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Exposure to Diethylstilbestrol during Sensitive Life Stages: A legacy of heritable health effects

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Abstract

Diethylstilbestrol (DES) is a potent estrogen mimic that was predominantly used from the 1940s to 1970s in hopes of preventing miscarriage in pregnant women. Decades later, DES is known to enhance breast cancer risk in exposed women, and cause a variety of birth related adverse outcomes in their daughters such as spontaneous abortion, second trimester pregnancy loss, preterm delivery, stillbirth, and neonatal death. Additionally, children exposed to DES in utero suffer from sub/fertility and cancer of reproductive tissues. DES is a pinnacle compound which demonstrates the fetal basis of adult disease. The mechanisms of cancer and endocrine disruption induced by DES are not fully understood. Future studies should focus on common target tissue pathways affected and the health of the DES grandchildren.

Keywords

diethylstilbestrol; DES; cancer; development; fetal basis of adult disease; breast; pregnancy

INTRODUCTION

Diethylstilbestrol (DES) is a synthetic estrogen, developed in 1938, that is estimated to be five times more potent than the naturally occurring estrogen, estradiol (Korach et al., 1978; IARC, 2012). It shares structural similarity with other xenoestrogens (Figure 1), and is known to activate the estrogen receptor- α (ER α), with a similar affinity for the receptor as estradiol (Korach et al., 1978). DES is well absorbed in the body and is lipid soluble. Once in the human body, DES reaches peak concentration within 20–40 min, having a primary half-life of 3–6 hr. It has a terminal half-life of 2–3 days due to entero-hepatic circulation, and is primarily excreted in urine (summarized in Giusti et al., 1995). DES has a short, biphasic half-life in dogs of approximately 1 hr, followed by a terminal half-life of 24 hr (Page 1991). Upon oral absorption and whole body distribution, DES can be metabolized in all species evaluated to produce either hormonally inactive compounds (such as α -di-enestrol) or compounds that retain estrogenic activity (like DES-epoxide or quinone metabolites) (Korach et al., 1978; IARC, 2012), and is exclusively eliminated through biliary excretion in the feces of rats, hamsters and mice (IARC 2012).

It is estimated that DES was prescribed to between 2 and 10 million pregnant women worldwide as pills, injections, suppositories, and creams to prevent miscarriage between 1947–

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1971 (Harremoes et al., 2001; Rubin 2007; Newbold 2008). Few toxicological studies were done on the drug before it was produced and given to women in non-standardized doses worldwide (Harremoes et al., 2001; Rubin 2007). Although the use of the pharmaceutical DES has been discontinued since the U.S. Food and Drug Administration (FDA) advised physicians to cease prescribing the drug in 1971, the adverse health outcomes discovered in women who took DES, and the many reproductive problems caused in their offspring and subsequent generations, continue to be characterized.

Latent health effects in DES-exposed offspring provide strong evidence for study regarding the fetal basis of adult disease. The developing fetus is uniquely susceptible to latent health effects due to the several underdeveloped systems at birth (i.e., DNA repair mechanisms, liver metabolism, blood/brain barrier, and immune system). Therefore, low doses of chemicals thought to be safe for use in pregnant women may seriously impact the adult health of their offspring. The data on DES have shown that dose and timing of exposure may matter –cumulative dose, time since exposure, or age at diagnosis of disease are considered important variables in cohort data analyses.

The purpose of this review is to summarize the accumulated data on birth related outcomes, cancer and endocrine related effects of DES in exposed mothers and their offspring, and to discuss current ideas on mechanisms of action and paths forward to insure that another product like DES is never prescribed to pregnant women again.

DES EXPOSURE MAGNITUDE

DES was prescribed to pregnant women in the first trimester (typically between weeks 7–8 post last menstrual cycle) to prevent miscarriages induced by a progesterone deficiency, and later in pregnancy to prevent premature labor or to treat break-through bleeding (Smith 1948; NTP 2011). It has also been used to treat prostate and breast cancer, inhibit lactation and post-partum engorgement, control gynecological bleeding, stunt abnormal height in girls, and as hormone-replacement therapy and a post-coital contraceptive (NTP 2011; Harris and Waring 2012). The exact number of women/fetuses prenatally-exposed to DES world-wide is unknown (IARC 2012). The majority of reports of DES use are from the U.S. where it is believed that between the 1940s and 1970s, 5 to 10 million people either consumed DES during pregnancy or experienced in utero exposures (IARC 2012). DES use was also popular in Europe and Australia, and like the U.S., many women did not know that they were taking DES. Therefore, estimated numbers of people reporting exposure during pregnancy or in utero are around 300,000 in the United Kingdom and 200,000 in France (Harris and Waring 2012).

Total doses of DES prescribed ranged between less than 100 mg (in the majority of cases) up to 46,600 mg, with a median U.S. dose of 3,650 to 4,000 mg (IARC 2012). Most women started off with low doses (i.e., 5 mg), that increased (up to 125 mg) as symptoms or pregnancy progressed, translating to doses of about 100 µg/kg to 2 mg/kg DES per day (Hilakivi-Clarke et al., 2013). The peak use of DES in the U.S. occurred between 1946 and 1964, whereas France, and potentially other parts of Europe, experienced a later peak – between 1966 and 1972 (Tournaire et al., 2012; Hilakivi-Clarke et al., 2013; (<http://diethylstilbestrol.co.uk>). Although limited data is available in a French cohort, the median total dose was 4,050 mg, with a 95% CI [3,000–4,500]. This is in contrast to the “high dose cohorts” with median doses (mg) of 12,442, 8,575, and 7,550 from Chicago, Boston and California, respectively (Tournaire et al., 2012). “Low dose cohorts” also existed in the U.S., with median doses (mg) of 3,175, 2,572, and 1,520 in Wisconsin, Texas, and Minnesota, respectively. Thus, comparison of effects based on dose may vary within the U.S. and across countries.

DES was not only used as a human pharmaceutical, it was also used as a feed additive for cattle, poultry, and sheep between 1954 and 1979 (and possibly later in other countries) to improve weight gain and produce leaner meat (Harris and Waring 2012). The high-dose (24–36 mg) subcutaneous pellets of DES were meant to last for extended periods in feedstock, and resulted in systemic release of 56–74 µg DES per day, with a half-life of 80–90 days (Rumsey et al., 1975). As a single oral dose, DES demonstrated a much shorter biphasic half-life of 17 hr and a later phase of 5.5 d in cattle (Rumsey et al., 1975; IARC 2012). Therefore, DES was a contaminant in food sources for 8 or more years after the FDA banned its use in humans. Because of this, it is unknown to what extent the general population was exposed.

Mice and rats exposed prenatally or neonatally to DES provide excellent models for human intrauterine exposure. Rodent models have been utilized to evaluate DES-induced infertility, breast cancer susceptibility, reproductive tract abnormalities and cancer development, as well as investigate the mechanisms involved in later life disease. Murine genital tract development at birth is similar to human fetal development at the end of the first trimester (Sato et al., 2004; Yamashita 2006; Ma 2009). Doses of DES given to pregnant mice or rats varied between 0.2 to 12,000 µg/day, or approximately 1 µg/kg to 60 mg/kg DES per day in the rat (Hilakivi-Clarke et al., 2013), and these doses were typically oral or via subcutaneous injection in oil.

HEALTH EFFECTS

DES is a transplacental carcinogen (a cancer-causing agent that crosses the placenta and causes reproductive cancer in offspring), a teratogen able to induce developmental defects, and an endocrine disrupting compound (EDC) that alters appropriate hormonal responses in a number of reproductive target tissues (Harremoes et al., 2001; Newbold 2008; NTP 2011; Harris and Waring 2012; IARC 2012). Cohort populations of DES-exposed individuals exist in multiple countries and provide vital information on the long-reaching effects of DES. The overall risk of neoplasia in ‘DES mothers’ (the women who were prescribed DES treatment) is low, but there are numerous reproductive and structural issues found at high frequency in gestationally exposed ‘DES daughters’ and ‘DES sons’ (Harremoes et al., 2001; Troisi et al., 2007; Newbold 2008; Palmer et al., 2009; Hoover et al., 2011; Kalfa et al., 2011; Virtanen and Adamsson 2012). It is not completely clear if a dose-response association for DES-exposed individuals and their health outcomes exists for some end points, but there is an association for timing of exposure in utero, suggesting that there is life stage susceptibility for maximal detrimental later-life health effects (Harris and Waring 2012). These long term abnormalities are primarily associated with exposure early in gestation (before gestation week 11) and result in changes that typically do not manifest until after the onset of puberty (Newbold et al., 1990; Walker and Haven 1997; Ma 2009).

Animal models are a vital source of information as well. Animal models have confirmed human disease endpoints and more importantly predicted changes such as malformations of the oviduct and increased incidence of fibroids which were later found in DES-exposed women (Newbold 2008). Animal models exploring in utero DES exposure at doses modeling internal human exposures showed tumor risk values within the range of the calculated values for humans, giving further credibility to using animal models and extrapolating findings to human health outcomes (Anderson 2004). These studies provided translational research and informed policy to help regulate the drug’s use. DES is no longer used in pregnant women, making DES-exposed animal models essential in trying to predict future health issues and the mechanistic pathways that are targeted in multiple generations. These areas are worthy of more in depth discussion.

Effects in DES Mothers

DES mothers are the women who were prescribed DES in some form during their pregnancy. A large case-control study with approximately 6,000 participants (Colton et al., 1993) reported that 20% of DES mothers vs. only 5% of age-adjusted control women had one or more miscarriages before their first term delivery, suggesting that DES mothers had reason for seeking help in pregnancy maintenance. Although women were prescribed DES to improve the outcomes of their given pregnancy, the results of a double-blind clinical trial of over 1500 women at the University of Chicago by Dieckmann and coworkers in 1953 demonstrated that DES did not reduce the incidence of spontaneous abortion, prematurity or postmaturity, and the study suggested that DES enhanced premature labor (Dieckmann et al., 1953). However, it continued to be used for another nearly 20 years.

Numerous studies have evaluated the possible health effects in cohorts of DES mothers. Only a slight increase of 10% has been reported for overall neoplasia in DES mothers (Titus-Ernstoff et al., 2001). However, several well powered case-control studies (see Table 1), that included evaluation of patient records, have found that there is a slight, but consistent and significant 30–50% increase in risk for developing breast cancer in DES mothers, with the relative risk (RR) varying by model adjustments (Bibbo et al., 1978; Greenberg et al., 1984; Hadjimichael et al., 1984; Titus-Ernstoff et al., 2001; Colton et al., 1993). In a 1984 study of over 5,000 women that were seen at numerous medical institutes primarily in the northeast region of the U.S. (but including Mayo Clinic), Greenberg and co-workers (Greenberg et al., 1984) reported an adjusted RR for breast cancer of 1.47 [95% CI, 1.10–1.98], that increased further for the oldest women in their study (30–39 yr since exposure, RR=2.5). Another well-powered follow-up study of northeast U.S. women also reported (9 years later) an adjusted RR of 1.35 [95% CI, 1.05–1.74] for breast cancer in DES mothers, but did not report an increased risk for women 30 or more years since exposure (Colton et al., 1993). Titus-Ernstoff *et al.* also found a modest association with breast cancer risk and DES use during pregnancy with a RR of 1.27 [95% CI, 1.07–1.52; (Titus-Ernstoff et al., 2001)]. This study was analyzed from two combined cohorts of women and the nearly 30% increased risk that was observed is consistent with previous studies. Additionally, in the combined cohort analyses, there was a significant increase in breast cancer risk for women 30–39 years since exposure RR=1.52 [95%CI, 1.11–2.07], which also happened to be the largest group of women.

These analyses taken together suggest that one in six women prescribed DES will develop breast cancer, versus one in eight women in the general population (not prescribed DES; Titus-Ernstoff et al., 2001). The increased risk, however, would not occur until at least 20 years after exposure, and as two of the three reviewed studies report (Greenberg et al., 1984; Titus-Ernstoff et al., 2001), it would take over 30 years post-exposure to see the adverse effects of DES in the breast. For instance, in the women 0–9 and 10–19 years since DES exposure, there was an insignificant RR of 1.0 and 1.1, respectively, and in the women 20–29 and 30–39 yr since exposure, there was increased RR of 1.6 and 2.5, respectively (Greenberg et al., 1984). Similarly, the Titus-Ernstoff analyses (Titus-Ernstoff et al., 2001) demonstrated that the highest RR for breast cancer (1.52) was found once the women were 30–39 yr since exposure. These data indicate that DES may act as a cancer initiator and a weak human carcinogen. Although a couple of earlier studies (Hoover et al., 1976; Hoover et al., 1977) suggested relative risks of endometrial and ovarian cancers among women who took DES, the more recent and robust cohort analyses to date have found no increased risk for ovarian, endometrial, or other cancers in women exposed as adults (Titus-Ernstoff et al., 2001).

Effects in animal research models mirrored the human effects of adult DES exposures, and provided additional sensitive endpoints of study. Adult oral DES exposure in experimental

rodent models demonstrated mammary gland tumors in CD-1 and genetically modified mouse lines (IARC 2012). Adult, orally-exposed mice were also found to have cancer of the ovary, cervix, uterus, vagina, testes, and bone, and some studies determined that life stage at exposure affected outcomes. Little oral exposure testing was performed on the parental generation in the rat model. The differences in health outcomes across the rodent species were significant and were likely due to the strain of rodent chosen for the studies. Some strains (i.e., Tg.AC) are more prone to one type of tumor vs. another and animals were not kept until all spontaneous tumors could develop in these studies.

Effects in DES Daughters

A critical point in the discussion of effects in DES offspring is the fact that exposure during sensitive life stages led to a variety of permanent adverse health outcomes in fairly large fractions of the exposed populations of both rodents and humans. These exposures were transplacental. The only example of adult exposure related to adverse outcomes was in the DES mothers, who were exposed during a period of rapid breast development (pregnancy), which is often regarded as a sensitive life stage (IBCERCC 2013). The result was an increased RR for breast cancer, with few other exceptions. That was not the case in the offspring.

In 1970, Herbst and Scully (Herbst and Scully 1970) reported the first conclusive evidence of vaginal clear-cell adenocarcinoma (CCA) in seven young women between the ages of 15–22. This very rare cancer is generally only found in older women (>40 yr), and typically in squamous cell, not clear cell form. This finding was confirmed multiple times between 1970 and 1972 in women as young as 7 years old, and was consistently linked to in utero DES exposures [for review see Laronda et al., 2012]. These results led to the development of registries of women and men exposed to DES (5 major cohorts; DESAD – Diethylstilbestrol Adenosis Project, Women’s Health Study, Mayo Clinic, Dieckmann, and Horne). The National Cancer Institute developed and funded the DES Follow-up Study, which continues to explore long-term health effects in over 21,000 exposed individuals in those cohorts.

DES daughters have about a 40-fold increase in the risk of vaginal or cervical CCA and an estimated cumulative incidence rate of 1.6 per 1,000 (0.2%) exposed women (Troisi et al., 2007; Hoover et al., 2011). The startling association of CCA and in utero DES exposures has led to numerous studies (in mice and humans) aimed at evaluating prenatal DES exposure of second generation females and the related DES-induced reproductive anomalies and malformations.

Structural abnormalities/changes—There are a range of adverse reproductive tract abnormalities seen in DES daughters (see Table 2). Upper and lower genital tract structural changes have been documented in 25–33% of this population (Kaufman 1982). These include morphological changes in the cervix such as collars, hoods, septae, and cockscombs (Kaufman 1982; Giusti et al., 1995; Goldberg and Falcone 1999; Kaufman et al., 2000). Uterine malformations that have been diagnosed include hypoplastic cavity, T-shaped uterus, constriction bands, wide lower segment, and irregular borders (Goldberg and Falcone 1999; CDC 2012). Many of these structural changes are harmless and have no effect on development, risk of disease, or the ability to conceive. Mouse models using perinatal DES exposure have reproduced these effects and found additional reproductive tract abnormalities that include the absence of corpus luteum, hypertrophy-hyperplasia of interstitial tissues, induction of polyovular follicles in the ovary, and vaginal cornification (Newbold et al., 1998; Newbold et al., 2006; Kakuta et al., 2012).

Other abnormalities documented in cohorts of women exposed in utero include paraovarian cysts, lesions in multiple reproductive tract tissues, benign vaginal adenosis, and uterine

fibroids (Giusti et al., 1995; Newbold et al., 2006; Rubin 2007; CDC 2012; D'Aloisio et al., 2012). In fact, a recent study found that *in utero* DES exposure was strongly associated with early onset leiomyomata (fibroids) in black women (D'Aloisio et al., 2012). It is estimated that DES daughters are more than twice as likely as unexposed women to have abnormal cellular changes in the lower reproductive tract upon cytological examination (Senekjian et al., 1988). It is not clear if these abnormal cellular changes are precursors to cancer. Benign cervical or vaginal adenosis, a congenital anomaly of the surface epithelium, has been estimated to affect between 34–91% of DES-exposed daughters (Laronda et al., 2012), but often goes undetected. In fact, one study determined that only 20–40% of patients with a histologically-determined lesion had it detected in routine cytological screening. This benign lesion affecting upwards of 90% of the DES-exposed women, is also found in a small percentage of unexposed women (4%). Adenosis is not currently thought of as a precursor to CCA, as DES exposure increases the incidence of both health outcomes by a similar 30–40 fold over the unexposed populations (Laronda et al., 2012).

Functional abnormalities—Possibly the most extensive effect of *in utero* DES exposure is on functional parameters of reproduction. In the largest and most recent cohort analyses, Hoover *et al.* determined that DES daughters have an increased risk for many pregnancy-related issues including spontaneous abortion (<14 weeks gestation), ectopic pregnancy, loss of pregnancy in the second trimester (14–27 weeks), preeclampsia, preterm delivery (<37 weeks), stillbirth (at >27 weeks), and neonatal death within the first month of life (Hoover et al., 2011; Table 2). Many of these outcomes including ectopic pregnancy, miscarriage, and premature delivery have been reported in more than one study (Senekjian et al., 1988; Goldberg and Falcone 1999; Kaufman et al., 2000; Newbold et al., 2006; CDC 2012), and appear to be exacerbated effects for which DES was prescribed to prevent.

The effects of prenatal DES exposure on the ability to reproduce are substantial. The risk for infertility (defined as 12 months of trying to conceive) among DES daughters is reported to be 33% compared to 14% in unexposed women ($p<0.001$) (Senekjian et al., 1988), and full-term infants were delivered in the first pregnancies of 84.5% of unexposed women compared with 64.1% of DES exposed women (RR=0.76, 95% CI, 0.72–0.80) (Kaufman et al., 2000). The Dutch DES cohort (Verloop et al., 2010) reports that 33% of DES daughters are nulliparous at the age of 40 yr, compared with only 17% in the Dutch population. Kaufman and co-workers (Kaufman et al., 2000) also reported that that once pregnant, 20% of DES daughters experience preterm delivery (versus 8% of unexposed population (RR=2.93; 95% CI, 2.23–3.86)), their risk of ectopic pregnancy was 3 to 5 times higher than unexposed women (RR=3.84; 95% CI, 2.26–6.54), and 20% of the DES-exposed group had a miscarriage during the first pregnancy (versus 10% unexposed (RR=2.00; 95% CI, 1.54–2.60)). These adverse pregnancy-related outcomes in DES daughters are also experienced by unexposed women, but the excess risk in those outcomes (not stillbirth) owing to *in utero* DES exposure was significant (Hoover et al., 2011). Also, there are strong data (Hoover et al., 2011) suggesting that the presence of vaginal epithelial changes at cohort entry examination adds to the cumulative risk for DES-induced infertility, spontaneous abortion, preterm delivery, and ectopic pregnancy.

Data from mouse studies have confirmed poor reproductive outcomes and reduced fertility following *in utero*/neonatal DES exposures in humans (McLachlan et al., 1982; Newbold et al., 1998; Newbold et al., 2006). DES-exposed female mice had both fewer litters over their lifetime and fewer pups per litter, than unexposed controls. This effect was dose dependent. McLachlan and colleagues (McLachlan et al., 1982) also reported numerous structural/cellular reproductive tract abnormalities in mice following prenatal DES exposure, along with reduced ovarian response. A major component of the decreased fertility of DES

exposed female mice was related to a significant decrement in the number of ova recovered following induced ovulation (30% of control level).

Control of ovarian function is also a long-term deleterious effect of DES in women. There is now evidence for DES effects at the beginning, middle and end of ovarian cyclicity. A recent evaluation of data from the Sister Study (information from over 33,000 women; D'Aloisio et al., 2013) indicates that prenatal DES exposure was significantly related to very early menarche (< 10 yr; [RR=1.56; 95% CI, 1.24–1.96]). This study also indicated that very early menarche was related to pregnancy-related hypertension, small birth weight and being a firstborn; risk factors which may all be linked to DES exposure and may be inter-related. Exposure to DES *in utero* may alter menstrual function in females; another factor that may alter reproductive ability. There are reports of hirsutism and irregular menses in large percentages of small cohorts of DES daughters, and increased serum prolactin and testosterone levels in other small cohorts of DES-exposed patients compared with controls [reviewed in Goldberg and Falcone 1999].

Multiple studies have evaluated menstrual irregularity in larger cohort studies (Bibbo et al., 1978; Herbst et al., 1981; Barnes et al., 1984; Senekjian et al., 1988). Some analyses (Bibbo et al., 1978; Herbst et al., 1981) reported increased menstrual irregularity in DES-exposed women compared with unexposed controls, whereas other analyses (Barnes et al., 1984; Senekjian et al., 1988) found no significant difference between the exposure groups. According to one report (Goldberg and Falcone 1999), the overall rate of menstrual irregularity, including four uncontrolled studies, was 32% in the 1,192 DES-exposed women and 15% in the 619 controls. The reasons for irregular menses are not understood, but contributing issues may include a reduction in the duration and qualitative assessment of volume in menstrual flow, a smaller endometrial cavity area, reduction in the endometrial thickness, or hormonal dysmenorrhea in DES-exposed women (Kaufman 1982; Goldberg and Falcone 1999).

Along with irregular menses, DES daughters commonly undergo menopause before 45 years of age, which is earlier than the average unexposed woman (Hoover et al., 2011). Early menopause, along with early menarche, is a known risk factor for breast cancer and other adverse health outcomes (IBCERCC 2013). In fact, in the most recent cohort analyses (Hoover et al., 2011), DES daughters had a 2–3 fold increased risk of early menopause, compared to unexposed, age-matched controls (RR=2.35; 95% CI, 1.67–3.31). Evaluation of data from the Sister Study confirmed that DES-exposed women underwent menopause at 1.45 times the rate of unexposed women, which translates to about one year earlier than unexposed women (Steiner et al., 2010).

Cancer Incidence—The spark that caused regulators to act on the safety of DES was not the fact that DES mothers developed breast cancer, but the early finding (Herbst and Scully 1970) of seven cases of CCA in young women who were prenatally exposed to DES [reviewed in Harremoës et al., 2001]. Seven of eight cases of vaginal CCA, but none of the 32 controls, had confirmed prenatal DES exposure. It is now well established that gestational exposure to DES increases the risk of CCA (Giusti et al., 1995; Hatch et al., 1998; Troisi et al., 2007; Verloop et al., 2010; Hoover et al., 2011). Troisi and colleagues reported that approximately 1.6 in every 1,000 (nearly 0.2%) of DES daughters will be diagnosed with CCA, while the risk is almost non-existent among unexposed premenopausal women (Troisi et al., 2007). They also revealed that CCA risk does not seem to be related to gestational age at exposure or DES dose. Even in the Dutch DES cohort (Verloop et al., 2010), where there are a small percentage of exposures confirmed by medical record, there is a highly significant standardized incidence ratio of 24.23 for CCA (95% CI, 8.89–52.74).

While the link between DES exposure *in utero* and CCA is indisputable, there is some disagreement on the risks for other cancers. Early studies suggested that there was no increase in risk of breast cancer in DES daughters (Hatch et al., 1998), but recent follow-up and more careful adjustment for age (since it takes decades for cancer to develop) have revealed consistent findings. Large cohort studies reported that DES daughters have a significant 2-fold elevation of risk for breast cancer at age 40 or older (Palmer et al., 2006; Troisi et al., 2007; Hoover et al., 2011), while women over the age of 50 may have an even greater risk (age-adjusted incidence rate ratio=3.00; 95% CI, 1.01–8.98; Palmer et al., 2006). The Dutch DES cohort (Verloop et al., 2010) has not reported a significant effect of exposure on breast cancer risk in daughters; the most recent standardized incidence ratio of 1.94 (95% CI, 0.97–3.57) for the lowest dose group approaches significance. It is possible that results in Europe and the U.S. will only be comparable if based on similar “dose” and time since peak use or exposure. It has been hypothesized that DES changes the hormone profile that the fetus is exposed to, which may enhance receptor activation or increase the total number of ductal stem cells that are at risk for additional carcinogen insult (Palmer et al., 2006). However, the cohort of DES daughters is still relatively young (mean of 44 years old) so the link between exposure and breast cancer incidence may become stronger as they age (Hoover et al., 2011).

As far as all other cancers are concerned, there is little risk due to prenatal DES exposure in female offspring. Hoover *et al.* found an increased risk of cervical intraepithelial neoplasia grade 2+ (CIN2+) (Hoover et al., 2011). Troisi *et al.* evaluated cancer risk in the DES follow up study and they found no increased risk for endometrial cancer or ovarian cancer (Troisi et al., 2007). Likewise, after following a cohort of DES daughters for 16 years, Hatch *et al.* found no correlation between DES exposure and increased risk for 80 different types of cancer, presumably because the women were still fairly young at that time (Hatch et al., 1998). Further follow-up is needed.

Rodent models have found increases in the incidence of malignant reproductive tract tumors including uterine adenocarcinoma, cervical cancer, vaginal cancer, and mammary tumors (Newbold et al., 1998; IARC 2012), in addition to many other types of abnormalities noted earlier (see Table 3). Umekita *et al.* treated rats neonatally with DES (1 µg to 1000 µg) and found that the treatment significantly increased the number of terminal end buds (TEBs), the rapidly proliferating duct ends, at postnatal day 50 (Umekita et al., 2011). The early development of TEBs and the slow development of alveolar buds indicate the predisposition for an increased number of terminal ductal lobular units (TDLUs) in the breast tissue of DES daughters. The TEBs in rodents and TDLUs in humans are the primary sites for carcinogen initiation and action so the earlier appearance and longer life span of these structures increases the time period that carcinogens can initiate malignant growth (Umekita et al., 2011; Hilakivi-Clarke et al., 2013).

Effects in DES Sons

Most of the research associated with prenatal exposure to DES has been focused on female reproductive outcomes. The studies that have been reported on male *in utero* DES exposure have primarily focused on neoplasia or genital anomalies (see Table 4 for summary). Genital abnormalities in DES sons are increased, and include elevated risk for non-cancerous epididymal cysts (21%–31% of exposed men versus 5%–8% of unexposed men) (Giusti et al., 1995; Palmer et al., 2009; NCI 2012). The main developmental issues that have been noted in the DES son population are cryptorchidism (undescended testicles), hypospadias (misplaced urethral opening), and micropallus (Klip et al., 2002; Palmer et al., 2009; Kalfa et al., 2011; Virtanen and Adamsson 2012). It is estimated that 15%–32% of the DES sons' population have these abnormalities versus 5%–8% of the general population (Klip et al., 2002; Newbold et al., 2006; CDC 2012). These studies are in agreement with meta-analyses

that have found doubled risk ratios for cryptorchidism and hypospadias in men exposed *in utero* to DES (Klip et al., 2002; Martin et al., 2008; Kalfa et al., 2011; Virtanen and Adamsson 2012). In fact, in their meta-analysis of three large studies Martin et al (2008) report a 3.7-fold increased risk of hypospadias following in utero DES exposure. An in-depth study looking at the urogenital abnormalities in the DES sons cohort found that exposure is not associated with varicocele (widening of veins along spermatic cord); structural abnormalities of the penis; urethral stenosis; benign prostatic hypertrophy; or inflammation/infection of the prostate, urethra, or epididymis (Palmer et al., 2009).

In rodents, neonatal DES exposure causes increased thickness of the smooth muscle layer of the seminal vesicles as well as a permanent inability of the tissue to reach and maintain a normal size (Walker et al., 2012). The seminal vesicles become refractory to androgen stimulation during adulthood and they become feminized (as indicated by expression of lactoferrin, *Itf*, an estrogen-responsive gene; Walker et al., 2012). These findings have not been shown in the human population. There are also reports of DES impacting circulating hormone levels necessary for proper reproduction. In a validated assay of human, mouse, and rat Leydig cells, DES significantly decreased relative testosterone secretion in the cultures of mouse and rat, but not human Leydig cells when compared to controls (N'Tumba-Byn et al., 2012).

Studies regarding sexual function and fertility in DES sons are inconsistent. Some studies report lower than average sperm density and decreased sperm counts [summarized in Guisti et al., 1995; Rubin 2007; CDC 2012], while others have seen no impairment in fertility or sexual function (Wilcox et al., 1995). In some individual cases there may be decreases in fertility caused by hypospadias due to misdirected ejaculate (Klip et al., 2002).

The association between DES exposure and testicular cancer is uncertain. Some studies have shown no association while others have found an increased risk. The overall cancer rate for testicular cancer is increased in DES sons versus the national rate but this increase is not statistically significant (Strohsnitter et al., 2001; NCI 2012). One meta-analysis, however, found that the risk ratio for testicular cancer after DES exposure was doubled (Martin et al., 2008). This lack of consistency may be related to age since exposure, as was the case for breast cancer. One proposed mechanism for DES-induced testicular cancer has to do with the reduction of Müllerian inhibiting hormone caused by DES. Müllerian inhibiting hormone degrades the Müllerian ducts (the female structures) in the male fetus; however the incomplete breakdown of these structures caused by DES may become cancerous later in life (Strohsnitter et al., 2001; Newbold et al., 2006). Mouse studies have found an association between DES exposure and an increased rate of rete testis cancer and prostate cancer (Newbold 1995). No human studies have found similar risks but these increased rates were found in older animals and the DES sons' cohort may not be old enough to see these effects (NCI 2012). Therefore, diligent follow up and individual screening are needed to detect early reproductive cancers.

EFFECTS OF DES IN A 3rd GENERATION

Walker and Haven (1997) predicted that “if the high intensity of DES multigenerational carcinogenicity seen in mice is applicable to the human population, this is a health problem of major proportions.” They go on to say that it “could take over 50 years” to detect the effects in future generations, due to the length of time required for diseases such as cancer to manifest. It is predicted that cross-generational responses to DES exposure are possible due to epigenetic changes in the DNA and that the “germ cell pool could have become massively contaminated”. For example, early exposure to EDCs, like DES, is thought to reprogram mouse female reproductive tract development and affect how the reproductive tract responds

to endogenous estrogens later in life (Ma 2009; Hilakivi-Clarke et al., 2013). They (Walker and Haven 1997) suggest that “environmental estrogens may be more potent than previously suspected, due to synergistic action from concurrent exposures.”

The studies on the cohort of men (grandsons) and women (granddaughters) whose mothers were exposed prenatally to DES (grandchildren had no direct exposure) are limited as they are just beginning to reach the age when relevant health problems can be studied (CDC 2012). Studies that have been performed contain preliminary data, as the power is low. Therefore, the main sources of information for third generation effects are rodent studies. In general, multi-generational mouse studies have shown an increased susceptibility to tumor formation in the third generation which suggests that DES grandchildren are also at an increased risk for cancer (Newbold et al., 1998; Newbold et al., 2006).

Granddaughters

Currently there are no human studies that definitively show any adverse effects of DES for the third generation of females. A small cohort study of 28 DES granddaughters found no abnormalities in the lower genital tract and no cases of CCA (Kaufman et al., 2002). These results led authors to conclude that third generation effects were unlikely even after they acknowledged that the number of participants was too small and the women were too young for the findings to have any real significance.

Multigenerational rodent studies, as a primary source for information on the effects of DES exposure, disagree with those preliminary findings in humans. Although severe effects of DES were apparent in the first round of CD-1 mouse offspring (second generation), the third generation did not exhibit the same subfertility, regardless of exposure timing or dose (Newbold et al., 1998). However, these studies have found an increased susceptibility to tumor formation in the third generation. Aged third generation female mice had increased risks for uterine cancers, benign ovarian tumors, and lymphomas (Newbold et al., 1998). One study found cervical adenocarcinomas, which are not generally seen in untreated mice, in third generation females similar to those induced by direct prenatal DES exposure (DES daughters; Walker and Haven 1997). In the same study, third generation female mice had increases in ovarian, uterine, and mammary tumors with the total number of reproductive tumors being statistically significant from the control mice.

Grandsons

The early reports of DES grandsons show an increase in hypospadias in this population. Hypospadias occurred twenty times more frequently in the DES grandsons' cohort, which suggests that their mothers (DES daughters) may have had a disturbed hormonal balance during their reproductive life that interfered with the genital development of the male fetus. The prevalence of hypospadias was found to be >3% in DES grandsons but the risk of the defect is still low (Klip et al., 2002; Kalfa et al., 2011). Mouse studies in the third generation DES-exposed male population have found an increased susceptibility for reproductive tumor formation (Newbold 1995; Newbold et al., 1998), specifically in the testes, prostate, and seminal vesicles. No effect on reproductive capacity or other deformities was seen in DES grandsons.

GENE CHANGES AND PROPOSED MECHANISMS OF ACTION

The fact that DES causes developmental changes in the second generation through gestational exposure has required evaluation of the mechanisms involved in several target tissues. DES is classified as a carcinogen by the World Health Organization, U.S. Environmental Protection Agency, National Toxicology Program, and the International Agency for Cancer Research. Studies on the genotoxicity of DES in humans have not

revealed striking outcomes; to date, it does not change ploidy patterns, cause specific mutations known to induce high risk of breast cancer, or induce loss of heterozygosity of allelic imbalance [reviewed in IARC 2012]. In directed in vitro tests, the data on induction of sister chromatid exchange, induction of micronuclei, and unscheduled DNA synthesis, were negative or equivocal. However, DES caused aneuploidy, induced adduct formation in mitochondrial DNA, and altered the ability of microtubules to form (IARC 2012).

DES is also known to affect endocrine sensitive tissues and may have hereditary effects due to DNA modifications (IARC 2012); like many other breast cancer risk factors, it may have multiple mechanisms of action, depending on the target tissue (see Figure 2). Through molecular studies many potential mechanisms of action have been proposed among them are different genetic and epigenetic pathways that have been implicated in the DES-induced carcinogenesis and reproductive developmental abnormalities seen in humans and animals. These effects appear to occur in specific target tissues and may be related to gene expression of the ER- at the time of exposure (Korach et al., 1978; Newbold et al., 1990; Yamashita 2006; IARC 2012). DES may create an environment conducive to the development of cancer over time.

In mouse models, pre-and neonatal DES exposure induces a wide range of gene expression changes that persist into adulthood (Newbold 1995; Newbold et al., 2007; LeBaron et al., 2010). Molecular mechanistic studies have shown that many of the changes caused by DES, including structural and cellular abnormalities, are caused by altered programming of *hox* and *wnt* genes which play roles in reproductive tract differentiation (Newbold et al., 2006; Newbold 2008). DES potentially inhibits the expression of *wnt7a*, *hoxa10*, and *hoxa11* during critical periods of reproductive tract development (Yamashita 2006; Ma 2009). Changes in *hox* gene expression have led to abnormalities in tissues that depend on their expression for normal developmental signaling (Newbold et al., 2007; Bromer et al., 2009). Down regulation of *hoxa11* (which is found in the stroma and epithelial cells of the uterus) may be partly responsible for DES-induced uterine malformations, as similar malformations are seen in *hoxa11*-null mice (Yamashita 2006). *Hoxa10* (which is expressed in the uterine horns) controls uterine organogenesis and its expression is increased in cultured human endometrial cells but repressed in mice after *in utero* exposure to DES (Newbold et al., 2007; Bromer et al., 2009). Female mice exposed to DES *in utero* had aberrant methylation in the promoter and intron of *hoxa10*, which persisted into adulthood (Bromer et al., 2009).

Genetic modifications by DES have also been implicated in the initiation and progression of neoplasms and cancer. Neonatal DES exposure in mice can reprogram uterine differentiation by changing genetic pathways controlling uterine morphogenesis and/or altering gene expression in stem cells (Newbold 1995; Sato et al., 2004; Ma 2009). DES affects the methylation patterns of genes that are associated with proliferation (*c-jun*, *c-fos*, *c-myc*, *Itf*); genes associated with apoptosis (*bcl-2*, *bcl-x*); and growth factors associated with proliferation and differentiation (EGF, TGF-) (Sato et al., 2004; Newbold 2006; LeBaron et al., 2010). This change in methylation is referred to as estrogen imprinting (Yamashita 2006). Estrogen imprinting is an epigenetic mechanism where early-life exposure to estrogens (i.e., DES, Bisphenol A) permanently alters DNA methylation and gene expression of estrogen-responsive genes. Once changed, the altered gene profiles can continue to be expressed without further hormonal stimulation.

Proto-oncogenes help regulate normal cell proliferation and differentiation. When these genes are changed through mutation or methylation they can cause neoplastic cell transformation. Studies have shown changes in patterns of expression of estrogen-related proto-oncogenes in the genital tract of female mice exposed to DES (Yamashita 2006). Changes in the proto-oncogenes and growth factors that cause elevations in their expression

are associated with increased proliferation in the tissues (like the uterus and vagina) which can lead to cancer (Newbold et al., 2007; Ma 2009). Genetic modifications of the apoptotic genes that cause decreases in apoptosis are also associated with an increased incidence of cancer (Newbold 1995; Sato et al., 2004; Ma 2009).

DES treatment affects male mice at the genomic level. DES altered *Ins13* mRNA expression in male mice exposed *in utero* (Emmen et al., 2000). Emmen *et al.* found a threefold decrease in *Ins13* mRNA, which is expressed in fetal Leydig cells and is associated with the transabdominal phase of testis descent and development of the gubernaculum (Emmen et al., 2000). This finding may provide a mechanism for DES-induced cryptorchidism. Another group found that gestational DES exposure in C57Bl/6 mice decreased the expression of two transcription factors (GATA4 and ID2) in the testes of adult males (LaRocca et al., 2011). GATA4 (expressed in Sertoli cells, Leydig cells, and other testicular somatic cells) is required for the correct expression of *Sry* and all the steps in testicular organogenesis that follow (LaRocca et al., 2011). ID2 is associated with the inhibition of differentiation of different cell types, and the decrease in GATA4 and ID2 may be associated with fertility problems later in life.

The research into tissue-specific mechanisms of action for DES is still underway. There are other unique attributes of DES that likely lead to its long-term effects following brief periods of exposure. A study of metabolism and disposition of DES in the pregnant rat, demonstrated enhanced disposition of DES and DES oxidative metabolites to the fetal reproductive tissues vs. liver following a single maternal exposure (Miller et al., 1982). Studies in mice demonstrate an accumulation of DES in the fetal reproductive tract, where it can reach levels three times higher than fetal blood (IARC 2012). These findings of accumulated DES in reproductive tissues relate specifically to the location of ER- α , the known receptor for DES (Korach et al., 1978). The fact that there are multiple metabolic DES products has complicated the understanding of its effects. DES metabolites (especially quinines) are reactive [reviewed in IARC 2012]; they are formed *in vivo*, bind DNA and have been found in mammary tissue of rat, adult mouse reproductive tract, and mouse fetal tissues. These oxidative metabolites affect CYP gene activation and likely play a role in cancer mediation.

DES is no longer used in the human population which makes research less of a priority for funding organizations. However, for individuals/families already exposed, DES seems to be an initiating event in an initiation/promotion model for hormonal carcinogenesis (Newbold et al., 2007) and there is ample reason to fund research on effects in their unexposed children. Therefore, thoughtful follow-up of all generations and justified/planned use of stored samples (blood) will be critical in the future to determine those at highest risk for adverse health consequences.

SUMMARY

The legacy of the adverse effects that stem from DES administration to pregnant women in the 1950s to 1970s has not completely formed. The male and female offspring of those women have reported significant fertility, cancer, and birth-related outcomes, but the cancer outcomes are not completely understood, with few exceptions (CCA and breast cancer in women over 40 yr old). Information on DES mothers and daughters, in addition to substantial animal data, earned DES a place in the *First Annual Report on Carcinogens*, a critical review of carcinogenic compounds produced by the National Toxicology Program, in 1980 and was noted by the International Agency for Research on Cancer in their Monographs (IARC 1974). As the male and female offspring of those women age, the overall effect of DES on reproductive cancers will be better understood. Even more

important to understand is the potential effect of this endocrine disruptor and carcinogen on the 3rd generation offspring who were not directly exposed, but may be affected in a heritable way through estrogen reprogramming and DNA modification. Further research is needed to indicate the mechanisms of action on the target tissues, so that future pharmaceuticals/environmental estrogen mimics will avoid these pathways, leading to healthier future generations.

Abbreviations

CCA	clear-cell adenocarcinoma
CI	confidence interval
DES	diethylstilbestrol
EDC	endocrine disrupting compound
ER	estrogen receptor-alpha
FDA	U.S. Food and Drug Administration
RR	relative risk
TDLU	terminal ductal lobular units
TEB	terminal end buds

References

- Anderson LM. Predictive values of traditional animal bioassay studies for human perinatal carcinogenesis risk determination. *Toxicol Appl Pharmacol.* 2004; 199:162–74. [PubMed: 15313588]
- Barnes AB. Menstrual history and fecundity of women exposed and unexposed in utero to diethylstilbestrol. *J Reprod Med.* 1984; 29:651–5. [PubMed: 6492030]
- Bibbo M, Haenszel WM, Wied GL, Hubby M, Herbst AL. A twenty-five-year follow-up study of women exposed to diethylstilbestrol during pregnancy. *N Engl J Med.* 1978; 298:763–7. [PubMed: 628409]
- Bromer JG, Wu J, Zhou Y, Taylor HS. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. *Endocrinology.* 2009; 150:3376–82. [PubMed: 19299448]
- CDC. Center for Disease Control and Prevention. DES Update: Consumers. 2012. <http://www.cdc.gov/des/consumers/index.html>
- Colton T, Greenberg ER, Noller K, Resseguie L, Van Bennekom C, Heeren T, et al. Breast cancer in mothers prescribed diethylstilbestrol in pregnancy. Further follow-up. *JAMA.* 1993; 269:2096–100. [PubMed: 8468763]
- D'Aloisio AA, Baird DD, DeRoo LA, Sandler DP. Early-life exposures and early-onset uterine leiomyomata in black women in the Sister Study. *Environ Health Perspect.* 2012; 120:406–12. [PubMed: 22049383]
- D'Aloisio AA, Deroo LA, Baird DD, Weinberg CR, Sandler DP. Prenatal and infant exposures and age at menarche. *Epidemiology.* 2013; 24:277–84. [PubMed: 23348069]
- Dieckmann WJ, Davis ME, Rynkiewicz LM, Pottinger RE. Does the administration of diethylstilbestrol during pregnancy have therapeutic value? *Amer J Obstet Gynecol.* 1953; 66:1062–81. [PubMed: 13104505]
- Emmen JM, McLuskey A, Adham IM, Engel W, Verhoef-Post M, Themmen AP, et al. Involvement of insulin-like factor 3 (InsI3) in diethylstilbestrol-induced cryptorchidism. *Endocrinology.* 2000; 141:846–9. [PubMed: 10650968]
- Giusti RM, Iwamoto K, Hatch EE. Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med.* 1995; 122:778–88. [PubMed: 7717601]

- Goldberg JM, Falcone T. Effect of diethylstilbestrol on reproductive function. *Fertil Steril*. 1999; 72:1–7. [PubMed: 10428139]
- Greenberg ER, Barnes AB, Resseguie L, Barrett JA, Burnside S, Lanza LL, et al. Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med*. 1984; 311:1393–8. [PubMed: 6493300]
- Hadjimichael OC, Meigs JW, Falcier FW, Thompson WD, Flannery JT. Cancer risk among women exposed to exogenous estrogens during pregnancy. *J Nat Cancer Inst*. 1984; 73:831–4. [PubMed: 6592380]
- Harremoes, P.; Gee, D.; MacGarvin, M.; Stirling, A.; Keys, J.; Wynne, B., et al. Environmental issue report no. 22. Vol. Chapter 8. Copenhagen: Official Publications of the European Communities; 2001. Late lessons from early warnings: the precautionary principle 1896–2000. European Environment Agency. http://www.eea.europa.eu/publications/environmental_issue_report_2001_22
- Harris RM, Waring RH. Diethylstilboestrol--a long-term legacy. *Maturitas*. 2012; 72:108–12. [PubMed: 22464649]
- Hatch EE, Palmer JR, Titus-Ernstoff L, Noller KL, Kaufman RH, Mittendorf R, et al. Cancer risk in women exposed to diethylstilbestrol in utero. *JAMA*. 1998; 280:630–4. [PubMed: 9718055]
- Herbst AL, Hubby MM, Azizi F, Makii MM. Reproductive and gynecologic surgical experience in diethylstilbestrol-exposed daughters. *Am J Obstet Gynecol*. 1981; 141:1019–28. [PubMed: 7315913]
- Herbst AL, Scully RE. Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). *Cancer*. 1970; 25:745–757. [PubMed: 5443099]
- Hilakivi-Clarke L, de Assis S, Warri A. Exposures to synthetic estrogens at different times during the life, and their effect on breast cancer risk. *J Mammary Gland Biol Neoplasia*. 2013; 18:25–42. [PubMed: 23392570]
- Hoover R, Fraumeni JF Jr, Everson R, Myers MH. Cancer of the uterine corpus after hormonal treatment for breast cancer. *Lancet*. 1976; 307:885–887. [PubMed: 58149]
- Hoover R, Gray LA, Fraumeni JF Jr. Stilbestrol (Diethylstilbestrol) and the risk of ovarian cancer. *Lancet*. 1977; 2:533–534. [PubMed: 95735]
- Hoover RN, Hyer M, Pfeiffer RM, Adam E, Bond B, Cheville AL, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. *N Engl J Med*. 2011; 365:1304–14. [PubMed: 21991952]
- IARC. Diethylstilbestrol (stilboestrol). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Lyon, France: International Agency for Research on Cancer; 1974. p. 55–76.
- IARC. International Agency for Research on Cancer. Pharmaceuticals. Diethylstilbestrol. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum. 2012; 100A:175–218.
- IBCERCC; Inter-agency Breast Cancer and the Environment Research Coordinating Committee. Dept of Health and Human Services. Breast Cancer and the Environment: Prioritizing Prevention. 2013. http://www.niehs.nih.gov/about/assets/docs/ibcercc_full_508.pdf
- Kakuta H, Tanaka M, Chambon P, Watanabe H, Iguchi T, Sato T. Involvement of gonadotropins in the induction of hypertrophy-hyperplasia in the interstitial tissues of ovaries in neonatally diethylstilbestrol-treated mice. *Reprod Toxicol*. 2012; 33:35–44. [PubMed: 22100434]
- Kalfa N, Paris F, Soyer-Gobillard MO, Daures JP, Sultan C. Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. *Fertil Steril*. 2011; 95:2574–7. [PubMed: 21458804]
- Kaufman RH, Adam E, Hatch EE, Noller K, Herbst AL, Palmer JR, et al. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. *Obstet Gynecol*. 2000; 96:483–9. [PubMed: 11004345]
- Kaufman RH, Adam E. Findings in female offspring of women exposed in utero to diethylstilbestrol. *Obstet Gynecol*. 2002; 99:197–200. [PubMed: 11814496]
- Kaufman RH. Structural changes of the genital tract associated with in utero exposure to diethylstilbestrol. *Obstet Gynecol Ann*. 1982; 11:187–202.

- Klip H, Verloop J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet*. 2002; 359:1102–7. [PubMed: 11943257]
- Korach KS, Metzler M, McLachlan JA. Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs. *Proc Natl Acad Sciences USA*. 1978; 75:468–71.
- LaRocca J, Boyajian A, Brown C, Smith SD, Hixon M. Effects of in utero exposure to Bisphenol A or diethylstilbestrol on the adult male reproductive system. *Birth Defects Res B Dev Reprod Toxicol*. 2011; 92:526–33. [PubMed: 21922642]
- Laronda MM, Unno K, Butler LM, Kurita T. The development of cervical and vaginal adenosis as a result of diethylstilbestrol exposure in utero. *Differentiation*. 2012; 84:252–60. [PubMed: 22682699]
- LeBaron MJ, Rasoulpour RJ, Klapacz J, Ellis-Hutchings RG, Hollnagel HM, Gollapudi BB. Epigenetics and chemical safety assessment. *Mutat Res*. 2010; 705:83–95. [PubMed: 20399890]
- Li HQ, Xue JY, Shi L, Gui SY, Zhu HL. Synthesis, crystal structure and antimicrobial activity of deoxybenzoin derivatives from genistein. *Eur J Med Chem*. 2008; 43:662–7. [PubMed: 17624635]
- Ma L. Endocrine disruptors in female reproductive tract development and carcinogenesis. *Trends Endocrinol Metab*. 2009; 20:357–63. [PubMed: 19709900]
- Martin O, Shialis T, Lester J, Scrimshaw M, Boobis A, Voulvoulis N. Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative meta-analysis. *Cien Saude Colet*. 2008; 13:1601–18. [PubMed: 18813661]
- McLachlan JA, Newbold RR, Shah HC, Hogan MD, Dixon RL. Reduced fertility in female mice exposed transplacentally to diethylstilbestrol (DES). *Fertil Steril*. 1982; 38:364–71. [PubMed: 7117561]
- Miller RK, Heckmann ME, McKenzie RC. Diethylstilbestrol: placental transfer, metabolism, covalent binding and fetal distribution in the Wistar rat. *J Pharmacol Exp Ther*. 1982; 220:358–65. [PubMed: 7057397]
- NCI. National Cancer Institute. Clinician Information: DES Sons - Men Exposed in Utero. 2012. <http://www.cancer.gov/cancertopics/causes/des/sons-exposed-to-des>
- Newbold R. Cellular and molecular effects of developmental exposure to diethylstilbestrol: implications for other environmental estrogens. *Environ Health Perspect*. 1995; 103(Suppl 7):83–7. [PubMed: 8593881]
- Newbold RR. Prenatal exposure to diethylstilbestrol (DES). *Fertil Steril*. 2008; 89:e55–6. [PubMed: 18308064]
- Newbold RR, Bullock BC, McLachlan JA. Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res*. 1990; 50:7677–81. [PubMed: 2174729]
- Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*. 1998; 19:1655–63. [PubMed: 9771938]
- Newbold RR, Jefferson WN, Grissom SF, Padilla-Banks E, Snyder RJ, Lobenhofer EK. Developmental exposure to diethylstilbestrol alters uterine gene expression that may be associated with uterine neoplasia later in life. *Mol Carcinog*. 2007; 46:783–96. [PubMed: 17394237]
- Newbold RR, Padilla-Banks E, Jefferson WN. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology*. 2006; 147:S11–7. [PubMed: 16690809]
- NTP National Toxicology Program. Diethylstilbestrol. 12th Report on Carcinogens. 2011; 12:159–61.
- N'Tumba-Byn T, Moison D, Lacroix M, Lecureuil C, Lesage L, Prud'homme SM, et al. Differential effects of bisphenol A and diethylstilbestrol on human, rat and mouse fetal leydig cell function. *PLoS One*. 2012; 7:e51579. [PubMed: 23284716]
- Page SW. Diethylstilboestrol—clinical pharmacology and alternatives in small animal practice. *Aust Vet J*. 1991; 68:226–30. [PubMed: 1929989]
- Palmer JR, Herbst AL, Noller KL, Boggs DA, Troisi R, Titus-Ernstoff L, et al. Urogenital abnormalities in men exposed to diethylstilbestrol in utero: a cohort study. *Environ Health*. 2009; 8:37. [PubMed: 19689815]

- Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohnsitter W, et al. Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:1509–14. [PubMed: 16896041]
- Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, et al. In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol.* 2007; 24:199–224. [PubMed: 17683900]
- Rubin MM. Antenatal exposure to DES: lessons learned...future concerns. *Obstet Gynecol Surv.* 2007; 62:548–55. [PubMed: 17634156]
- Rumsey TS, Oltjen RR, Kozak AS, Daniels FL, Aschbacher PW. Fate of radiocarbon in beef steers implanted with 14C-diethylstilbestrol. *J Anim Sci.* 1975; 40:550–60. [PubMed: 1116976]
- Sato T, Fukazawa Y, Ohta Y, Iguchi T. Involvement of growth factors in induction of persistent proliferation of vaginal epithelium of mice exposed neonatally to diethylstilbestrol. *Reprod Toxicol.* 2004; 19:43–51. [PubMed: 15336711]
- Senekjian EK, Potkul RK, Frey K, Herbst AL. Infertility among daughters either exposed or not exposed to diethylstilbestrol. *Am J Obstet Gynecol.* 1988; 158:493–8. [PubMed: 3348310]
- Smith OW. Diethylstilbestrol in the prevention and treatment of complications of pregnancy. *Amer J Obstet Gynecol.* 1948; 56:821–34. [PubMed: 18888213]
- Steiner AZ, D'Aloisio AA, DeRoo LA, Sandler DP, Baird DD. Association of intrauterine and early-life exposures with age at menopause in the Sister Study. *Am J Epidemiol.* 2010; 172:140–8. [PubMed: 20534821]
- Strohnsitter WC, Noller KL, Hoover RN, Robboy SJ, Palmer JR, Titus-Ernstoff L, et al. Cancer risk in men exposed in utero to diethylstilbestrol. *J Natl Cancer Inst.* 2001; 93:545–51. [PubMed: 11287449]
- Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, et al. Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer.* 2001; 84:126–33. [PubMed: 11139327]
- Tournaire M, Devouche E, Epelboin S, Cabau A. Diethylstilbestrol exposure: evaluation of the doses received in France. *Eur J Epidemiol.* 2012; 27:315–6. [PubMed: 22618853]
- Troisi R, Hatch EE, Titus-Ernstoff L, Hyer M, Palmer JR, Robboy SJ, et al. Cancer risk in women prenatally exposed to diethylstilbestrol. *Int J Cancer.* 2007; 121:356–60. [PubMed: 17390375]
- Umekita Y, Souda M, Hatanaka K, Hamada T, Yoshioka T, Kawaguchi H, et al. Gene expression profile of terminal end buds in rat mammary glands exposed to diethylstilbestrol in neonatal period. *Toxicol Lett.* 2011; 205:15–25. [PubMed: 21658437]
- Verloop J, van Leeuwen FE, Helmerhorst TJ, van Boven HH, Rookus MA. Cancer risk in DES daughters. *Cancer Causes Control.* 2010; 21:999–1007. [PubMed: 20204493]
- Virtanen HE, Adamsson A. Cryptorchidism and endocrine disrupting chemicals. *Mol Cell Endocrinol.* 2012; 355:208–20. [PubMed: 22127307]
- Walker BE, Haven MI. Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis.* 1997; 18:791–3. [PubMed: 9111216]
- Walker VR, Jefferson WN, Couse JF, Korach KS. Estrogen receptor-alpha mediates diethylstilbestrol-induced feminization of the seminal vesicle in male mice. *Environ Health Perspect.* 2012; 120:560–5. [PubMed: 22275727]
- Wilcox AJ, Baird DD, Weinberg CR, Hornsby PP, Herbst AL. Fertility in men exposed prenatally to diethylstilbestrol. *N Engl J Med.* 1995; 332:1411–6. [PubMed: 7723797]
- Yamashita S. Expression of estrogen-regulated genes during development in the mouse uterus exposed to diethylstilbestrol neonatally. *Curr Pharm Des.* 2006; 12:1505–20. [PubMed: 16611131]

Highlights

1. DES is both an endocrine disruptor and human carcinogen
2. In utero exposure caused increased birth-related outcomes in DES daughters
3. DES increased the risk of breast cancer in mothers and daughters
4. Exposure period and dose determine extent of many health effects

Figure 1.

Similarity in chemical structure of (A) the endogenous estrogen, 17- estradiol (Korach et al., 1978), (B) the pharmaceutical, diethylstilbestrol (DES) (IARC 1974), (C) a bioactive phytoestrogen, genistein (Li et al., 2008), and (D) an estrogenic component of plastics, Bisphenol A (BPA) (Richter et al., 2007).

Mechanisms for Cancer

Figure 2.

Mechanisms involved in breast cancer etiology. Numerous mechanisms are reported to be associated with increased breast cancer risk. These mechanisms fall into two main categories: DNA mediated and non-DNA mediated. Mutagenic factors affect the DNA sequence, whereas epigenetic factors modify the DNA conformation; both lead to heritable changes in breast cancer risk. Non-DNA mediated factors act in a more indirect manner, causing altered inflammatory response, changes in stromal tissue, or modified hormone actions. More than one mechanism may be involved in an individual's cancer risk. Reproduced from IBCERCC Report (2013).

Table 1

Summary of breast cancer risk in women prescribed diethylstilbestrol (DES) during pregnancy. Data from numerous sources suggest an increased relative risk (RR) and significant 95% confidence interval (CI), especially when age at diagnosis, time since exposure, or cumulative dose are taken into consideration.

Study	Number exposed	Number unexposed	RISK (95% CI)
Bibbo et al., 1978 [30]	693	668	t=1.46, p=0.16 CT Tumor Registry as standard
Hadjimichael et al., 1984 [29]	1531	1404	RR 1.37 (0.83–2.28) RR 2.28 for dose mean 2100 mg RR 1.46 (1.07–2.00) all cancers
Greenberg et al., 1984 [28]	2885	2816	RR 1.4 (1.1–1.9) unadjusted RR increased with time since exposure and age at diagnosis
Colton et al., 1993 [25]	2864	2760	RR 1.35 (1.05–1.74) Reviewed obstetric records RR 1.47 (1.02–2.13) 60+y @ diagn
Titus-Ernstoff et al., 2001 [27]	2434	2402	RR 1.27* (1.07–1.52)

* combined RR for Mothers Study and Dieckmann Study cohorts

Table 2

Summary of hazard ratios for significant adverse health outcomes in women with in utero DES exposure compared to those without exposure. Reproduced from [21].

Adverse outcome	Exposed women (#/ total #)	Unexposed women (#/ total #)	Hazard Ratio (95% Confidence Interval)
Infertility	1144/3769	252/1654	2.37 (2.05–2.75)
Spontaneous abortion	916/2690	328/1291	1.64 (1.42–1.88)
Ectopic pregnancy	255/2692	36/1293	3.72 (2.58–5.38)
Loss of second-trimester pregnancy	201/2692	35/1293	3.77 (2.56–5.54)
Preterm delivery	624/2385	100/1238	4.68 (3.74–5.86)
Preeclampsia	216/2412	80/1159	1.42 (1.07–1.89)
Stillbirth	54/2385	16/1239	2.45 (1.33–4.54)
Neonatal death	57/2383	7/1238	8.12 (3.53–18.65)
Early menopause	181/3993	49/1682	2.35 (1.67–3.31)
Cervical intraepithelial neoplasia (CIN; grade 2)	208/4120	40/1785	2.28 (1.59–3.27)
Breast cancer at 40 yr	61/3693	21/1647	1.82 (1.04–3.18)
Clear-cell adenocarcinoma	4/4652	0/1926	

Table 3

Comparative developmental effects of prenatal exposure to DES in female offspring of humans and mice. Reproduced from [61] and updated.

Developmental effect	Observed in humans	Observed in mice
Immune dysfunction	?	Yes
Mammary tumors	Yes	Yes
Ectopic pregnancy	Yes	?
Subfertility and infertility	Yes	Yes
Uterine tumors	?	Yes
Ovarian cysts	?	Yes
Ovarian tumors	?	Yes
Elevated serum testosterone levels	Yes	?
Salpingitis isthmica nodosa of oviduct	Yes	Yes
Structural abnormalities of uterus	Yes	Yes
Malformed cervical canal	Yes	Yes
Cervical and vaginal hood and polyps	Yes	Yes
Vaginal adenocarcinoma	Yes	Yes
Vaginal adenosis	Yes	Yes
Persistent vaginal cornification	?	Yes

Table 4

Comparative developmental effects of prenatal exposure to DES in male offspring of humans and mice. Reproduced from [61].

Developmental effect	Observed in humans	Observed in mice
Subfertility	Yes	Yes
Sperm abnormalities	Yes	Yes
Decreased sperm counts	Yes	Yes
Epididymal cysts	Yes	Yes
Hypoplastic and cryptorchid testes	Yes	Yes
Testicular tumors	Yes	Yes
Anatomical feminization	Yes	Yes
Microphallus	Yes	Yes
Hypospadias	Yes	Yes
Retention of Müllerian duct remnants	Yes	Yes
Seminal vesicle tumors	?	Yes
Prostatic inflammation	Yes	Yes
Prostatic tumors	?	Yes
Immune dysfunction	?	Yes