were associated with the lowest scores on both the mental and the psychomotor scales. **CONCLUSIONS:** Prenatal exposure to p,p'DDE was associated with a delay in mental and psychomotor development at 13 months. No association was found for exposure to HCB. Long-term breastfeeding was found to be beneficial to neurodevelopment, potentially counterbalancing the impact of exposure to these chemicals through breast milk.

**Preterm birth in relation to maternal organochlorine serum levels.**

Torres-Arreola L, Berkowitz G, Torres-Sanchez L, Lopez-Cervantes M, Cebrian ME, Uribe M, Lopez-Carrillo L.  
Ann Epidemiol. 2003 Mar;13(3):158-62.

**PURPOSE:** To evaluate the associations of serum levels of p,p'-DDE and two other persistent organochlorine pesticides, beta-HCH and HCB, in relation to preterm birth. **METHODS:** During 1995 we performed a case-cohort study and 233 mothers were recruited at three large maternity hospitals in Mexico City. Serum levels were obtained shortly after delivery. **RESULTS:** A non-significant increased risk of preterm birth in relation to serum p,p'-DDE levels was observed. There was also a suggestion of an

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increased risk of preterm birth among women in the highest tertile of beta-HCH (adjusted odds ratio 1.85, 95% CI = 0.94-3.66, p value for test of trend  $p = 0.08$ ) compared with the lowest tertile. No association was found between HCB serum levels and preterm births. CONCLUSIONS: These findings suggest that p,p'-DDE and other organochlorine pesticides may pose a risk to preterm birth in countries that continue to use such insecticides for malaria control.

**Persistent chlorinated pesticides and intra-uterine foetal growth retardation: a possible association.**

Siddiqui MK, Srivastava S, Srivastava SP, Mehrotra PK, Mathur N, Tandon I.

Int Arch Occup Environ Health. 2003 Feb;76(1):75-80. Epub 2002 Oct 17.

OBJECTIVE: To examine the association between DDT (dichlorodiphenyl trichloroethane) and HCH (hexachlorocyclohexane) exposure and intra-uterine growth retardation (IUGR, <10th percentile of birth weight for gestational age). METHOD: We detected p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE and alpha-HCH, beta-HCH, gamma-HCH, delta-HCH in maternal blood, placenta and cord blood, collected at parturition, from mothers with IUGR babies (n=30) and from those with babies of normal weight (n=24), using gas-liquid chromatography equipped with electron capture detector ( $^{63}\text{Ni}$ ). The adjusted odds ratios (ORs) for these pesticides in mothers and infants were determined by multiple logistic regression. RESULTS: There were statistically significant associations ( $P < 0.05$ ) between maternal blood levels of alpha-HCH (OR=1.22; 95% CI: 1.02-1.46), gamma-HCH (OR=1.38; 95% CI: 1.05-1.80), delta-HCH (OR=1.61; 95% CI: 1.01-2.54), total HCH (OR=1.07; 95% CI: 1.01-1.13) and p,p'-DDE (OR=1.21; 95% CI: 1.03-1.42) and IUGR after adjustment for potential confounders. Also, significant association ( $P < 0.05$ ) between cord blood levels of gamma-HCH (OR=1.14; 95% CI: 1.00-1.31), delta-HCH (OR=1.31; 95% CI: 1.00-1.75), total HCH (OR=1.07; 95% CI: 1.00-1.14) and IUGR were found after adjustment for potential confounders. A significant negative correlation between body weight of newborn babies and p,p'-DDE in maternal blood ( $r = -0.25$ ;  $P < 0.05$ ) and delta-HCH and p,p'-DDE in the cord blood ( $r = -0.27$  and  $-0.26$ ;  $P < 0.05$ ) was noticed after gestational age had been accounted for. CONCLUSION: Exposure of pregnant women to organochlorine pesticides may increase the risk of IUGR, which is a contributing factor for infant mortality in India.

**Environmental organochlorines and semen quality: results of a pilot study.**

Hauser R, Altshul L, Chen Z, Ryan L, Overstreet J, Schiff I, Christiani DC.

Environ Health Perspect. 2002 Mar;110(3):229-33.

There have been numerous studies that suggest that sperm concentrations (sperm counts) are declining in men. However, other studies suggest that sperm counts are not declining or may be increasing in some areas. Although there is disagreement on whether there is a downward temporal trend in sperm counts, the studies provide evidence that sperm counts vary by geographic location. It has been hypothesized that the geographic

variation in sperm concentrations may be due to environmental exposures, lifestyle factors, or some unknown causes. To determine whether contemporary ambient levels of polychlorinated biphenyls (PCBs) and p,p-DDE are associated with altered semen quantity and quality, we selected a study population without specific exposure to PCBs or p,p-DDE. The present study presents the results from a pilot study on the relationship between serum PCBs and p,p-DDE and semen quality in 29 subjects recruited from the Massachusetts General Hospital Andrology Laboratory. Of the 29 subjects, 3 had sperm concentrations < 20 million/mL, 7 had < 50% motile sperm, 9 had < 4% normal morphology, and 6 were below normal in more than one semen parameter. The 18 subjects with normal spermatozoa concentration, motility, and morphology were used as comparison subjects. The mean (SE) concentration of the sum of PCBs and p,p-DDE was 242 ng/g lipids (34.0) and 354 ng/g lipids (120), respectively, for men with below normal motility as compared to 202 ng/g lipids (16.6) and 240 ng/g lipids (31.1), respectively, for the comparison subjects. The data showed general trends that were suggestive of an association between PCBs and p,p-DDE and abnormal motility, as well as with sperm concentration and morphology. A full-scale study is currently in progress.

**\* Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth.**

Longnecker MP, Klebanoff MA, Zhou H, Brock JW.  
Lancet. 2001 Jul 14;358(9276):110-4.

**BACKGROUND:** DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) is highly effective against most malaria-transmitting mosquitoes and is being widely used in malaria-endemic areas. The metabolite, DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), has been linked to preterm birth in small studies, but these findings are inconclusive. Our aim was to investigate the association between DDE exposure and preterm birth. **METHODS:** Our study was based on the US Collaborative Perinatal Project (CPP). From this study we selected a subset of more than 44000 eligible children born between 1959 and 1966 and measured the DDE concentration in their mothers' serum samples stored during pregnancy. Complete data were available for 2380 children, of whom 361 were born preterm and 221 were small-for-gestational age. **FINDINGS:** The median maternal DDE concentration was 25 mg/L (range 3-178)-several fold higher than current US concentrations. The adjusted odds ratios (OR) of preterm birth increased steadily with increasing concentrations of serum DDE (ORs=1, 1.5, 1.6, 2.5, 3.1; trend p<0.0001). Adjusted odds of small-for-gestational-age also increased, but less consistently (ORs=1, 1.9, 1.7, 1.6, 2.6; trend p=0.04). After excluding preterm births, the association of DDE with small-for-gestational-age remained. **INTERPRETATION:** The findings strongly suggest that DDT use increases preterm births, which is a major contributor to infant mortality. If this association is causal, it should be included in any assessment of the costs and benefits of vector control with DDT.

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\* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.



**\* Association of DDT with spontaneous abortion: a case-control study.**

Korrick SA, Chen C, Damokosh AI, Ni J, Liu X, Cho SI, Altshul L, Ryan L, Xu X.  
Ann Epidemiol. 2001 Oct;11(7):491-6.

**PURPOSE:** Spontaneous abortion (SAB), the most common adverse pregnancy outcome, affects approximately 15% of clinically recognized pregnancies. Except for advanced maternal age and smoking, there are not well-established risk factors for SAB. Animal models associate increased fetal resorption or abortion with exposure to the pesticide dichlorodiphenyl trichloroethane (DDT), but epidemiologic investigations of DDT and SAB are inconsistent. We undertook a pilot investigation of the hypothesized association of DDT with SAB. **METHODS:** Participants in this case-control study were selected from a longitudinal study of reproductive effects of rotating shifts among female Chinese textile workers who were married, ages 22-34, nulliparous without history of SAB or infertility, and planning pregnancy. From 412 pregnancies, 42 of which ended in SAB, 15 SAB cases and 15 full-term controls were randomly selected and phlebotomized. Serum was analyzed for p,p'-DDT, o,p'-DDT, their metabolites (DDE and DDD), and other organochlorines including polychlorinated biphenyls. **RESULTS:** Cases and controls were nonsmokers and did not differ in age (mean 25 years), body mass index (BMI), passive smoke exposure, or workplace exposures. Cases had significantly ( $p < 0.05$ ) higher serum levels of p,p'-DDE (22 vs. 12 ng/g) and o,p'-DDE (0.09 vs. 0.05 ng/g) than controls. After adjustment for age and BMI, each ng/g serum increase in p,p'-DDE was associated with a 1.13 (CI, 1.02-1.26) increased odds of SAB. With adjustment of serum DDE levels for excretion via breastfeeding, DDE-associated increased odds of SAB remained significant with up to 7% declines in maternal serum DDE levels for each month of breastfeeding. **CONCLUSIONS:** A potential increased risk of SAB is associated with maternal serum DDE levels.

**\* Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene.**

Gladen BC, Ragan NB, Rogan WJ.  
J Pediatr. 2000 Apr;136(4):490-6.

**OBJECTIVES:** Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) are ubiquitous toxic environmental contaminants. Prenatal and early life exposures affect pubertal events in experimental animals. We studied whether prenatal or lactational exposures to background levels of PCBs or DDE were associated with altered pubertal growth and development in humans. **Study design:** Follow-up of 594 children from an existing North Carolina cohort whose prenatal and lactational exposures had previously been measured. Height, weight, and stage of pubertal development were assessed through annual mail questionnaires. **RESULTS:** Height of boys at puberty increased with transplacental exposure to DDE, as did weight adjusted for height;

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\* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

adjusted means for those with the highest exposures (maternal concentration 4+ ppm fat) were 6.3 cm taller and 6.9 kg larger than those with the lowest (0 to 1 ppm). There was no effect on the ages at which pubertal stages were attained. Lactational exposures to DDE had no apparent effects; neither did transplacental or lactational exposure to PCBs. Girls with the highest transplacental PCB exposures were heavier for their heights than other girls by 5.4 kg, but differences were significant only if the analysis was restricted to white girls. CONCLUSIONS: Prenatal exposures at background levels may affect body size at puberty.

### **Susceptibility to infections and immune status in Inuit infants exposed to organochlorines.**

Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R.  
Environ Health Perspect. 2000 Mar;108(3):205-11.

We investigated whether organochlorine exposure is associated with the incidence of infectious diseases in Inuit infants from Nunavik (Arctic Quebec, Canada). We compiled the number of infectious disease episodes during the first year of life for 98 breast-fed and 73 bottle-fed infants. Concentrations of organochlorines were measured in early breast milk samples and used as surrogates to prenatal exposure levels. Immune system parameters were determined in venous blood samples collected from infants at 3, 7, and 12 months of age. Otitis media was the most frequent disease, with 80.0% of breast-fed and 81.3% of bottle-fed infants experiencing at least one episode during the first year of life. During the second follow-up period, the risk of otitis media increased with prenatal exposure to p,p'-DDE, hexachlorobenzene, and dieldrin. The relative risk (RR) for 4- to 7-month-old infants in the highest tertile of p, p'-DDE exposure as compared to infants in the lowest tertile was 1.87 [95% confidence interval (CI), 1.07-3.26]. The RR of otitis media over the entire first year of life also increased with prenatal exposure to p,p'-DDE (RR, 1.52; CI, 1.05-2.22) and hexachlorobenzene (RR, 1.49; CI, 1.10-2.03). Furthermore, the RR of recurrent otitis media ([Greater/equal to] 3 episodes) increased with prenatal exposure to these compounds. No clinically relevant differences were noted between breast-fed and bottle-fed infants with regard to immunologic parameters, and prenatal organochlorine exposure was not associated with immunologic parameters. We conclude that prenatal organochlorine exposure could be a risk factor for acute otitis media in Inuit infants.

### **DDE and shortened duration of lactation in a northern Mexican town.**

Gladen BC, Rogan WJ.

Am J Public Health. 1995 Apr;85(4):504-508. [American journal of public health].

OBJECTIVES. Worldwide declines in the duration of lactation are cause for public health concern. Higher levels of dichlorodiphenyl dichloroethene (DDE) have been associated with shorter durations of lactation in the United States. This study examined whether this relationship would hold in an agricultural town in northern Mexico.

METHODS. Two hundred twenty-nine women were followed every 2 months from

childbirth until weaning or until the child reached 18 months of age. DDE was measured in breast milk samples taken at birth, and women were followed to see how long they lactated. RESULTS. Median duration was 7.5 months in the lowest DDE group and 3 months in the highest. The effect was confined to those who had lactated previously, and it persisted after statistical adjustment for other factors. These results are not due to overtly sick children being weaned earlier. Previous lactation lowers DDE levels, which produces an artifactual association, but simulations using best estimates show that an effect as large as that found here would arise through this mechanism only 6% of the time. CONCLUSIONS. DDE may affect women's ability to lactate. This exposure may be contributing to lactation failure throughout the world.

### **Neonatal effects of transplacental exposure to PCBs and DDE.**

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M.

J Pediatr. 1986 Aug;109(2):335-41.

Neonatal effects of transplacental exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) were examined in a study of 912 infants. Birth weight, head circumference, and neonatal jaundice showed no relationship to PCBs or DDE. We also administered the Brazelton Neonatal Behavioral Assessment Scales, which are psychologic and neurologic tests designed for use in newborn infants. The results of these tests showed that higher PCB levels were associated with hypotonicity and hyporeflexia and that higher DDE levels were associated with hyporeflexia.

## **B. Meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes**

### **Maternal Exposure To DDE May Increase Weight And Body-Mass In Adult Female Offspring.**

Karmaus W, Eneli I

Epidemiology 2004 Jul;15(4):S117-S118

Introduction: To investigate the effect of in utero exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenylethylene (DDE) on weight, height and body mass index (BMI) in adult female offspring. Methods: We identified 213 daughters, 20-50 year of age, of mothers with DDE and PCBs determinations from the first generation in the Michigan fish eater cohort. Of the adult female offspring 71% participated in our study (n=151). Maternal serum levels (DDE, PCBs) were ascertained between 1973 and 1991 in repeated investigations. We extrapolated maternal serum levels backward to the time of birth. To estimate the effect of in utero PCB and DDE exposure on weight, height, and BMI, we used linear regression analyses. We controlled for maternal height and weight, as well as for factors of the adult offspring such as birth weight, breastfed or not, number

of pregnancies, and age of the offspring. Results: The average age of the adult female offspring was 36.6 years; 17% had a BMI of 30 kg/m<sup>2</sup> and higher (obese) and 20.5% a BMI between 25 and > 30 kg/m<sup>2</sup> (overweight). Maternal height and BMI were significant predictors of their daughters' height, weight, and BMI. Low birth weight (> 2,500 g) was significantly associated with reduced adult offspring height, weight, and BMI. High birth weight (< 4,000 g) significantly predicted adult offspring height, but not weight or BMI. Maternal DDE levels predicted the weight and body-mass of adult offspring. The weight of the adult offspring, controlling their height, increased 6.08 kg per 10microg/L increase in maternal serum DDE levels (p=0.047). Body-mass of the offspring increased by 2.0 kg/m<sup>2</sup> per 10microg/L maternal DDE (p=0.058). Maternal PCB concentrations during pregnancy were not related to these anthropometric measures. Discussion: In utero exposure to the estrogenic endocrine-disrupting chemical DDE may explain a part of the obesity epidemic in the United States. Further research is needed to better understand the relationship.

### **Effects of Endocrine Disruptors During Pregnancy on Neonatal Growth.**

Campoy C; Jiménez-Torres M; Machado I; García-Vena E; Fernández JM; Bayés R; Olea MF; Olea N  
Horm Res 2004;62(Suppl 2):83

The present study tries to confirm the presence of OP in 199 samples from umbilical cord (UV) (n:66) and in mother's serum (S) (n: 66) & adipose tissue (AT) (n:67), and to invest the relationship between the OP content in UV, S & AT and the fetal growth parameters. Healthy volunteers women, aged between 17-35 years, with pregnancy finished by caesarea due to different causes were recruited. Their neonates were borned at term with a weight (W) appropriated for gestational age (GA). OP: Gas chromatography [electron capture detector (GC/ECD)] & gas chromatography/mass spectrometry (GC/MS) were used. Neonatal anthropometric data (2 days post-partum): W & height (H), and ponderal index (PI) were measured. Statistical analysis: ANOVA, Bonferroni correction and correlation analysis were done. Serum data: ng/ml; tissue data: ng/g of fat. \*:p<0.01. The mean±SD in serum from mother & UV were different [p,p'DDT (5,3±7,2 vs 3,4±6,3), p,p'DDE (31,4±34,7 vs 23,0±48,1), Endosulfan eter (7,2±13,0 vs 7,9±18,7)]. The OP analysed in the AT presented different concentrations: pp'DDE (2603.3±1917.7), endosulfan-lactone (7.7±16.8 ng/g of fat); the frequency of presence was variable from 12.4% for the endosulfan alpha to 39.1% for the endosulfan eter or 100% for p,p'DDE or Aldrin. Inverse significant correlations were found between the different OP determined in UV, S & AT and the neonatal W & GA (Aldrin S-PI: r:-0.40\*; Dieldrin VU-GA: r:-0.41\*; HBC AT-PI: r:-0.44\*; p,p'DDE VU-W: r:-0.46\*; o,pDDT VU-W: r:-0.42\*). Conclusions: The present study confirmed, once more, that fetal exposure to these molecules and a relationship between the majority of them with the neonatal W, H & PI. The correlations established in this study demonstrate the capacity for endocrine disruption of these molecules that could interfere the fetal metabolism and even are able to determine prematurity or low birth weight infants.

### C. Studies reporting no increased risk of adverse developmental or reproductive outcomes

#### **In utero exposure to DDT and performance on the Brazelton neonatal behavioral assessment scale.**

Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. Neurotoxicology. 2007 May;28(3):471-7.

We investigated whether decrements in neonatal neurodevelopment, as determined by the Brazelton neonatal behavioral assessment scale (BNBAS), were associated with in utero exposure to dichlorodiphenyltrichloroethane (DDT): p,p'-dichlorodiphenyl trichloroethane (p,p'-DDT), o,p'-dichlorodiphenyl trichloroethane (o,p'-DDT) and p,p'-DDT's primary breakdown product p,p'-dichlorodiphenyl dichloroethylene (p,p'-DDE) (heretofore collectively referred to as DDT/DDE). Our subjects were a birth cohort of 303 infants whose mothers were low-income Latinas living in the Salinas Valley, an agricultural community in California. We assessed neonates  $\leq$  2 months old using the seven BNBAS clusters (habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and reflex) and examined performance in relationship to DDT/DDE measures in maternal serum samples collected during pregnancy. We did not find any detrimental associations between in utero DDT/DDE levels and neonatal performance on the BNBAS. In this same cohort, we previously demonstrated that exposures to DDT/DDE were related to decrements in neurodevelopment at 6-24 months of age. The failure to observe effects on the BNBAS in these same children may be due to limited sensitivity of a single BNBAS assessment or a delay in the manifestations of neurodevelopmental effects of DDT/DDE until after the neonatal period.

#### **In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, Mexico.**

Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, Hernandez-Avila M. Am J Epidemiol. 2007 May 1;165(9):1015-1022.

The insecticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) is still used for disease control in some areas, resulting in high levels of human exposure. The main degradation product of DDT is 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), an antiandrogen. In animal experiments, in utero exposure to DDE decreases anogenital distance in male offspring. In these models, anogenital distance serves as a measure of fetal androgen action. The authors designed the present study to examine the hypothesis that in utero exposure to DDE decreases anogenital distance in newborn human males. A cross-sectional study of 781 newly delivered male infants was conducted in 2002-2003 in Chiapas, Mexico, where DDT had recently been used for malaria control. Measurements of anogenital distance and penile dimensions were taken, and a sample of the mother's blood was drawn. In this population, the range of serum DDE levels was large (0.8-398

microg/liter). The authors, using two-sided tests, found no evidence that exposure in utero to DDE was related to reduced androgen action as reflected by anogenital distance or penile dimensions at birth. If DDE has important antiandrogenic action in humans, it may be manifest only at higher levels of exposure or via effects on other outcomes.

**Organochlorine exposures during pregnancy and infant size at birth.**

Sagiv SK, Tolbert PE, Altshul LM, Korrick SA.

Epidemiology. 2007 Jan;18(1):120-129.

**BACKGROUND:** Organochlorines, including polychlorinated biphenyls (PCBs) and pesticides, are environmentally persistent contaminants that concentrate in the food chain as well in human adipose tissue and readily cross the placenta. **METHODS:** To follow up on studies suggesting an association of organochlorine exposure with reduced birth size, we investigated the association of PCBs and organochlorine pesticides (including p,p'-dichlorodiphenyl dichloroethene [p,p'-DDE], the major degradation product of p,p'-dichlorodiphenyl trichloroethane [p,p'-DDT], and hexachlorobenzene [HCB]), with birth weight, crown-heel length, and head circumference. We evaluated a cohort of 722 infants born between 1993 and 1998 to mothers residing near a PCB-contaminated harbor and Superfund site in New Bedford, Massachusetts. **RESULTS:** Small negative associations were observed for PCBs and birth weight; associations were weaker for birth length and head circumference. There was evidence for effect modification by smoking during pregnancy on the association between PCBs and birth weight. No associations were found with p,p'-DDE or HCB for any measures of birth size. **CONCLUSIONS:** This study supports the growing literature that demonstrates at most a weak association between very low-level organochlorine exposure and birth size.

**Maternal DDT exposures in relation to fetal and 5-year growth.**

Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I.

Epidemiology. 2006 Nov;17(6):692-700.

**BACKGROUND:** Dichlorodiphenyltrichloroethane (DDT) is an organochlorine pesticide still used in areas of the world where malaria vector control is needed. Few studies have examined in utero exposures to DDT in relation to fetal and early childhood growth in populations with substantial exposure to DDT. Furthermore, only a portion of these studies have investigated in utero exposures and growth during childhood. **METHODS:** To assess the role of in utero exposures to DDT on fetal and early childhood growth, we analyzed data from mothers and children who participated in the Child Health and Development Study (CHDS), a cohort study of 20,754 women and their pregnancies conducted in the San Francisco Bay area during the 1960s. We measured p,p'-DDE, o,p'-DDT, and p,p'-DDT concentrations from the stored sera of 399 women collected during pregnancy. Outcomes were measured at the child's birth and at 5 years of age. **RESULTS:** Maternal p,p'-DDE concentrations were considerable in this study, averaging

6.9 micrograms per gram lipid. After covariate adjustment, a small increase in gestational age was observed with increases in p,p'-DDT and o,p'-DDT, but there was no association with p,p'-DDE. At 5 years of age, an increase from the 25th to the 75th percentile in p,p'-DDE was related to a 2-mm increase in head circumference (95% confidence interval = 0 to 4). Overall effect sizes were small and imprecise. Furthermore, there was little evidence of specificity for a given outcome or exposure at either age. **CONCLUSIONS:** At the concentrations studied in this sample, DDT compounds did not appear to impair fetal or 5-year growth.

**In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers.**

Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, Sunyer J. *Am J Epidemiol.* 2006 Nov 15;164(10):955-962.

p,p'-DDT (bis[p-chlorophenyl]-1,1,1-trichloroethane) is a persistent organochlorine compound that has been used worldwide as an insecticide. The authors evaluated the association of cord serum levels of DDT and its metabolite, 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE), with neurodevelopment at age 4 years. Two birth cohorts in Ribera d'Ebre and Menorca (Spain) were recruited between 1997 and 1999 (n = 475). Infants were assessed at age 4 years by using the McCarthy Scales of Children's Abilities. Organochlorine compounds were measured in cord serum. Children's diet and parental sociodemographic information was obtained through questionnaire. Results showed that DDT cord serum concentration at birth was inversely associated with verbal, memory, quantitative, and perceptual-performance skills at age 4 years. Children whose DDT concentrations in cord serum were >0.20 ng/ml had mean decreases of 7.86 (standard error, 3.21) points in the verbal scale and 10.86 (standard error, 4.33) points in the memory scale when compared with children whose concentrations were <0.05 ng/ml. These associations were stronger among girls. Prenatal exposure to background, low-level concentrations of DDT was associated with a decrease in preschoolers' cognitive skills. These results should be considered when evaluating the risk and benefits of spraying DDT during antimalaria and other disease-vector campaigns.

**Persistent pesticides in human breast milk and cryptorchidism.**

Damgaard IN, Skakkebaek NE, Toppari J, Virtanen HE, Shen H, Schramm KW, Petersen JH, Jensen TK, Main KM. *Environmental Health Perspectives.* 2006 Jul;114(7):1133-1138.

**INTRODUCTION:** Prenatal exposure to some pesticides can adversely affect male reproductive health in animals. We investigated a possible human association between maternal exposure to 27 organochlorine compounds used as pesticides and cryptorchidism among male children. **DESIGN:** Within a prospective birth cohort, we performed a case-control study; 62 milk samples from mothers of cryptorchid boys and 68 from mothers of healthy boys were selected. Milk was collected as individual pools

between 1 and 3 months postpartum and analyzed for 27 organochlorine pesticides. RESULTS: Eight organochlorine pesticides were measurable in all samples (medians; nanograms per gram lipid) for cases/controls: 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (p,p -DDE) : 97.3/83.8; beta-hexachlorocyclohexane (beta-HCH) : 13.6/12.3; hexachlorobenzene (HCB) : 10.6/8.8; alpha-endosulfan: 7.0/6.7; oxychlordane: 4.5/4.1; 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (p,p -DDT) : 4.6/4.0; dieldrin: 4.1/3.1 ; cis-heptachloroepoxide (cis-HE) : 2.5/2.2. Five compounds [octachlorostyrene (OCS); pentachlorobenzene, 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane(p,p -DDD) ; o,p -DDT ; mirex] were measurable in most samples (detection rates 90.8-99.2%) but in lower concentrations. For methoxychlor, cis-chlordane, pentachloroanisole (PCA), gamma-HCH, 1,1-dichloro-2-(2-chlorophenyl) -2,2(4-chlorophenyl) ethane, trans-chlordane, alpha-HCH, and o,p -DDE, both concentrations and detection rates were low (26.5-71.5%). Heptachlor, HCH (lc delta, epsilon), aldrin, beta-endosulfan and trans-heptachloroepoxide were detected at negligible concentrations and low detection rates and were not analyzed further. Seventeen of 21 organochlorine pesticides [p,p -DDT, p,p -DDE, p,p -DDD, o,p -DDT, HCH (alpha, beta, gamma), HCB, PCA, alpha-endosulfan, cis-HE, chlordane (cis-, trans-) oxychlordane, methoxychlor, OCS, and dieldrin] were measured in higher median concentrations in case milk than in control milk. Apart from trans-chlordane (p = 0.012), there were no significant differences between cryptorchid and healthy boys for individual chemicals. However, combined statistical analysis of the eight most abundant persistent pesticides showed that pesticide levels in breast milk were significantly higher in boys with cryptorchidism (p = 0.032). CONCLUSION: The association between congenital cryptorchidism and some persistent pesticides in breast milk as a proxy for maternal exposure suggests that testicular descent in the fetus may be adversely affected.

### **Maternal contamination with dichlorodiphenyltrichloroethane and reproductive outcomes in an Australian population.**

Khanjani N, Sim MR.

Environmental Research. 2006 Jul;101(3):373-379.

Persistent organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), are lipophilic environmental pollutants which accumulate in the food chain. These chemicals have long half-lives and can be detected in human milk, serum, and some other tissues. These chemicals have recently been under scrutiny for their possible health hazards such as cancer and reproductive outcomes including low birth weight. The aim of our study was to investigate whether mothers with a higher contamination of pesticides were different from mothers with low contamination in relation to their offspring's birth outcomes such as birth weight, small for gestation age, prematurity, head circumference, sex ratio, and previous miscarriage or still birth. We used data collected as part of a cross-sectional study of organochlorines in breast milk. This study did not show any association between low birth weight or small for gestation age and organochlorine contamination when comparing the higher to the low exposure group. Weak, significant correlations (-0.1) were detected between low birth weight and contamination levels of



dichlorodiphenyldichloroethylene (DDE) only in female offspring. There was a decrease in the percentage of female offspring for all chemicals in the high-contamination group. The baby's head circumference increased as the mother's contamination increased but the adjusted difference in means was not significant. We did not see any association between either miscarriage or stillbirth in the mother's previous pregnancies or prematurity of the first live (recent) baby when comparing the higher to the low exposure mothers. The organochlorines DDT and DDE were not found to be associated with adverse birth outcomes in contaminated mothers in the range of contamination of our population (<7.5 mg/kg lipid in maternal milk), although there is weak evidence that sex ratio may be affected.

**Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population.**

Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, Hubbard A, Barr DB.

Environmental Health Perspectives. 2006 Apr;114(4):597-602.

From 1940 through the 1970s, organochlorine compounds were widely used as insecticides in the United States. Thereafter, their use was severely restricted after recognition of their persistence in the environment, their toxicity in animals, and their potential for endocrine disruption. Although substantial evidence exists for the fetal toxicity of organochlorines in animals, information on human reproductive effects is conflicting. We investigated whether infants' length of gestation, birth weight, and crown-heel length were associated with maternal serum levels of 11 different organochlorine pesticides: p,p -dichlorodiphenyltrichloroethane (p,p -DDT), p,p -dichlorodiphenyldichloroethylene (p,p -DDE), o,p -dichlorodiphenyltrichloroethane (o,p -DDT), hexachlorobenzene (HCB), gamma-hexachlorocyclohexane (gamma-HCCH), gamma-hexachlorocyclohexane (gamma-HCCH), dieldrin, heptachlor epoxide, oxychlorane, trans-nonachlor, and mirex. Our subjects were a birth cohort of 385 low-income Latinas living in the Salinas Valley, an agricultural community in California. We observed no adverse associations between maternal serum organochlorine levels and birth weight or crown-heel length. We found decreased length of gestation with increasing levels of lipid-adjusted HCB (adjusted gamma = -0.47 weeks; p = 0.05). We did not find reductions in gestational duration associated with any of the other organochlorine pesticides. Our finding of decreased length of gestation related to HCB does not seem to have had clinical implications for this population, given its relatively low rate of preterm delivery (6.5%).

**Time to pregnancy as a function of male and female serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE).**

Axmon A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Zvyezday V, Ludwicki JK, Jonsson BA, Toft G, Bonde JP, Hagmar L.  
Hum Reprod. 2006 Mar;21(3):657-665.

**BACKGROUND:** Persistent organochlorine pollutants (POP) may affect both the female and male reproductive system in animals as well as in humans. **METHODS:** Blood samples were collected from pregnant women and their partners from Greenland, Warsaw and Kharkiv, and from a cohort of Swedish fishermen's wives. Blood samples were analysed for 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE). Information on the participants' fertility, measured as time to pregnancy (TTP), was collected. In total, 778 men and 1505 women were included in the analyses. **RESULTS:** The data from Warsaw, Kharkiv and the Swedish fishermen's wives indicated no effect of either male or female exposure to POP on TTP. However, among men and women from Greenland, there seemed to be an association between serum concentrations of CB-153 and p,p'-DDE and prolonged TTP. Due to the strong intra-individual correlation between CB-153 and p,p'-DDE in the Greenlandic population, it was not possible to determine whether the risk was associated with CB-153 or p, p'-DDE or was an interaction between the two compounds. **CONCLUSIONS:** The overall results of the present study create a somewhat ambiguous pattern, but give some support to the idea that dietary POP exposure might be harmful for couple fertility.

**In utero exposure to persistent organic pollutants in relation to testicular cancer risk.**

Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M.  
International Journal of Andrology. 2006 Feb;29(1):228-234.

Testicular cancer is the most common tumour type in young men. In Sweden the annual age-adjusted incidence increased significantly by 2.4% during the time period 1984-1993 and during 1994-2003 by 1.4%. Exposure to endocrine disrupting chemicals during the foetal period has been postulated to be a risk factor. In this investigation we studied the concentrations of chlorinated biphenyls (PCBs), p,p'-dichlorodiphenyl-dichloroethylene (pp'-DDE), hexachlorobenzene (HCB), chlordanes and polybrominated diphenylethers (PBDEs) in 58 cases with testicular cancer and 61 age-matched controls. Furthermore, case and control mothers were also asked to participate and 44 case mothers and 45 control mothers agreed. No significant differences were found between cases and controls. Case mothers had in general higher concentrations of these chemicals. For the sum of PCBs an odds ratio (OR) = 3.8, 95% confidence interval (CI) = 1.4-10 was calculated using the median concentration for the controls as cut-off value. For HCB OR = 4.4, CI = 1.7-12 and for PBDE OR = 2.5, 95% CI = 1.02-6.0 were obtained, whereas OR was not significantly increased for pp'-DDE and sum of chlordanes. The cases were born during a period with high concentrations of persistent organic pollutants (POPs) in

humans. The decline of the increasing incidence of testicular cancer during recent years may reflect decreasing body burden of certain POPs since the 1980s.

**Exposure to PCB and p, p'-DDE in European and Inuit populations: impact on human sperm chromatin integrity.**

Spano M, Toft G, Hagmar L, Eleuteri P, Rescia M, Rignell-Hydbom A, Tyrkiel E, Zvyezday V, Bonde JP.  
Hum Reprod. 2005 Dec;20(12):3488-3499.

**BACKGROUND:** Persistent organochlorine pollutants (POP), such as polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (p, p'-DDE), are widely found in the environment and considered potential endocrine-disrupting compounds (EDC). Their impact on male fertility is still unknown. **METHODS:** To explore the hypothesis that POP is associated with altered sperm chromatin integrity, a cross-sectional study involving 707 adult males (193 Inuits from Greenland, 178 Swedish fishermen, 141 men from Warsaw, Poland, and 195 men from Kharkiv, Ukraine) was carried out. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), as a proxy of the total PCB burden, and of p,p'-DDE were determined. Sperm chromatin structure assay (SCSA) was used to assess sperm DNA/chromatin integrity. **RESULTS:** We found a strong and monotonically increasing DNA fragmentation index with increasing serum levels of CB-153 among European but not Inuit men, reaching a 60% higher average level in the highest exposure group. No significant associations were found between SCSA-derived parameters and p, p'-DDE serum concentrations. **CONCLUSION:** These results suggest that human dietary PCB exposure might have a negative impact on the sperm chromatin integrity of adult males but additional issues, including differences in the genetic background and lifestyle habits, still need to be elucidated.

**DDT serum concentration and menstruation among young Chinese women.**

Chen A, Zhang J, Zhou L, Gao ES, Chen L, Rogan WJ, Wolff MS.  
Environmental Research. 2005 Nov;99(3):397-402.

High DDE and DDT concentrations were found to be associated with shortened menstrual cycle length in Laotian immigrants to the United States. We examined this issue in a sample of young Chinese women. A total of 60 women aged 20-24 years were enrolled in three maternal and child health clinics (20 from urban, 20 from suburban, 20 from rural) in Shanghai, China, and vicinity, in 1998. Of these women, 47 who did not use hormonal contraceptives and had valid menstrual cycle characteristics were included in the analysis for associations among serum DDE and DDT concentration and menstrual cycle length, duration of menses, and heaviness of menstrual flow. In univariate analysis, higher p,p'-DDE concentration was associated with longer menstrual cycle length (0.66 day per 10 microg/L, 95% confidence interval [CI]: 0.21, 1.11 day). With adjustment for age, body mass index, education, occupation, and resident location, the estimate was 0.42 day (95% CI: -0.35, 1.19 day). p,p'-DDE was not associated with duration of menses or

heaviness of menstrual flow. Neither p,p'-DDT nor o,p'-DDT were associated with menstrual cycle length, duration of menses or heaviness of menstrual flow. The study largely suggests no association between DDE and DDT concentrations and menstrual cycle characteristics in young Chinese women, though the weak-to-no correlation of DDE with menstrual cycle length merits further study.

**Association of DDT and DDE with birth weight and length of gestation in the Child Health and Development Studies, 1959-1967.**

Farhang L, Weintraub JM, Petreas M, Eskenazi B, Bhatia R.  
Am J Epidemiol. 2005 Oct 15;162(8):717-725.

The pesticide p,p'-dichlorodiphenyltrichloroethane (DDT) and its persistent metabolite p,p'-dichlorodiphenyldichloroethylene (DDE) are associated with negative reproductive outcomes in animals. In humans, however, the findings are inconsistent. Using data from the Child Health and Development Studies, a longitudinal study of 20,754 pregnancies among San Francisco Bay Area women from 1959 to 1967, the authors examined the effects of maternal serum DDT and DDE concentrations on preterm birth, small-for-gestational-age birth, birth weight, and gestational age in 420 male subjects. Data were analyzed using multivariate logistic regression for preterm and small-for-gestational-age birth and linear regression for birth weight and gestational age. Median serum concentrations of DDE were 43 mug/liter (interquartile range: 32-57; range: 7-153) and of DDT were 11 mug/liter (interquartile range: 8-16; range: 3-72), several times higher than current US concentrations. The adjusted odds ratio for preterm birth was 1.28 (95% confidence interval (CI): 0.73, 2.23) for DDE and 0.94 (95% CI: 0.50, 1.78) for DDT. For small-for-gestational-age birth, the adjusted odds ratio was 0.75 (95% CI: 0.44, 1.26) for DDE and 0.69 (95% CI: 0.73, 1.27) for DDT; none of the study results achieved statistical significance. Given the persistence of DDT in the environment and its continuing role in malaria control, studies using more robust data should continue to assess this relation.

**Maternal serum levels of polychlorinated biphenyls and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and time to pregnancy.**

Law DC, Klebanoff MA, Brock JW, Dunson DB, Longnecker MP.  
Am J Epidemiol. 2005 Sep 15;162(6):523-32.

Polychlorinated biphenyls (PCBs), once used widely in transformers and other applications, and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), the main metabolite of the pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), are hormonally active agents. Changes in menstrual cycle functioning associated with PCBs and DDE, and increased odds of spontaneous abortion associated with DDE, suggest that these compounds could affect fertility. The authors investigated the association between PCB and DDE exposure and time to pregnancy by using serum levels measured in 390 pregnant women in the Collaborative Perinatal Project enrolled at 12 study centers in the

United States from 1959 to 1965. They estimated adjusted fecundability odds ratios by using Cox proportional hazards modeling for discrete time data. Compared with time to pregnancy for women in the lowest exposure category (PCBs < 1.24 microg/liter, DDE < 14 microg/liter), time to pregnancy increased for women in the highest exposure category in terms of both PCBs (fecundability odds ratio for PCBs > or = 5.00 microg/liter = 0.65, 95% confidence interval: 0.36, 1.18) and DDE (fecundability odds ratio for DDE > or = 60 microg/liter = 0.65, 95% confidence interval: 0.32, 1.31). Overall, time to pregnancy increased with increasing serum PCB levels but was less suggestive of an association with DDE. Both trends were imprecise and attenuated when expressed on a lipid basis. Overall, evidence of an association between PCB or DDE exposure and time to pregnancy was weak and inconclusive.

**Comparative study of dichlorodiphenyldichloroethylene in blood and semen of two young male populations: lack of relationship to infertility, but evidence of high exposure of the mothers.**

Charlier CJ, Foidart JM.

Reproductive Toxicology. 2005 Jul-Aug;20(2):215-220.

The purpose of the study was to investigate the possible effect of an environmental organochlorine, dichlorodiphenyldichloroethylene (p,p'-DDE), on male fertility assessed by main sperm variables, conducted through a case-control study on young men attending the andrology laboratory in the context of infertility investigation in the couple. Blood and semen samples were obtained from 73 young men considered as fertile based on semen analysis (controls) and 82 classified as subfertile or infertile (cases). Standard clinical analysis of semen was performed and identification and quantification of p,p'-DDE in serum and in seminal plasma were done by gas chromatography. No p,p'-DDE was detected in the seminal plasma of either group. Blood concentration of p,p'-DDE in both groups was very low and did not differ between cases and controls; however, blood samples were obtained from 23 mothers in the control group, and from 19 in the case group, and p,p'-DDE serum level was significantly higher in the mothers of subfertile men. These data suggest that male infertility could be associated with exposure of the mothers to p,p'-DDE with deleterious effects restricted to intra-uterine life and thus undetected in blood or seminal plasma of subfertile men.

**Exposure to PCBs and p,p'-DDE and human sperm chromatin integrity.**

Rignell-Hydbom A, Rylander L, Giwercman A, Jonsson BA, Lindh C, Eleuteri P, Rescia M, Leter G, Cordelli E, Spano M, Hagmar L.

Environmental Health Perspectives. 2005 Feb;113(2):175-179.

Persistent organochlorine pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (p,p'-DDE), the major metabolite of dichlorodiphenyltrichloroethane (DDT), are stable lipophilic compounds widely found in the environment and in the general population. They can enter the food chain, and their

negative impact on male reproduction is currently under active scrutiny. To explore the hypothesis that environmental exposure to these compounds is associated with altered sperm chromatin structure integrity in human sperm, we conducted a study of 176 Swedish fishermen (with low and high consumption of fatty fish, a very important exposure source of POPs). We determined serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and p,p'-DDE, and we used the sperm chromatin structure assay (SCSA) to assess sperm DNA/chromatin integrity. When CB-153 serum levels (individual dose range, 39-1,460 ng/g lipid) were categorized into equally sized quintiles, we found an association with the DNA fragmentation index (%DFI). A significantly lower %DFI was found in the lowest CB-153 quintile (< 113 ng/g lipid) compared with the other quintiles; there was a similar tendency, although not statistically significant, between %DFI and p,p'-DDE. These results suggest that POP exposure may have a slight negative impact on human sperm chromatin integrity.

**Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls.**

Denham M, Schell LM, Deane G, Gallo MV, Ravenscroft J, DeCaprio AP. Pediatrics. 2005 Feb;115(2):e127-134.

**BACKGROUND:** Children are commonly exposed at background levels to several ubiquitous environmental pollutants, such as lead and persistent organic pollutants, that have been linked to neurologic and endocrine effects. These effects have prompted concern about alterations in human reproductive development. Few studies have examined the effects of these toxicants on human sexual maturation at levels commonly found in the general population, and none has been able to examine multiple toxicant exposures. The aim of the current investigation was to examine the relationship between attainment of menarche and levels of 6 environmental pollutants to which children are commonly exposed at low levels, ie, dichlorodiphenyldichloroethylene (p,p'-DDE), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), mirex, lead, and mercury. **METHODS:** This study was conducted with residents of the Akwesasne Mohawk Nation, a sovereign territory that spans the St Lawrence River and the boundaries of New York State and Ontario and Quebec, Canada. Since the 1950s, the St Lawrence River has been a site of substantial industrial development, and the Nation is currently adjacent to a US National Priority Superfund site. PCB, p,p'-DDE, HCB, and mirex levels exceeding the US Food and Drug Administration recommended tolerance limits for human consumption have been found in local animal species. The present analysis included 138 Akwesasne Mohawk Nation girls 10 to 16.9 years of age. Blood samples and sociodemographic data were collected by Akwesasne community members, without prior knowledge of participants' exposure status. Attainment of menses (menarche) was assessed as present or absent at the time of the interview. Congener-specific PCB analysis was available, and all 16 PCB congeners detected in >50% of the sample were included in analyses (International Union of Pure and Applied Chemistry numbers 52, 70, 74, 84, 87, 95, 99, 101 [+90], 105, 110, 118, 138 [+163 and 164], 149 [+123], 153, 180, and

187). Probit analysis was used to determine the median age at menarche for the sample. Binary logistic regression analysis was used to determine predictors of menarcheal status. Six toxicants (p,p'-DDE, HCB, PCBs, mirex, lead, and mercury) were entered into the logistic regression model. Age, socioeconomic status (SES), and BMI were tested as potential cofounders and were included in the model at  $P < .05$ . Interactions among toxicants were also evaluated. RESULTS: Toxicant levels were measured in blood for this sample and were consistent with long-term exposure to a variety of toxicants in multiple media. Mercury levels were at or below background levels, all lead levels were well below the Centers for Disease Control and Prevention action limit of 10 microg/dL, and PCB levels were consistent with a cumulative, continuing exposure pattern. The median age at menarche for the total sample was 12.2 years. The predicted age at menarche for girls with lead levels above the median (1.2 microg/dL) was 10.5 months later than that for girls with lead levels below the median. In the logistic regression analysis, age was the strongest predictor of menarcheal status and SES was also a significant predictor but BMI was not. The logistic regression analysis that corrected for age, SES, and other pollutants (p,p'-DDE, HCB, mirex, and mercury) indicated that, at their respective geometric means, lead (geometric mean: 0.49 microg/dL) was associated with a significantly lower probability of having reached menarche ( $\beta = -1.29$ ) and a group of 4 potentially estrogenic PCB congeners (E-PCB) (geometric mean: 0.12 ppb; International Union of Pure and Applied Chemistry numbers 52, 70, 101 [+90], and 187) was associated with a significantly greater probability of having reached menarche ( $\beta = 2.13$ ). Predicted probabilities at different levels of lead and PCBs were calculated on the basis of the logistic regression model. At the respective means of all toxicants and SES, 69% of 12-year-old girls were predicted to have reached menarche. However, at the 75th percentile of lead levels, only 10% of 12-year-old Mohawk girls were predicted to have reached menarche; at the 75th percentile of E-PCB levels, 86% of 12-year-old Mohawk girls were predicted to have reached menarche. No association was observed between mirex, p,p'-DDE, or HCB and menarcheal status. Although BMI was not a significant predictor, we tested BMI in the logistic regression model; it had little effect on the relationships between menarcheal status and either lead or E-PCB. In models testing toxicant interactions, age, SES, lead levels, and PCB levels continued to be significant predictors of menarcheal status. When each toxicant was tested in a logistic regression model correcting only for age and SES, we observed little change in the effects of lead or E-PCB on menarcheal status. CONCLUSIONS: The analysis of multichemical exposure among Akwesasne Mohawk Nation adolescent girls suggests that the attainment of menarche may be sensitive to relatively low levels of lead and certain PCB congeners. This study is distinguished by the ability to test many toxicants simultaneously and thus to exclude effects from unmeasured but coexisting exposures. By testing several PCB congener groupings, we were able to determine that specifically a group of potentially estrogenic PCB congeners affected the odds of reaching menarche. The lead and PCB findings are consistent with the literature and are biologically plausible. The sample size, cross-sectional study design, and possible occurrence of cofounders beyond those tested suggest that results should be interpreted cautiously. Additional investigation to determine whether such low toxicant levels may affect reproduction and disorders of the reproductive system is warranted.

### **Organochlorine pesticides and male genital anomalies in the child health and development studies.**

Bhatia R, Shiao R, Petreas M, Weintraub JM, Farhang L, Eskenazi B.  
Environ Health Perspect. 2005 Feb;113(2):220-4.

Increasing rates of cryptorchidism and hypospadias in human populations may be caused by exogenous environmental agents. We conducted a case-control study of serum levels of p,p'-dichlorodiphenyltrichloroethane (DDT) and its major metabolite, p,p'-dichlorodiphenyldichloroethylene (DDE), and cryptorchidism and hypospadias in the Child Health and Development Study, a longitudinal cohort of pregnancies that occurred between 1959 and 1967, a period when DDT was produced and used in the United States. Serum was available from the mothers of 75 male children born with cryptorchidism, 66 with hypospadias, and 4 with both conditions. We randomly selected 283 controls from the cohort of women whose male babies were born without either of these conditions. Overall, we observed no statistically significant relationships or trends between outcomes and serum measures. After adjusting for maternal race, triglyceride level, and cholesterol level, compared with boys whose mothers had serum DDE levels < 27.0 ng/mL, boys whose mothers had serum DDE levels > or = 61.0 ng/mL had odds ratios of 1.34 [95% confidence interval (CI), 0.51-3.48] for cryptorchidism and 1.18 (95% CI, 0.46-3.02) for hypospadias. For DDT, compared with boys whose mothers had serum DDT levels < 10.0 ng/mL, boys whose mothers had serum DDT levels > or = 20.0 ng/mL had adjusted odds ratios of 1.01 (95% CI, 0.44-2.28) for cryptorchidism and 0.79 (95% CI, 0.33-1.89) for hypospadias. This study does not support an association of DDT or DDE and hypospadias or cryptorchidism.

### **Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males.**

Gladen BC, Klebanoff MA, Hediger ML, Katz SH, Barr DB, Davis MD, Longnecker MP.

Environ Health Perspect. 2004 Dec;112(17):1761-7.

DDT (dichlorodiphenyltrichloroethane), a pesticide once used widely in agriculture and now limited to public health use, remains a controversial chemical because of a combination of benefits and risks. DDT or its breakdown products are ubiquitous in the environment and in humans. Compounds in the DDT family have endocrine actions and have been associated with reproductive toxicity. A previous study reported associations between prenatal exposure to p,p'-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene] and increased height and weight in adolescent boys. We examined a group with higher exposures to see whether similar associations would occur. Our study group was 304 males born in Philadelphia in the early 1960s who had participated in a previous study. Anthropometric and pubertal measures from one to six visits during their adolescent years were available, as were stored maternal serum samples from pregnancy. We measured p,p'-DDE, p,p'-DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane], and o,p'-DDT [1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)-ethane] in the maternal



serum. Outcomes examined in the boys were height, ratio of sitting height to height, body mass index, triceps skinfold thickness, ratio of subscapular to the sum of triceps and subscapular skinfold thicknesses, skeletal age, serum testosterone, and serum dehydroepiandrosterone sulfate. No associations between prenatal exposure to any of the DDT compounds and any outcome measure were seen.

**The long-term effects of DDT exposure on semen, fertility, and sexual function of malaria vector-control workers in Limpopo Province, South Africa.**

Dalvie MA, Myers JE, Thompson ML, Robins TG, Dyer S, Riebow J, Molekwa J, Jeebhay M, Millar R, Kruger P.

Environmental research. 2004 Sep;96(1):1-8.

Hormonally active chemicals in the environment such as DDT have been associated with declining male reproductive health, especially semen quality. A cross-sectional study of 60 workers was performed near the Malaria Control Center (MCC) in Tzaneen, Limpopo Province, South Africa. Tests included a questionnaire (sexual function, fertility, and job history), a physical examination of the reproductive system, and semen analysis (produced via coitus interruptus or masturbation). Sperm count, density, and motility using the World Health Organization criteria and morphology using the strict Tygerberg criteria were determined. Serum o'p' and p'p' isomers of DDE, DDT, and DDD were measured. Forty-eight (81.0%) participants produced a semen sample, while all completed the questionnaires and physical examination. The mean sperm count was 93.8+/-130.3 million, and sperm density was 74.6+/-85.1 million/mL. The mean normal morphology score was 2.5+/-1.8% of subjects. Eighty-four percent of morphology scores were below either the WHO or the Tygerberg criteria, with the highest individual score being 6%. Self-perceived current problems with sexual function ranged between 10% and 20%. The most prevalent genital abnormality was abnormal testis disposition at 71%. There were few significant associations between DDT exposure measures (measured as years worked at MCC and serum DDT) and reproductive outcomes. p'p'-DDT was negatively associated with semen count (beta=-3.7+/-1.7; P=0.04; R<sup>2</sup>=0.05 adjusted for age, abstinence, physical abnormality, and fever in last 2 months). While the semen quality in the study was less than normal, no strong evidence for a DDT effect was found.

**Serum sex hormones in men occupationally exposed to dichloro-diphenyl-trichloro ethane (DDT) as young adults.**

Cocco P, Loviselli A, Fadda D, Ibba A, Melis M, Oppo A, Serra S, Taberlet A, Tocco MG, Flore C.

J Endocrinol. 2004 Sep;182(3):391-397.

To explore endocrine effects in relation to para,para'-dichloro-diphenyl-dichloro ethylene (p,p'-DDE) body burden and past occupational exposure to its precursor dichloro-diphenyl-trichloro ethane (DDT), we assayed serum sex hormones, including serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17beta-estradiol (E2),

testosterone and sex hormone binding globulin (SHBG), and p,p'-DDE levels in 107 male participants in a 1946-1950 anti-malarial campaign in Sardinia, Italy. Cumulative DDT exposure during the anti-malarial operations was retrospectively estimated from detailed reports of the anti-malarial agency. Ortho,para-DDE, and its precursor ortho,para-DDT were always below the detection limit. p,p'-DDT was detected in 14/107 subjects, and p,p'-DDE in 106/107 subjects. The median lipid-adjusted p,p'-DDE serum concentration over the total study population was 396 parts per billion (interquartile range 157-1045), and it did not vary according to the job at the time of anti-malarial operations, nor was it affected by cumulative DDT exposure. LH, FSH, and SHBG, but not testosterone or E2, showed a significant positive correlation with age. Neither current serum p,p'-DDE nor past cumulative DDT exposure affected sex hormone concentrations. Our results suggest that (1) the low current p,p'-DDE serum concentration does not affect serum hormone levels, and (2) past cumulative DDT exposure is not correlated with the current p,p'-DDE serum level, nor does it show persistent effects on serum hormone levels.

**Exposure to CB-153 and p,p'-DDE and male reproductive function.**

Rignell-Hydbom A, Rylander L, Giwercman A, Jonsson BA, Nilsson-Ehle P, Hagmar L. Hum Reprod. 2004 Sep;19(9):2066-75.

**BACKGROUND:** During the last decades, there has been concern that exposure to endocrine disruptors, such as persistent organochlorine pollutants (POPs), may contribute to an impairment of male reproductive function. To investigate whether exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE) affects semen quantity and quality and reproductive hormones, 195 Swedish fishermen, aged 24-65 years, were investigated. **METHODS:** The men provided semen samples which were analysed in a mobile laboratory unit. Blood samples and information relating to lifestyle, medical and reproductive history were obtained.

**RESULTS:** The subjects had a median CB-153 serum level of 193 ng/g lipid (range 39-1460) and a median p,p'-DDE serum level of 240 ng/g lipid (range 334-2251). When CB-153 was categorized into quintiles, the subjects in the quintile with the highest concentration (> 328 ng/g lipid), tended to have decreased sperm motility compared with the subjects in the lowest quintile (< 113 ng/g lipid). The age-adjusted mean difference was 9.9% (95% confidence interval -1.0 to 21% P = 0.08). We found no significant associations between p,p'-DDE and semen characteristics or reproductive hormones.

**CONCLUSION:** The association between CB-153 and sperm motility, although not formally significant, is of interest considering the possible endocrine-disrupting effects of polychlorinated biphenyls (PCBs).

**Polychlorinated biphenyls in colostrum milk and visual function at 12 months of life.**

Riva E, Grandi F, Massetto N, Radaelli G, Giovannini M, Zetterstrom R, Agostoni C. Acta Paediatr. 2004 Aug;93(8):1103-1107.

**BACKGROUND AND AIMS:** Environmental contaminants such as persistent organic chlorines and heavy metals, which are supplied to the foetus by transplacental transfer

and to breastfed infants by the milk, may impair cognitive functions. Long-chain polyunsaturated fatty acids, which are known to enhance development during foetal life and early infancy, may counteract the toxic effect of environmental contaminants. In this study, we have investigated whether polychlorinated biphenyls (PCBs) impair early development of vision, and whether such impairment can be modulated by essential long-chain polyunsaturated fatty acids. MATERIAL: Healthy term infants born in Milan and its surroundings, and who were exclusively breastfed for at least 4 mo, were prospectively examined up to the age of 12 mo. METHODS: Samples from colostrums, the first 2 d after delivery, and of mature breast-milk after 1 and 3 mo were collected. The samples were analyzed for PCB 105, 118, 138, 153, 156 and 180 and for DDT and DDE. In all infants, the plasma levels of the long-chain polyunsaturated fatty acids (LC-PUFAs), C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3 and C22:6 n-3 were analysed within the first three postnatal days. The PCB levels in colostrum milk, as well as of LC-PUFAs in plasma, were considered to mirror perinatal supply. Visual function was evaluated by P100 with latency evoked potentials (VEPs) at 12 mo of age. Statistical analysis was based on simple and partial correlation coefficients ( $p < 0.05$ ). RESULTS: On bivariate analysis, wave latency VEP at 15 min was significantly related to the colostrum levels of DDT, DDE and all examined PCBs except PCB 105 (with correlation coefficient  $r = 0.401$  to  $0.618$ ), whereas P100 wave latency VEP at 60 min was related to DDT ( $r = 0.513$ ) and PCB 180 ( $r = 0.504$ ). Infant plasma levels of C22:6 n-3 were inversely associated with P100 wave latency at 60 min ( $r = -0.418$ ) and at 1Hz-2J ( $r = -0.466$ ). After controlling for C22:6 n-3, the partial correlation coefficient of P100 wave latency VEP at 15 min to the colostrum level of PCB 180 was  $0.403$  ( $p = 0.07$ ). CONCLUSION: Within the population of this study, a weak relation was found between impaired visual function at 12 mo of age of healthy infants and the levels of PCBs, DDT and DDE in colostrum milk. The effect of impairment was no longer evident after controlling for the plasma level of LC-PUFAs as found in the infant a few days after birth.

#### **[DDT/DDE concentrations and risk of hypospadias. Pilot case-control study]**

[Article in Spanish]

Flores-Luevano S, Farias P, Hernandez M, Romano-Riquer P, Weber JP, Dewailly E, Cuevas-Alpuche J, Romieu I.

Salud Publica Mex. 2003 Nov-Dec;45(6):431-8.

OBJECTIVE: To evaluate the association between maternal serum DDT/DDE levels and risk of hypospadias. MATERIAL AND METHODS: A case-control study in Mexico City was conducted during 1997-1999 among 41 subjects with hypospadias and 28 controls. Blood samples were obtained from study subjects. Information was obtained using a standardized questionnaire. Data were analyzed using multivariate logistic regression models. RESULTS: Maternal DDT and DDE levels showed no association with hypospadias (OR 1.13; 95% CI 0.24-5.29 and OR = 0.48; 95% CI 0.15-1.60, respectively). Associated factors were older age of mothers (OR 8.69; 95% CI 1.69-44.9), and working during pregnancy (OR 4.68; 95% CI 1.15-18.9). CONCLUSIONS:

Information about the endocrine effects of different levels of DDT/DDE in human subjects is scarce; dosage may be a determinant factor of the type of effect. The English version of this paper is available at:<http://www.insp.mx/salud/index.html>.

**PCBs, hexachlorobenzene and DDE are not associated with recurrent miscarriage.**

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K.

Am J Reprod Immunol. 2003 Dec;50(6):485-9.

**PROBLEM:** A case-control study was designed to evaluate any associations between high exposure to polychlorinated biphenyls (PCB), hexachlorobenzene (HCB) and the 1,1,1,-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) and recurrent miscarriage and immunoendocrine abnormalities. **METHODS OF STUDY:** A total of 18 kinds of co-planer PCBs, HCB, DDE, natural killer cell (NK) activity, antiphospholipid antibodies, antinuclear antibody, prolactin, progesterone, thyroid-stimulating hormone (TSH) and free T4 were examined in 45 patients with a history of three or more (3-11) consecutive first-trimester miscarriages and 30 healthy women with no history of live birth and infertility. **RESULTS:** There were no differences in mean +/- S.D. values in serum samples for PCBs, HCB and DDE between patients and controls. Hypothyroidism, hyperprolactinemia, luteal phase defects, NK cell activity and the presence of autoantibodies were also not associated with levels of any of the compounds in the patients. **CONCLUSION:** PCBs, HCB and DDE are not associated with miscarriage and immunoendocrine abnormalities in patients with a history of recurrent miscarriage.

**Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns.**

Ribas-Fito N, Sala M, Cardo E, Mazon C, De Muga ME, Verdu A, Marco E, Grimalt JO, Sunyer J.

Occupational and environmental medicine. 2003 Apr;60(4):301-303.

**AIMS:** To assess the association between prenatal exposure to organochlorine compounds and thyroid status in newborns from an area with high levels of hexachlorobenzene (HCB). **METHODS:** A total of 98 mother-infant pairs (83.1% of all children born during the period 1997-99 in a specific area polluted with HCB) were recruited. Levels of organochlorine compounds were measured in 70 cord serum samples. Concentrations of thyroid stimulating hormone (TSH) were measured in plasma of all newborns three days after birth. **RESULTS:** All newborns had concentrations of TSH within the range of normal reference values (<25 mU/l). Dichlorodiphenyl dichloroethylene (p,p'DDE), beta-hexachlorocyclohexane (beta-HCH), polychlorinated biphenyl (PCB) 138 and 118 were related to higher concentrations of TSH, although only significant for beta-HCH. Levels of HCB were not associated with TSH. **CONCLUSIONS:** Although this community is highly exposed to HCB, no association was found between this organochlorine and TSH concentrations at birth.

**Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study.**

Weisskopf MG, Anderson HA, Hanrahan LP; Great Lakes Consortium.

Environ Health. 2003 Mar 12;2(1):2.

**BACKGROUND:** Fish from the Great Lakes are contaminated with polychlorinated biphenyls, which have been found to have several adverse reproductive effects. Several environmental contaminants have been found to alter the sex ratio of offspring at birth, but the evidence of such an effect of polychlorinated biphenyls has been inconsistent. **METHODS:** We examined parental serum polychlorinated biphenyl concentration in relation to the sex ratio of 173 children of mothers and 208 children of fathers from the Great Lakes region of the United States between 1970 and 1995. We calculated odds ratios for a male child using logistic regression and generalized estimating equations with adjustment for the year of birth of the child, maternal and paternal age, the mother's parity at the child's birth, and whether the child had an older brother. **RESULTS:** The adjusted odds ratio for having a male child among mothers in the highest quintile of serum polychlorinated biphenyl concentration was 0.18 (95% CI: 0.06-0.59) compared to mothers in the lowest quintile. Treating exposure as a continuous variable, the adjusted odds ratio for having a male child was 0.54 per unit increase in the natural log of maternal serum polychlorinated biphenyl concentration (95% CI: 0.33-0.89). There was little evidence of an association with paternal exposure. We found no association between either maternal or paternal serum dichlorodiphenyl-dichloroethene concentration and the sex ratio. **CONCLUSIONS:** These findings suggest that maternal exposure to polychlorinated biphenyls may decrease the sex ratio of offspring. These data add to the growing body of evidence that exposure to particular chemicals can alter the sex ratio at birth.

**Persistent organochlorine compounds and birth weight.**

Gladen BC, Shkiryak-Nyzhnyk ZA, Chyslovska N, Zadorozhnaja TD, Little RE.

Ann Epidemiol. 2003 Mar;13(3):151-7.

Comment in:

Ann Epidemiol. 2004 Feb;14(2):151; author reply 152-3.

**PURPOSE:** To determine whether weight at birth is related to prenatal exposure to persistent organochlorine compounds. **METHODS:** Birth weight was obtained for 197 singleton infants drawn from the general population born in two cities in Ukraine in 1993 to 1994. Concentrations of seven organochlorine pesticides (p,p'-DDT, p,p'-DDE, beta-hexachlorocyclohexane, hexachlorobenzene, trans-nonachlor, oxychlorane, heptachlor epoxide) and 11 polychlorinated biphenyl congeners measured in maternal milk taken at four or five days after birth were used as an index of prenatal exposure. **RESULTS:** The greatest differences were seen for beta-hexachlorocyclohexane, with a pattern not suggestive of dose-response; infants in the lowest tertile were small, those in the central tertile were large, and those in the upper tertile were average. Adjustment for gestational age and other potential confounders had little effect on these patterns. Infants in the two

upper tertiles for p,p'-DDE were larger than those in the lower tertile, with the effect being more striking after adjustment for gestational age. Adjustment for potential confounders made the pattern disappear. Other chemicals showed no convincing evidence of effects. **CONCLUSIONS:** Prenatal exposure to the chemicals studied, at concentrations currently seen in this population, does not impact weight at birth.

**Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring.**

Longnecker MP, Klebanoff MA, Brock JW, Zhou H, Gray KA, Needham LL, Wilcox AJ.

Am J Epidemiol. 2002 Feb 15;155(4):313-22.

1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) is a metabolite of the insecticide 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and is a ubiquitous environmental contaminant. Nearly everyone in the United States has a detectable serum level of DDE. DDE was recently found to inhibit binding of androgen to its receptor and to block androgen action in rodents. Normal development of male genitalia in mammals depends on androgen action. The authors used stored serum samples to examine the relation between maternal DDE levels during pregnancy and adjusted odds of cryptorchidism (n = 219), hypospadias (n = 199), and polythelia (extra nipples) (n = 167) among male offspring, using a nested case-control design with one control group (n = 552). Subjects were selected from the Collaborative Perinatal Project, a US birth cohort study begun in 1959-1966, when DDE levels were much higher than they are at present. Compared with boys whose mother's recovery-adjusted serum DDE level was less than 21.4 microg/liter, boys with maternal levels greater than or equal to 85.6 microg/liter had adjusted odds ratios of 1.3 (95% confidence interval (CI): 0.7, 2.4) for crypt-orchidism, 1.2 (95% CI: 0.6, 2.4) for hypospadias, and 1.9 (95% CI: 0.9, 4.0) for polythelia. For cryptorchidism and polythelia, the results were consistent with a modest-to-moderate association, but in no instance was the estimate very precise. The results were inconclusive.

**Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence.**

Darvill T, Lonky E, Reihman J, Stewart P, Pagano J.

Neurotoxicology. 2000 Dec;21(6):1029-38.

A sample of infants was examined at conceptual age 67 weeks (6-month testing, N=230), and again at conceptual age 92 weeks (12-month testing, N=216) using the Fagan Test of Infant Intelligence (FTII). Analysis of the results revealed a dose-dependent relationship between total umbilical cord-blood PCB levels and poorer FTII performance at both ages. A similar relationship was observed using a subset of the persistent and heavily chlorinated PCB congeners associated with Lake Ontario fish consumption (septa-, octa-, and nonachlorinated biphenyls) in children tested at 12-months but not at 6-months.

These data replicate previous research (Jacobson et al., 1985) which demonstrated a dose-dependent relationship between prenatal PCB exposure and FTII performance in infants of Lake Michigan fish eaters. Analyses of FTII scores with cord-blood DDE and maternal hair methylmercury (MeHg) revealed no significant associations between FTII performance and either of these toxicants. The latter results replicate those of Myers et al., (1995) who found no effect of MeHg on FTII performance in Seychellois infants.

**Organochlorine pesticides and preterm labour in human beings.**

Sharma M, Bhatnagar P.

Curr Sci. 1996 Oct;71(8):628-631.

In this study, we compare organochlorine pesticides, viz. aldrin, isomers of HCH, metabolites of DDT and heptachlor in the circulating blood, cord blood and placenta of pregnant women undergoing full term normal delivery with those in premature labour. The data and the statistical outcome of the study show that the mean concentrations of organochlorine pesticides in maternal blood of cases of premature labour were not found to be significantly different from those of controls (full term normal delivery). Similar results were obtained from the residue levels of cord blood and placental tissue. An attempt has also been made to correlate the high concentration of chlorinated hydrocarbon pesticides with the initiation of premature labour on the basis of existing evidences.

**The role of DDE and polychlorinated biphenyl levels in preterm birth.**

Berkowitz GS, Lapinski RH, Wolff MS

Arch Environ Contam Toxicol 1996 Jan;30(1):139-41

Maternal serum levels of DDE and polychlorinated biphenyls (PCB) and their congeners were compared for 20 women who had a spontaneous preterm delivery and 20 matched women who had delivered at term at Mount Sinai Medical Center in New York between October 1990 and August 1993. Since no substantial case-control differences were evident, these findings do not indicate that increased DDE or PCB levels are associated with spontaneous preterm birth.

**Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development.**

Gladen BC, Rogan WJ.

J Pediatr. 1991 Jul;119(1 ( Pt 1)):58-63.

OBJECTIVE: Determining whether early developmental effects of perinatal exposure to polychlorinated biphenyls (PCBs) or dichlorodiphenyl dichloroethene (DDE) persist.

DESIGN: Cohort followed from birth; ages now 5 1/2 to 10 1/2 years. SETTING:

General community. PARTICIPANTS: Volunteer sample of 859 children, of whom 712

had been examined with the McCarthy Scales of Children's Abilities at 3, 4, or 5 years; 506 sent report cards. INTERVENTIONS: None. MEASUREMENTS AND RESULTS: Neither transplacental nor breast-feeding exposure to PCBs or DDE affected McCarthy scores at 3, 4, or 5 years. There was no statistically significant relationship between poorer grades and PCB or DDE exposure by either route. CONCLUSIONS: The deficits seen in these children on the Bayley Scales of Infant Development through 2 years of age are no longer apparent.

D. Meeting abstracts reporting no increased risk of adverse developmental or reproductive outcomes

**Prenatal Exposure To Organophosphates And Organochlorines And Performance On The Brazelton Neonatal Behavioral Assessment Scale.**

Engel SM, Berkowitz GS, Barr DB, Teitelbaum S, Siskind J, Meisel S, Wolff MS. Am J Epidemiol. 2006 Jun;163(11 Suppl):S69.

In order to evaluate the effect of in utero pesticide exposure on neurodevelopment, we enrolled a multiethnic cohort of primiparous mothers and their infants who were delivered at Mount Sinai Hospital in New York City between May 1998 and May 2002. Before hospital discharge, the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) was administered to 311 infants. Prenatal maternal urines collected in the third trimester were analyzed for the presence of dialkylphosphate (DAP) metabolites, including three diethylphosphate (DEP) and three dimethylphosphate (DMP) metabolites; and malathion dicarboxylic acid (MDA). A subset of maternal peripheral blood samples were analyzed for the presence of polychlorinated biphenyls (PCBs) and 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene (DDE). For each log unit increase in prenatal urine DEP level, the number of abnormal reflexes elicited increased by 1.38 (95% CI 1.01, 1.89) in an adjusted Poisson model. A similar increase was found for total DAPs. Similarly, MDA levels above the limit of detection (LOD) were associated with a 2.28 increase in the number of abnormal reflexes (95% CI 1.58, 3.27). There was no negative association with PCBs or DDE and any of the BNBAS domains. These results confirm a recently reported association between organophosphate metabolite levels and risk of abnormal reflexes in offspring. However, our results conflict with previous reports of neonatal hypotonicity associated with prenatal PCB exposure. We believe the latter may be explained by the relatively low levels of these contaminants in our population.



## **Measuring The Effects Of DDT And DDE On Preterm Delivery, Small-For-Gestational Age Birth And Low Birthweight Birth.**

Farhang L, Bhatia R, Weintraub J, Petreas M, Eskenazi B

Epidemiology 2004 Jul;15(4):S187

Objective: Though banned in the United States since 1972, the pesticide 1,1,1-trichloro-2,2,2'-bis(p-chlorophenyl)ethane (DDT) is used elsewhere for mosquito control. Its human health costs and benefits thus remain a current concern. DDT, and its persistent metabolite DDE, have been associated with negative reproductive outcomes in birds, rabbits and sea lions. However, there are inconsistencies regarding exposure to DDT and effects on human health. This study was designed to evaluate the effects of serum levels of DDT and its major metabolite DDE on three adverse human birth outcomes - preterm delivery, small-for-gestational-age birth and low birthweight. Methods: Nested case-control study. We studied a sample of male live births from a longitudinal cohort study of 20,754 pregnancies that occurred between 1959 and 1967 (a period of high domestic DDT usage in the United States). The 283 male subjects included in this analysis were randomly selected controls from another study of the effects of organochlorine pesticides on male genital anomalies. Birth outcome data and maternal demographic/behavioral data were collected through maternal interviews and abstracted medical records. Maternal serum samples were taken during pregnancy and post-partum. Serum DDT and DDE were determined by gas chromatography/electron capture detection (GC/ECD). Data were analyzed using multivariate logistic regression for preterm delivery and small-for-gestational age birth and linear regression for birthweight. Results: Mean serum levels for DDT and DDE were 1.6 and 5.9 ug/g lipid, respectively. Variables identified as significant in bivariate analysis against each outcome variable were included as covariates in multivariate analysis. Preliminary findings indicate that maternal DDT and DDE levels, controlling for a range of demographic and behavioral variables associated with birth outcomes (i.e., age, race, education, marital status, income, occupation, place of birth, parity, BMI, and smoking and alcohol use) do not predict preterm delivery (n=23); small-for-gestational age birth (n=28), or low birthweight (n=13). Final results of regression analyses, including odds ratios and confidence intervals, will be presented. Discussion: The effect of DDT and DDE on human birth outcomes in this study appears null. However, we were limited by the small size of the study and the fact that we were only able to examine live births. Considering the persistence of DDT in the environment and its role in malaria control, further studies using more robust data should continue to assess this relationship.

### E. Studies with unclear findings

#### **Reproductive outcomes following environmental exposure to DDT.**

Cocco P, Fadda D, Melis M.

Reproductive Toxicology. 2006 Jul;22(1):5-7.

We used official statistics of births and stillbirths in 1945-1954 to evaluate reproductive outcomes in the general population following use of DDT during a 1946-1950 anti-

malarial campaign in the Italian region of Sardinia. Due to the disruption of registration systems in the World War II years, data in the pre-DDT years were available only for 1945-1946. Such a short period of observation, and social conditions in the war and post-war years, do not allow exclusion of adverse effects of DDT on birth rate; however, we did not observe an effect. The stillbirth rate, infant mortality rate, and male/female ratio in newborns were apparently unaffected following widespread but focused use of DDT in Sardinia, Italy.

**Reproductive outcomes in DDT applicators.**

Cocco P, Fadda D, Ibba A, Melis M, Tocco MG, Atzeri S, Avataneo G, Meloni M, Monni F, Flore C.

Environmental Research. 2005 May;98(1):120-126.

**OBJECTIVES:** To explore reproductive outcomes in relation to occupational exposure to DDT. **METHODS:** We inquired into the reproductive history, including total number of children, sex distribution in the offspring, time-to-pregnancy, and number of spontaneous abortions and stillbirths, of the spouses of 105 men first exposed to DDT in a 1946-1950 anti-malarial campaign in Sardinia, Italy. The time-to-pregnancy in months at the first successful conception was estimated from population Registrars. Cumulative DDT exposure during the anti-malarial campaign was retrospectively estimated. **RESULTS:** The stillbirth rate was elevated and the male/female ratio in the offspring was reversed among DDT-exposed workers, and particularly among DDT applicators, compared to the unexposed subjects. Among DDT applicators, the stillbirth rate increased and the male/female ratio decreased by the tertile of cumulative DDT exposure. The fecundity ratio among spouses of DDT applicators was 0.72 (95% CI, 0.41,1.21) compared to the unexposed. The average number of children and abortion rate were unaffected by DDT exposure. **CONCLUSIONS:** The low statistical power of our study does not allow definitive conclusions. However, the results prompt further in-depth research into adverse reproductive outcomes and reduced fertility among men heavily exposed to DDT.

**The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p,p'-DDE.**

Hauser R, Chen Z, Pothier L, Ryan L, Altshul L.

Environmental Health Perspectives. 2003 Sep;111(12):1505-1511.

Scientific and public concern exists about potential reproductive health effects of persistent chlorinated organic chemicals, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE, the most stable daughter compound of DDT). To explore the hypothesis that environmental exposures to PCBs and DDE are associated with altered semen parameters, we conducted a cross-sectional study of 212 male partners of subfertile couples who presented to the Massachusetts General Hospital Andrology Laboratory. Semen parameters were analyzed as both a continuous measure and dichotomized based on World Health Organization reference values for sperm concentration (< 20 million/mL), motility (< 50% motile), and

Kruger strict criteria for morphology (< 4% normal). The comparison group for the dichotomized analysis was men with all three semen parameters above the reference values. In serum, 57 PCB congeners and p,p'-DDE were measured by congener-specific analysis using gas chromatography with electron capture detection. There were dose-response relationships among PCB-138 and sperm motility (odds ratio per tertile, adjusted for age, abstinence, and smoking, and p-value for trend were, respectively, 1.00, 1.68, 2.35, and p-value = 0.03) and morphology (1.00, 1.36, 2.53, p-value = 0.04). There was limited evidence of an inverse relationship between sum of PCBs, as well as those PCBs classified as cytochrome P450 enzyme inducers, with sperm motility and sperm morphology, as well as limited evidence of an inverse association between p,p'-DDE and sperm motility. The lack of a consistent relationship among semen parameters and other individual PCB congeners and groupings of congeners may indicate a difference in spermatotoxicity between congeners.

#### **DDT and DDE exposure in mothers and time to pregnancy in daughters.**

Cohn BA, Cirillo PM, Wolff MS, Schwingl PJ, Cohen RD, Sholtz RI, Ferrara A, Christianson RE, van den Berg BJ, Siiteri PK.

Lancet. 2003 Jun 28;361(9376):2205-6. Erratum in: Lancet. 2003 Nov 1;362(9394):1504.

Reproductive-tract anomalies after administration of the potent oestrogen, diethylstilboestrol, in pregnant women raised concerns about the reproductive effects of exposure to weakly oestrogenic environmental contaminants such as bis[4-chlorophenyl]-1,1,1-trichloroethane (p,p'-DDT) or its metabolites, such as bis[4-chlorophenyl]-1,1-dichloroethene (p,p'-DDE). We measured p,p'-DDT and p,p'-DDE in preserved maternal serum samples drawn 1-3 days after delivery between 1960 and 1963. We recorded time to pregnancy in 289 eldest daughters 28-31 years later. Daughters' probability of pregnancy fell by 32% per 10 microg/L p,p'-DDT in maternal serum (95% CI 11-48). By contrast, the probability of pregnancy increased 16% per 10 microg/L p,p'-DDE (6-27). The decreased fecundability associated with prenatal p,p'-DDT remains unexplained. We speculate that the antiandrogenic activity of p,p'-DDE may mitigate harmful androgen effects on the ovary during gestation or early life.

#### **F. Related Studies**

#### **The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study.**

Noakes PS, Taylor P, Wilkinson S, Prescott SL.

Chemosphere. 2006 May;63(8):1304-1311.

BACKGROUND: Modern persistent organic pollutants (POPs) contamination are logical candidates in the investigation of the, as yet, unexplained association between allergic disease and progressive industrialisation. POPs have been detected in human cord blood, placental tissues and breast milk, and the reported association between cord blood IgE levels and cord/placental POP levels has raised concerns about potential immunological

effects in early life. **METHODS:** The initial aim of this study was to determine if POPs were detectable in maternal blood, cord blood, placental tissues, adipose tissue and breast milk samples from randomly selected Western Australian women (n = 31), where allergic disease is epidemic. Gas chromatography was used to detect polychlorinated biphenyl compounds [PCBs] (as Aroclor 1232, 1254, 1260) and organochlorine (OC) pesticides, including p,p'-DDT, p,p'-DDE, hexachlorobenzene (HCB), lindane, heptachlor epoxide, dieldrin and chlordane. Secondly, we assessed the relationship between POP levels detected in vivo and maternal and neonatal responses (cytokine and lymphoproliferation) to allergens and mitogens. **RESULTS:** Low level POP contamination was detected in adipose tissue and breast milk (but not in cord blood, maternal blood or placental tissues). The most ubiquitous compound found in over 90% of adipose tissues samples was a OC metabolite of DDT, p,p'-DDE (median 0.07 mg/kg; interquartile range [IQR] 0.05-0.12). However, the majority of other OC compounds were not detectable and PCB were not detectable in any samples. The three main residues detected in breast milk were p,p'-DDE (0.003 mg/l; 0.001-0.009), dieldrin (0.001 mg/l; 0.001-0.046) and HCB (0.001 mg/l; 0.001-0.001). These levels are significantly lower than reported over 20 years ago. There were no consistent relationships between POP levels in vivo and maternal or infant responses, with the exception of a significant inverse association (Spearman rank correlation:  $r = -0.406$ ,  $p = 0.049$ ) between maternal adipose tissue levels of OC p,p'-DDE and maternal T helper cell Type 1 interferon [IFN] gamma to mitogens. **CONCLUSION:** This study provides the first evidence (in Australia) since the early 1990's that adipose OC levels have continued to fall. The negligible levels in this randomly selected group are significantly lower than those previously recorded, suggesting that POP contamination (at biologically relevant levels) is not likely to be a major contributing factor in the increasing rates of allergy in Western Australia. However, the relationship between Th1 immune function and OC contamination is consistent with other reports and is worth investigating as a relevant factor in populations where OC contamination is greater.

**Rapid action of pesticides on cytosolic calcium concentrations in cultures of human umbilical vein endothelial cells.**

Younglai EV, Wu Y, Foster WG.

Reproductive Toxicology. 2006 Apr;21(3):271-279.

Persistent metabolites of pesticides such as p,p'-DDE, at environmentally relevant concentrations, have been shown to have a rapid effect on intracellular calcium  $[Ca^{2+}]_i$  concentrations in human granulosa-lutein cells. Since endocrine disrupting substances can be transferred from the maternal circulation to the fetus the present study examined whether the pesticides, kepone, o,p'-DDE, p,p'-DDE and methoxychlor, could alter cytoplasmic calcium  $[Ca^{2+}]_{cyt}$  concentrations in human umbilical vein endothelial (HUVE) cells. Cultured HUVE cells were loaded with Fura-2 AM and changes in  $[Ca^{2+}]_{cyt}$  of single cells were studied using a dynamic digital  $Ca^{2+}$  imaging system. Kepone and methoxychlor consistently increased  $[Ca^{2+}]_{cyt}$  concentrations, similar to the effects of estradiol and progesterone. p,p'-DDE increased  $[Ca^{2+}]_{cyt}$  concentrations in

80% of experiments whereas o,p-DDE stimulated its increases in 42%. Estrone, estriol, pregnenolone and cortisol were not effective. These results demonstrate that pesticides can have a rapid effect on HUVE cells probably through a nongenomic mechanism of action.

**Environmental pathways of exposure to DDT for children living in a malarious area of Chiapas, Mexico.**

Herrera-Portugal C, Ochoa H, Franco-Sanchez G, Yanez L, Diaz-Barriga F.  
Environmental Research. 2005 Oct;99(2):158-163.

Considering that DDT was used for control of malaria vectors in Mexico, and taking into account that the information regarding children in areas exposed to DDT is scarce, we started a research program for the assessment of health effects in children living in DDT sprayed areas. In this first report, we present information about pathways of exposure in two communities with a different history of exposure to DDT. Environmental pathways such as outdoor soils, indoor soils and household dust were assessed comparing a community highly exposed to DDT (HEC) and a community less exposed to DDT (LEC). Also in these communities, a cross-sectional study of 60 children (30 in each community) aged 6--12 years was conducted. Tests included a questionnaire and the measurement of whole blood DDT and DDE. Results show that in children living in the HEC, DDT and DDE mean blood levels were higher (15.9+/-8.2 and 58.2+/-29.2 microg/L) than in the LEC (1.9+/-3.6 and 9.2+/-5.7 microg/L) (P<0.01). Concentrations of DDT, DDE and DDD in indoor soil were higher in the HEC (10.3+/-10; 4.9+/-5.8; and 4.4+/-9.1mg/kg) than in the LEC (0.3+/-0.3; 0.04+/-0.06; and 0.03+/-0.04 mg/kg) (P<0.001). Similar results were obtained for outdoor soils; in the HEC, levels for DDT, DDE and DDD were 3.1+/-3.0; 1.0+/-0.8; and 0.3+/-0.2mg/kg; whereas levels in the LEC were 0.16+/-0.2; 0.02+/-0.03; and 0.02+/-0.03 mg/kg (P<0.001). High concentrations of DDT, DDE, and DDD were obtained in samples of indoor dust collected from the walls in the HEC (17.5+/-10.0; 5.5+/-6.2; and 9.8+/-16.8 mg/kg); levels in the LEC were lower (0.6+/-0.9; 0.07+/-0.1; and 0.05+/-0.07 mg/kg) (P<0.001). We did not find any correlation between blood levels of DDE and total DDT with environmental concentrations but there levels increased in LEC and HEC as the frequency of fish consumption increased (P<0.01).

**Effect of single and repeated in vitro exposure of ovarian follicles to o,p'-DDT and p,p'-DDT and their metabolites.**

Wójtowicz AK, Gregoraszczyk EL, Ptak A, Falandysz J.  
Pol J Pharmacol. 2004 Jul-Aug;56(4):465-72.

The aim of the presented study was to compare the effect of o,p'-DDT [1,1-dichloro-2,2-bis-(p,p'-chlorophenyl)-ethylene] and p,p'-DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)-ethane] and their metabolites DDE and DDD on estradiol secretion by ovarian follicles, the target organs of environmental estrogens. Theca interna (Tc) and granulosa cells (Gc) were collected from medium size porcine follicles and cultured as a monolayer. The cells

were initially cultured for 24 h to allow attachment to the plates and then media were changed for the new ones and o,p'-DDT and p,p'-DDT and their metabolites: o,p'-DDE, p,p'-DDE and o,p'-DDD were added at doses of 4, 40, 400 ng and 4 microg/ml medium to investigate dose-dependent effects. Media were collected after 24 h and frozen for estradiol content determination. When the effect of single and repeated exposure was investigated, the lowest dose of 4 ng/ml and the highest one of 4 microg/ml were chosen on the basis of the results of Experiment 1. o,p'-DDT exerted antiestrogenic action at all doses used while its metabolites and p,p'-DDT and its metabolites decreased estradiol secretion only when present in the medium at a dose of 4 ng/ml. The highest doses caused the increase in estradiol secretion. Parent o,p'-DDT and its metabolites showed antiestrogenic action after single exposure to 4 ng/ml while parent p,p'-DDT and its metabolites caused estrogenic action. All investigated compounds, except o,p'-DDT, increased estradiol secretion after single exposure to the dose of 4 microg/ml. Repeated exposure resulted in a massive antiestrogenic action of all investigated chemicals. In conclusion, our study points to time-dependent effect of DDT and its metabolites on ovarian follicles with the strongest estrogenic properties observed after single exposure and antiestrogenic action caused by repeated exposure. Given the duration of folliculogenesis, one can imagine many different potential mechanisms by which DDT could influence steroidogenesis.

**Synergistic effects between FSH and 1,1-dichloro-2,2-bis(P-chlorophenyl)ethylene (P,P'-DDE) on human granulosa cell aromatase activity.**

Younglai EV, Holloway AC, Lim GE, Foster WG.

Hum Reprod. 2004 May;19(5):1089-1093.

**BACKGROUND:** 1,1-Dichloro-2,2-bis(P-chlorophenyl)ethylene (P,P'-DDE, DDE), a metabolite of DDT, is a persistent hormonally active environmental toxicant which has been found in human serum and follicular fluid. The objective of this study was to investigate the interaction between FSH and ppDDE on aromatase activity in primary cultures of human granulosa cells. **METHODS:** Granulosa cells were obtained at the time of oocyte retrieval for IVF procedures and cultured in defined medium containing FSH and environmentally relevant concentrations of DDE. Aromatase activity was measured by incubating the cells with 1beta-[(3)H]androstenedione and measuring the release of (3)H(2)O. **RESULTS:** The granulosa cell response to FSH was highly dependent on the basal level of aromatase activity ( $r = -0.703$ ,  $P = 0.001$ ,  $n = 17$ ) with the highest activity occurring at low basal levels of aromatase activity. Enzyme activity was significantly stimulated at 100 ng DDE/ml. A synergistic effect on aromatizing activity was observed when cells were co-cultured with DDE and FSH. **CONCLUSIONS:** Concentrations of DDE similar to those present in human follicular fluid enhance basal and FSH-stimulated granulosa cell aromatizing enzyme activity.

**Contamination of human ovarian follicular fluid and serum by chlorinated organic compounds in three Canadian cities.**

Jarrell JF, Villeneuve D, Franklin C, Bartlett S, Wrixon W, Kohut J, Zouves CG.  
CMAJ. 1993 Apr 15;148(8):1321-7.

**OBJECTIVES:** To determine the extent of contamination of ovarian follicular fluid and serum samples in women undergoing in-vitro fertilization and to study the effect of the contaminants on reproductive outcome. **DESIGN:** Inception cohort study. **PATIENTS:** Seventy-four women undergoing in-vitro fertilization at three regional clinics in Halifax, Hamilton, Ont., and Vancouver. **MAIN OUTCOME MEASURES:** Follicular fluid and serum levels of contaminants, cleavage rates and time to cleavage of first egg. **RESULTS:** Five chlorinated organic chemicals were frequently found in the two types of samples: alpha-chlordane (ALCH), dichlorochlorophenylethylene (DDE), heptachloroepoxide-oxychlordane (OXCH), hexachlorobenzene (HCB) and polychlorinated biphenyl (PCB). The levels were generally low. Regional differences between the three clinics were present. Samples from the Halifax clinic had the lowest frequency and level of contamination. The source of drinking water (well, bottled or municipal) was an important confounder. The concentrations of the five contaminants did not affect the cleavage rate or the time to cleavage of the first egg. **CONCLUSION:** Trace amounts of toxic and persistent chlorinated organic chemicals found in the follicular fluid of Canadian women undergoing in-vitro fertilization did not seem to have any adverse biologic effect on the rate of fertilization and the time to cleavage. Reasons for regional differences in the concentrations of contaminants require

G. Studies without abstracts

**Persistent organochlorine pesticides levels in blood serum lipids in women bearing babies with undescended testis.**

Waliszewski SM, Infanzon RM, Arroyo SG, Pietrini RV, Carvajal O, Trujillo P, Hayward-Jones PM.  
Bull Environ Contam Toxicol. 2005 Nov;75(5):952-959.

## II. Animal DART Studies

### A. Studies reporting developmental or reproductive toxicity

#### **Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat**

Gray LE Jr., Wolf C, Lambright C, Mann P, Price M, Cooper RL, and Ostby J  
Toxicol Ind Health 1999;15(1-2):94-118

Antiandrogenic chemicals alter sexual differentiation by a variety of mechanisms, and as a consequence, they induce different profiles of effects. For example, in utero treatment with the androgen receptor (AR) antagonist, flutamide, produces ventral prostate agenesis and testicular nondescent, while in contrast, finasteride, an inhibitor of 5 alpha-dihydrotestosterone (DHT) synthesis, rarely, if ever, induces such malformations. In this regard, it was recently proposed that dibutyl phthalate (DBP) alters reproductive development by a different mechanism of action than flutamide or vinclozolin (V), which are AR antagonists, because the male offsprings display an unusually high incidence of testicular and epididymal alterations--effects rarely seen after in utero flutamide or V treatment. In this study, we present original data describing the reproductive effects of 10 known or suspected anti-androgens, including a Leydig cell toxicant ethane dimethane sulphonate (EDS, 50 mg kg<sup>-1</sup> day<sup>-1</sup>), linuron (L, 100 mg kg<sup>-1</sup> day<sup>-1</sup>), p,p'-DDE (100 mg kg<sup>-1</sup> day<sup>-1</sup>), ketoconazole (12-50 mg kg<sup>-1</sup> day<sup>-1</sup>), procymidone (P, 100 mg kg<sup>-1</sup> day<sup>-1</sup>), chlozolate (100 mg kg<sup>-1</sup> day<sup>-1</sup>), iprodione (100 mg kg<sup>-1</sup> day<sup>-1</sup>), DBP (500 mg kg<sup>-1</sup> day<sup>-1</sup>), diethylhexyl phthalate (DEHP, 750 mg kg<sup>-1</sup> day<sup>-1</sup>), and polychlorinated biphenyl (PCB) congener no. 169 (single dose of 1.8 mg kg<sup>-1</sup>). Our analysis indicates that the chemicals discussed here can be clustered into three or four separate groups, based on the resulting profiles of reproductive effects. Vinclozolin, P, and DDE, known AR ligands, produce similar profiles of toxicity. However, p,p'-DDE is less potent in this regard. DBP and DEHP produce a profile distinct from the above AR ligands. Male offsprings display a higher incidence of epididymal and testicular lesions than generally seen with flutamide, P, or V even at high dosage levels. Linuron treatment induced a level of external effects consistent with its low affinity for AR [reduced anogenital distance (AGD), retained nipples, and a low incidence of hypospadias]. However, L treatment also induced an unanticipated degree of malformed epididymides and testis atrophy. In fact, the profile of effects induced by L was similar to that seen with DBP. These results suggest that L may display several mechanisms of endocrine toxicity, one of which involves AR binding. Chlozolate and iprodione did not produce any signs of maternal or fetal endocrine toxicity at 100 mg kg<sup>-1</sup> day<sup>-1</sup>. EDS produced severe maternal toxicity and a 45% reduction in size at birth, which resulted in the death of all neonates by 5 days of age. However, EDS only reduced AGD in male pups by 15%. Ketoconazole did not demasculinize or feminize males but rather displayed anti-hormonal activities, apparently by inhibiting ovarian hormone synthesis, which resulted in delayed delivery and whole



litter loss. In summary, the above in vivo data suggest that the chemicals we studied alter male sexual differentiation via different mechanisms. The anti-androgens V, P, and p,p'-DDE produce flutamide-like profiles that are distinct from those seen with DBP, DEHP, and L. The effects of PCB 169 bear little resemblance to those of any known anti-androgen. Only in depth in vitro studies will reveal the degree to which one can rely upon in vivo studies, like those presented here, to predict the cellular and molecular mechanisms of developmental toxicity.

**Interactive effects of TCDD and p,p'-DDE on male reproductive tract development in utero and lactationally exposed rats.**

Loeffler IK and Peterson RE

Toxicol Appl Pharmacol 1999;154(1):28-39

The developing male rat reproductive system is highly sensitive to low doses of TCDD and p,p'-DDE (DDE), which exert antiandrogenic effects via different mechanisms. This study investigates the interactive effects of in utero and lactational exposure to a mixture of these compounds. Pregnant Holtzman rats received one of the following: vehicle on gestation day (GD) 14-18, 0.25 microgram/kg TCDD on GD15, 100 mg/kg DDE on GD 14-18, or 0.25 microgram/kg TCDD on GD15 and 100 mg/kg DDE on GD 14-18. Male offspring were euthanized on postnatal day (PND) 21 (weaning), PND 32 (prepuberty), PND 49 (puberty), and PND 63 (postpuberty). Coadministration of these doses of TCDD and DDE appeared to potentiate their individual actions on prostate weight on PND 21, while immunostaining for the prostatic androgen receptor exhibited patterns characteristic of the effects of both compounds individually. Cauda epididymal sperm number was reduced by each compound but was not further reduced by exposure to TCDD and DDE in combination. Anogenital distance, age at onset of puberty, daily sperm production, testicular and accessory sex organ weight (nonprostate), and levels of prostatic androgen-regulated gene transcripts are affected at higher doses of both compounds, but not at the doses used in the present study. Only DDE-treated animals retained nipples on PND 13. Serum androgen levels did not differ between treatment groups. In conclusion, the developing rat prostate is uniquely sensitive to the effects of TCDD and DDE, which may augment one another's effects in this organ.

**In utero exposure to antiandrogens alters the responsiveness of the prostate to p,p'-DDE in adult rats and may induce prostatic inflammation.**

You L, Brenneman KA, and Heck H

Toxicol Appl Pharmacol 1999;161(3):258-66

DDE is an environmental pollutant with antiandrogenic properties. Following administration to pregnant rats, DDE was shown to cause feminization in the male offspring at the neonatal stages but did not affect the pubertal growth of accessory sex organs. In this study, we examined the potential of in utero exposure to antiandrogens to alter the responsiveness of the male rats to subsequent DDE challenge. Pregnant Long-

Evans rats were dosed by gavage from Gestation Day 14 to 18 at 0, 10 (low dose), or 100 (high dose) mg DDE, or 40 mg flutamide/kg body wt (bw)/day (in utero treatment). At approximately 80 days of age, the male offspring from each of the four in utero treatment groups were divided into two groups. One group received the adult treatment of four daily gavage administrations of DDE at 70 mg/kg bw (adult treatment), while the second group served as the adult treatment control (adult control). The in utero treatment resulted in 18, 31, and 53% reductions of ventral prostate weights at approximately 85 days of age compared to the control for the low- and high-dose DDE and flutamide groups, respectively. These results suggest that the in utero antiandrogen treatments produced a latent effect on prostate growth that became pronounced only in the postpubertal stage. The in utero treatment also altered the responsiveness of the prostate to the adult treatment, indicated by a significant reduction in ventral prostate weight that was seen only in the control group of the in utero treatment but not in the other groups. The in utero treatment was also associated with expression of testosterone-repressed prostatic message-2 in the adult ventral prostate. In addition, a few prostates in the high-dose DDE- and flutamide-treated groups of the in utero treatment were found to have chronic suppurative prostatitis. While other types of hormonal manipulations have been shown to incite similar responses in rat prostate, the possible linkage between in utero antiandrogen treatment and prostatic inflammation needs to be further evaluated.

**Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE.**

You L, Casanova M, Archibeque-Engle S, Sar M, Fan LQ, and Heck HA  
Toxicol Sci 1998;45(2):162-73

Although the pesticide DDT has been banned in the United States for decades, it remains at low levels in the environment. p,p'-DDE, a metabolite of DDT, was recently shown to inhibit the binding of androgens to the androgen receptor and to exert antiandrogenic effects in perinatal Long-Evans (LE) rats at a dose of 100 mg/kg/day administered to pregnant dams. In this study, we compared the effects of p,p'-DDE on male sexual development in offspring of Sprague-Dawley (SD) and LE rats. The chemical was dosed by gavage to pregnant dams at 10 or 100 mg/kg body wt from gestation day 14 to 18. The developing male rats were examined for sexual developmental landmarks, while the effects of p,p'-DDE on androgen receptor expression were evaluated in the testis and other reproductive organs. The tissue dosimetry of p,p'-DDE was also determined at different stages of development following in utero and lactational exposures. The higher p,p'-DDE dose induced a reduction in the male anogenital distance, an increase in retention of male thoracic nipples and alterations in expression of the androgen receptor in either one or both strains. A much weaker response was seen in the lower dose groups. Tissue and body fluid concentrations of p,p'-DDE were similar in the two strains in some tissues but dissimilar in others, particularly in the serum levels. Higher serum p,p'-DDE levels in the LE strain during pregnancy corresponded with an overall greater sensitivity of the LE strain to the antiandrogenic effects of p,p'-DDE. These results support the previous findings of p,p'-DDE antiandrogenicity in LE rats, extend the findings to SD

rats, and suggest that the developmental effects of p,p'-DDE on male rat sexual differentiation are minimal at maternal doses below 10 mg/kg/day.

## B. Studies reporting no developmental or reproductive toxicity

### **Gene expression profiling of androgen receptor antagonists in the rat fetal testis reveals few common gene targets.**

Mu X, Liu K, Kleymenova E, Sar M, Young SS, Gaido KW.

Journal of biochemical and molecular toxicology. 2006;20(1):7-17.

The androgen receptor (AR) is expressed in the fetal testis; however, the role of AR in fetal testicular development is poorly understood. Disrupted AR activity and subsequent gene expression alterations may disturb developmental programming of the fetal testis and result in testicular abnormalities later in life. The present study was performed to examine global gene expression patterns in rat fetal testis following in utero exposure to various AR antagonists. Pregnant Sprague-Dawley rats were treated with flutamide (50 mg/kg/day), linuron (50 mg/kg/day), vinclozolin (200 mg/kg/day), p,p'-DDE (100 mg/kg/day) or corn oil vehicle by gavage daily from gestation day (GD) 12-19. Testes were isolated on GD 19, and AR immunostaining, histology, and global changes in gene expression were determined. There were no alterations in the pattern or expression level of AR and no apparent histological changes in the fetal testes in any treatment group. Microarray analysis using Dunnett's test with multiple testing correction revealed no significant gene expression alterations following exposure to flutamide, linuron, vinclozolin, and p,p'-DDE. A less stringent analysis yielded some chemical specific effects on gene expression, and these effects were further evaluated by real-time RT-PCR. Vinclozolin treatment reduced the expression of several genes involved in cholesterol biosynthesis, though the testosterone levels were unchanged in the fetal testes in any treatment group. In flutamide, linuron, and p,p'-DDE treatment groups, the expression of hemoglobin Y, beta-like embryonic chain (Hbb-y) was reduced. Myomesin 2 (Myom2) expression was increased following linuron treatment. Given the lack of a common set of genes and the absence of overt histopathology, we conclude that the fetal testis is not a major target for AR activity at this stage of development although some cell-type specific gene expression changes cannot be ruled out.

### **Effects of perinatal combined exposure to 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene and tributyltin on male reproductive system.**

Makita Y, Omura M.

Basic & clinical pharmacology & toxicology. 2006 Aug;99(2):128-32.

Prenatal or early postnatal exposure to some synthetic chemicals may affect the later reproductive system of the offspring. There may also be unique responses observed due to exposure to combinations of chemicals that are not observed when the chemicals are

present individually. 1,1-Dichloro-2,2 bis (p-chlorophenyl) ethylene (p,p'-DDE) is a persistent metabolite of DDT and tributyltin (TBT) compounds are used primarily as antifouling agents, as they exert biocidal actions. p,p'-DDE and TBT are ubiquitously distributed in the environment. Oral p,p'-DDE and TBT intake through marine products is demonstrated to be high in Japan. Consequently, the foetus and neonate are supposed to be exposed much more to p,p'-DDE and TBT via the maternal body. Therefore, effects of perinatal exposure to p,p'-DDE and/or TBT on the reproductive system after maturation have been investigated in rat male offspring of dams orally administered 125 ppm p,p'-DDE (approximately 10 mg/kg) and 25 ppm TBT (approximately 2 mg/kg) during the gestational and lactational period. In this study, growth retardation attributed to TBT has sustained in rat male offspring after perinatal exposure. However, perinatal exposure to p,p'-DDE and TBT failed to affect the male reproductive organs and sperm parameters in matured male offspring.

**Two-generation reproduction toxicity study in rats with 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT).**

Hojo H, Aoyama H, Takahashi KL, Shimizu N, Araki M, Takizawa Y, et al. *Congenital Anomalies*. 2006;46(2):105-14.

DDT, an organochlorine pesticide, has been cited as a representative chemical suspected of having endocrine disrupting effects. In this study, the potential endocrine disrupting activities of p,p'-DDT, a major component of DDT, were investigated in rats in a 2-generation reproduction toxicity study in accordance with the most current test guidelines of the Ministry of Agriculture, Forestry and Fisheries in Japan, Organization for Economic Cooperation and Development (OECD) and United States Environmental Protection Agency (USEPA) with some modifications and additions. p,p'-DDT was given to parental rats at dietary levels of 0, 5, 50 or 350 ppm. Systemic toxicities in the parental animals consisted of tremors and subsequent deaths (females only) and/or pathological alterations of the liver (both sexes of animals) of the 2 higher dose groups. Reproductive and postnatal developmental toxicities were not evident up to the highest dose level except for the decreased pup viability index on postnatal day 21 in the 350 ppm group. Changes in serum estradiol and progesterone levels and/or a delay in male sexual maturation were noted in the 2 higher dose groups in a dose-dependent fashion, suggesting alterations of endogenous endocrine functions. However, these changes never resulted in substantial reproductive disorders.

**Effects of concurrent exposure to tributyltin and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (p,p'-DDE) on immature male Wistar rats.**

Makita Y, Omura M, Tanaka A, Kiyohara C. *Basic & clinical pharmacology & toxicology*. 2005 Dec;97(6):364-8.

Tributyltin and 1, 1-dichloro-2, 2 bis (p-chlorophenyl) ethylene (p,p'-DDE) have been ubiquitously distributed over the world. In Japan, p,p'-DDE and tributyltin are ingested

through marine products, in which these substances are accumulated through bio-concentration and the food chain. However, the consequence of potential combined hazards of these substances remains unknown. Therefore, the effects of concurrent exposure to 125 ppm p,p'-DDE and 25 ppm tributyltin were investigated in immature male Wistar rats by oral administration during puberty. In this study, tributyltin promoted the growth of pubertal male rats, while p,p'-DDE itself did not affect the growth but inhibited the growth enhancement by tributyltin. Furthermore, tributyltin reduced thymus weight but p,p'-DDE also prevented this weight reduction. Neither development of male sexual accessory organs nor sexual maturation was affected even by concurrent exposure to p,p'-DDE and tributyltin. No significant changes of serum testosterone, luteinizing hormone, follicle-stimulating hormone concentrations, and epididymal sperm numbers were observed with the administration of p,p'-DDE and/or tributyltin. These results indicate that sexual maturation, male reproductive organ development and sperm production is scarcely affected in immature male Wistar rats even by concurrent exposure to p,p'-DDE and tributyltin at a daily dose of ca. 2 mg/kg tributyltin and 10 mg/kg p,p'-DDE. Moreover, the simultaneous administration of p,p'-DDE with tributyltin counterbalanced the effects that were attributed to tributyltin alone.

**Effects of perinatal combined exposure to 1,4-dichlorobenzene and 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene on rat male offspring.**

Makita Y.

Basic & clinical pharmacology & toxicology. 2005 May;96(5):361-5.

1,4-Dichlorobenzene (DCB) is used as an air deodorant or a moth repellent and 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (p,p'-DDE) is a persistent metabolite of 1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane (DDT) which was used as a pesticide before. DCB concentrations of residential air and oral p,p'-DDE intake through marine products are demonstrated to be very high in Japan and consequently, fetuses and neonates may be exposed much more to DCB and/or p,p'-DDE via the maternal body. It has recently been reported that DCB is oestrogenic and that p,p'-DDE is antiandrogenic. Therefore, the combined effects of perinatal exposure to DCB and p,p'-DDE have been investigated in rat male offspring of dams ingesting these contaminants during the perinatal period from gestational day 1 to postpartum day 21 for 42 days. In this study, no obvious developmental effects on male offspring have been recognized until 6 weeks of age, following oral administration of 25 ppm DCB (approximately 2 mg/kg) and/or 125 ppm p,p'-DDE (approximately 10 mg/kg) to dams. In contrast to female offspring, the thymus weight in male offspring was not affected by DCB at 6 weeks of age, but there might be sexual differences concerning the effects of DCB on the thymus.

**Effects of perinatal combined exposure to 1,4-dichlorobenzene and 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (p,p'-DDE) on rat female offspring.**

Makita Y

Basic Clin Pharmacol Toxicol 2004;95(3):139-43

1,4-Dichlorobenzene (DCB) is used as an air freshener and a moth repellent and 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (p,p'-DDE) is a persistent metabolite of 1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane (DDT) previously used as a pesticide. DCB concentrations of residential air and oral p,p'-DDE intake via marine products are demonstrated to be higher in Japan than elsewhere. Consequently, human foetuses and neonates may be exposed to DCB and p,p'-DDE via the mother. Therefore, the combined effects of DCB and p,p'-DDE have been investigated in rat female offspring of dams after ingestion of these contaminants. No deteriorated reproductive outcomes of dams and developmental effects on female offspring were observed following oral administration of 25 ppm DCB (approximately 2 mg/kg) and/or 125 ppm p,p'-DDE (approximately 10 mg/kg) to dams. In this study, the thymus weight of female offspring was preserved by DCB at 6 weeks of age though the biological relevance remains unknown. Simultaneous administration of p,p'-DDE with DCB inhibited this phenomenon, through a mechanism still to be elucidated.

**Systemic Effects of Orally Administered p, p'-DDE on Immature Male Wistar Rats during Pubertal Period.**

Makita Y, Matsuura T, Ogata R, Romero Y, Omura M, Tanaka A, Hirata M, and Inoue N  
Journal of Occupational Health 2003;45(4):223-7

Systemic effects of p,p'-DDE (1,1-dichloro-2,2bis(p-chlorophenyl) ethylene ; DDE) on immature male rats were investigated in pubertal Wistar rats after oral administration of DDE. Special rat chow containing 125 ppm DDE (approximately 10 mg/kg DDE) had been administered daily for 42 d since 6 wk of age and its effects had been observed until 12 wk of age. The administration of DDE did not produce any overt signs of toxicity. Neither physical development nor sexual maturation was affected, and serum biochemistry was not impaired at the dose used in this experiment. Moreover, the male reproductive organs and epididymal sperm count were not affected by the administration of DDE during the pubertal period. Our results showed that even immature male rats were resistant to DDE exposure at the daily dose of ca. 10 mg/kg, but metabolic and immunological changes still remained uncertain. Further investigation should be conducted to reveal all the effects of DDE on immature male rats.

**Effects of p,p'-DDE on male reproductive organs in peripubertal Wistar rats following a single intraperitoneal injection.**

Romero YE

Fukuoka Igaku Zasshi 1998;89(2):64-77

The effects of p,p'-DDE on male reproductive organs were investigated in detail in peripubertal Wistar rats following a single intraperitoneal injection. 220 mg/kg of p,p'-DDE (1/4 of LD50) were injected once into prepubertal and postpubertal Wistar rats and its effects were observed until 20 weeks of age. Weights of the body and reproductive organs in p,p'-DDE-injected rats were similar to those in control rats, who were injected with corn oil only. Sperm profile parameters such as spermatid number within the testis, sperm number within the epididymis, sperm motility and its morphology were not different between the prepubertal or postpubertal p,p'-DDE-exposed group and the control group. Like-wise, the histopathological examination at stage VII of the seminiferous epithelium cycle, when the germ cells are sensitive to testosterone, was similar in all three groups during the observation period. Serum levels of testosterone also showed no significant changes by exposure to p,p'-DDE under the conditions of this study. From these results, the antiandrogenic or estrogenic activity attributed to p,p'-DDE was not confirmed in male reproductive organs and no impairment of sperm profile was observed. This study confirmed that the reproductive functions of matured animals are scarcely affected by p,p'-DDE exposure during the peripubertal period and revealed that they might be relatively resistant to exogenous endocrine-disrupting chemicals. p,p'-DDE may threaten the hormonal equilibrium required for normal gonadal development during the organogenesis period, at an earlier stage of life. Further studies are necessary to fully reveal all the effects of p,p'-DDE on male reproductive organs and sperm profile.

**Effects of 1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene (DDE) on lactation in rats.**

Kornbrust D, Gillis B, Collins B, Goehl T, Gupta B, and Schwetz B

J Toxicol Environ Health 1986;17(1):23-36

An inverse correlation between the concentration of DDE in human breast milk samples and the duration of breast feeding prompted the present study of the effects of DDE administration on the lactational performance of primiparous rats. Daily doses of 10 mg p,p'-DDE/kg body weight were given to virgin female Sprague-Dawley rats 5 d/wk for 5 wk prior to mating and continued throughout the gestation and lactation periods. Lactation capacity was determined by monitoring neonatal growth and by measuring milk production, milk composition (total protein, total lipid, and lactose), and mammary-gland weight and nucleic acid content on d 9 and 20 postpartum. Gross toxicity was assessed by monitoring clinical signs and body weight of the dams, and by measuring organ weights of the dams on lactation d 9 and 20. Histopathological evaluation of the mammary glands and selected organs in the dams and pups was also performed. The dose level of DDE employed was apparently not toxic to the dams and did not have a pronounced effect on neonatal mortality. No significant differences between DDE-treated and control groups were observed for any of the lactation parameters, even though the

concentration of DDE in the milk of treated rats was approximately two orders of magnitude greater than the upper range of the DDE levels measured in human milk samples. These findings indicate that DDE does not adversely affect lactation or neonatal growth in Sprague-Dawley rats at the dose level used in this study.

**Effects of DDT homologs administered to female rats during the perinatal period.**

Gellert RJ and Heinrichs WL

Biol Neonate 1975;26(3-4):283-90

The administration of estrogen to female rats during the perinatal period induces a masculine pattern of hypothalamic differentiation resulting in persistent vaginal estrus (PVE) and anovulation. 10 mg day of the estrogenic homologs o,p-DDT, o,p-DDE, and DDA, as well as allegedly nonestrogenic forms of the pesticide, p,p-DDT and o,p-DDD, were given by gavage from days 15-19 of pregnancy. 1-mg doses of the homologs were also given SQ daily to female rats on the second, third, and fourth days of life. Vaginal opening was delayed by 2 days in rats derived from the dams gavaged with o,p-DDD. Periodic examination of estrous cycles in the rats treated neonatally with o,p-DDD revealed PVE by 209 days of age, and absence of corpora lutea by 258 days of age. Prior to 4 months of age, estrous cycles were normal in these animals. The remaining pesticide homologs tested, regardless of the mode of administration, had no significant effect on the estrous cycles or on endocrine gland weights.

**Effects of neonatally-administrated DDT homologues on reproductive function in male and female rats.**

Gellert RJ, Heinrichs WL, and Swerdloff RS

Neuroendocrinology 1974;16:84-94

The possibility of altering neuroendocrine differentiation by administering homologs of the pesticide DDT to neonatal male and female rats was investigated. The estrogenic isomer o,p'-DDT produced precocious puberty with an inverse relationship between age at vaginal opening and dose of DDT. 0.1 mg o,p'-DDT on the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> days of life was the minimum effective dose for inducing persistent vaginal estrus and anovulation. In addition, as the dosage was increased, the syndrome appeared earlier in life. Uterine histology in adult rats treated as neonates with high doses of o,p'-DDT was markedly altered, i.e. patches of stratified squamous epithelium were evident. The feedback rise in serum gonadotropin concentration in response to ovariectomy was reduced in rats treated with high doses of o,p'-DDT neonatally when compared to vehicle-treated controls. Male rats treated as neonates with high doses of the DDT homologs, o,p'-DDT, DDA, methoxychlor, p,p'-DDE and p,p'-DDT, had normal reproductive organ weights and motile sperm as adults in spite of the known estrogenic activity of the first three compounds. It is concluded that o,p'-DDT given to newborn female rats may, in a dose-dependent fashion, permanently alter neuroendocrine differentiation, but that male rats are unaffected by such treatment.



### C. Related articles

#### **Effects of p,p'-dichlorodiphenyldichloroethylene on the expressions of transferrin and androgen-binding protein in rat Sertoli cells.**

Xiong X, Wang A, Liu G, Liu H, Wang C, Xia T, et al.  
Environmental research. 2006 Jul;101(3):334-9.

The mechanisms of reproductive malfunction of male mammals caused by 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE, hereafter DDE) remain unknown. To explore the effects of DDE on the expressions of transferrin (Tf) and androgen-binding protein (ABP), we isolated Sertoli cells from healthy immature rats (18-20 days SD rats), set up Sertoli cell cultures, evaluated the toxicity, and measured the expression levels of mRNA of Tf and ABP genes by the one-step reverse transcriptase polymerase chain reaction method after cultured Sertoli cells were in vitro exposed to DDE at different concentrations for 24 h. The results showed that the number and survival rate of Sertoli cells decreased sharply with increased doses of DDE. The expression level of Tf mRNA decreased, whereas ABP mRNA increased gradually with increased DDE doses. There existed an obvious dose-effect relationship between the concentration of DDE and the expression levels of Tf mRNA and ABP mRNA. These findings suggest that DDE may inhibit the expression of Tf and up-modulate expression of ABP in cultured rat Sertoli cells.

#### **Effects of postnatal exposure to a mixture of polychlorinated biphenyls, p,p'-dichlorodiphenyltrichloroethane, and p-p'-dichlorodiphenyldichloroethene in prepubertal and adult female Sprague-Dawley rats.**

Desaulniers D, Cooke GM, Leingartner K, Soumano K, Cole J, Yang J, et al.  
International journal of toxicology. 2005 Mar-Apr;24(2):111-27.

The postnatal period is a critical phase of development and a time during which humans are exposed to higher levels of persistent organic pollutants (POPs), than during subsequent periods of life. There is a paucity of information describing effects of postnatal exposure to environmentally relevant mixtures of POPs, such as polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichloroethane (DDT), and p,p'-dichlorodiphenyldichloroethene (DDE). To provide data useful for the risk assessment of postnatal exposure to POPs, mixtures containing 19 PCBs, DDT, and DDE were prepared according to their concentrations previously measured in the milk of Canadian women, and dose-response effects were tested on the proliferation of MCF7-E3 cells in vitro, and in vivo experiments. Female neonates were exposed by gavage at postnatal days (PNDs) 1, 5, 10, 15, and 20 with dosages equivalent to 10, 100, and 1000 times the estimated human exposure level over the first 24 days of life. The MCF7-E3 cells showed a 227% increase in the AlamarBlue proliferation index, suggesting estrogen-like properties of the mixture, but this was not confirmed in vivo, given the absence of uterotrophic effects at PND21. An increase (511%) in hepatic ethoxyresorufin-o-deethylase activity at the dose 100 x was the most sensitive endpoint among those measured at PND21 (organ weight,

mammary gland and ovarian morphometry, hepatic enzyme inductions, serum thyroxine and pituitary hormones). In liver samples from older female rats (previously involved in a mammary tumor study [Desaulniers et al., Toxicol. Sci. 75:468-480, 2001]), hepatic metabolism of <sup>14</sup>C-estradiol-17beta (E2) at PND55 to PND62 was significantly higher in the 1000x compared to the control group, but hepatic detoxification enzyme activities had already returned to control values. The production of hepatic 2-hydroxy-E2 decreased, whereas that of estrone increased with age. In conclusion, the smallest dose of the mixture to induce significant effects was 100x, and mixture-induced changes in the hepatic metabolism of estrogens might be a sensitive indicator of persistent effects.

**Effects of perinatal simultaneous exposure to tributyltin (TBT) and p,p'-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene] on male offspring of Wistar rats.**

Makita Y, Omura M, and Ogata R

J Toxicol Environ Health A 2004;67(5):385-95

p,p'-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene; DDE] and tributyltin (TBT) are ubiquitous in the environment and in Japan were shown to bioaccumulate in marine products. Thus these chemicals serve as a source of contaminant in the mammalian food chain. Fetuses and neonates through maternal ingestion may be exposed to DDE and TBT. Therefore, the effects of concurrent exposure to DDE and TBT were investigated in male Wistar rat offspring of dams ingesting these two contaminants. In this study, TBT suppressed the growth and delayed eye opening. However, both growth retardation and delayed eye opening produced by TBT failed to occur in the presence of DDE. Unexpectedly, the prostate weight of male rat offspring was significantly reduced with the administration of TBT but restored in the presence of DDE. These results indicate that TBT and DDE affected the development of male rat offspring following maternal exposure, and simultaneous administration of DDE prevented some of the observed effects of TBT, especially of an antagonistic nature, through a mechanism, still to be determined.

**Effects of simultaneous administration of tributyltin (TBT) and p,p'-DDE on female offspring of Wistar rats.**

Makita Y, Tanaka A, Omura M, and Ogata R

J Toxicol Environ Health A 2003;66(24):2337-47

p,p'-DDE (DDE) and tributyltin (TBT) occur globally and in Japan were shown to bioaccumulate in marine products, thus serving as a source of contamination in the mammalian food chain. Consequently, fetuses and neonates, through maternal ingestion, may be exposed to DDE and TBT. Therefore, the effects of combined DDE and TBT were investigated in female Wistar rat offspring of dams ingesting these two contaminants. In this study, TBT suppressed the growth of female offspring and delayed eye opening. However, both growth retardation and delayed eye opening produced by TBT failed to occur in the presence of DDE. These results indicated that TBT or DDE

affected the development of female rat offspring following maternal exposure and simultaneous administration of DDE prevented some of the observed effects of TBT through a mechanism that remains to be elucidated.

**Comparison of prostate gene expression and tissue weight changes as monitors of antiandrogen activity in GNRH-inhibited rats**

Nellemann C, Lefevre PA, and Ashby J

Birth Defects Res B Dev Reprod Toxicol 2003;68(4):344-54

**BACKGROUND:** The Hershberger assay for antiandrogens and modifiers of steroid biosynthesis uses surgically-castrated rats. We described an adaptation of the assay using the GnRH inhibitor Antarelix in place of surgical castration [Ashby J, Lefevre PA, Deghenghi R, Wallis N. Regulatory Toxicology and Pharmacology 34:188-203, 2001], and concomitantly described changes in expression of the androgen-dependent prostatic genes PBP C3, TRPM-2, and ODC as a possible complement to gravimetric analysis of the sex accessory tissues (SAT) [Nellemann C, Vinggaard AM, Dalgaard M, Hossaini A, Larsen J-J. Toxicology 163:29-38, 2001].

**METHODS:** The present study describes the results of combining these two modifications into a single assay. During the course of these experiments it was shown that SD rats gave similar results to AP rats and that the higher stimulatory dose of testosterone propionate (TP) used in our experiments gave stronger assay responses to FLU than the lower dose of TP used by some earlier investigators. The potent antiandrogen flutamide (FLU) and the weak antiandrogen DDE were used to evaluate this modified assay.

**RESULTS:** For all parameters studied (SAT weights and changes in expression of the 3 prostatic genes) FLU gave the expected positive results. The weak antiandrogen DDE gave variable and mainly non-reproducible responses. Use of DDE as a weak antiandrogen accelerated assessment of the new assay.

**CONCLUSIONS:** Possible reasons for this failure to detect DDE are discussed, and it is concluded that the modified assay is unsuitable for use in its present form. The use of gene expression analyses together with evaluation of SAT weights is a promising tool as an early and sensitive marker of antiandrogen action, but more work is needed on the choice of time frame as well as the selection of genes to monitor.

**Lack of estrogenic or (anti-)androgenic effects of d-phenothrin in the uterotrophic and Hershberger assays.**

Yamada T, Ueda S, Yoshioka K, Kawamura S, Seki T, Okuno Y, and Mikami N.

Toxicology 2003;186(3):227-39

Synthetic pyrethroids are among the most common insecticides and pesticides currently in use worldwide. Recently, d-phenothrin, a synthetic pyrethroid, is suspected to have endocrine activities through the estrogen and androgen receptors. However, no study has been conducted to evaluate its potential for hormonal activity using an in vivo test

specifically focused on estrogenic and androgenic activities. In this study, we evaluated the interaction of d-phenothrin (0, 100, 300 or 1000 mg/kg per day, p.o.) with estrogen- or androgen-mediated mechanisms using in vivo short-term assays. While internationally standardized protocols for the uterotrophic and Hershberger assays have not yet been fully developed, both are widely used and are being considered by the OECD as short-term screening assays for hormonal activity. The highest dose level tested for d-phenothrin was a limit dose (1000 mg/kg per day) designated in the current draft protocol by the OECD, and in fact there was no excessive systemic toxicity in both assays; slightly increased liver weight but no change of serum androgen levels in accessing anti-androgenicity. Potential estrogenic effect of d-phenothrin was evaluated by means of 3-day uterotrophic assay using immature Crj:CD(SD)IGS rats (20 days of age). No increase in uterine weight (wet or blotted) was observed following oral exposure to d-phenothrin. Reference control ethynyl estradiol (0.001 mg/kg per day) showed a significant effect in this assay protocol. A 10-day Hershberger assay using castrated peripubertal male rats measures the androgenic or anti-androgenic effects of the test chemicals on several accessory glands/tissues (the ventral prostate, dorso-lateral prostate, seminal vesicles with coagulating glands, levator ani plus bulbocavernosus muscles, glans penis and Cowper's glands). d-Phenothrin was administered by oral gavage for 10 days to castrated male Crj:CD(SD)IGS rats (7 weeks of age, rats were castrated at 6 weeks of age) with or without co-administration of 0.2 mg/kg per day testosterone propionate (subcutaneous injection on the dorsal surface). Reference controls of methyltestosterone and p,p'-DDE (100 mg/kg per day) provided significant effects in this assay protocol, whereas d-phenothrin did not show any androgenic or anti-androgenic effects. It is concluded that, based on the results of these two reliable in vivo assays, d-phenothrin exhibits no potential to cause adverse estrogenic or (anti-)androgenic effects even at dose of 1000 mg/kg per day, the limit dose designated in the current draft protocol by the OECD.

**Lack of antiandrogenic effects in adult male rats following acute exposure to 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE).**

Leavens TL, Sparrow BR, and Devito MJ.  
Toxicology 2002;174(2):69-78

Although the insecticide dichlorodiphenyltrichloroethane (DDT) was banned in the US in 1972, DDT and its major metabolite 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) are still persistent in the environment. DDE at high doses is antiandrogenic in fetal and adult rats and, therefore, is of concern in humans exposed environmentally. The objective of this work was to determine the dose-response relationship between DDE and its antiandrogenic effect in adult, male rats and to quantitate the concentration of DDE in tissues following oral exposures. Adult, male, Long-Evans rats (11-13 weeks) were castrated, implanted with testosterone capsules, and dosed by oral gavage with 0, 5, 12.5, 25, 50, or 100 mg DDE per kg body weight (BW) per day in corn oil for 4 days. On day 5 the rats were euthanized and liver, adrenals, ventral prostate, and seminal vesicles were weighed as a measure of response to DDE exposure. Blood, adrenals, brain, fat, kidney, lung, liver, muscle, ventral prostate, seminal vesicles, and skin were analyzed for DDE

concentrations. Testosterone and dihydrotestosterone were measured in serum. There was a decrease in prostate weight that was not dose dependent; only the prostate weights in rats treated with 12.5 mg DDE per kg BW per day were reduced significantly compared to controls. The liver displayed a dose-dependent increase in weight that was significantly greater than control at DDE doses of 25, 50, and 100 mg/kg BW per day. Blood concentrations of DDE ranged from 0.32 to 11.3 ppm, while tissue concentrations ranged from 0.72 to 2620 ppm with the highest concentration in fat. Although DDE concentrations in the androgen-responsive tissues were higher than concentrations previously shown in vitro to inhibit androgen-receptor transcriptional activity, these concentrations did not appear to be antiandrogenic in vivo. The doses administered to the rats in this study are at least 10(5)-fold greater than the daily, average of human dietary intake of DDE.

**Effects of subchronic exposure to a complex mixture of persistent contaminants in male rats: systemic, immune, and reproductive effects.**

Wade MG, Foster WG, Younglai EV, McMahon A, Leingartner K, Yagminas A, Blakey D, Fournier M, Desaulniers D, and Hughes CL  
Toxicol Sci. 2002;67(1):131-43

Human populations throughout the world are exposed daily to low levels of environmental contaminants. The consequences of potential interactions of these compounds to human endocrine, reproductive, and immune function remain unknown. The current study examines the effects of subchronic oral exposure to a complex mixture of ubiquitous persistent environmental contaminants that have been quantified in human reproductive tissues. The dosing solution used in this study contained organochlorines (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD], polychlorinated biphenyls [PCBs], p,p'-dichlorodiphenoxydichloroethylene [p,p'-DDE], p,p-dichlorodiphenoxytrichloroethane [p,p'-DDT], dieldrin, endosulfan, methoxychlor, hexachlorobenzene, and other chlorinated benzenes, hexachlorocyclohexane, mirex and heptachlor) as well as metals (lead and cadmium). Each chemical was included in the mixture at the minimum risk level (MRL) or tolerable daily intake (TDI) as determined by the U.S. EPA or ATSDR or, for TCDD, at the no observable effect level (NOEL) used to calculate the TDI. Sexually mature male rats were exposed to this complex mixture at 1, 10, 100, and 1000 times the estimated safe levels daily for 70 days. On day 71, all animals were sacrificed and a variety of physiological systems assessed for toxic effects. Evidence of hepatotoxicity was seen in the significant enlargement of the liver in the 1000x group, reduced serum LDH activity (100x), and increased serum cholesterol and protein levels (both 1000x). Hepatic EROD activities were elevated in animals exposed to 10x and above. The mixture caused decreased proliferation of splenic T cells at the highest dose and had a biphasic effect on natural killer cell lytic activity with an initial increase in activity at 1x followed by a decrease to below control levels in response to 1000x. No treatment-related effects were seen on bone marrow micronuclei, daily sperm production, serum LH, FSH, or prolactin levels or weights of most organs of the reproductive tract. The weights of the whole epididymis and of the caput epididymis were significantly

decreased at 10x and higher doses, although no effect was seen on cauda epididymal weight. The sperm content of the cauda epididymis was increased at the 1x level but not significantly different from control at higher dose levels. A slight, but significant, increase in the relative numbers of spermatids was seen in the animals from the 1000x group with a trend towards reduced proportion of diploid cells at the same dose. Only minor, nondose related changes were seen in parameters related to condensation of chromatin, as determined by flow cytometry, in epididymal sperm. We conclude that the mixture induced effects on the liver and kidney and on general metabolism at high doses but caused only minor effects on immune function, reproductive hormone levels, or general indices of reproductive function measures. These data suggest that additive or synergistic effects of exposure to contaminants resulting in residue levels representative of contemporary human tissue levels are unlikely to result in adverse effects on immune function or reproductive physiology in male rats.

### **Effects of an environmental anti-androgen on erectile function in an animal penile erection model.**

Brien SE, Heaton JP, Racz WJ, and Adams MA  
J Urol 2000;163(4):1315-21

**PURPOSE:** Erectile function is testosterone dependent. For example, interference with either the levels or receptor binding of this steroid hormone may induce erectile dysfunction. Several environmental contaminants can interfere with the actions of endogenous hormones and have been termed 'endocrine disrupters.' p,p-DDE, a prominent and persistent metabolite of the insecticide DDT, has been shown to be an androgen receptor antagonist. The objective was to determine whether endocrine disrupters, as exemplified by p,p-DDE, are factors in the etiology of erectile dysfunction. **MATERIALS AND METHODS:** Using the established rat model of apomorphine-induced (80 microg./kg, s.c.) erections we assessed the dose-response effects of p,p-DDE in comparison to the known androgen receptor antagonist flutamide in acute (0.5 to 12 hours) and short-term (up to 8 weeks) experiments in both intact (Study 1) and castrated (Study 2) rats. As a follow up (Study 3), castrated rats treated with p,p-DDE were given increasing doses of testosterone (0.48 to 2.4 mg./kg., i.p.), eight weeks after p,p-DDE administration, to assess reversibility of p,p-DDE effect. **RESULTS:** A single dose of flutamide (50 mg./kg., i.p.) was found to significantly decrease apomorphine-induced erections to less than 50% over 12 hours following flutamide administration with recovery of erectile response within 48 hours. In comparison, a single dose of p,p-DDE (500 mg./kg., i.p.) decreased apomorphine-induced erections for at least two weeks (1.15+/-0.3 versus 2.5+/-1.1). Castration significantly decreased apomorphine-induced erections to approximately 0.5 erections/30 minutes. Flutamide (50 mg./kg.; i.p.) or p,p-DDE (50 mg./kg.; i.p.) did not further suppress the apomorphine erections in castrated rats. Testosterone supplementation (480 microg./kg; s.c.) in vehicle treated castrated rats recovered erectile response to pre-castrated levels, whereas p,p-DDE treated castrated rats required 4 times the dose of testosterone (2 mg./kg.; s.c.) given to vehicle treated rats to recover erections. **CONCLUSIONS:** The endocrine disrupter p,p-DDE can markedly

interfere with erectile function and demonstrates persistence after a single dose. This supports our novel concept that environmental hormones may cause erectile dysfunction.

**Evaluation of a 5-day Hershberger assay using young mature male rats: methyltestosterone and p,p'-DDE, but not fenitrothion, exhibited androgenic or antiandrogenic activity in vivo.**

Sunami O, Kunimatsu T, Yamada T, Yabushita S, Sukata T, Miyata K, Kamita Y, Okuno Y, Seki T, Nakatsuka I, and Matsuo M  
J Toxicol Sci 2000;25(5):403-15

A 5-day Hershberger assay using young mature male rats to detect compounds interfering with androgen receptor (AR)-mediated mechanisms was evaluated for ability to identify p,p'-DDE (a weak AR antagonist) and methyltestosterone (MT, an AR agonist). Fenitrothion, an organophosphate pesticide, was also evaluated in this validated assay. Castrated male Crj:CD(SD)IGS rats (1 week after castration, 11 weeks of age) were subjected to experiments. To determine a suitable value of testosterone propionate (TP) as a reference androgen for detection of antiandrogenic chemicals, castrated male rats were treated daily with TP (0, 0.06, 0.25, 1, 4, or 16 mg/kg/day, s.c.). TP produced increases in weights of ventral prostate, seminal vesicles and levator ani plus bulbocavernosus muscles. Serum androgen level measured by RIA kit (mostly TP) were elevated in a dose-related manner, while the weights of organs with 1 mg/kg/day of TP were nearly equivalent to the maximum responses (i.e., sub-maximal). One hundred mg/kg/day of p,p'-DDE significantly attenuated TP 0.1 mg/kg-induced increases in weights of seminal vesicles and muscles, and TP 1 mg/kg-induced increases in weights of ventral prostate, seminal vesicles and muscles, but did not affect the weight of these organs in either TP 16 mg/kg-treated or intact rats, demonstrating that the dose range of 0.1-1 mg/kg TP is suitable for reference androgen. Oral treatment with 100 mg/kg of MT increased the weights of ventral prostate, seminal vesicles and muscles as strongly as did subcutaneous injection of 1 mg/kg of TP. These findings demonstrate that the 5-day Hershberger assay using young mature as well as immature male rats is a sensitive and valid short-term screening method for the detection of chemicals interfering with AR-mediated mechanisms. To determine whether fenitrothion interferes with AR-mediated mechanisms in vivo, fenitrothion (0, 0.75, 1.5 or 3 mg/kg/day) was administered by gavage for 5 days to castrated rats for androgenicity, or to castrated rats treated with 1 mg/kg TP for antiandrogenicity. Treatment with fenitrothion had no adverse effects on clinical signs, body weight, or liver or kidney weights, but cholinesterase activities in the brain and erythrocytes were significantly suppressed by fenitrothion to, respectively, 77-81% and 66-67% of control levels. In the antiandrogenicity experiment, serum androgen levels of TP-treated, castrated rats did not differ among groups. Treatment with 100 mg/kg of p,p'-DDE as a positive control again significantly attenuated TP-induced increases in weights of the ventral prostate and seminal vesicles, while fenitrothion had no effect on the weights of any organs. In the androgenicity experiment, treatment with 100 mg/kg of MT significantly increased weights of ventral prostate, seminal vesicles and muscles, but fenitrothion had no effects on the weights of any of these organs. These

findings yield no evidence that fenitrothion interferes with AR-mediated mechanisms in vivo, consistent with the result of several toxicological bioassays.

**Detection of the environmental antiandrogen p,p'-DDE in CD and Long-Evans rats using a tier I screening battery and a Hershberger assay.**

O'Connor JC, Frame SR, Davis LG, and Cook JC

Toxicol Sci 1999;51(1):44-53

In this report, p,p'-DDE, a weak androgen receptor (AR) antagonist, has been examined in a Tier I screening battery designed to detect endocrine-active compounds (EACs). The screening battery that was used to examine p,p'-DDE was an abbreviated version of a proposed Tier I screening battery (Cook et al., 1997, Regul. Toxicol Pharmacol. 26, 60-68) that consisted of a 15-day intact male in vivo battery and an in vitro yeast transactivation system (YTS). In addition, strain sensitivity differences were evaluated using male Crl:CDIGS BR (CD) and Long-Evans (LE) rats. Finally, p,p'-DDE was examined in a Hershberger assay designed to detect AR agonists. In the in vivo male battery using CD rats, responses to p,p'-DDE included organ weight changes (increased relative liver weight and decreased absolute epididymis weight) and hormonal alterations (increased serum estradiol [E2] levels and decreased serum FSH and T4 levels). Responses to p,p'-DDE in LE rats included organ weight changes (increased relative liver weight, absolute epididymis weight, relative accessory sex gland [ASG] unit weight, as well as the individual component weights of the ASG [prostate and seminal vesicles]), and hormonal alterations (increased serum testosterone [T], E2, dihydrotestosterone [DHT], thyroid-stimulating hormone [TSH], and decreased T4 levels). These data demonstrate that there are considerable strain-sensitivity differences to p,p'-DDE exposure. The described in vivo male battery using CD rats did not identify p,p'-DDE as an EAC. In contrast, the in vivo male battery using LE rats identified p,p'-DDE as a EAC. Evaluation of the data for the LE rats demonstrate that p,p'-DDE appears to be acting as an AR antagonist whose primary effects are more potent centrally than peripherally. In the YTS for the AR, p,p'-DDE had an EC50 value of  $3.5 \times 10^{-4}$  M; however, in the AR YTS competition assay, p,p'-DDE did not inhibit DHT binding to the AR. p,p'-DDE was inactive in the YTS containing the estrogen receptor or progesterone receptor at the concentrations evaluated. In the Hershberger assay, p,p'-DDE administration caused antiandrogen-like effects characterized by attenuation of the testosterone propionate-induced increases in reproductive-organ weights. In summary, these data suggest that strain selection will affect the ability to detect certain weak EACs. However, a Tier I screening battery consisting of both in vivo and in vitro endpoints would reduce the chance that weak-acting compounds such as p,p'-DDE would not be identified as potential EACs.



### **Transplacental and lactational transfer of p,p'-DDE in Sprague-Dawley rats.**

You L, Gazi E, Archibeque-Engle S, Casanova M, Conolly RB, and Heck HA  
Toxicol Appl Pharmacol 1999;157(2):134-44

p,p'-DDE (hereafter DDE), a persistent metabolite of p,p'-DDT, is a widespread environmental contaminant that can induce antiandrogenic developmental effects in rats. Quantitative measurements of the transfer of DDE from pregnant or lactating dams to the fetus or suckling neonate were performed, and physiologically based pharmacokinetic (PBPK) models for the transplacental and lactational transfer of DDE were developed. Pregnant Sprague-Dawley rats were dosed by gavage in corn oil with either 10 or 100 mg DDE per kg body wt per day from Gestation Day (gd) 14 to 18. DDE was analyzed in several maternal tissues as well as in fetal and neonatal tissues from gd 15 to Postnatal Day (pnd) 21. Fetal DDE concentrations were about threefold lower than corresponding placental concentrations. By adopting a cross-fostering design, the contributions of transplacental and lactational transfer were compared. In the pup liver, where DDE was detectable in the 100 mg/kg groups on pnd 10, the lactationally exposed group had DDE concentrations about 50 times higher than those of the in utero only exposure group; the lactation only exposure groups had DDE tissue dose profiles very similar to those of the in utero plus lactation exposure groups, indicating that the lactational route is far more important than the in utero route quantitatively. The PBPK models postulated initial absorption of DDE into both the blood circulation and lymphatic system with the primary storage sites being maternal and neonatal adipose tissues. Mobilization of DDE from its storage sites is postulated to occur via its association with mobilized fatty acids and lipoproteins. The results provide an overall framework for evaluating the tissue dosimetry of DDE and for understanding how maternal exposure to DDE could affect perinatal sexual development in utero or in the early postnatal period. Copyright 1999 Academic Press.

### **Modulation of testosterone-metabolizing hepatic cytochrome P-450 enzymes in developing Sprague-Dawley rats following in utero exposure to p,p'-DDE.**

You L, Chan SK, Bruce JM, Archibeque-Engle S, Casanova M, Corton JC, and Heck H  
Toxicol Appl Pharmacol 1999;158(2):197-205

1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) causes sexual developmental aberrations in male rats through a likely mechanism of androgen receptor antagonism. DDE is also known to induce liver cytochrome P-450 (CYP). The expression of CYP enzymes is regulated by steroid hormones, which, in turn, are inactivated in the liver by CYP-catalyzed hydroxylations and subsequent conjugations. This study was undertaken to examine the potential of in utero DDE exposure to affect the developmental expression of the hepatic CYP enzymes that are responsible for testosterone hydroxylations. Pregnant Sprague-Dawley rats were dosed daily by gavage with DDE at 0, 10, or 100 mg/kg body weight or with flutamide at 40 mg/kg body weight from gestation day 14 to 18. Additional adult male rats were given seven daily doses of DDE at 100 mg/kg. Liver samples were collected from the offspring of the dosed dams on postnatal days (PND) 10

and 21 and from the adult rats a day after the last dosing. Assays for regioselective and stereospecific testosterone hydroxylase activities were performed using hepatic microsomal preparations. Specific liver CYP proteins were detected by immunoblotting. While the CYP2B1 and 3A1 and their hydroxylated testosterone products were highly elevated by the DDE treatments in both adult and developing rats, the responses of 2C11 and 2A1 were development-dependent. The flutamide treatment had little effect on CYP enzyme expression. This study demonstrated that developing offspring rats are susceptible to the hepatic CYP enzyme-modulating action of DDE following its administration to the pregnant dams.

**Inhibition of androgen receptor-dependent transcriptional activity by DDT isomers and methoxychlor in HepG2 human hepatoma cells.**

Maness SC, McDonnell DP, and Gaido KW  
Toxicol Appl Pharmacol 1998;151( 1):135-42

Recent reports have raised new concerns that chemicals in our environment may disrupt normal reproduction and development through inhibition of androgen receptor function. This heightened concern has also increased our need for methods that allow us to characterize chemical interaction with the androgen receptor. In this report we describe an androgen receptor assay that utilizes the HepG2 human hepatoma cell line transiently transfected with the human androgen receptor and an androgen-responsive reporter. We used this assay to characterize the interaction with the androgen receptor of several steroidal and nonsteroidal chemicals, including isomers of DDT and methoxychlor. Chemicals were tested either in the absence (for determining agonist activity) or presence of  $10^{-7}$  M dihydrotestosterone (for determining antagonist activity). Testosterone and dihydrotestosterone were equally potent agonists in this assay. Estradiol and progesterone displayed partial agonist/antagonist activity. Flutamide was a complete agonist, whereas its hydroxylated metabolite, hydroxyflutamide, only partially antagonized and displayed some agonist activity at  $10^{-6}$  M and above. o,p'-DDT, o,p'-DDE, o,p'-DDD, p,p'-DDT, p,p'-DDE, and p, p'-DDD all behaved as antagonists at concentrations above  $10^{-6}$  M. p,p'-DDE also showed some agonist activity at  $10^{-5}$  M. Methoxychlor was only weakly antagonistic while its hydroxylated metabolite, HPTE, was approximately 10-fold more potent. Our results demonstrate that the HepG2 assay is a sensitive and specific method for detecting chemical interaction with the androgen receptor. This reporter gene assay, which we have used to characterize interaction with both the estrogen and androgen receptors, coupled with more extensive in vivo studies, should be useful for determining the role of multiple steroid receptors in the mechanism of action of endocrine active chemicals.

## **Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells**

Chen CW, Hurd C, Vorobjkina DP, Arnold SF, and Notides AC  
Biochem Pharmacol 1997;53(8):1161-72

In this study, we determined whether the DDT isomers p,p'-DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane], o,p'-DDT [1,1,1-trichloro-2(p-chlorophenyl)-2-(o-chlorophenyl)ethane], and their metabolites p,p'-DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane], o,p'-DDD [1,1-dichloro-2-(p-chlorophenyl)-2-(o-chlorophenyl)ethane], p,p'-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], o,p'-DDE [1,1-dichloro-2-(p-chlorophenyl)-2-(o-chlorophenyl)ethylene], and p,p'-DDA [2,2-bis(p-chlorophenyl)acetic acid], could bind to and transcriptionally activate the human estrogen receptor (hER). Novel results from competitive binding assays showed that o,p'-DDD, o,p'-DDE, and p,p'-DDT, as well as the established environmental estrogen o,p'-DDT, were able to bind specifically to the hER with approximately 1000-fold weaker affinities for the hER than that of estradiol. In contrast, only o,p'-DDT, but not p,p'-DDT, bound to the rat estrogen receptor. Moreover, two yeast expression-reporter systems, constructed to test if the DDT isomers and metabolites could transcriptionally activate the hER, demonstrated that an o,p'-DDT metabolite could transactivate the hER or LexA-hER fusion protein with just a 140- to 300-fold weaker potency than that of estradiol. The DDT isomers and metabolites that bound the hER *in vitro* triggered estrogen receptor-mediated transcription of the lacZ reporter gene in the yeast systems. Furthermore, the DDT isomers and metabolites that transactivated the hER elicited an additive response when given together or with estradiol. The DDT isomers and metabolites that triggered transcription of the yeast expression-reporter systems also stimulated two estrogenic endpoints in estrogen-responsive MCF-7 cells: the induction of the progesterone receptor and the down-regulation of the hER. Thus, in MCF-7 cells and in yeast expression-reporter systems, certain DDT isomers and metabolites act directly as agonists and transactivate the hER at concentrations found in human tissues.

## **Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist.**

Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, and Wilson EM.  
Nature 1995;375(6532):581-5

The increase in the number of reports of abnormalities in male sex development in wildlife and humans coincided with the introduction of 'oestrogenic' chemicals such as DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) into the environment. Although these phenotypic alterations are thought to be mediated by the oestrogen receptor, they are also consistent with inhibition of androgen receptor-mediated events. Here we report that the major and persistent DDT metabolite, p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), has little ability to bind the oestrogen receptor, but inhibits androgen binding to the androgen receptor, androgen-induced transcriptional activity, and androgen action in developing, pubertal and adult male rats. The results suggest that abnormalities in male sex development induced by p,p'-DDE and related environmental

chemicals may be mediated at the level of the androgen receptor.

**Differential effects of dichlorodiphenyltrichloroethane analogs, chlordecone, and 2,3,7,8-tetrachlorodibenzo-p-dioxin on establishment of pregnancy in the hypophysectomized rat.**

Johnson DC, Sen M, and Dey SK

Proc Soc Exp Biol Med 1992;199(1):42-8

Many of the organochlorine pesticides have been shown to elicit estrogenic responses in laboratory animals. Two estrogenic actions, initiation of implantation and maintenance of pregnancy, were examined in progesterone-primed, delayed-implanting, hypophysectomized rats exposed to several polychlorinated hydrocarbons. The insecticide P,P'-dichlorodiphenyltrichloroethane (DDT) was nearly devoid of estrogenic activity for initiating implantation, as was a dichloro analog, 1,1-dichloro-2-[p-chlorophenyl],2-[o-chlorophenyl]ethane (O,P'-DDD), but another such analog, 1,1-dichloro-2-(p-chlorophenyl),2-(o-chlorophenyl)ethylene (O,P'-DDE), was nearly as estrogenic as the O,P'-DDT isomer of DDT and the methoxylated analog methoxychlor. The latter three compounds not only initiated implantation, but maintained pregnancy when given in large (200 mg/kg) and repeated doses. Another insecticide, chlordecone (Kepone) was more estrogenic than any of the DDT analogs and maintained pregnancy with a single dose of 50 mg/kg. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a toxic contaminant of herbicide production, did not induce implantation at a dose of 125 micrograms/kg, but inhibited the implantation initiated by estrone in 35% of the animals. The mechanism of this antiestrogenicity is unknown but most probably does not involve direct action via the classical estrogen receptor. The possible interference with the normal blastocyst-uterine interactions of these polychlorinated xenobiotics may be an important factor in their being considered reproductive toxins.

**[The influence on reproduction in rats of DDE mobilization induced by ethanol administration during pregnancy and lactation] [Article in German]**

Bleyl DW, Nickel B, and Kujawa M

Nahrung 1984;28(4):357-69

The mobilization of xenobiotics is, in common with their persistence and accumulation, a latent problem of our chemicalized society. Possible sequels connected with the chronic consumption of alcohol were demonstrated by results from animal experiments. After a 10-week exposition to DDE (50 p.p.m. given with the diet), followed by a time of nonexposition, rats were mated and their reproductiveness was brought in relation to the results from residue analyses. Half of the animal received 20% alcohol instead of drinking water during pregnancy and lactation. An alcohol-induced increase in the mobilization of DDE was evidenced on determining residues in the maternal brain, the placenta and the foetus, and also by the excretion in milk and urine. There was a significance increase in prenatal and postnatal mortality (reduced survival index). By

analogy to DDT, an oestrogenic action is assumed to be causative.

**Estrogenic behavior of 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane and its homologues.**

Forster MS, Wilder EL, and Heinrichs WL  
Biochem Pharmacol 1975;24(1777-1780)

Eight chlorinated hydrocarbons were tested for their ability to compete with 3H-estradiol-17B for specific binding proteins in uterine cytoplasm from immature rats. The binding was assayed on 5-20% sucrose density gradients. 2(o-Chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane (o,p'-DDT) and 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1-trichloroethylene (o,p'-DDE) ( $1.4 \times 10^{-4}$  M) competed with 3H-estradiol-17B ( $8.7 \times 10^{-9}$  M) for binding to the "8S receptor" in the cytoplasm. 2,2-Bis(p-chlorophenyl)-1,1,1-trichlorophenylethane (p,p'-DDT), 2,2-bis(p-chlorophenyl)-1,1-trichloroethylene (p,p'-DDE), 2,2-bis(p-chlorophenyl)-1,1-trichloroethane (p,p'-DDD), 2(m-chlorophenyl)-2-(p-chlorophenyl)-1,1-dichlorophenylethane (m,p'-DDD), 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1-dichlorophenylethane (o,p'-DDD), and bis-(p-chlorophenyl)-1,1-dichloroacetate (DDA) did not compete at  $1.4 \times 10^{-4}$ M. Transfer of the receptor-bound compounds into the nuclei was examined using similar competition techniques. o,p'-DDT and o,p'-DDE at high concentrations act as estrogens as measured by their ability to compete with estradiol-17B for binding to the uterine cytoplasmic receptor and in the transfer and binding of estradiol in the nuclei of cells.

**DDT homologues: estrogen like effects on the vagina, uterus and pituitary of the rat.**

Gellert RJ, Heinrichs WL, and Swerdloff RS  
Endocrinology 1972;91:1095-1100

The estrogenic activity of DDT and some of its homologues was evaluated in female rats by using three experimental models: 1) young rats treated for 27 days, 2) mature ovariectomized rats treated for one week, and 3) mature hemiovariectomized rats treated for one week. In the first model, 500 ug o,p'-DDT injected daily produced advanced vaginal opening and heavier ovaries and uteri. Utilizing the second model, 10.0 mg/day of o,p'-DDT and p,p'-DDA produced increased uterine weight and a thickened, vacuolated uterine epithelium. The former compound also induced cornified vaginal smears and reduced serum LH concentrations without significantly affecting FSH titers. Neither compound affected the compensatory hypertrophy of the remaining ovary in the hemicastrated rat model. The p,p'-DDD and p,p'-DDE homologues failed to show estrogenic activity. These results demonstrate that o,p'-DDT and one of its major metabolites, DDA, exert estrogenic activity by stimulating uterine and vaginal tissue and reduce serum LH, probably via an inhibitory feedback action at the pituitary or hypothalamic level.

## **Distribution of DDT, DDD, and DDE in tissues of neonatal rats and in milk and other tissues of mother rats chronically exposed to DDT**

Woolley DE and Talens GM

Toxicol Appl Pharmacol 1971;18(907-916)

Female rats were chronically exposed to 25, 100, or 200 ug/g dietary DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] during pregnancy and lactation. In offspring sacrificed within 1 hr after birth before suckling has occurred, DDT concentrations in the whole rat averaged from 1.3 to 2.2 ug/g fresh tissue, and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] concentrations were 1.0 ug/g or less in the 3 groups. Average concentrations of DDT and metabolites were higher in liver (1.3-4.3 ug/g) than in brain (0.9-2.5 ug/g). In neonatal rats, sacrificed about 10 hr after birth, after suckling had occurred and stomachs were full of milk, concentrations of DDT in the liver were about 5-fold greater than in the littermates sacrificed 1 hr after birth. The results indicate that the retention of DDT in tissues of the neonate during the lactational period was greater than in fetal tissues during gestation. DDT concentrations were 33 ug/g fresh tissue in milk from dams on the 25 ug/g dietary DDT, 92 ug/g in milk from dams on 100 ug/g dietary DDT and 174 ug/g in milk from dams on 200 ug/g dietary DDT, showing that the level of output of DDT in the milk may be higher than the level of DDT intake on the lower levels of DDT. In the maternal tissues, fat contained by far the highest concentrations of DDT and metabolites. Liver, kidneys, the various brain areas and spinal cord had approximately similar levels of DDT. Average plasma levels of DDT were low, ranging from 0.7 to 1.7 ug/ml among the 3 groups. Liver was the only tissue in which DDD concentrations were as high or higher than DDT concentrations. DDD concentrations in the liver were higher than DDE concentrations, whereas the reverse was true for all other tissues.

## **Estrogenic action of DDT and its analogs**

Welch RM, Levin W, and Conney AH

Toxicol Appl Pharmacol 1969;14(2):358-367

The intraperitoneal injection of 50 mg/kg of purified o,p'-DDT, technical grade DDT, purified methoxychlor, or purified p,p'-DDT increased the uterine wet weight by 49, 43, 37, and 28%, respectively, 6 hours after injection. o,p'-DDD, m,p'-DDD, p,p'-DDD, and p,p'-DDE exhibited little or no activity. The intraperitoneal injection of as little as 5 mg/kg or 1 mg/kg of technical grade DDT or o,p'-DDT, respectively, caused a significant increase in the uterine wet weight in immature female rats. The injection of technical grade DDT or o,p'-DDT into ovariectomized adult rats, also increased uterine wet weight, indicating that the effect of the DDT analogs was not mediated through the ovaries. Treatment of immature female rats with a 50 mg/kg injection of technical grade DDT or purified o,p'-DDT caused a several fold stimulation in the incorporation in vitro of glucose-U-14C into lipid, protein, RNA and acid-soluble constituents in the uterus 6 hours after the dose of DDT. A smaller stimulatory effect was observed with purified p,p'-DDT. Treatment of rats with technical grade DDT, purified o,p'-DDT,

methoxychlor or p,p'-DDT 2 hours before an injection of estradiol-17B-6,7-3H inhibited the uptake of estradiol-17B-6,7-3H by the uterus in vivo, possibly by competing for sites that bind estradiol-17B in the uterus. o,p'-DDD, p,p'-DDD, m,p'-DDD, and p,p'-DDE did not inhibit the uptake of estradiol-17B-6,7-3H by the uterus. Pretreatment of rats with carbon tetrachloride inhibited the uterotrophic action of o,p'-DDT and technical grade DDT. This suggests the possibility that the action of these substances on the uterus may depend on the conversion of the analogs of DDT to estrogenic metabolites.