IARC MONOGRAPHS

IARC MONOGRAPHS

PHARMACEUTICALS

VOLUME 100 A
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

LYON, FRANCE - 2012

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

DIETHYLSTILBESTROL

Diethylstilbestrol was considered by previous IARC Working Groups in 1978 and 1987 (IARC, 1979a, 1987a). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 56–53–1
Chem. Abstr. Name: Phenol, 4,4′-[(1*E*)-1,2-diethyl-1,2-ethenediyl]bis-
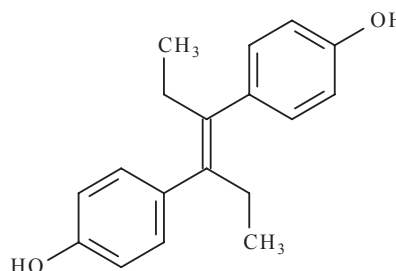*IUPAC Systematic Name*: 4-[(*E*)-4-(4-Hydroxyphenyl)hex-en-3-yl]phenol
Synonyms: (*E*)-3,4-Bis(4-hydroxyphenyl)-3-hexene; (*E*)-4,4′-(1,2-diethyl-1,2-ethenediyl)bisphenol; (*E*)-diethylstilbestrol; α,α′-diethyl-4,4′-stilbenediol; α,α′-diethylstilbenediol; 4,4′-dihydroxy-α,β-diethylstilbene; 4,4′-dihydroxydiethylstilbene; phenol, 4,4′-(1,2-diethyl-1,2-ethenediyl)bis-, (*E*)-; 4,4′-stilbenediol, α,α′-diethyl-, *trans*-;
Description: white, odourless, crystalline powder (McEvoy, 2007)

(a) Structural and molecular formulae, and relative molecular mass

$C_{18}H_{20}O_2$
Relative molecular mass: 268.35

1.2 Use of the agent

Information for Section 1.2 is taken from IARC (1979a), McEvoy (2007), Royal Pharmaceutical Society of Great Britain (2007), and Sweetman (2008).

1.2.1 Indications

Diethylstilbestrol is a synthetic non-steroidal estrogen that was historically widely used to prevent potential miscarriages by stimulating the synthesis of estrogen and progesterone in the placenta (in the United States of America, especially from the 1940s to the 1970s)

([Rogers & Kavlock, 2008](#)). It was also used for the treatment of symptoms arising during the menopause and following ovariectomy, and for senile (atrophic) vaginitis and vulvar dystrophy. Diethylstilbestrol was used as a postcoital emergency contraceptive ('morning-after pill'). It has also been used for the prevention of postpartum breast engorgement, for dysfunctional menstrual cycles, and for the treatment of female hypogonadism.

Diethylstilbestrol is now rarely used to treat prostate cancer because of its side-effects. It is occasionally used in postmenopausal women with breast cancer.

Diethylstilbestrol was also used as a livestock growth stimulant.

1.2.2 Dosage

Historically, diethylstilbestrol was used for the treatment of symptoms arising during the menopause (climacteric) and following ovariectomy in an oral daily dose of 0.1–0.5 mg in a cyclic regimen. For senile vaginitis and vulvar dystrophy, it was given in an oral daily dose of 1 mg, or, for vulvar dystrophies and atrophic vaginitis, in suppository form in a daily dose of up to 1 mg. As a postcoital emergency contraceptive ('morning-after pill'), it was given as an oral dose of 25 mg twice a day for 5 days starting within 72 hours of insemination. An oral dose of 5 mg 1–3 times per day for a total of 30 mg was typically given in combination with methyltestosterone for the prevention of postpartum breast engorgement. For dysfunctional uterine bleeding, diethylstilbestrol was given in an oral dose of 5 mg 3–5 times per day until bleeding stopped. It was also used for the treatment of female hypogonadism, in an oral dose of 1 mg per day ([IARC, 1979a](#); [McEvoy, 2007](#)).

The typical dosage of diethylstilbestrol is 10–20 mg daily to treat breast cancer in postmenopausal women, and 1–3 mg daily to treat prostate cancer. Diethylstilbestrol has also been given to treat prostate cancer in the form of its diphosphate salts (Fosfestrol).

When used as pessaries in the short-term management of menopausal atrophic vaginitis, the daily dose was 1 mg ([Royal Pharmaceutical Society of Great Britain, 2007](#); [Sweetman, 2008](#)).

Diethylstilbestrol is available as 1 mg and 5 mg tablets for oral administration in several countries ([Royal Pharmaceutical Society of Great Britain, 2007](#)).

Diethylstilbestrol is no longer commercially available in the USA ([McEvoy, 2007](#)).

1.2.3 Trends in use

Most reports about diethylstilbestrol use are from the USA. The number of women exposed prenatally to diethylstilbestrol worldwide is unknown. An estimated 5 to 10 million US citizens received diethylstilbestrol during pregnancy or were exposed to the drug *in utero* from the 1940s to the 1970s ([Giusti *et al.*, 1995](#)).

A review of 51000 pregnancy records at 12 hospitals in the USA during 1959–65 showed geographic and temporal variation in the percentage of pregnant women exposed: 1.5% of pregnancies at the Boston Lying-In Hospital, and 0.8% at the Children's Hospital in Buffalo were exposed to diethylstilbestrol; at the remaining ten hospitals, 0.06% of pregnant women were exposed ([Heinonen, 1973](#)). At the Mayo Clinic during 1943–59, 2–19% (mean, 7%) of pregnancies per year were exposed ([Lanier *et al.*, 1973](#)).

The peak years of diethylstilbestrol use in the USA varied from 1946–50 at the Mayo Clinic, 1952–53 at the General Hospital in Boston, and 1964 at the Gundersen Hospital in Wisconsin ([Nash *et al.*, 1983](#)). Over 40% of the women in the US National Cooperative Diethylstilbestrol Adenosis (DESAD) cohort were exposed during the early 1950s (1950–55) ([Herbst & Anderson, 1990](#)). Among cases of clear cell adenocarcinoma (CCA) of the cervix and vagina recorded in the Central Netherlands Registry, born

during 1947–73, the median year of birth was 1960 (Hanselaar *et al.*, 1997). In the Registry for Research on Hormonal Transplacental Carcinogenesis, which registers cases of CCA of the vagina and cervix in the USA, Australia, Canada, Mexico and Europe, most of the exposed women from the USA were born during 1948–65 (Herbst, 1981; Melnick *et al.*, 1987).

Diethylstilbestrol doses varied by hospital. Based on the record review at 12 hospitals in the USA, the highest doses were administered at the Boston Lying-in, where 65% of treated pregnant women received total doses higher than 10 g, up to 46.6 g, for a duration of up to 9 months. At all the other hospitals, most women (74%) received < 0.1 g (Heinonen, 1973). Data available from the DESAD project indicate that median doses were 3650 mg (range 6–62100 mg) for women identified through the record review, whereas the median dose exceeded 4000 mg for women who entered the cohort through referral (self or physician), more of whom were affected by diethylstilbestrol-related tissue changes (O'Brien *et al.*, 1979). Diethylstilbestrol doses may have varied over time, but this has not been reported.

The use of diethylstilbestrol and other estrogens during pregnancy is now proscribed in many countries (Anon, 2008), and diethylstilbestrol use is no longer widespread for other indications.

Until the 1970s, it was common practice to stimulate the fattening of beef cattle and chickens by mixing small amounts of diethylstilbestrol into the animal feed or by implanting pellets of diethylstilbestrol under the skin of the ears of the animals. In the early 1970s, concern over trace amounts of the hormone in meat led to bans on the use of diethylstilbestrol as a livestock growth stimulant (Anon, 2008).

2. Cancer in Humans

The previous *IARC Monograph* (IARC, 1987a) states that there is sufficient evidence of a causal association between CCA of the vagina/cervix and prenatal exposure to diethylstilbestrol. That *Monograph* also cited clear evidence of an increased risk of testicular cancer in prenatally diethylstilbestrol-exposed male offspring, an association that is now uncertain due to the publication of recent studies. The association between diethylstilbestrol administered during pregnancy and breast cancer was considered established, but the latent period remained uncertain. Evidence was mixed for an association between diethylstilbestrol exposure during pregnancy and cancer of the uterus, cervix, and ovary. Finally, the *IARC Monograph* states that there is sufficient evidence of a causal relationship between uterine cancer and use of diethylstilbestrol as hormonal therapy for menopausal symptoms.

The studies cited in this review represent key historical reports relevant to the association between diethylstilbestrol and human cancer. Only studies of key cancer end-points published since the most recent *IARC Monograph* in 1987 are shown in the tables, available online.

2.1 Women exposed to diethylstilbestrol during pregnancy

2.1.1 Breast cancer incidence

Historically, nearly all of the studies assessing diethylstilbestrol in relation to invasive breast cancer incidence or mortality involve the retrospective and/or prospective follow-up of women with verified exposure to diethylstilbestrol during pregnancy. The results of some early studies suggested modestly increased risk, with relative risks (RR) ranging from 1.37 to 1.47

(Clark & Portier, 1979; Greenberg et al., 1984; Hadjimichael et al., 1984). However, a standardized incidence ratio (SIR) of 2.21 was reported from the Dieckmann clinical trial cohort (Hubby et al., 1981), despite null results from an earlier analysis of the same cohort (Bibbo et al., 1978). Historically, null results were also reported from a small US cohort (eight cases) (Brian et al., 1980), and two small cohorts arising from separate clinical trials in London, the United Kingdom (four and 13 cases, respectively) (Beral & Colwell, 1981; Vessey et al., 1983).

Two reports published since the previous *IARC Monograph* are consistent with a modest association between diethylstilbestrol exposure during pregnancy and breast cancer incidence (see Table 2.1 available et http://monographs. iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.1.pdf). The first of these (Colton et al., 1993) was based on further follow-up of the Women's Health Study (WHS) (Greenberg et al., 1984). The WHS cohort was originally assembled at three US medical centres (Mary Hitchcock Memorial Hospital in Hanover; Boston Lying-in Hospital in Boston; Mayo Clinic in Rochester) and a private practice in Portland (Greenberg et al., 1984). At all participating WHS centres, diethylstilbestrol exposure (or lack of exposure) during pregnancy was based on a review of obstetrics records during 1940–60. Although exact diethylstilbestrol doses administered to women in the WHS are largely unknown, they are believed to have been relatively low. In the 1989 WHS follow-up, health outcomes, including breast cancer diagnosis and mortality, were retrospectively and prospectively ascertained in 2864 exposed and 2760 unexposed women. The data produced a relative risk of 1.35 for breast cancer risk based on 185 exposed and 140 unexposed cases (Colton et al., 1993), whereas the earlier study reported a relative risk of 1.47 (Greenberg et al., 1984).

The second report was based on the US National Cancer Institute (NCI) Combined Cohort Study, which in 1994 combined and extended follow-up of the WHS cohort (by 5 years), and the Dieckmann clinical trial cohort (by 14 years). The Dieckmann clinical trial was conducted in 1951–52 (Dieckmann et al., 1953) to assess the efficacy of diethylstilbestrol for preventing adverse pregnancy outcomes. Administered diethylstilbestrol doses were high, with a cumulative dose of 11–12 g (Bibbo et al., 1978). The combined WHS and Dieckmann cohorts produced a modestly elevated relative risk of 1.25 for breast cancer (Titus-Ernstoff et al., 2001).

Based on data from the Dieckmann clinical trial cohort (Hubby et al., 1981) and the NCI Combined Cohort Study (Titus-Ernstoff et al., 2001), the influence of diethylstilbestrol on breast cancer risk did not differ according to family history of breast cancer, reproductive history, prior breast diseases, or oral contraceptive use. Although the first follow-up of the Dieckmann clinical trial cohort suggested breast cancer occurred sooner after trial participation in the diethylstilbestrol-exposed women (Bibbo et al., 1978), this was not seen in the subsequent follow-up (Hubby et al., 1981), in the WHS cohort (Greenberg et al., 1984; Colton et al., 1993), in the NCI Combined Cohort Study (Titus-Ernstoff et al., 2001), or the Connecticut study (Hadjimichael et al., 1984). In both the NCI Combined Cohort Study (Titus-Ernstoff et al., 2001) and the Connecticut study (Hadjimichael et al., 1984), the elevated risk associated with diethylstilbestrol was not apparent 40 or more years after exposure.

Data from the WHS (Greenberg et al., 1984) and the Dieckmann clinical trial cohort (Bibbo et al., 1978; Hubby et al., 1981) did not show systematic differences in breast tumour size, histology or stage at diagnosis for the diethylstilbestrol-exposed and -unexposed women. No differences between exposed and unexposed women with regard to breast self-examination or mammography screening were noted in follow-up data from the WHS (Colton et al., 1993).

[The Working Group noted it seemed unlikely the increased risk in diethylstilbestrol-exposed women was due to an increased surveillance of exposed women or to confounding by lifestyle factors.]

Historically, a few studies have suggested an association between exposure to diethylstilbestrol during pregnancy and an increased risk of breast cancer mortality; these include an analysis based on the first follow-up report of women in the Dieckmann clinical trial (RR, 2.89; 95%CI: 0.99–8.47) (Clark & Portier, 1979), and a study in Connecticut (RR, 1.89; 95%CI: 0.47–7.56) (Hadjimichael et al., 1984). More recent studies are consistent with a modest association, including an analysis of fatal breast cancer in a large American Cancer Society (ACS) cohort of gravid women (RR, 1.34; 95%CI: 1.06–1.69) (Calle et al., 1996), the second follow-up of women in the WHS (RR, 1.27; 95%CI: 0.84–1.91) (Colton et al., 1993), and the NCI Combined Cohort Study, which for this analysis combined and extended the follow-up of the WHS women by 8 years and the Dieckmann women by 17 years (hazard ratio [HR] 1.38; 95%CI: 1.03–1.85) (Titus-Ernstoff et al., 2006a). Similarly to the NCI study of breast cancer incidence (Titus-Ernstoff et al., 2001), the ACS study showed that risk of breast cancer mortality did not differ by family history of breast cancer, reproductive history, or hormone use; also, the elevated risk was no longer evident 40 or more years after exposure (Calle et al., 1996).

In summary, evidence from large, recent cohort studies suggests a modest association between diethylstilbestrol exposure during pregnancy and increased breast cancer incidence and mortality. Notably, these associations were apparent in women participating in the Dieckmann clinical trial cohort, minimizing the possibility of distortion due to confounding by the clinical indication for diethylstilbestrol use. The increased risk of breast cancer mortality also argues against an artefactual association stemming from the heightened surveillance of diethylstilbestrol-exposed women.

Diethylstilbestrol was also prescribed for the treatment of menopausal symptoms, but the use of diethylstilbestrol in menopause has not been assessed systematically in relation to breast cancer risk, and the association is unclear.

2.1.2 Other cancer sites

An early study suggested a relationship between the use of diethylstilbestrol to treat gonadal dysgenesis and an increased risk of endometrial cancer in young women (Cutler et al., 1972). An increased risk of endometrial cancer was also reported in association with the use of diethylstilbestrol to treat symptoms of menopause (Antunes et al., 1979).

Two follow-up studies indicated (Hoover et al., 1977) or suggested (Hadjimichael et al., 1984) an increased risk of ovarian cancer among women exposed to diethylstilbestrol during pregnancy, but the number of exposed cases was small. Similarly, early attempts to assess the risk of cervical and other cancers were limited by small case numbers (Hadjimichael et al., 1984). The large and more recent NCI Combined Cohort study did not show an association between diethylstilbestrol exposure during pregnancy and the incidence of cancer of the endometrium, ovary, or cervix (Titus-Ernstoff et al., 2001).

Although relative risks were elevated for brain and lymphatic cancers in the Connecticut study (Hadjimichael et al., 1984) and for stomach cancer in the NCI Combined Cohort Study (Titus-Ernstoff et al., 2001), confidence intervals were wide. A recent report from the large ACS study showed no association between diethylstilbestrol taken during pregnancy and pancreatic cancer mortality (1959 deaths in 387981 women) (Teras et al., 2005). The NCI Combined Cohort study did not find associations between diethylstilbestrol exposure during pregnancy and death due to cancers other than breast cancer (Titus-Ernstoff et al., 2006a).

2.2 Women exposed *in utero*

2.2.1 *Clear cell adenocarcinoma of the vagina and cervix*

Substantial evidence indicates that women exposed *in utero* to diethylstilbestrol have a markedly increased risk of CCA of the vagina and cervix. The earliest report, published in 1970, described seven cases of adenocarcinoma (six CCA) in women of ages 15–22 years who had been exposed prenatally to diethylstilbestrol (Herbst & Scully, 1970). The following year, a case–control study based on these seven cases plus an additional case (eight cases) and 32 matched controls showed a strong statistical association between prenatal diethylstilbestrol exposure and risk of vaginal CCA based on seven exposed cases and zero exposed controls ($P < 0.00001$) (Herbst *et al.*, 1971). A second case–control study published the same year, involving five cases identified through the New York State Cancer Registry and eight matched controls, also supported an association between prenatal exposure to synthetic estrogens and vaginal CCA based on five exposed cases and zero exposed controls (Greenwald *et al.*, 1971). The strength of this evidence was based primarily on the rarity of CCA, particularly in young women, and on the high proportion of cases that were exposed to a medication that was used relatively infrequently. Based on these reports, the FDA issued a bulletin against prescribing diethylstilbestrol during pregnancy in late 1971 (Anon, 1972).

Additional evidence published in 1972 established a link between prenatal diethylstilbestrol exposure and CCA. That study identified seven cases of CCA occurring in girls aged 7–19 years; of the four mothers who were successfully contacted, three reported diethylstilbestrol use during the first trimester of pregnancy and one reported taking a hormone of unknown type for vaginal bleeding (Noller *et al.*, 1972). A study of the California Tumor Registry during 1950–69 showed an increase of vaginal tumours in girls aged 10–19 years (Linden & Henderson, 1972). Subsequent case series, two of which were based in California, supported the link between prenatal diethylstilbestrol exposure and CCA at both sites (Henderson *et al.*, 1973; Hill, 1973).

The only follow-up study of prenatal diethylstilbestrol exposure in relation to risk of CCA is the NCI Combined Cohort Study, which combined pre-existing US cohorts with verified diethylstilbestrol exposure (or lack of exposure), including:

- daughters of women who participated in the Dieckmann clinical trial (Dieckmann *et al.*, 1953),
- daughters of women enrolled in the WHS (Greenberg *et al.*, 1984),
- daughters of women treated with diethylstilbestrol at a Boston infertility clinic and their unexposed sisters (the Horne cohort), and
- more than 5000 women (including more than 4000 exposed) who were initially identified through medical records or referral (self or physician), and enrolled during the 1970s in the multicentre DESAD study (Labarthe *et al.*, 1978).

Follow-up of the NCI Combined Cohort through 1994 ascertained three diethylstilbestrol-exposed cases of vaginal CCA, producing an SIR of 40.7 (95%CI: 13.1–136.2). Continued follow-up through 2001 ascertained an additional exposed case of cervical CCA, producing an SIR of 39 (95%CI: 15–104) (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.2.pdf), and indicating a cumulative risk of 1.6 per 1000 of CCA of the vagina/cervix from birth through 39 years of age (Troisi *et al.*, 2007).

An early study comparing internationally ascertained diethylstilbestrol-exposed CCA cases, recorded in the Registry for Research on Transplacental Carcinogenesis at the University of Chicago, to diethylstilbestrol-exposed non-cases

in the DESAD study suggested that CCA risk is influenced by early gestational exposure, but not by dose. Evidence was unclear for an influence of prior miscarriage (Herbst et al., 1986). Another University of Chicago registry-based study published since the previous *IARC Monograph* found that maternal vaginal bleeding during pregnancy was not associated with case status, reducing the likelihood that pregnancy complications confounded the association between diethylstilbestrol and CCA (Sharp & Cole, 1990). The same study also found that CCA occurring in diethylstilbestrol-exposed women was associated with earlier gestational exposure and with greater body weight and greater height at ages 14–15 years (Sharp & Cole, 1991). [The Working Group noted that, possibly, greater body weight and height in the early teenage years was a proxy for early puberty, which may have increased the time at risk.] A recent study comparing diethylstilbestrol-exposed CCA cases to diethylstilbestrol-controls did not identify postnatal factors that influenced the risk of this developing cancer (Palmer et al., 2000).

Vaginal adenosis is an established, although non-obligatory, precursor of CCA that affects between 34–88% of diethylstilbestrol-exposed women (Antonioli & Burke, 1975; Bibbo et al., 1975; Herbst et al., 1975; Kaufman & Adam, 1978; O'Brien et al., 1979) and fewer than 4% of unexposed women (Bibbo et al., 1975; Herbst et al., 1975). The lower prevalence (34–35%) was found in diethylstilbestrol-exposed women who were identified through a medical record review (Herbst et al., 1975; Robboy et al., 1979); also, in these studies, tissues were biopsied only when changes were seen upon clinical examination or colposcopy. The higher prevalence (88%) was reported in women many of whom had been referred for study because of other diethylstilbestrol-related vaginal anomalies (Antonioli & Burke, 1975). Several studies suggested the likelihood of vaginal epithelial changes, including adenosis, is greater in women who

received higher diethylstilbestrol doses (O'Brien et al., 1979), women of young ages (aged 13–26 years in Mattingly & Stafl, 1976), and women who were exposed early in gestation (defined variously as before Weeks 16, 19 or 20, or during the first trimester) (Herbst et al., 1975; Mattingly & Stafl, 1976; Kaufman & Adam, 1978; O'Brien et al., 1979). A decreasing prevalence with age has been seen in case series (Kaufman et al., 1982), in the DESAD study (Robboy et al., 1981), and in prospective follow-up studies of diethylstilbestrol-exposed women, suggesting possible regression (Burke et al., 1981; Noller et al., 1983). Although most women affected by adenosis do not develop CCA, adenosis is present in up to 100% of vaginal CCA (Herbst et al., 1972; Herbst et al., 1974; Robboy et al., 1984a).

2.2.2 Squamous neoplasia of the cervix

Around the time of puberty, the outer cervical epithelium undergoes a transition from the original columnar epithelium to squamous epithelium. The area affected by this change (squamous metaplasia), known as the cervical transformation zone (squamo-columnar junction), is at increased risk of malignancy. Early clinical series suggested the extended transformation zone associated with prenatal diethylstilbestrol exposure might increase susceptibility for squamous neoplasia/dysplasia in these women (Stafl & Mattingly, 1974; Fetherston, 1975; Fowler et al., 1981). A study comparing diethylstilbestrol-exposed and -unexposed women showed a higher percent of dysplastic squamous cells in the exposed (11%) than in the unexposed (7%) based on cytology; the prevalence was greater (27%) in exposed women with pathologically confirmed adenosis (Herbst et al., 1975). In a subsequent study of 280 women exposed to diethylstilbestrol in the first trimester, 82% were affected by adenosis and nearly all (96%) of these had abnormal colposcopic findings (Mattingly & Stafl, 1976).

The baseline examination of the DESAD study women who were identified through medical record review did not find elevated rates of squamous dysplasia in the diethylstilbestrol-exposed group (Robboy et al., 1981), but the 7-year follow-up of 1488 (744 exposed) women noted higher rates of cervical squamous cell dysplasia and carcinoma *in situ* in the diethylstilbestrol-exposed compared to the unexposed women (15.7 versus 7.9 cases per 1000 person–years) based on cytology or biopsy (Robboy et al., 1984b). The difference between exposed and unexposed was more apparent when the analyses were confined to cases identified through biopsy (as opposed to cytology) (5.0 versus 0.4 cases per 1000 person–years) (Robboy et al., 1984b). [The Working Group noted that studies relying on selective biopsy may exaggerate the association between prenatal diethylstilbestrol exposure and risk of cervical neoplasia.] A recent analysis of the NCI Combined Cohort Study showed a doubling of the risk of high-grade intraepithelial neoplasia (squamous cell dysplasia) in the women exposed prenatally to diethylstilbestrol compared to the unexposed; the risk appeared to be higher for those with intrauterine exposure within 7 weeks of the last menstrual period (RR, 2.8; 95%CI: 1.4–5.5) (Hatch et al., 2001). There were not enough confirmed cases of invasive cervical cancer for a meaningful analysis.

A study of 5421 questionnaire respondents (representing 41% of 13350 queried) who had been enrolled previously in the Netherlands Diethylstilbestrol Information Centre (NDIC), in which prenatal diethylstilbestrol exposure was validated using medical records, found evidence of a 5-fold risk (prevalence ratio [PrR]: 5.4; 95%CI: 2.8–9.5) of confirmed non-clear-cell-adenocarcinoma cervical cancer in comparison to the number of cases expected based on age and calendar year rates derived from a cancer registry (Verloop et al., 2000). [The Working Group noted that because a low proportion of women returned their questionnaires, participation bias may have inflated the PrR.]

2.2.3 Cancer of the breast

A study in the Netherlands based on 5421 questionnaires returned to the NDIC found a modestly elevated risk of breast cancer for diethylstilbestrol-exposed women, but the confidence intervals were wide (PrR, 1.5; 95%CI: 0.7–2.9) (Verloop et al., 2000). Findings based on the 1994 and 2001 follow-up of the NCI Combined Cohort Study did not show an overall increase of breast cancer rates in prenatally exposed women (Hatch et al., 1998; Troisi et al., 2007) (see Table 2.3 available at http://monographs. iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.3.pdf). Relative risks from the two reports were 1.18 (95%CI: 0.56–2.49) (Hatch et al., 1998) and 1.35 (95%CI: 0.85–2.10) (Troisi et al., 2007). A more detailed analysis of the 2001 follow-up data gave an incidence rate ratio (IRR) of 2.05 (95%CI: 1.12–3.76) in women aged 40 years or more, and 0.57 (95%CI: 0.24–1.34) in women aged less than 40 years. The data also showed an elevated risk for women aged 50 years or more (IRR, 3.85; 95%CI: 1.06–14.0) (Palmer et al., 2006). [The Working Group noted that women aged 50 years or more contributed 3% of the person–years in these analyses.] While speculative, women approaching the age of 50 years in this cohort would have been exposed during the peak years (1952–53 for the Dieckmann clinical trial and DESAD cohort members), which might have involved higher doses. If the association is real, the increased risk in older women might reflect higher exposure rather than age-related risk. In the same study, risk appeared to be elevated for older women with high (versus low) diethylstilbestrol exposure classified using known dose (38%) or assumed dose based on geographic region. There was no evidence that the risk in women aged 40 years or more was influenced by the timing of gestational exposure, which was known for 75%

of the exposed subjects. Also, there was no indication of effect modification by known breast cancer risk factors. Diethylstilbestrol exposure did not influence the receptor status of the breast tumour or lymph node involvement, but the association was evident in women with larger tumours (≥ 2 cm), arguing against screening bias (Palmer *et al.*, 2006).

2.2.4 Other sites

The study based on the NDIC produced a prevalence ratio of 2.9 (95%CI: 0.8–7.5) based on four cases of ovarian cancer observed in women prenatally exposed to diethylstilbestrol (1.36 cases expected) (Verloop *et al.*, 2000). The NCI Combined Cohort Study, however, showed no evidence of an association between prenatal diethylstilbestrol exposure and ovarian cancer in the 1994 or 2001 follow-up (Hatch *et al.*, 1998; Troisi *et al.*, 2007). The SIR was 0.88 (95%CI: 0.44–1.80) based on eight cases in the exposed at the time of the 2001 follow-up (Troisi *et al.*, 2007).

Based on one case, the NDIC study suggested an association between prenatal diethylstilbestrol exposure and cancer of the vulva (PrR, 8.8; 95%CI: 0.2–49.0) but confidence intervals were wide (Verloop *et al.*, 2000).

The NCI Combined Cohort Study found no evidence of an association between prenatal diethylstilbestrol exposure and endometrial cancer (SIR, 1.04; 95%CI: 0.52–2.10) based on eight cases in the exposed (Troisi *et al.*, 2007).

The NCI Combined Cohort Study suggested possible increases of lymphoma, lung and brain/nervous system cancers in prenatally exposed women, but the estimates were imprecise and compatible with chance (Troisi *et al.*, 2007). Sites for which there was no indication of increased risk included the thyroid and colorectum (Troisi *et al.*, 2007).

Based on the present studies of women, there is scant evidence to support an association between prenatal exposure to diethylstilbestrol and tumours other than the established relationship with CCA affecting the cervix and vagina.

2.3 Men exposed to diethylstilbestrol

2.3.1 Men exposed through cancer therapy

Early case reports of breast cancer occurring in prostate cancer patients treated with diethylstilbestrol implied a possible link; however, the extent to which some of these tumours represented metastatic prostate cancer is uncertain (Bülow *et al.*, 1973).

2.3.2 Men exposed in utero

(a) Cancer of the testes

Several studies have examined prenatal diethylstilbestrol exposure in relation to testicular cancer, but findings have been inconsistent. Because the diethylstilbestrol-exposed men now have passed the age of highest risk for testicular cancer, the question of an association is likely to remain unanswered.

Based on the findings from several case–control studies examining this relationship, most of which relied completely (Henderson *et al.*, 1979; Schottenfeld *et al.*, 1980; Depue *et al.*, 1983; Brown *et al.*, 1986) or partly (Moss *et al.*, 1986) on self-reported hormone use, the previous *IARC Monograph* concluded there is sufficient evidence of a relationship between prenatal diethylstilbestrol exposure and testicular cancer. Three of the contributing studies found possible evidence of an association (Henderson *et al.*, 1979; Schottenfeld *et al.*, 1980; Depue *et al.*, 1983) and two did not (Brown *et al.*, 1986; Moss *et al.*, 1986). Of the three studies that found possible evidence, the association was not of statistical significance in two (Henderson *et al.*, 1979; Schottenfeld *et al.*, 1980). The strongest association arose from a study in California that assessed hormone use during the first

trimester of pregnancy with a relative risk of 8.00 (95%CI: 1.3–4.9); 2/9 case mothers (and none of the control mothers) specified using diethylstilbestrol (Depue *et al.*, 1983). Data from some studies showed (Brown *et al.*, 1986) or suggested (Schottenfeld *et al.*, 1980) an increased risk for the sons of women who had experienced spotting or bleeding during the index pregnancy, a possible marker for diethylstilbestrol use not recalled by the mother. Four of the contributing studies relied partly (Schottenfeld *et al.*, 1980) or entirely (Henderson *et al.*, 1979; Depue *et al.*, 1983; Moss *et al.*, 1986) on neighbourhood controls. [The Working Group noted both of these approaches may have resulted in overmatching and attenuation of a possible relationship between prenatal diethylstilbestrol exposure and risk of testicular cancer.] It is also possible the mothers' reporting was inaccurate, in part because of the amount of time that had passed since the pregnancy and in part because women of the diethylstilbestrol era were not always given complete information about their medical care. [The Working Group noted that errors of recall or recall bias may have influenced the results of these studies.]

Early cohort studies of men exposed *in utero* to diethylstilbestrol also have been largely inconclusive. No testicular cancer cases were identified in the sons of women exposed to high doses of diethylstilbestrol through participation in the Dieckmann clinical trial (11–12 g) (Gill *et al.*, 1979), or a clinical trial involving diabetic women in the UK (mean of 17.9 g) (Beral & Colwell, 1980), although both cohorts were small. One case of fatal teratoma was ascertained in the 138 exposed (no cases in the unexposed) sons of women who participated in a separate high-dose (mean of 11.5 g) clinical trial at the University College Hospital in London (Vessey *et al.*, 1983).

Two studies have been published since the previous *IARC Monograph*. The first study, a case–control design, matched controls to cases by an obstetrician (Gershman & Stolley, 1988) (see Table 2.4 available at http://monographs. iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.4.pdf). The source of diethylstilbestrol exposure status was unclear, but apparently was not based on the medical record. The analysis did not show an association between prenatal diethylstilbestrol exposure and testicular cancer. The NCI Combined Cohort Study assessed 2759 (1365 exposed, 1394 unexposed) sons born to women in the WHS study, the Dieckmann clinical trial, and the Horne cohort, as well as sons identified through the Mayo Clinic with retrospective follow-up for an average of 16.9 years (1978–94) (Strohsnitter *et al.*, 2001) (see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.5.pdf). For all participants, diethylstilbestrol exposure (or lack of exposure) was verified by the medical or clinical trial record. In this study, the SIR for prenatally exposed men was 2.04 (95%CI: 0.82–4.20) based on seven cases observed in the exposed and 3.4 expected. The relative risk was 3.05 (95%CI: 0.65–22.0) in the internal comparison (two unexposed cases). None of the cases in the NCI Combined Cohort study arose from the Dieckmann clinical trial cohort in which women were consistently given high doses of diethylstilbestrol (cumulative dose of 11–12 g) during the first trimester, although the subcohort was small in size (205 exposed, 187 unexposed). All of the elevated risk was due to an excess of exposed cases arising in the Mayo cohort (five cases in 660 exposed, one case in 592 unexposed). Among those for whom diethylstilbestrol dose was known, the mothers of cases and noncases received 12.5 and 10 mg/day, respectively, doses that are lower than those received by the Dieckmann clinical trial or Horne cohorts (Strohsnitter *et al.*, 2001). The relative risk was unchanged when the analyses were confined to 138 men whose mothers were given diethylstilbestrol during the first trimester of pregnancy but increased to 5.91 (95%CI: 1.05–46.1) after excluding from the analysis men who were

exposed prenatally to both diethylstilbestrol and progestogen.

Cryptorchidism increases the risk for testicular cancer (Sarma *et al.*, 2006). An increased prevalence of cryptorchidism was not seen in the exposed men in either of the two small cohort studies involving the sons of women who received high doses through participation in separate clinical trials in the UK (a mean of 17.9 g in Beral & Colwell, 1980; mean of 11.5 g in Vessey *et al.*, 1983). However, an increased prevalence of cryptorchidism (17/308 exposed versus 1/307 unexposed; *P* < 0.005) was seen in the sons of women exposed to high doses of diethylstilbestrol through participation in the Dieckmann clinical trial (Gill *et al.*, 1979), suggesting a possible pathway linking diethylstilbestrol and testicular cancer (no cases were noted). In the case–control study that addressed this connection, only 1/22 testicular cancer cases affected by cryptorchidism was also exposed to diethylstilbestrol (Schottenfeld *et al.*, 1980).

(b) Other sites

In the NCI Combined Cohort Study, findings were suggestive for bone and thyroid cancer, but estimates were imprecise.

2.4 Offspring (third generation) of women who were exposed to diethylstilbestrol *in utero*

2.4.1 Third-generation women

Follow-up of the prenatally exposed and unexposed second-generation women participating in the NCI Combined Cohort in 1994, 1997, and 2001 included inquiries about cancers occurring in their offspring (Titus-Ernstoff *et al.*, 2008; see Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-11-Table2.6.pdf). Based on the mothers' unconfirmed reports, two cases of

ovarian cancer occurred (diagnoses at ages 7 and 20 years) in the 2539 daughters of prenatally exposed women. The SIR in the exposed was 5.3 (95%CI: 1.3–21) based on 0.38 cases expected. No cases were reported in the 1423 unexposed third-generation daughters.

In 2001, the NCI Combined Cohort Study initiated a follow-up study of the adult daughters of women who either had or had not been exposed to diethylstilbestrol *in utero* (Titus-Ernstoff *et al.*, 2008). The results of the baseline survey, which enrolled 793 third-generation women (463 exposed, 330 unexposed), confirmed two cases of ovarian cancer in exposed women (diagnosis ages of 20 and 22 years), including one of the cases that had been reported by the mother. No cases of ovarian cancer were observed in the daughters of women who were not exposed to diethylstilbestrol *in utero*. The SIR was 14.68 (95%CI: 3.67–58.71) based on 0.14 expected cases. Because only half of the second-generation women had allowed contact with their daughters, participation bias was a possible explanation for this finding. However, the SIR remained elevated (6.6; 95%CI: 1.7–26) when based on all adult daughters of prenatally exposed women, regardless of whether they participated in the third-generation study (0.30 cases expected).

Only one study involved clinical examinations of third-generation women (Kaufman & Adam, 2002). Most of the mothers had a history of diethylstilbestrol-related changes, but no vaginal or cervical anomalies were noted upon colposcopic examination of 28 third-generation daughters. Although the study was based on small numbers and did not include hysterosalpingography, the absence of anomalies is inconsistent with the high prevalence of diethylstilbestrol-related vaginal epithelial changes affecting prenatally exposed women.

2.4.2 Third-generation men

In the NCI Combined Cohort Study and based on the mothers' reports, the SIR provided no evidence of increased cancer risk in men born to women exposed prenatally to diethylstilbestrol.

2.5 Synthesis

Diethylstilbestrol is associated with cancer of the breast in women who were exposed while pregnant. Diethylstilbestrol also causes CCA in the vagina and cervix of women who were exposed *in utero*. Finally, a positive association has been observed between exposure to diethylstilbestrol and cancer of the endometrium, and between in-utero exposure to diethylstilbestrol and squamous cell carcinoma of the cervix, and cancer of the testis.

3. Cancer in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

Dietary exposure of diethylstilbestrol induced tumours in many sites, such as the ovary, endometrium and cervix of the uterus, and mesothelioma (origin not indicated) (Greenman *et al.*, 1986). Mammary adenocarcinoma incidence was increased in C3H/HeN-MTV+ female mice (Greenman *et al.*, 1987). Dietary diethylstilbestrol induced thyroid follicular cell adenoma in C57BL/6 mice (Greenman *et al.*, 1990).

Diethylstilbestrol was considered negative in the oral studies in Tg.AC mouse, which is one of the models selected for examination by topical application of either mutagenic or non-mutagenic carcinogens with papilloma formation at the site of application (Eastin *et al.*, 2001). Effect of dietary diethylstilbestrol was studied in p53$^{\pm}$ mice. Interstitial cell hyperplasia and tumours were observed in the testis, and pituitary hyperplasia and adenomas were observed in females; however, the incidences of these lesions were not statistically significant (Storer *et al.*, 2001). When diethylstilbestrol was given to CB6F1-rasH2 transgenic mice, benign tumours and hyperplasia of the Leydig cells in the testes were noted. The incidence of Leydig cell tumours in the rasH2 males at high dose was significantly higher than in vehicle control males (4/15 versus 0/15; $P < 0.05$) (Usui *et al.*, 2001). Carcinogenicity of dietary diethylstilbestrol was investigated in two mouse knockout models, the Xpa homozygous knockout, and the combined Xpa homozygous and p53 heterozygous knockout. The incidence of osteosarcoma and testicular interstitial cell adenomas was higher in male Xpa/p53 mice. One Xpa male had osteosarcoma, which was not observed in wild-type mice. Xpa mice were no more sensitive than wild-type mice for compounds like diethylstilbestrol. The Xpa/p53 mouse model nevertheless showed an increased susceptibility to diethylstilbestrol in inducing osteosarcoma and testicular cell adenoma in males (McAnulty & Skydsgaard, 2005).

See Table 3.1.

3.2 Subcutaneous and/or intramuscular administration

3.2.1 Mouse

The effects of diethylstilbestrol on urethane-induced lung carcinogenesis were assessed in the mouse. Results indicate that diethylstilbestrol promotes lung carcinogenesis (Jiang *et al.*, 2000).

See Table 3.2.

Table 3.1 Studies of cancer in experimental animals exposed to diethylstilbestrol (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse C3H/HeN-MTV (F) 133 wk Greenman et al. (1986)	640 ppb DES in diet from 3, 5, 7 or 9 wk of age until 133 wk of age DES-free diet for controls 192/group, 96 controls	Ovary (granulosa cell tumours): Control–6/75 (8%) DES–0/181; 1/180 (0.5%); 3/183 (2%); 0/183 Ovary (tubular adenomas): Control–16/75 (21%) DES–1/181 (1%); 24/180 (13%); 44/183 (24%); 59/183[a] (32%) Pituitary gland (adenomas): Control–1/67 (1%) DES–8/173 (5%); 10/180 (6%); 16/180 (9%); 21/179[b] (12%) Uterus (endometrial carcinomas): Control–(0/77) DES–0/182; 6/189 (3%); 9/191[c] (5%); 8/192 (4%) Uterus (cervical carcinomas): Control–(0/77) DES–0/182; 5/189 (3%); 13/191[d] (7%); 9/192[e] (5%) Mesothelioma:[1] Control–(0/77) DES–0/182; 13/189 (7%); 28/191 (15%); 29/192[f] (15%) Mammary gland (adenocarcinomas): Control–(0/73) DES–0/182; 0 189; 4/185 (2%); 3/182 (2%)	[P < 0.0001][a] [P < 0.0065][b] [P < 0.0449][c] [P < 0.05][d,e] [P < 0.0001][f]	Age at the start of DES treatment was a major factor in susceptibility of mice to mammary carcinogenesis of C3H/HeN-MTV female mice
Mouse C3H/HeN-MTV (F) 61 wk Greenman et al. (1987)	320 ppb DES in diet for 4, 8, 26 and 140 wk 640 ppb DES in diet for 4, 8, 26, and 140 wk DES-free diet for controls 48–72, 312 controls	Mammary (adenocarcinomas): Control–234/295 (79%) 320 ppb–59/71 (83%); 60/72 (83%); 69/72[a] (96%); 68/72[a] (94%) 640 ppb–45/48 (94%); 46/48[a] (96%); 46/48[a] (96%); 47/48[a] (98%)	[P < 0.05][a]	
Mouse C57BL/6 (M, F) 153 wk (M), 143 wk (F) Greenman et al. (1990)	DES 0, 5, 10, 20, 40, 160, 320, 640 ppb DES-free diet for controls 72/sex/group	Thyroid (follicular cell adenomas): M–2/48 (4%); 0/51; 1/47 (2%); 3/48 (6%); 6/51 (11%); 27/51 (50%); 3/58 (5%); 0/43 F–13/64 (20%); 11/56 (20%); 10/51 (20%); 10/55 (18%); 16/61 (26%); 14/48 (29%); 4/60 (7%); 0/50	P < 0.001 (negative trend) P < 0.001 (negative trend)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse Tg.AC[2] (M, F) 27 wk Eastin *et al.* (2001)	DES 0, 30, 240, or 480 μg/ kg bw in corn oil i.g. twice weekly for 26 wk then once during Week 27; control corn oil 15/sex/group	No effect on the incidences of either forestomach papillomas or skin tumours in either sex		No table available because of negative data
Mouse p53[±] transgenic (M, F) 26 wk Storer *et al.* (2001)	M– p53[±] (0, 50, 250 ppm), wild type (0, 50, 250 ppm) F– p53[±] (0, 500, 1000 ppm), wild type (0, 500, 1000 ppm) 15/sex/group	Testis (interstitial cell tumours): M–p53[±] 0/15; 0/15; 2/15 (13%) M–wild type 0/15; 0/13; 0/15 Pituitary gland (adenomas): F–p53[±] 0/15; 2/15 (13%); 2/15 (13%) F–wild type 0/15; 0/15; 0/14	Incidences of interstitial cell and pituitary tumours were not statistically significant	
Mouse CB6F1-rasH2 transgenic (M, F) 26 wk Usui *et al.* (2001)	M– rasH2 (0, 0.1, 0.3, 1.0 ppm), wild type (0, 0.1, 0.3, 1.0 ppm) F– rasH2 (0, 0.1, 0.3, 1.0 ppm), wild type (0, 0.1, 0.3, 1.0 ppm)	Leydig cell (tumours): M–rasH2 0/15; 0/14; 0/15; 4/15[a] (27%) M–wild type 0/15; 0/15; 1/15 (7%); 2/15 (14%) Lung (adenomas): F–rasH2 1/14 (7%); 0/14; 4/14 (28%); 2/14 (14%) F–wild type NR	$P < 0.05$[a]	

188

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse Xpa/p53[4] (M, F) 39 wk McAnulty & Skydsgaard (2005)	M– Xpa (0, 100, 300, 1500 ppb), wild type (0, 1500 ppb) Xpa/p53 (0, 1500 ppb) F– Xpa (0, 100, 300, 1500 ppb), wild type (0, 1500 ppb) Xpa/p53 (0, 1500 ppb) 15/sex/group	Osteosarcomas: M–Xpa 0/15; 0/14; 0/13; 1/6 (17%) M–wild type 0/15; 0/15 M–Xpa/p53 0/13; 5/6[a] (83%) Testis (interstitial cell adenomas): M–Xpa 0/15; 0/14; 3/13 (23%); 1/6 (17%) M–wild type 0/15; 1/15 (7%) M–Xpa/p53 0/13; 4/6[b] (67%) Osteosarcomas: F–Xpa 0/15; 0/15; 0/15; 0/2 F–wild type 0/15; 0/15 F–Xpa/p53 0/13; 2/2 (100%) Mammary (carcinomas): F–Xpa 0/15; 0/15; 0/15; 0/2 F–wild type 0/15; 0/15 F–Xpa/p53 1/13 (8%); 0/2	$P < 0.05^a$ $P < 0.05^b$	

[1] Origin was not described.
[2] The Tg.AC genetically engineered mouse, carrying a v-Ha-ras oncogene fused to the promoter of the gamma-globin gene.
[3] CB6F1-rasH2 transgenic mice with human c-Ha-ras proto-oncogene
[4] Xpa homozygous and p53 heterozygous deficient condition
d, day or days; DES, diethylstilbestrol; F, female; i.g., intragastric; M, male; NR, not reported; wk, week or weeks

Table 3.2 Studies of cancer in experimental animals exposed to diethylstilbestrol (intramuscular injection)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse Kunming (F) 18 wk Jiang et al. (2000)	Single i.p. injection of U in saline (50 mg/kg) + DES, i.m. injections one wk later 5 or 50 mg/kg bw once every wk for 18 wk Control: saline and DMSO + saline 26–28, 58 controls	Lung (macroscopic tumours): U alone–(9/27 (33%), 0.69 ± 1.04) U + DES 5 mg–(17/28[a] (61%), 1.80 ± 1.79[b]) U + DES 50 mg–(20/26[b] (77%), 3.81 ± 2.83[b]) Lung (malignant tumours):[1] U alone–(5/27) (18%) U + DES 5 mg–(9/28) (32%) U + DES 50 mg–(17/26[b]) (65%)	$P < 0.05$[a, b], $P < 0.01$ vs U alone group, respectively	DES is a promoter of lung carcinogenesis Age at start NR, animal weight 17–20 g

[1] Malignant tumours were combinations of adenocarcinoma, papillocarcinoma (author's translation), and mixed type cancer
DES, diethylstilbestrol; i.m., intramuscular; i.p., intraperitoneal; NR, not reported; U, urethane; vs, versus; wk, week or weeks

3.3 Subcutaneous implantation

3.3.1 Rat

Diethylstilbestrol pellets were implanted in lactating Wistar-MS rats after irradiation (260 cGy). A significantly higher incidence of mammary tumours was observed in the 260 cGy plus diethylstilbestrol group compared with the 260 cGy-alone group. The latency period was shortest in the diethylstilbestrol-treated group irradiated during the late lactation period. Diethylstilbestrol treatment alone in virgin rats, without irradiation ($n = 20$), did not produce any tumours (Suzuki et al., 1994).

Implanted diethylstilbestrol silastic tubes induced significantly larger and highly haemorrhagic pituitary tumours in female F344 rats but not in Brown Norway (BN) rats. The female F1 (F344 x BN) rats exhibited significantly increased pituitary growth after 10 weeks of diethylstilbestrol treatment, but the pituitary was not haemorrhagic. The haemorrhagic pituitaries in F2 rats were mostly massive, indicating that some genes regulate both phenotypes (Wendell et al.,

1996). Diethylstilbestrol increased pituitary mass to 10.6-fold in male ACI rats, and only to 4.4-fold in male Copenhagen (COP) rats. The pituitary growth response of the diethylstilbestrol-treated (5 mg at 63 ± 4 days until 12 weeks of age) in F1 (COPxACI) rats was intermediate (6.9-fold) to that exhibited by the parental ACI and COP strains (Strecker et al., 2005).

See Table 3.3.

3.4 Perinatal exposure

3.4.1 Mouse

Methylcholanthrene treatment induced vaginal tumours (squamous cell carcinoma and mixed carcinoma (squamous cell carcinoma plus adenocarcinoma)) with significantly higher incidence in the CD-1 mice after prenatal exposure to diethylstilbestrol (Walker, 1988). Prenatal exposure to diethylstilbestrol with a high-fat diet increased the incidence of uterine glandular tumours but not of mammary tumours (Walker, 1990). Prenatal diethylstilbestrol induced

Table 3.3 Studies of cancer in experimental animals exposed to diethylstilbestrol (subcutaneous implantation)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat Wistar-MS (F) 1 yr Suzuki *et al.* (1994)	Irradiated with 260 cGy of gamma rays on 21 d after parturition[a] + CHOL pellets containing 5 mg DES were implanted 1 mo after lactation. DES pellets[b] remained for 1 yr and were replaced every 8 wk 17–28 rats/group	Mammary tumours (no histological information) *Incidence, latency period (month)* 260 cGy + CHOL (6/17 (35%), 10.5 ± 0.2); O cGy + DES (3/11 (27%), 10.0 ± 1.2); 260 cGy + DES (27/28 (96%), 7.4 ± 0.5); Virgin rats: 0 cGy + DES (0/20)	*P* < 0.001 in the incidence and latency period, 260 cGy + DES vs 260 cGy + CHOL *P* < 0.001 in the incidence and latency period 260 cGy + DES vs DES alone	DES promoted radiation-induced mammary tumorigenesis

[a] Detailed location was not described
[b] The release of DES from the pellet was estimated to be 1 µg/day
CHOL, cholesterol; d, day or days; DES, diethylstilbestrol; F, female; mo, month or months; vs, versus; wk, week or weeks; yr, year or years

pituitary tumours in female CD-1 mice (Walker & Kurth, 1993).

In the CBA female descendants of mothers treated with prenatal diethylstilbestrol exposure, described as F2m, the incidence of uterine sarcomas, lymphomas, and ovarian tumours was significantly higher than in controls (Turusov *et al.*, 1992). The persistence of diethylstilbestrol effects was studied in one further generation (diethylstilbestrol-lineage-2 mice). Diethylstilbestrol-lineage-2 mice, exposed to low- or high-fat maternal diets, had significantly more tumours in their reproductive system and liver than control mice with the same dietary fat exposure (Walker & Haven, 1997). The incidence of uterine adenocarcinomas in F2 females with prenatal diethylstilbestrol exposure was significantly higher than in controls, whereas the incidence of tumours of the liver, lung or other organs examined in this study was not significantly different from that in control animals (Newbold *et al.*, 1998). In F2 males, a significant increase in the incidences of proliferative lesions of the rete testis (hyperplasia and tumours) was observed,

suggesting that the rete testis is a target for the transgenerational effects of diethylstilbestrol in males (Newbold *et al.*, 2000).

Prenatal diethylstilbestrol treatment of female CBA mice increased the incidence of DMH-induced colon carcinoma (Turusov *et al.*, 1997). Effects of perinatal diethylstilbestrol exposure on mammary tumorigenesis were studied in female C3H/HeN/MTV+ mice. Neonatal treatment with a low dose of diethylstilbestrol increased the probability of mammary tumour formation (Lopez *et al.*, 1988). Effects of perinatal exposure to estrogens during the development stage of organs in the reproductive tract were studied in CD-1 mice. Uterine adenocarcinomas were induced in a time- and dose-related manner after diethylstilbestrol treatment (Newbold *et al.*, 1990). Male offspring of CD-1 mice with transplacental exposure to arsenite were treated with diethylstilbestrol neonatally. Total liver tumour incidence, the number of mice with multiple liver tumours, and urinary bladder proliferative lesions was higher in the arsenite plus diethylstilbestrol mice compared to the arsenite-alone

group (Waalkes et al., 2006b). In female offspring CD-1 mice, the incidence of carcinoma of the cervix and of urinary bladder total proliferative lesions (hyperplasia plus papilloma plus carcinoma) in the arsenite plus diethylstilbestrol group was significantly higher than in the arsenite-alone group (Waalkes et al., 2006a).

CD-1 and diethylstilbestrol induced-TGFα transgenic mice were neonatally treated with diethylstilbestrol. The presence of the TGFα transgene significantly increased the incidence of endometrial hyperplasia and benign ovarian cysts, whereas it did not promote uterine adenocarcinoma (Gray et al., 1996). Transgenic MT-mER mice, which overexpress the estrogen receptor, driven by the mouse metallothionein I promoter were neonatally treated with diethylstilbestrol. The diethylstilbestrol-treated MT-mER mice demonstrated a significantly higher incidence of uterine adenocarcinomas (Couse et al., 1997). Diethylstilbestrol-treated wild-type mice exhibited a relatively high frequency of uterus endometrial hyperplasia and granulosa cell tumours in the ovary, while αERKO mice (estrogen receptor α knockout mice) showed a complete lack of these lesions (Couse et al., 2001). Lymphoma-prone Mlh1 or Msh2 knockout mice were treated with diethylstilbestrol. The combination of Mlh1 deficiency with diethylstilbestrol exposure was shown to accelerate lymphomagenesis (Kabbarah et al., 2005). In murine PTEN (mPTEN) heterozygous mutant mice, demonstrated that neonatal diethylstilbestrol treatments exerted an inhibitory, rather than an enhancing, effect on PTEN-associated endometrial carcinogenesis via stromal alterations (Begum et al., 2006).

3.4.2 Rat

Mammary tumours are induced in female ACI rats by either prenatal injections or by postnatal pellet implantation of diethylstilbestrol. The combination of both yielded significantly greater tumour multiplicity, and decreased tumour latency (Rothschild et al., 1987). Vaginal epithelial tumours were induced in a dose-related manner in female Wistar rat following in-utero diethylstilbestrol exposure (Baggs et al., 1991). Prenatal exposure to diethylstilbestrol produced uterine adenocarcinomas and pituitary adenomas in female Donryu rats, as reported in an earlier study in mice (Kitamura et al., 1999). In Sprague Dawley rats, neonatal diethylstilbestrol exposure at a relatively low dose (1 μg/kg body weight) caused an increase in the incidence of mammary carcinomas induced by 1,2-dimethylbenz[a] anthracene (Ninomiya et al., 2007). Female rats carrying the Eker mutation (Tsc-$2^{Ek/+}$) administered diethylstilbestrol neonatally had a significantly greater multiplicity of leiomyoma in the uterus (Cook et al., 2005).

3.4.3 Hamster

The subcutaneous implantation of diethylstilbestrol pellets caused renal tumours in young Syrian hamsters (Liehr & Wheeler, 1983), and diethylstilbestrol pellets, implanted after orchiectomy, induced kidney tumours in the same species (Goldfarb & Pugh, 1990). Diethylstilbestrol-treated castrated hamsters exhibited interstitial lesions in the kidney as well as kidney tumours (Oberley et al., 1991). In male and female Armenian hamsters, diethylstilbestrol pellets applied subcutaneously induced hepatocellular carcinomas (Coe et al., 1990).

See Table 3.4.

3.5 Synthesis

The oral administration of diethylstilbestrol induced tumours of the ovary, endometrium and cervix, and mammary adenocarcinomas in female mice. Osteosarcomas and Leydig cell tumours were induced in rasH2 and Xpa/p53 male mice, respectively.

Table 3.4 Studies of cancer in experimental animals exposed to diethylstilbestrol (perinatal exposure)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse CD-1 (F), gestation Day 17 Walker (1988)	Prenatal: s.c. injection 1 μg/g bw DES in olive oil + 2% ethanol at Day 17 of pregnancy + Postnatal: sponges impregnated with MCA in beeswax were lodged against the cervix and vaginal fornices of the mice at 6 months of age Controls received olive oil Number/group at start NR	Vaginal tumours (SCC and mixed (SCC + adenocarcinoma) carcinomas): DES + MCA–(10/35, 29%) Vehicle + MCA–(2/28, 7%) DES + beeswax–(1/12, 8%) Vehicle + beeswax–(0/8, 0%)	$P < 0.05$ DES+MCA vs Vehicle+MCA	
Mouse CD-1 (F), gestation Day 16 Lifespan Walker & Kurth (1993)	i.p. injection 1 or 2 μg/g bw at 16–17 days postconception 132; 64 controls	Pituitary tumours: Control–(1/57, 2%) DES 1 μg–(19/34, 56%) DES 2 μg–(3/3, 100%)	$P < 0.01$	
Mouse CBA (F), gestation Day 17 Lifespan Turusov et al. (1992)	Prenatal: i.p. injection 1 μg/g bw DES in olive oil + 2% ethanol at Day 17 of pregnancy (F2m,[a] the descendants of DES-treated grandmothers were used in this study) Number/group at start NR	Uterine (sarcomas): F2m female (17/84, 20%), control (6/108, 6%) Lymphomas:[b] F2m female (17/84, 20%), control (10/108, 9%) Benign ovarian tumours: F2m female (16/84, 19%), control (9/108, 8%)	$P = 0.0022$ $P = 0.037$ $P = 0.004$	
Mouse CD-1 (F), gestation Day 17 Lifespan Walker & Haven (1997)	1 μg/g bw of DES in olive oil + 0.1% ethanol at Day 17 of pregnancy. DES-lineage2[c] mice were exposed to low– (LF, 2.6%) or high-fat (HF, 29%) diets Number/group at start NR	Reproductive system tumours: combination of ovarian, uterine, cervical (adenocarcinoma) and mammary tumours (adenocarcinoma + sarcoma) DES+LF (31/61, 51%), LF (11/66, 17%) DES+HF (25/54, 46%), HF (18/68, 26%) Liver tumours: DES+LF (17/61, 28%), LF (32/66, 48%) DES+HF (16/54, 30%), HF (22/68, 32%)	$P < 0.001$ $P < 0.05$ $P = 0.03$ NS	The multi-generational effect of DES was observed in mice

Table 3.4 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse CD-1 (F), Day 1 for F2 Up to 24 mo Newbold et al. (1998)	s.c. injection G1: 2.5, 5, 10 µg/kg bw on Days 9–16 of gestation G2: 1000 µg/kg bw on Day 18 of gestation G3: 0.002 µg/pup/day on Days 1–5 after birth Female F2 mice[d] were examined in this study. Animals were held for 17–19 or 22–24 mo Number at start NR	Uterine adenocarcinomas in F2: Groups / at 17–19 mo / at 22–24 mo / total Control 0/32 0/23 0/55 G1 2.5 g 2/29 (7%) 3/35 (9%) 5/64 (8%) G1 5 g 2/35 (6%) 6/37 (16%) 8/72 (11%) G1 10 g 0/16 0/24 0/40 G2 0/33 1/15 (7%) 1/48 (2%) G3 1/29 (3%) 4/36 (11%) 5/65 (8%)	vs. concurrent controls;[e] vs. historical controls[f] – ; – $P < 0.05$; $P < 0.001$ $P < 0.01$; $P < 0.001$ NS; NS NS; NS $P < 0.05$; $P < 0.001$	Pups, 1–3.5 g
Mouse CD-1 (M) Day 1 for F2 Up to 24 mo Newbold et al. (2000)	s.c. injection G1: 2.5, 5, 10 g/kg bw on Days 9–16 of gestation G2: 1000 µg/kg bw on Day 18 of gestation G3: 0.002 µg/pup/d on Days 1–5 after birth DES was dissolved in corn oil Male F2 mice[g] examined in this study were killed at 17–24 mo Number at start NR	Rete testis proliferative lesions (hyperplasia or tumours) in testis of DES-lineage (F2) male mice: Control (3/53, 6%) G1 2.5 µg (15/73, 21%) G1 5 µg (27/83, 32%) (1 tumour) G1 10 µg (17/49, 35%) G2 (5/52, 10%) (1 tumour) G3 (7/23, 30%)	– $P < 0.05$ $P < 0.01$ $P < 0.01$ NS $P < 0.01$	Pups, 1–3.5 g
Mouse, C3H (F) CBA (F) 2–3 months for F1 50 wk Turusov et al. (1997)	s.c. injection 0, 0.1, 0.3 mg/kg bw DES in olive oil + 0.1% ethanol at Day 17 of pregnancy and then descendants were treated with 1,2-dimethyl hydrazine (DMH)[h] Killed at Week 50 after the beginning of DMH 35–50; 84–101 controls/group	Colon (carcinomas): DMH / 0.1-DES+DMH / 0.3-DES+DMH C3H 9/37 (24%) 6/45 (13%) 4/32 (12%) CBA 2/38 (5%) 10/54 (18%) 10/27 (37%) Uterus (sarcomas): C3H 0/37 4/45 (9%) 0/32 CBA 21/38 (55%) 29/54 (53%) 16/27 (59%)	NS $P<0.01$ (0.3-DES+DMH) NS NS	

Table 3.4 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse CD-1 (F) Day 1 of life Up to 18 mo Newbold et al. (1990)	s.c. injection of 0.002 or 2 µg/pup/d DES in corn oil for Days 1–5 of age Controls received corn oil Killed 1, 2, 4, 6, 8, 12, 18 mo of age Number at start NR	Uterus adenocarcinomas of 2 µg group at 1, 2, 4, 6, 8, 12, 18 mo: DES–0/18, 0/10, 0/10, 0/12, 0/5, 8/17 (47%), 9/10 (90%) Controls–0/15, 0/10, 0/10, 0/12, 0/5, 0/17, 0/10	$P = 0.0026$ at 12 mo, $P = 0.0002$ at 18 mo	
Mouse CD-1 (F) Day 1 of life (female offspring) 90 wk Waalkes et al. (2006a)	Transplacental exposure to arsenite (ARS)[i] 85 ppm from Days 8–18 of gestation + s.c. injection 2 µg/pup/d DES Days 1–5 of age Killed at 90 wk 35/group (offspring)	Cervix (carcinomas): Control (0/33), ARS (0/34), DES (6/33, 18%), ARS+DES (8/33, 24%) Urinary bladder (total proliferative lesions):[j] Control (1/33, 3%), ARS-alone (5/34, 15%), DES-alone (1/33, 3%), ARS+DES (13/33, 38%)	$P = 0.01$ (DES vs control) $P < 0.05$ (ARS+DES vs ARS) $P < 0.05$ (ARS+DES vs ARS)	Sodium arsenite in the drinking-water
Mouse CD-1 (F) TGF α[k] (F) Day 1 of life Gray et al. (1996)	s.c. injection 2 µg/pup/d DES in sesame oil on Days 1 – 5 of age Control remained untreated maintained 39, 52 wk or for life Number/group at start NR	Uterus (adenocarcinomas): Vehicle in CD-1, (0/26) Vehicle in TGF α (0/25) DES in CD-1 (7/16, 44%) DES in TGF α (7/15, 47%)	– – $P<0.01$ $P<0.01$	
Mouse, Wild type fvb/N (F) MT-mER[l] (F) Day 1 of life Up to 18 mo Couse et al. (1997)	s.c. injection 2 µg/pup/d DES in corn oil on Days 1 – 5 of age Control: untreated Killed at 4, 8, 12, and 18 mo of age Number/group at start NR	Uterus adenocarcinomas at 4, 8, 12, 18 mo: MT-mER +DES: 0/19, 19/26 (73%), 13/15 (87%), 12/13 (92%) Wild type+DES: 0/19, 11/24 (46%), 11/15 (73%), 13/14 (93%) MT-mER control: 0/14, 0/10, 0/15, 1/19 (5%) Wild type control: 0/15, 0/11, 0/15, 0/19	$P < 0.05$ at 8 months (MT-mER+DES vs Wild type+DES) $P < 0.05$ at 8, 12, 18 mo (Wild type+DES vs wild type)	

Table 3.4 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, ACI (F) gestation Day 15 Rothschild et al. (1987)	Prenatal: s.c. injection of 0.8 µg (Low) or 8.0 µg (High) DES in sesame oil on Days 15 and 18 of gestation Postnatal:ᵐ 2.5 mg DES pellet at 12 wk Control: sesame oil (vehicle/DES) Killed 7 mo post pellet 32–47, 32 controls	*Mean time to appearance of first mammary tumour mean No. of palpable mammary tumours/rat:* Vehicle/DES: 22.2 wk, 2.2 Low DES/DES: 19.4 wk,[1] 3.0 High DES/DES: 15.2 wk,[1] 4.3[2]	[1] $P < 0.05$ vs Vehicle/DES [2] $P < 0.05$ vs Vehicle/DES	
Rat, Wistar (F) gestation Day 18 > 120 d Baggs et al. (1991)	i.p. injection 0, 0.1, 0.5, 25, 50 mg/kg bw on Days 18, 19, and 20 of gestation 11–80, 147 controls	Vaginal epithelial tumours:ⁿ 2/147 (1%), 2/49 (4%), 4/80 (5%), 1/63 (2%), 3/11 (27%)	$P < 0.001$ (dose response)	
Rat, Donryu (F) gestation Day 17 18 mo Kitamura et al. (1999)	s.c. injection 0, 0.01, 0.1 mg/kg bw DES on Days 17 and 19 of gestation Control: olive oil 35–72, 23 controls	Uterus tumours (endometrial adenocarcinomas): 1/20 (5%), 4/39 (10%), 9/29 (31%) Pituitary (adenomas): 1/20 (5%), 0/39, 6/29 (20%)	$P < 0.05$, 0.1 mg vs control $P < 0.01$, 0.1 mg vs control	
Rat, Sprague Dawley (F) Day 1 of life Up to 300 d Ninomiya et al. (2007)	s.c injection 0, 0.1, 1, 10, 100 µg/kg bw DES in sesame oil at birth. 50 days after birth all rats received 1 mg DMBA in 1 mL sesame oil by gastric intubation Killed 50–300 d after birth 13–21, 22 controls	*Mammary tumour incidence and multiplicity (adenocarcinomas) at 300 d after birth:* 0.0 µg: 17/22 (77%), 1.50 ± 1.19 0.1 µg: 13/15 (86%), 2.80 ± 1.61[1] 1 µg: 21/21[1] (100%), 3.00 ± 1.22[2] 10 µg: 11/13 (85%), 2.54 ± 1.66[1] 100 µg: 12/16 (75%), 1.63 ± 1.45	[1] $P < 0.05$ vs control [2] $P < 0.01$ vs control	
Rat, Eker Rats carrying a germ-line defect in the tuberous sclerosis complex 2 (Tsc-2) tumour suppressor gene (F) Day 3 of life Up to 16 mo Cook et al. (2005)	s.c. injections 10 µg/rat/d DES in sesame oil on Days 3, 4, and 5 after birth Controls: 50 µl sesame oil Killed up to 16 mo of age Number at start NR	Uterus (leiomyomas): *Incidence, multiplicity and size* In Ekar rats $Tsc\text{-}2^{Ek/+}$ Vehicle: 18/28 (64%), 0.82 ± 0.15, 2.30 ± 1.10 DES: 22/24 (92%), 1.33 ± 0.17, 10.50 ± 2.70[1] In wild type $Tsc\text{-}2^{+/+}$ Vehicle: 0/34, 0, 0 DES: 0/34, 0, 0	[1] $P < 0.02$ in multiplicity, and size in Eker rats (DES vs vehicle)	

Table 3.4 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Hamster Syrian (M), 4 wk 172 d Liehr & Wheeler (1983)	Two 40 mg pellets containing 90% DES + 10% cholesterol were implanted at Day 27 and one more (same composition) 3 mo later 15/group	Renal tumours: Control (0/9) DES (11/13, 85%)	$P < 0.05$	
Hamster, Syrian (M), 7 wk 2.5–6.2 mo Goldfarb & Pugh (1990)	Orchiectomy and 4 wk later 20 mg DES pellets every 3 mo, 9–13; 8–12 controls	Renal tumours: Controls at 2.5–3; 5–6.2 mo 0/8; 0/12 DES at 2.5; 4.6; 5.6; 6.2 mo 0/13; 2/12 (17%); 8/12 (67%); 7/9 (78%)	$P < 0.05$, DES vs control at 6.2 mo	
Hamster, Syrian (M) 2 mo Up to 9 mo Oberley et al. (1991)	Castrated and 20 mg of DES pellet every 2.5 mo 57, 4 controls	Interstitial foci in the kidney: DES at 1, 2, 3, 4, 5, 6, 7, 8, 9 mo 0/7; 0/7; 0/7; 0/7; 4/12 (33%); 5/8 (62%); NR; 2/3 (66%); 1/6 (17%) Kidney tumours:[m] DES at 1, 2, 3, 4, 5, 6, 7, 8, 9 mo 0/7; 0/7; 0/7; 0/12; 3/8 (37%); NR; 2/3 (67%); 4/6 (67%) Controls: at 9 mo 0/4	NS	Age NR, Animal weight 90–100 g

[a] The descendants of DES-treated mothers, described as F1DES, were mated among each other or with untreated animals. F1DES males were successfully mated with untreated females (F2m).

[b] Both grossly visible tumours and microscopic cancers included in this category.

[c] The descendants of DES-treated mothers, described as DES-lineage were mated with control animals. DES-lineage2 mice were generated by mating DES-lineage female mice with control males.

[d] Female mice (F1) in each group were raised to sexual maturity and bred with control males. Female offspring (DES lineage or F2) from these matings were raised to maturity and housed with control males for 20 weeks.

[e] versus concurrent controls; relative to concurrent control rate of 0/55.

[f] versus historical controls; relative to historical control rate of 0.4% (2/482) in 21–24 month old female Charles River CD-1 mice.

[g] DES-exposed female mice (F(1)) were raised to maturity and bred with control males to generate DES-lineage (F(2)) descendants. The F(2) males obtained from these matings are the subjects of this report.

[h] The descendants, starting from the age of 2–3 months, received weekly s.c. injections of 1,2-dimethylhydrazine (DMH) (8 mg/kg bw), for a total of 20 injections.

[i] Pregnant CD-1 mice received 85 ppm arsenite in the drinking-water from gestation Days 8 to 18

[j] Total proliferative lesion (hyperplasia+ papilloma+ carcinoma).

[k] Homozygous TGF α transgenic female mice from the MT42 line.

[l] The transgenic construct consisted of a fragment of the mouse ER cDNA encoding the full-length ER protein driven by the mouse metallothionein I promoter.

[m] Pellets containing 2.5 mg DES+17.5 mg cholesterol (DES pellet) or 20 mg cholesterol were implanted s.c. into 12-week-old female offsprings.

[n] The types of epithelial tumours of the vagina were adenocarcinomas, squamous cell carcinomas, and mixed carcinomas.

bw, body weight; d, day or days; DES, diethylstilbestrol; DMBA, 7,12 dimethylbenz[a]anthracene; F, female; i.p., intraperitoneal; M, male; MCA, methylcholanthrene; mo, month or months; NR, not reported; NS, not significant; s.c., subcutaneous; vs, versus; wk, week or weeks

Subcutaneous implantation of diethylstilbestrol induced mammary tumours in female Wistar rats.

Perinatal exposure to diethylstilbestrol induced lymphomas, uterine sarcomas, adenocarcinomas and pituitary, vaginal, and ovarian tumours in female mice. Uterine adenocarcinomas and mammary and vaginal tumours were also induced in female rats. In hamsters, diethylstilbestrol perinatal exposure induced kidney tumours. In castrated hamsters, kidney tumours were also induced following implantation of diethylstilbestrol.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The toxicokinetics and metabolism of diethylstilbestrol (diethylstilbestrol) were reviewed by IARC, (1979b), and by Metzler & Fischer (1981).

Diethylstilbestrol is readily absorbed and distributed in the whole organism after oral administration (Marselos & Tomatis, 1992). In animal models used for the pharmacokinetics of diethylstilbestrol (with the exception of primates), it is apparent that the drug is almost exclusively eliminated through biliary excretion into the intestine, where it undergoes extensive enterohepatic circulation before being excreted in the faeces (Marselos & Tomatis, 1992). Only traces of diethylstilbestrol can be detected in urine (McMartin et al., 1978).

Whole animal autoradiography experiments showed that radiolabelled diethylstilbestrol injected intravenously into rats is accumulated in the liver and small intestine within 4 hours, and radioactivity can still be detected in these organs after 4 days (Bengtsson, 1963). Peak plasma levels of radioactivity were found within 16 hours in sheep given radiolabelled diethylstilbestrol at single oral doses. Radioactivity disappeared almost completely after 120 hours (Aschbacher, 1972). Ten days after a single oral dose of radiolabelled diethylstilbestrol to steers, residues could be detected in the small intestine, the faeces, and the urine (Aschbacher & Thacker, 1974). In the rat, it was demonstrated that after intestinal intubation of diethylstilbestrol or diethylstilbestrol-glucuronide, free diethylstilbestrol is readily absorbed through the epithelium, whereas the conjugated form requires prior hydrolysis by the intestinal microflora (Fischer et al., 1973).

Studies on diethylstilbestrol transfer across the placenta in mice have shown that it accumulates in the fetal genital tract, where it reaches levels that are three times higher than that found in the fetal plasma (Shah & McLachlan, 1976).

The kinetics of a single oral dose of radiolabelled diethylstilbestrol (10 mg) in cattle followed a biphasic depletion curve, attributed to hepatic clearance. An initial steeper slope represented a biological half-life of 17 hours, while the half-life for the later phase was 5.5 days (Rumsey et al., 1975a). Furthermore, pellets of 24–36 mg diethylstilbestrol implanted subcutaneously in cattle or steers resulted in a systematic release of about 56–74 μg of diethylstilbestrol per day; the half-life was 80–90 days (Rumsey et al., 1975b).

Subsequently, the oxidative quinone metabolite of diethylstilbestrol (4′,4″-diethystilbestrol quinone) was found to be reactive in vitro, binding to DNA (Liehr et al., 1983, 1985a). The formation of the quinone is mediated by microsomal mono-oxygenase (Degen et al., 1986; Roy et al., 1991a), in particular cytochrome P450(CYP)1A1 (Roy et al., 1992), by prostaglandin synthase (Ross et al., 1985; Degen, 1993), and by peroxidases (Liehr et al., 1983, 1985a; Metzler, 1984). The quinone metabolite is reduced by P450 reductase and xanthine oxidase, via the semiquinone and non-enzymatically, directly to diethylstilbestrol (Roy & Liehr, 1988; Roy et al., 1991b). Diethylstilbestrol quinone is also formed in vivo, in the kidney of diethylstilbestrol-treated

male Syrian hamsters (Roy & Liehr, 1988), in the mammary gland tissue of diethylstilbestrol-treated ACI rats (Thomas *et al.*, 2004), and in the liver of diethylstilbestrol-treated rats (Green *et al.*, 2003). Diethylstilbestrol quinone is formed in the liver, kidney, uterus, and placenta of pregnant diethylstilbestrol-treated Syrian hamsters, and in the liver and kidney of their fetuses (Roy & Liehr, 1989). Diethylstilbestrol metabolites are also found in the female genital tract of adult mice and pregnant mice, and in tissues of their fetuses (Gottschlich & Metzler, 1984; Maydl *et al.*, 1985). The quinone metabolite was found to undergo a CYP-mediated process of redox cycling (Liehr *et al.*, 1985a), via a semiquinone intermediate (Kalyanaraman *et al.*, 1989).

During redox cycling of diethylstilbestrol, superoxide radicals are formed *in vitro* (Epe *et al.*, 1986; Roy & Liehr, 1988). In the kidney of diethylstilbestrol-treated hamsters, elevated levels of 8-hydroxy deoxyguanosine were found, indicating that diethylstilbestrol can induce oxidative DNA damage *in vivo* (Roy *et al.*, 1991c). Furthermore, increased levels of lipid hydroperoxides and of malondialdehyde-DNA adducts were also detected (Wang & Liehr, 1995a). Lipid hydroperoxides were also found to be increased in the mammary gland tissue of diethylstilbestrol-treated ACI rats (Gued *et al.*, 2003). These lipid hydroperoxides co-activate the CYP1A1-mediated oxidation of diethylstilbestrol to its quinone metabolite (Wang & Liehr, 1994). Diethylstilbestrol treatment reduced the activity of enzymes that protect against diethylstilbestrol-induced oxidative stress, such as glutathione peroxidase, quinone reductase, and superoxide dismutase (Segura-Aguilar *et al.*, 1990). In the mammary gland tissue of female rats, expression of the *Cyp1A1* gene was increased by diethylstilbestrol treatment, whereas the expression of the genes encoding glutathione-*S*-transferase and superoxide dismutase were depressed (Green *et al.*, 2007).

The oxidative metabolism of diethylstilbestrol almost certainly plays a central role in the induction of kidney tumours in Syrian hamsters, of genetic changes in various in-vitro assays, and probably also of other tumours in animals perinatally exposed to diethylstilbestrol *in utero*. Whether these events occur in target tissues of transplacental exposure to diethylstilbestrol in humans has not been determined.

4.2 Genetic and related effects

4.2.1 Direct genotoxicity

(a) Humans

No changes in DNA ploidy pattern and no mutations were found in specific cancer-related genes (H-*RAS* and K-*RAS* proto-oncogenes, *TP53* and the Wilms tumour (*WT-1*) tumour-suppressor genes) or in the coding region of the *estrogen receptor-α* (*ERα*) gene (Welch *et al.*, 1983; Boyd *et al.*, 1996; Waggoner *et al.*, 1996). The frequency of some known polymorphisms (exon 1, 3, and 8) in the *ERα* gene was not different from that expected in the general population (Boyd *et al.*, 1996).

In cervico-vaginal biopsies and smears from 19 women who had been exposed to diethylstilbestrol *in utero* and 19 controls, the frequencies of trisomy of chromosomes 1, 7, 11, and 17 were evaluated by the FISH technique. The trisomy frequencies were elevated in 4/19 (21%) diethylstilbestrol-exposed women. Trisomy of chromosomes 1, 7, and/or 11 was found, which frequently occurs in gynaecological tumours, but trisomy of chromosome 17 did not occur. No chromosomal trisomy was observed in samples from the control women (Hajek *et al.*, 2006).

In neoplastic and preneoplastic lesions of the breast, loss of heterozygosity and allelic imbalance at 20 microsatellite markers on nine chromosomal arms was comparable between women exposed *in utero* to diethylstilbestrol and control women (Larson *et al.*, 2006).

There are no data on the effects of diethylstilbestrol on cell proliferation or apoptosis in human target tissues of diethylstilbestrol-induced carcinogenicity.

Women with documented in-utero exposure to diethylstilbestrol had a higher mitogen-induced proliferation of peripheral blood lymphocytes compared to age- and menstrual-cycle phase-matched control women (Ways *et al.*, 1987; Burke *et al.*, 2001), suggestive of an increased cellular immune response. A hyperactive immune system may be related to the reported higher frequency of autoimmune disease, and immune-related inflammatory disorders such as arthritis following in-utero exposure to diethylstilbestrol, compared with control women (Wingard & Turiel, 1988; Noller *et al.*, 1988). However, natural killer-cell activity was not found to be altered in women exposed to diethylstilbestrol *in utero* (Ford *et al.*, 1983).

The developmental abnormalities and the disturbance of menstrual activity found in sons and daughters, respectively, of diethylstilbestrol daughters suggest that third-generation (F2) effects of human prenatal diethylstilbestrol exposure, including cancer development, are conceivable. However, there are no mechanistic data on this point in animal models, nor data about germ-line mutations or other heritable alterations.

Vaginal adenosis is an established, although non-obligatory, precursor of CCA. Although most women affected by vaginal adenosis do not develop CCA, adenosis is present in up to 100% of women with CCA (Herbst *et al.*, 1972, 1974; Robboy *et al.*, 1984a).

Other effects of in-utero exposure to diethylstilbestrol include infertility in female offspring, as reported in most but not all studies (Palmer *et al.*, 2001), and possibly in males (Perez *et al.*, 2005).

In most studies, changes in menstrual activity by decreasing the duration of menstrual bleeding were observed in comparison with control women (Hornsby *et al.*, 1994). Young women whose mothers had been exposed to diethylstilbestrol *in utero* had a 1.5- to 2-fold increased risk for self-reported menstrual irregularities and fertility problems (Titus-Ernstoff *et al.*, 2006b).

In a meta-analysis (Martin *et al.*, 2008) of three studies (Klip *et al.*, 2002; Palmer *et al.*, 2005; Pons *et al.*, 2005), in-utero exposure to diethylstilbestrol was associated with a 3.7-fold increased risk for hypospadias in men.

(b) Experimental systems

(i) In vivo

Diethylstilbestrol induced chromosomal aberrations in bone-marrow cells of mice treated *in vivo*, but data on in-vivo induction of sister chromatid exchange and micronuclei were equivocal (IARC, 1987b); it induced sister chromatid exchange in one study in rats (Gloser & Cerni, 1984). Diethylstilbestrol induced micronuclei in early haploid mouse spermatids 17 days after a single subcutaneous injection (Pylkkänen *et al.*, 1991a); chromosomal aberrations in cells of the renal cortex in male Syrian golden hamsters (the target tissue of diethylstilbestrol-induced carcinogenicity) (Banerjee *et al.*, 1994); sister chromatid exchange (but no changes in chromosome number) in uterine cervical epithelial cells, but not in the epithelium of the uterus or kidneys (Forsberg, 1991), and sister chromatid exchange, but no aneuploidy in mouse bone-marrow cells (Zijno *et al.*, 1989). Markedly increased aneuploidy was found in proximal tubular kidney cells of male Syrian hamsters with subcutaneously implanted diethylstilbestrol pellets (Li *et al.*, 1993, 1999).

In hamsters, diethylstilbestrol-induced kidney tumour point mutations were detected in the catalylic domain of the DNA polymerase β gene compared to control tissue (Yan & Roy, 1995), and at 44/365 random loci, seven of which were also present in non-tumorous kidney tissue (Singh & Roy, 2004). The expression of the DNA

polymerase β gene and a novel gene, *Etrg-1*, was reduced in tumorous and non-tumorous kidney tissues of diethylstilbestrol-treated hamsters compared to controls (Singh & Roy, 2008). Microsatellite instability was increased in early lesions induced by neonatal treatment of mice (Kabbarah *et al.*, 2003). In host-mediated assays using mice, no DNA-repair response was detected in *E. coli* strains (Kerklaan *et al.*, 1986).

Using [^{32}P]-postlabelling, adducted nucleotides were found in the kidney DNA of hamsters chronically treated with diethylstilbestrol but not in the kidneys of untreated animals (Liehr *et al.*, 1985b). Some adducts were chromatographically identical to those induced by estradiol and other estrogenic compounds, suggesting that some of these adducts may not be diethylstilbestrol-derived (Liehr *et al.*, 1986). The major diethylstilbestrol adduct formed *in vivo* in the hamster kidney and liver DNA was chromatographically identical to that observed after in-vitro reaction of DNA with 4′,4″-diethystilbestrol quinone in the presence of microsomes and hydroperoxide cofactors, suggesting that this metabolite is responsible for DNA damage by diethylstilbestrol *in vivo*, and that oxidative metabolism of diethylstilbestrol is required for its formation (Gladek & Liehr, 1989; Bhat *et al.*, 1994). The adduct was unstable with an in-vitro half-life of 4–5 days at 37°C, and an estimated in-vivo half-life of 14 hours, which is suggestive of in-vivo repair (Gladek & Liehr, 1989). Importantly, diethylstilbestrol adducts were also found in the mammary gland tissue of diethylstilbestrol-treated adult female rats (Green *et al.*, 2005), and in hamster fetal tissues after injection of their mothers with diethylstilbestrol, but the major adduct found was different from that identified in the kidneys of adult diethylstilbestrol-treated hamsters (Gladek & Liehr, 1991). The precise structures of the diethylstilbestrol-induced DNA adducts have not been elucidated, but it is probable that some are oxidative-stress-generated lipid–hydroperoxide– and malondialdehyde–DNA adducts (Wang &

Liehr, 1995a, b). Although feeding of vitamin C reduced the incidence of kidney tumours, the generation of diethylstilbestrol quinone and the formation of adducts in the kidney of diethylstilbestrol-treated male Syrian hamsters (Liehr *et al.*, 1989), the biological significance of the diethylstilbestrol-generated adducts has not been determined, and specific mutations generated by exposure to diethylstilbestrol have not been identified thus far.

(ii) In vitro

Diethylstilbestrol induces aneuploidy and DNA strand breaks in human cells *in vitro* (IARC, 1987a, b; Rupa *et al.*, 1997; Schuler *et al.*, 1998; Quick *et al.*, 2008). Data on in-vitro induction of sister chromatid exchange, chromosomal aberrations, and mutations in human cells were inconclusive (IARC, 1987a, b). More recent studies found additional evidence of diethylstilbestrol-induced sister chromatid exchange in cultured human lymphocytes, but at cytotoxic diethylstilbestrol concentrations (Lundgren *et al.*, 1988; Konac *et al.*, 2005). Data on induction of micronuclei by diethylstilbestrol remain equivocal (Fauth *et al.*, 2000; Clare *et al.*, 2006), while studies on the induction of unscheduled DNA synthesis in human cells *in vitro* were mostly negative (IARC, 1987a, b). Diethylstilbestrol inhibited the polymerization of microtubules in human fibroblasts and prostate cancer cells, inducing metaphase arrest (Parry *et al.*, 1982; Hartley-Asp *et al.*, 1985), an effect that may underlie the induction of aneuploidy.

Diethylstilbestrol inhibited the in-vitro growth of human primary cervical cell strains, and inhibited colony formation at high concentrations (Johnstone *et al.*, 1984; Stanley *et al.*, 1985). Short-term exposure to diethylstilbestrol stimulated the growth of SV40-immortalized human endometrial stromal cells in soft agar, an effect that was inhibited by the anti-estrogen tamoxifen (Xu *et al.*, 1995). Chronic exposure of these cells to low concentrations of diethylstilbestrol

markedly increased growth in soft agar (Siegfried et al., 1984; Rinehart et al., 1996). Thus, diethylstilbestrol caused the transformation of human endometrial stromal cells.

Repeated treatment with low doses of diethylstilbestrol of MCF-10F immortalized, non-tumorigenic, human epithelial breast cells increased colony formation in a soft agar assay at diethylstilbestrol concentrations ranging from 0.007–70 nM (Russo et al., 2001, 2003). Growth of these cells in collagen changed from differentiated ductular growth to solid spherical masses with the same dose–response relationship. Invasive growth in a Boyden chamber assay was increased more than 10-fold at a diethylstilbestrol concentration of 70 nM (Russo et al. 2001, 2003). Different effects are seen with high doses of diethylstilbestrol. Estrogen-receptor-positive MCF-7 human breast cancer cells growth in soft agar was inhibited by diethylstilbestrol at concentrations of 2 μM and higher (Brandes & Hermonat, 1983).

Block et al. (2000) found effects of exposure to diethylstilbestrol in Ishikawa (endometrial carcinoma) cells, HeLa (cervical carcinoma) cells, and SKOV-3 (ovarian carcinoma) cells on mRNA expression of homeobox (HOX) genes that are involved in the development of the reproductive tract and other tissues.

Tests for in-vitro transformation in rat and Syrian hamster embryo cells gave positive results, while results in mouse cells were negative (IARC, 1987b). No mutations were found in BALB/C 3T3 cells transformed by diethylstilbestrol (Fitzgerald et al., 1989).

Aneuploidy and DNA strand breaks were induced in rodent cells in vitro (IARC, 1987b), as confirmed in additional studies (Hayashi et al., 1996; Tsutsui & Barrett, 1997; Tsutsui et al., 1997). Results for chromosomal aberrations, micronuclei, and sister chromatid exchange were equivocal (IARC, 1987b), but in more recent studies, chromosomal aberrations, micronuclei, and sister chromatid exchange, as well as

aneuploidy were found in a variety of rodent cell lines (de Stoppelaar et al., 2000; Aardema et al., 2006; Wakata et al., 2006; Tayama et al., 2008).

In a comparison of diethylstilbestrol-induced aneuploidy in human foreskin fibroblasts and Syrian hamster embryo fibroblasts, the hamster cells appeared significantly more sensitive than the human cells (Tsutsui et al., 1990).

The ability of diethylstilbestrol to bind covalently to tubulin in cell-free systems in the presence of an activating system (Sharp & Parry, 1985; Epe et al., 1987), and to inhibit the polymerization of microtubules in vitro (Sharp & Parry, 1985; Sato et al., 1987; Albertini et al., 1993; Metzler & Pfeiffer, 1995), in Chinese hamster V79 cells and in Syrian hamster embryo cells (Tucker & Barrett, 1986; Sakakibara et al., 1991; Ochi, 1999) may underlie the induction of aneuploidy. This microtubule-damaging property appears to be unique to diethylstilbestrol because it is not shared with estradiol or 17α-ethinyl estradiol, which are otherwise equally strong estrogens, and can be similarly genotoxic in some systems (Metzler & Pfeiffer, 1995).

Exposure to diethylstilbestrol did not induce mutations or unscheduled DNA synthesis (IARC, 1987b), except in a single study in Syrian hamster embryo cells, and in the presence of liver postmitochondrial supernatant from male rats pretreated with aroclor (Tsutsui et al., 1984). Diethylstilbestrol did not inhibit intercellular communication and most studies did not find positive results for diethylstilbestrol in the mouse lymphoma assay using L5178 tk+/+ cells (IARC, 1987b; Sofuni et al., 1996). Exposure of phage and plasmid DNA to diethylstilbestrol quinone resulted in a variety of mutations and, under certain conditions, recombinations in LacZ(α) following transfection into E. coli (Korah & Humayun, 1993).

Diethylstilbestrol did not induce mutation in a variety of bacterial and insect systems, but it was mutagenic in plants (IARC, 1987b). In assays with Saccharomyces cervisiae and other yeasts,

diethylstilbestrol caused aneuploidy (IARC, 1987b), but it had mixed effects on induction of chromosomal losses (Albertini *et al.*, 1993), and, in most studies, it did not induce mutation, recombinations, or gene conversion (IARC, 1987b; Carls & Schiestl, 1994). DNA damage was not induced in fungi (yeasts) or bacteria, but diethylstilbestrol induced single-strand breaks in bacteriophage DNA in the presence of a horseradish peroxidase activation system (IARC, 1987b).

In vitro, rat liver and mammary gland mitochondria were able to oxidatively metabolize diethylstilbestrol to 4′,4″-diethylstilbestrol quinone and to reduce diethylstilbestrol quinone to diethylstilbestrol (Thomas & Roy, 1995; Thomas *et al.*, 2004). Treatment of Syrian hamsters with diethylstilbestrol resulted in the formation of adducts in kidney mitochondrial DNA by [32P]-postlabelling detection (Thomas & Roy, 2001a), and diethylstilbestrol treatment of rats induced similar adducts in liver mitochondrial DNA at higher levels than in nuclear DNA (Thomas & Roy, 2001b). In addition, both functional ERα and ERβ have been identified in mitochondria (Yager & Chen, 2007). Thus, mitochondria may be a target of diethylstilbestrol, and its mitochondrial effects conceivably play a role in its carcinogenic activity.

4.2.2 Indirect effects related to genotoxicity

(a) Cell proliferation and apoptosis

Diethylstilbestrol increased the mitotic rate in Chinese hamster embryo cells, and in primary male hamster kidney tubular epithelial cells *in vitro* (Stopper *et al.*, 1994; Li *et al.*, 1995; Chen *et al.*, 1996). Chronic diethylstilbestrol treatment increased DNA synthesis in renal tubular cells isolated from male Syrian hamsters (Li *et al.*, 1993); this effect was blocked by co-treatment with a pure anti-estrogen (ICI 182780) (Chen *et al.*, 1996).

In-utero treatment of rats resulted in increased DNA synthesis in both the epithelium and stroma of the proximal portion of the Müllerian duct (which differentiate into the oviduct) on the last day of gestation, but not in the caudal portion (which differentiate into the upper vagina) where epithelial cell proliferation was actually depressed (Okada *et al.*, 2001). Neonatal exposure of mice to diethylstilbestrol resulted in markedly elevated DNA synthesis in epithelial, but not stromal cells of the vagina, whereas it increased the percentage of apoptotic stromal cells, but not epithelial cells at 90 days of age (Sato *et al.*, 2004). Following diethylstilbestrol treatment of pre-pubertal mice, DNA synthesis was markedly increased in the uterine and vaginal epithelium after 16–42 hours (Takahashi *et al.*, 1994). This effect was first apparent at 5 days of age and was still observed at 70 days (Suzuki *et al.*, 2006).

(b) Immune modulatory effects

There are several studies in mice that indicate some immune modulatory effects of diethylstilbestrol treatment. These appear to target the thymus, are highly dose-dependent, and differ in male and female animals (Calemine *et al.*, 2002; Utsuyama *et al.*, 2002; Brown *et al.*, 2006).

(c) Estrogen-receptor-mediated effects

(i) Female animals

Diethylstilbestrol exposure *in utero* reduced the response of the mouse uterus weight and morphology to estrogenic stimulation by diethylstilbestrol on Days 22–25 of life, but not on Day 21 (Maier *et al.*, 1985). Neonatal diethylstilbestrol treatment reduced the responsiveness of uterus weight to ovariectomy, with or without subsequent estrogen stimulation in young adult mice (Medlock *et al.*, 1992), and reduced vaginal weight (Suzuki *et al.*, 1996).

The morphological appearance of the mammary glands of 2- to 11-month-old mice neonatally treated with diethylstilbestrol

(0.1 µg daily for 5 days) was not different from that of untreated controls, but they developed hyperplasia more often in response to stimulation with estradiol. They showed the same response to stimulation with estradiol plus progesterone. The severity of the hyperplasia was increased in diethylstilbestrol-treated mice in response to both hormonal stimuli (Bern et al., 1992).

Overexpression of ERα accelerated the onset of squamous metaplasia, atypical hyperplasia and adenocarcinoma of the uterus induced by neonatal diethylstilbestrol exposure by at least 4 months (Couse et al., 1997). In αERKO mice, no uterine abnormalities, persistent vaginal cornification, or oviduct lesions were found following neonatal diethylstilbestrol treatment, and uterine weight was the same as in vehicle-treated αERKO mice (Couse et al., 2001). This finding strongly suggests that the ERα is the mediator of the effects of neonatal diethylstilbestrol exposure in the female mouse genital tract (Couse & Korach, 2004). ERβ knockout mice (βERKO mice) had a normal morphological response to neonatal diethylstilbestrol treatment (Couse & Korach, 1999), related to the very low to absent expression of ERβ in the female mouse genital tract (Jefferson et al., 2000).

In-utero diethylstilbestrol exposure caused persistent Müllerian duct structures resulting in a range of male and female genital tract abnormalities in mice, which are remarkably similar to those found in diethylstilbestrol-exposed humans (IARC, 1979a). Besides alterations in the uterus, cervix, and vagina, diethylstilbestrol also caused ovarian abnormalities in mice aged 3–14 months, exposed *in utero* (on Days 9–16 of gestation), and markedly increased ex-vivo ovarian production of progesterone, estradiol, and testosterone (Haney et al., 1984).

(ii) Male animals

Neonatal diethylstilbestrol treatment of mice caused persistent decreases in weight of the male accessory sex glands at 12 months of age and the development of inflammation and dysplastic lesions in the posterior periurethral region of the accessory sex gland complex at 2, 12, and 18 months of age (Pylkkänen et al., 1991b, 1993). After 12 and 18 months, there were also morphological changes in the testes (Pylkkänen et al., 1991a, 1993). Treatment of these diethylstilbestrol-exposed mice at 2 months of age with estradiol caused squamous metaplasia in the periurethral prostatic ducts (Pylkkänen et al., 1991b), and adult treatment with estradiol and 5α-dihydrotestosterone (via silastic implants) from 9–12 months of age exacerbated the inflammation and dysplasia at 12 months (Pylkkänen et al., 1993). In contrast, prenatal diethylstilbestrol treatment did not have any lasting effects on the male accessory sex glands, except for occasional dysplasia in the ventral prostate lobe (Pylkkänen et al., 1993). The prostatic weight decrease and lesion development were also found in mice exposed neonatally to diethylstilbestrol (Edery et al., 1990). Neonatal exposure of rats to diethylstilbestrol enhanced the induction of prostatic dysplasia and cancer by subsequent chronic adult treatment with estradiol and testosterone (Yuen et al., 2005). Diethylstilbestrol treatment of rats for 16 weeks with or without concomitant testosterone treatment resulted in increased levels of lipid peroxidation products, and altered antioxidant activity in the ventral and dorsolateral prostate (Tam et al., 2003).

Neonatal diethylstilbestrol treatment of male mice also resulted in a decrease in size of male accessory sex glands, particularly the seminal vesicles. Inflammation and dysplastic lesions developed in the glands of the ventral and dorsolateral prostate between 6–18 months of age and increased in severity with time (Prins et al., 2001). When the same treatment was given to αERKO mice, no morphological effects were found after 6–18 months, whereas the neonatal diethylstilbestrol effects in βERKO mice were indistinguishable from those in wild-type mice (Prins et al., 2001).

(d) Effects on gene expression (hormonal imprinting)

(i) Female animals

In-utero treatment with diethylstilbestrol caused changes in the expression of several genes, including the estrogen-responsive lactoferrin gene and the developmental *Hox* and *Wnt* genes, in the Müllerian duct/uterus of the developing murine fetus and of mice on the first days of life (Newbold *et al.*, 1997; Ma *et al.*, 1998; Miller *et al.*, 1998; Okada *et al.*, 2001).

The expression of a range of genes in the mouse uterus and/or vagina was permanently altered by neonatal exposure to diethylstilbestrol on the first 4–5 days of life up to postnatal Days 60–90, and included alterations in developmental *Hox* and *Wnt* genes (Miller *et al.*, 1998; Block *et al.*, 2000; Couse *et al.*, 2001; Li *et al.*, 2003a; Miyagawa *et al.*, 2004a, b; Sato *et al.*, 2004; Huang *et al.*, 2005; Newbold *et al.*, 2007; Tang *et al.*, 2008).

A single injection of diethylstilbestrol in pre-pubertal mice acutely altered the expression of genes coding for 3 TGFß isoforms in the uterus (Takahashi *et al.*, 1994). Treatment of young adult mice also altered the expression of several genes in the vagina and uterus (Klotz *et al.*, 2000; Miyagawa *et al.*, 2004a; Suzuki *et al.*, 2006).

The persistently increased expression of lacto-ferrin, c-*fos*, and *Nsbp1* in mice that were treated neonatally with diethylstilbestrol was associated with the persistent hypomethylation of CpG sequences in the promoter regions of these genes (Li *et al.*, 1997, 2003a; Tang *et al.*, 2008). Other mechanisms may also be involved in gene expression (Miyagawa *et al.*, 2004a, Tang *et al.*, 2008). The persistently decreased expression of *Hox* genes found in the uterus after 5 days of neonatal treatment with diethylstilbestrol (Couse *et al.*, 2001) was not associated with changes in the methylation status of these genes (Li *et al.*, 2001). The decreased expression of most but not all developmental *Hox* and *Wnt* genes required

the presence of ERα, because the expression of these genes is not affected when mice that lacked this estrogen receptor subtype are neonatally exposed to diethylstilbestrol (Couse *et al.*, 2001). The dose of diethylstilbestrol may be a major determinant of the size and direction of the effects on DNA methylation in the mouse uterus (Alworth *et al.*, 2002).

The mRNA expression of nucleosomal binding protein-1 (*Nsbp1*), which plays a role in chromatin remodelling, was permanently increased in mice treated neonatally with diethyl-stilbestrol for up to 18 months in a dose-related fashion (Tang *et al.*, 2008). A low-dose treatment resulted in a response in the expression and meth-ylation pattern of the uterine *Nsbp*1 gene to the estrogen surge at puberty that was the opposite of that in control mice, but this phenomenon was dose-specific because a high diethylstilbestrol dose did not have this effect (Tang *et al.*, 2008). Ovarian hormones are important in the induc-tion of uterine adenocarcinomas in mice treated neonatally with diethylstilbestrol, because pre-pubertally ovariectomized mice did not develop these tumours (Newbold *et al.*, 1990).

(ii) Male animals

Neonatal administration of diethylstilbestrol of mice caused a persistent upregulation of the c-*fos* and c-*myc* proto-oncogenes in all male accessory sex glands (Pylkkänen *et al.*, 1993; Salo *et al.*, 1997), and a marked increase in the response of c-*fos* expression to estradiol injection at 3–5 months (Salo *et al.*, 1997). In 30-day-old F344 rats treated neonatally with diethylstilbestrol, the expression of both ERα and ERβ was increased as well as circulating prolactin (Khurana *et al.*, 2000). Neonatal treat-ment of mice caused changes in the expression of several other genes and in DNA methylation patterns (Sato *et al.*, 2006).

Neonatal exposure of mice to diethyl-stilbestrol resulted in a persistent reduction of androgen-receptor-protein expression in the

ventral and dorsolateral prostate, ERβ expression was persistently decreased, and ERα expression (in stromal cells around prostatic ducts) was upregulated at postnatal Day 10 but not later in life (Prins *et al.*, 2001). This treatment also resulted in a persistent downregulation of a secretory protein, DLP_2, in the dorsolateral prostate. These effects of neonatal treatment with diethylstilbestrol were not seen in αERKO mice, whereas they were identical to those in wild-type mice in βERKO mice (Prins *et al.*, 2001).

4.3 Synthesis

Following exposure *in utero*, the oxidative metabolism of diethylstilbestrol can occur in fetal mouse tissues. There is some evidence that diethylstilbestrol binds covalently to DNA in fetal target tissue (uterus). In animal cells and tissues, diethylstilbestrol binds covalently to DNA and causes oxidative damage to DNA and lipids; some of these tissues are known targets of diethylstilbestrol-induced cancer in animals.

There is some evidence that diethylstilbestrol alters the expression of enzymes involved in diethylstilbestrol metabolism in rat.

Diethylstilbestrol causes aneuploidy in human and animal cells, most likely because of interference with microtubules, which requires oxidative metabolic activation. Diethylstilbestrol also induces chromosomal breaks and other chromosomal aberrations; this is likely to be a major mechanism of diethylstilbestrol-induced carcinogenicity.

Diethylstilbestrol can immortalize primary animal embryo cells *in vitro* and transform human breast cell lines. Diethylstilbestrol also increases the proliferation of human and animal cervical and uterine cells, and increases cell proliferation in diethylstilbestrol target tissues (uterus) in animals following neonatal and pre-pubertal exposure.

Neonatal exposure to diethylstilbestrol causes persistent changes in gene expression and DNA methylation patterns in diethylstilbestrol target tissues (prostate and uterus), and there is some evidence that hormone responsiveness is permanently altered in the mammary and prostate tissue of exposed mice.

Inflammatory and dysplastic prostate lesions are also observed in mice after neonatal exposure to diethylstilbestrol.

Several of the above effects of diethylstilbestrol, including mitogenic, gene expression, and prostatic effects, are mediated at least in large part by ERα.

There is some evidence of modulatory effects of perinatal exposure to diethylstilbestrol on the immune system in animals and humans.

It is likely that two or more of these factors in combination are responsible for the carcinogenic effects of diethylstilbestrol; estrogen-receptor-mediated effects and genotoxicity conceivably both being involved, while other factors may be contributory. The early developmental changes in the female and male genital tract caused by exposure to diethylstilbestrol *in utero* or – in rodents – neonatally, may result in epigenetic events that create a tissue and cellular environment conducive for the mechanisms responsible for the transplacental carcinogenic effects of diethylstilbestrol in humans and animals.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of diethylstilbestrol. Diethylstilbestrol causes cancer of the breast in women who were exposed while pregnant. Diethylstilbestrol also causes CCA in the vagina and cervix of women who were exposed *in utero*. Also, a positive association has been observed between exposure to diethylstilbestrol and cancer of the endometrium, and between in-utero exposure to diethylstilbestrol and squamous cell carcinoma of the cervix and cancer of the testis.

There is *sufficient evidence* in experimental animals for the carcinogenicity of diethylstilbestrol.

Diethylstilbestrol is *carcinogenic to humans (Group 1).*

References

Aardema MJ, Snyder RD, Spicer C *et al.* (2006). SFTG international collaborative study on in vitro micronucleus test III. Using CHO cells. *Mutat Res*, 607: 61–87. PMID:16797224

Albertini S, Brunner M, Würgler FE (1993). Analysis of the six additional chemicals for in vitro assays of the European Economic Communities' EEC aneuploidy programme using Saccharomyces cerevisiae D61.M and the in vitro porcine brain tubulin assembly assay. *Environ Mol Mutagen*, 21: 180–192. doi:10.1002/em.2850210211 PMID:8444145

Alworth LC, Howdeshell KL, Ruhlen RL *et al.* (2002). Uterine responsiveness to estradiol and DNA methylation are altered by fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: effects of low versus high doses. *Toxicol Appl Pharmacol*, 183: 10–22. doi:10.1006/taap.2002.9459 PMID:12217638

Anon (2008) Diethylstilbestrol. *Encyclopædia Britannica Online*

Anon (1972). Selected item from the FDA drug bulletin-november 1971: diethylstilbestrol contraindicated in pregnancy. *Calif Med*, 116: 85–86. PMID:18730697

Antonioli DA & Burke L (1975). Vaginal adenosis. Analysis of 325 biopsy specimens from 100 patients. *Am J Clin Pathol*, 64: 625–638. PMID:1190123

Antunes CMF, Strolley PD, Rosenshein NB *et al.* (1979). Endometrial cancer and estrogen use. Report of a large case-control study. *N Engl J Med*, 300: 9–13. doi:10.1056/NEJM197901043000103 PMID:213722

Aschbacher PW (1972). Metabolism of 14 C-diethylstilbestrol in sheep. *J Anim Sci*, 35: 1031–1035. PMID:5085294

Aschbacher PW & Thacker EJ (1974). Metabolic fate of oral diethylstilbestrol in steers. *J Anim Sci*, 39: 1185–1192. PMID:4443319

Baggs RB, Miller RK, Odoroff CL (1991). Carcinogenicity of diethylstilbestrol in the Wistar rat: effect of postnatal oral contraceptive steroids. *Cancer Res*, 51: 3311–3315. PMID:2040004

Banerjee SK, Banerjee S, Li SA, Li JJ (1994). Induction of chromosome aberrations in Syrian hamster renal cortical cells by various estrogens. *Mutat Res*, 311: 191–197. PMID:7526183

Begum M, Tashiro H, Katabuchi H *et al.* (2006). Neonatal estrogenic exposure suppresses PTEN-related endometrial carcinogenesis in recombinant mice. *Lab Invest*, 86: 286–296. doi:10.1038/labinvest.3700380 PMID:16402032

Bengtsson G (1963). Autoradiographic distribution studies of injected 32p-labelled polydiethylstilboestrol phosphate. *Acta Endocrinol (Copenh)*, 43: 581–586. PMID:14055731

Beral V & Colwell L (1980). Randomised trial of high doses of stilboestrol and ethisterone in pregnancy: long-term follow-up of mothers. *BMJ*, 281: 1098–1101. doi:10.1136/bmj.281.6248.1098 PMID:7000292

Beral V & Colwell L (1981). Randomised trial of high doses of stilboestrol and ethisterone therapy in pregnancy: long-term follow-up of the children. *J Epidemiol Community Health*, 35: 155–160. doi:10.1136/jech.35.3.155 PMID:7035598

Bern HA, Mills KT, Hatch DL *et al.* (1992). Altered mammary responsiveness to estradiol and progesterone in mice exposed neonatally to diethylstilbestrol. *Cancer Lett*, 63: 117–124. doi:10.1016/0304-3835(92)90061-Y PMID:1562988

Bhat HK, Han X, Gladek A, Liehr JG (1994). Regulation of the formation of the major diethylstilbestrol-DNA adduct and some evidence of its structure. *Carcinogenesis*, 15: 2137–2142. doi:10.1093/carcin/15.10.2137 PMID:7955045

Bibbo M, Ali I, Al-Naqeeb M *et al.* (1975). Cytologic findings in female and male offspring of DES treated mothers. *Acta Cytol*, 19: 568–572. PMID:1061475

Bibbo M, Haenszel WM, Wied GL *et al.* (1978). A twenty-five-year follow-up study of women exposed to diethylstilbestrol during pregnancy. *N Engl J Med*, 298: 763–767. doi:10.1056/NEJM197804062981403 PMID:628409

Block K, Kardana A, Igarashi P, Taylor HS (2000). In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing müllerian system. *FASEB J*, 14: 1101–1108. PMID:10834931

Boyd J, Takahashi H, Waggoner SE *et al.* (1996). Molecular genetic analysis of clear cell adenocarcinomas of the vagina and cervix associated and unassociated with diethylstilbestrol exposure in utero. *Cancer*, 77: 507–513. doi:10.1002/(SICI)1097-0142(19960201)77:3<507::AID-CNCR12>3.0.CO;2-8 PMID:8630958

Brandes LJ & Hermonat MW (1983). Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol. *Cancer Res*, 43: 2831–2835. PMID:6850594

Brian DD, Tilley BC, Labarthe DR *et al.* (1980). Breast cancer in DES-exposed mothers: absence of association. *Mayo Clin Proc*, 55: 89–93. PMID:7354650

Brown LM, Pottern LM, Hoover RN (1986). Prenatal and perinatal risk factors for testicular cancer. *Cancer Res*, 46: 4812–4816. PMID:3731127

Brown N, Nagarkatti M, Nagarkatti PS (2006). Diethylstilbestrol alters positive and negative selection of T cells in the thymus and modulates T-cell repertoire in the periphery. *Toxicol Appl Pharmacol*, 212: 119–126. doi:10.1016/j.taap.2005.07.012 PMID:16122773

Bülow H, Wullstein HK, Böttger G, Schröder FH (1973). Mamma-carinom bei oestrogenbehandeltem prostata-carcinom. *Urologe A*, 12: 249–253. PMID:4357698

Burke L, Antonioli D, Friedman EA (1981). Evolution of diethylstilbestrol-associated genital tract lesions. *Obstet Gynecol*, 57: 79–84. PMID:7454179

Burke L, Segall-Blank M, Lorenzo C *et al.* (2001). Altered immune response in adult women exposed to diethylstilbestrol in utero. *Am J Obstet Gynecol*, 185: 78–81. doi:10.1067/mob.2001.113873 PMID:11483908

Calemine JB, Gogal RM Jr, Lengi A *et al.* (2002). Immunomodulation by diethylstilbestrol is dose and gender related: effects on thymocyte apoptosis and mitogen-induced proliferation. *Toxicology*, 178: 101–118. doi:10.1016/S0300-483X(02)00201-9 PMID:12160618

Calle EE, Mervis CA, Thun MJ *et al.* (1996). Diethylstilbestrol and risk of fatal breast cancer in a prospective cohort of US women. *Am J Epidemiol*, 144: 645–652. PMID:8823060

Carls N & Schiestl RH (1994). Evaluation of the yeast DEL assay with 10 compounds selected by the International Program on Chemical Safety for the evaluation of short-term tests for carcinogens. *Mutat Res*, 320: 293–303. doi:10.1016/0165-1218(94)90082-5 PMID:7508555

Chen CW, Oberley TD, Roy D (1996). Inhibition of stilbene estrogen-induced cell proliferation of renal epithelial cells through the modulation of insulin-like growth factor-I receptor expression. *Cancer Lett*, 105: 51–59. doi:10.1016/0304-3835(96)04263-2 PMID:8689633

Clare MG, Lorenzon G, Akhurst LC *et al.* (2006). SFTG international collaborative study on in vitro micronucleus test II. Using human lymphocytes. *Mutat Res*, 607: 37–60. PMID:16765631

Clark LC & Portier KM (1979). Diethylstilbestrol and the risk of cancer. *N Engl J Med*, 300: 263–264. doi:10.1056/NEJM197902013000519 PMID:759877

Coe JE, Ishak KG, Ross MJ (1990). Estrogen induction of hepatocellular carcinomas in Armenian hamsters. *Hepatology*, 11: 570–577. doi:10.1002/hep.1840110408 PMID:2328952

Colton T, Greenberg ER, Noller K *et al.* (1993). Breast cancer in mothers prescribed diethylstilbestrol in pregnancy. Further follow-up. *JAMA*, 269: 2096–2100. doi:10.1001/jama.269.16.2096 PMID:8468763

Cook JD, Davis BJ, Cai SL *et al.* (2005). Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci USA*, 102: 8644–8649. doi:10.1073/pnas.0503218102 PMID:15937110

Couse JF, Davis VL, Hanson RB *et al.* (1997). Accelerated onset of uterine tumors in transgenic mice with aberrant expression of the estrogen receptor after neonatal exposure to diethylstilbestrol. *Mol Carcinog*, 19: 236–242. doi:10.1002/(SICI)1098-2744(199708)19:4<236::AID-MC4>3.0.CO;2-A PMID:9290700

Couse JF, Dixon D, Yates M *et al.* (2001). Estrogen receptor-alpha knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. *Dev Biol*, 238: 224–238. doi:10.1006/dbio.2001.0413 PMID:11784006

Couse JF & Korach KS (1999). Estrogen receptor null mice: what have we learned and where will they lead us? [Erratum in: Endocr Rev 1999 Aug;20] [4]*Endocr Rev*, 20: 358–417. doi:10.1210/er.20.3.358 PMID:10368776

Couse JF & Korach KS (2004). Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. *Toxicology*, 205: 55–63. doi:10.1016/j.tox.2004.06.046 PMID:15458790

Cutler BS, Forbes AP, Ingersoll FM, Scully RE (1972). Endometrial carcinoma after stilbestrol therapy in gonadal dysgenesis. *N Engl J Med*, 287: 628–631. doi:10.1056/NEJM197209282871302 PMID:5076457

de Stoppelaar JM, Faessen P, Zwart E *et al.* (2000). Isolation of DNA probes specific for rat chromosomal regions 19p, 19q and 4q and their application for the analysis of diethylstilbestrol-induced aneuploidy in binucleated rat fibroblasts. *Mutagenesis*, 15: 165–175. doi:10.1093/mutage/15.2.165 PMID:10719043

Degen GH (1993). SEMV cell cultures: a model for studies of prostaglandin-H synthase-mediated metabolism and genotoxicity of xenobiotics. *Toxicol Lett*, 67: 187–200. doi:10.1016/0378-4274(93)90055-3 PMID:8451760

Degen GH, Metzler M, Sivarajah KS (1986). Co-oxidation of diethylstilbestrol and structural analogs by prostaglandin synthase. *Carcinogenesis*, 7: 137–142. doi:10.1093/carcin/7.1.137 PMID:3080250

Depue RH, Pike MC, Henderson BE (1983). Estrogen exposure during gestation and risk of testicular cancer. *J Natl Cancer Inst*, 71: 1151–1155. PMID:6140323

Dieckmann WJ, Davis ME, Rynkiewicz LM, Pottinger RE (1953). Does the administration of diethylstilbestrol during pregnancy have therapeutic value? *Am J Obstet Gynecol*, 66: 1062–1081. PMID:13104505

Eastin WC, Mennear JH, Tennant RW *et al.* (2001). Tg.AC genetically altered mouse: assay working group overview of available data. *Toxicol Pathol*, 29: Suppl60–80. doi:10.1080/019262301753178483 PMID:11695563

Edery M, Turner T, Dauder S *et al.* (1990). Influence of neonatal diethylstilbestrol treatment on prolactin receptor levels in the mouse male reproductive system. *Proc Soc Exp Biol Med*, 194: 289–292. PMID:2388902

Epe B, Hegler J, Metzler M (1987). Site-specific covalent binding of stilbene-type and steroidal estrogens to tubulin following metabolic activation in

vitro. *Carcinogenesis*, 8: 1271–1275. doi:10.1093/carcin/8.9.1271 PMID:3304691

Epe B, Schiffmann D, Metzler M (1986). Possible role of oxygen radicals in cell transformation by diethylstilbestrol and related compounds. *Carcinogenesis*, 7: 1329–1334. doi:10.1093/carcin/7.8.1329 PMID:3015447

Fauth E, Scherthan H, Zankl H (2000). Chromosome painting reveals specific patterns of chromosome occurrence in mitomycin C- and diethylstilboestrol-induced micronuclei. *Mutagenesis*, 15: 459–467. doi:10.1093/mutage/15.6.459 PMID:11076996

Fetherston WC (1975). Squamous neoplasia of vagina related to DES syndrome. *Am J Obstet Gynecol*, 122: 176–181. PMID:1155501

Fischer LJ, Kent TH, Weissinger JL (1973). Absorption of diethylstilbestrol and its glucuronide conjugate from the intestines of five- and twenty-five-day-old rats. *J Pharmacol Exp Ther*, 185: 163–170. PMID:4693182

Fitzgerald DJ, Piccoli C, Yamasaki H (1989). Detection of non-genotoxic carcinogens in the BALB/c 3T3 cell transformation/mutation assay system. *Mutagenesis*, 4: 286–291. doi:10.1093/mutage/4.4.286 PMID:2674607

Ford CD, Johnson GH, Smith WG (1983). Natural killer cells in in utero diethylstilbesterol-exposed patients. *Gynecol Oncol*, 16: 400–404. doi:10.1016/0090-8258(83)90168-3 PMID:6654182

Forsberg JG (1991). Estrogen effects on chromosome number and sister chromatid exchanges in uterine epithelial cells and kidney cells from neonatal mice. *Teratog Carcinog Mutagen*, 11: 135–146. doi:10.1002/tcm.1770110303 PMID:1686821

Fowler WC Jr, Schmidt G, Edelman DA *et al.* (1981). Risks of cervical intraepithelial neoplasia among DES-exposed women. *Obstet Gynecol*, 58: 720–724. PMID:7312237

Gershman ST & Stolley PD (1988). A case-control study of testicular cancer using Connecticut tumour registry data. *Int J Epidemiol*, 17: 738–742. doi:10.1093/ije/17.4.738 PMID:3225080

Gill WB, Schumacher GF, Bibbo M *et al.* (1979). Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. *J Urol*, 122: 36–39. PMID:37351

Giusti RM, Iwamoto K, Hatch EE (1995). Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med*, 122: 778–788. PMID:7717601

Gladek A & Liehr JG (1989). Mechanism of genotoxicity of diethylstilbestrol in vivo. *J Biol Chem*, 264: 16847–16852. PMID:2777810

Gladek A & Liehr JG (1991). Transplacental genotoxicity of diethylstilbestrol. *Carcinogenesis*, 12: 773–776. doi:10.1093/carcin/12.5.773 PMID:2029740

Gloser H & Cerni C (1984). Increase of sister chromatid exchange formation induced by diethylstilbestrol. *Oncology*, 41: 285–288. doi:10.1159/000225839 PMID:6462605

Goldfarb S & Pugh TD (1990). Morphology and anatomic localization of renal microneoplasms and proximal tubule dysplasias induced by four different estrogens in the hamster. *Cancer Res*, 50: 113–119. PMID:2152770

Gottschlich R & Metzler M (1984). Oxidative metabolites of the teratogen and transplacental carcinogen diethylstilbestrol in the fetal Syrian golden hamster. *J Environ Pathol Toxicol Oncol*, 5: 329–338. PMID:6520735

Gray K, Bullock B, Dickson R *et al.* (1996). Potentiation of diethylstilbestrol-induced alterations in the female mouse reproductive tract by transforming growth factor-alpha transgene expression. *Mol Carcinog*, 17: 163–173. doi:10.1002/(SICI)1098-2744(199611)17:3<163::AID-MC9>3.0.CO;2-G PMID:8944077

Green M, Newell O, Aboyade-Cole A *et al.* (2007). Diallyl sulfide induces the expression of estrogen metabolizing genes in the presence and/or absence of diethylstilbestrol in the breast of female ACI rats. *Toxicol Lett*, 168: 7–12. doi:10.1016/j.toxlet.2006.10.009 PMID:17129689

Green M, Thomas R, Gued L, Sadrud-Din S (2003). Inhibition of DES-induced DNA adducts by diallyl sulfide: implications in liver cancer prevention. *Oncol Rep*, 10: 767–771. PMID:12684656

Green M, Wilson C, Newell O *et al.* (2005). Diallyl sulfide inhibits diethylstilbesterol-induced DNA adducts in the breast of female ACI rats. *Food Chem Toxicol*, 43: 1323–1331. doi:10.1016/j.fct.2005.02.005 PMID:15989972

Greenberg ER, Barnes AB, Resseguie L *et al.* (1984). Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med*, 311: 1393–1398. doi:10.1056/NEJM198411293112201 PMID:6493300

Greenman DL, Highman B, Chen J *et al.* (1990). Estrogen-induced thyroid follicular cell adenomas in C57BL/6 mice. *J Toxicol Environ Health*, 29: 269–278. doi:10.1080/15287399009531390 PMID:2313739

Greenman DL, Highman B, Chen JJ *et al.* (1986). Influence of age on induction of mammary tumors by diethylstilbestrol in C3H/HeN mice with low murine mammary tumor virus titer. *J Natl Cancer Inst*, 77: 891–898. PMID:3020299

Greenman DL, Kodell RL, Highman B *et al.* (1987). Mammary tumorigenesis in C3H/HeN-MTV + mice treated with diethylstilboestrol for varying periods. *Food Chem Toxicol*, 25: 229–232. doi:10.1016/0278-6915(87)90087-1 PMID:3570111

Greenwald P, Barlow JJ, Nasca PC, Burnett WS (1971). Vaginal cancer after maternal treatment with synthetic estrogens. *N Engl J Med*, 285: 390–392. doi:10.1056/NEJM197108122850707 PMID:5556578

Gued LR, Thomas RD, Green M (2003). Diallyl sulfide inhibits diethylstilbestrol-induced lipid peroxidation in breast tissue of female ACI rats: implications in breast cancer prevention. *Oncol Rep*, 10: 739–743. PMID:12684652

Hadjimichael OC, Meigs JW, Falcier FW *et al.* (1984). Cancer risk among women exposed to exogenous estrogens during pregnancy. *J Natl Cancer Inst*, 73: 831–834. PMID:6592380

Hajek RA, King DW, Hernández-Valero MA *et al.* (2006). Detection of chromosomal aberrations by fluorescence in situ hybridization in cervicovaginal biopsies from women exposed to diethylstilbestrol in utero. *Int J Gynecol Cancer*, 16: 318–324. doi:10.1111/j.1525-1438.2006.00338.x PMID:16445652

Haney AF, Newbold RR, McLachlan JA (1984). Prenatal diethylstilbestrol exposure in the mouse: effects on ovarian histology and steroidogenesis in vitro. *Biol Reprod*, 30: 471–478. doi:10.1095/biolreprod30.2.471 PMID:6704476

Hanselaar A, van Loosbroek M, Schuurbiers O *et al.* (1997). Clear cell adenocarcinoma of the vagina and cervix. An update of the central Netherlands registry showing twin age incidence peaks. *Cancer*, 79: 2229–2236. doi:10.1002/(SICI)1097-0142(19970601)79:11<2229::AID-CNCR22>3.0.CO;2-X PMID:9179071

Hartley-Asp B, Deinum J, Wallin M (1985). Diethylstilbestrol induces metaphase arrest and inhibits microtubule assembly. *Mutat Res*, 143: 231–235. doi:10.1016/0165-7992(85)90086-7 PMID:2862579

Hatch EE, Herbst AL, Hoover RN *et al.* (2001). Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). *Cancer Causes Control*, 12: 837–845. doi:10.1023/A:1012229112696 PMID:11714112

Hatch EE, Palmer JR, Titus-Ernstoff L *et al.* (1998). Cancer risk in women exposed to diethylstilbestrol in utero. *JAMA*, 280: 630–634. doi:10.1001/jama.280.7.630 PMID:9718055

Hayashi N, Hasegawa K, Komine A *et al.* (1996). Estrogen-induced cell transformation and DNA adduct formation in cultured Syrian hamster embryo cells. *Mol Carcinog*, 16: 149–156. doi:10.1002/(SICI)1098-2744(199607)16:3<149::AID-MC5>3.0.CO;2-C PMID:8688150

Heinonen OP (1973). Diethylstilbestrol in pregnancy. Frequency of exposure and usage patterns. *Cancer*, 31: 573–577. doi:10.1002/1097-0142(197303)31:3<573::AID-CNCR2820310312>3.0.CO;2-# PMID:4693585

Henderson BE, Benton B, Jing J *et al.* (1979). Risk factors for cancer of the testis in young men. *Int J Cancer*, 23: 598–602. doi:10.1002/ijc.2910230503 PMID:37169

Henderson BE, Benton BD, Weaver PT *et al.* (1973). Stilbestrol and urogenital-tract cancer in adolescents and young adults. *N Engl J Med*, 288: 354 doi:10.1056/NEJM197302152880708 PMID:4682947

Herbst AL (1981). Clear cell adenocarcinoma and the current status of DES-exposed females. *Cancer*, 48: Suppl 484–488. doi:10.1002/1097-0142(19810715)48:1+<484::AID-CNCR2820481308>3.0.CO;2-X PMID:7272973

Herbst AL & Anderson D (1990). Clear cell adenocarcinoma of the vagina and cervix secondary to intrauterine exposure to diethylstilbestrol. *Semin Surg Oncol*, 6: 343–346. doi:10.1002/ssu.2980060609 PMID:2263810

Herbst AL, Anderson S, Hubby MM *et al.* (1986). Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: a case-control study. *Am J Obstet Gynecol*, 154: 814–822. PMID:3963071

Herbst AL, Kurman RJ, Scully RE, Poskanzer DC (1972). Clear-cell adenocarcinoma of the genital tract in young females. Registry report. *N Engl J Med*, 287: 1259–1264. doi:10.1056/NEJM197212212872501 PMID:4636892

Herbst AL, Poskanzer DC, Robboy SJ *et al.* (1975). Prenatal exposure to stilbestrol. A prospective comparison of exposed female offspring with unexposed controls. *N Engl J Med*, 292: 334–339. doi:10.1056/NEJM197502132920704 PMID:1117962

Herbst AL, Robboy SJ, Scully RE, Poskanzer DC (1974). Clear-cell adenocarcinoma of the vagina and cervix in girls: analysis of 170 registry cases. *Am J Obstet Gynecol*, 119: 713–724. PMID:4857957

Herbst AL & Scully RE (1970). Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). *Cancer*, 25: 745–757. doi:10.1002/1097-0142(197004)25:4<745::AID-CNCR2820250402>3.0.CO;2-2 PMID:5443099

Herbst AL, Ulfelder H, Poskanzer DC (1971). Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med*, 284: 878–881. doi:10.1056/NEJM197104222841604 PMID:5549830

Hill EC (1973). Clear cell carcinoma of the cervix and vagina in young women. A report of six cases with association of maternal stilbestrol therapy and adenosis of the vagina. *Am J Obstet Gynecol*, 116: 470–484. PMID:4709483

Hoover R, Gray LA Sr, Fraumeni JF Jr (1977). Stilboestrol (diethylstilbestrol) and the risk of ovarian cancer. *Lancet*, 2: 533–534. doi:10.1016/S0140-6736(77)90667-5 PMID:95735

Hornsby PP, Wilcox AJ, Weinberg CR, Herbst AL (1994). Effects on the menstrual cycle of in utero exposure to diethylstilbestrol. *Am J Obstet Gynecol*, 170: 709–715. PMID:8141188

Huang WW, Yin Y, Bi Q *et al.* (2005). Developmental diethylstilbestrol exposure alters genetic pathways of uterine cytodifferentiation. *Mol Endocrinol*, 19: 669–682. doi:10.1210/me.2004-0155 PMID:15591538

Hubby MM, Haenszel WM, Herbst AL (1981) Effects on mother following exposure to diethylstilbestrol during pregnancy. In: Herbst AL, Bern HA (eds) *Developmental effects of diethylstilbestrol (DES) in pregnancy*. New York: Thieme-Stratton.

IARC (1979b). Diethylstilboestrol and diethylstilboestrol dipropionate. *IARC Monogr Eval Carcinog Risk Chem Hum*, 21: 173–231. PMID:397181

IARC (1979a). Sex Hormones (II). *IARC Monogr Eval Carcinog Risk Chem Hum*, 21: 1–583.

IARC (1987b). Genetic and related effects: An updating of selected IARC Monographs from Volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 6: 1–729. PMID:3504843

IARC (1987a). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

Jefferson WN, Couse JF, Banks EP *et al.* (2000). Expression of estrogen receptor beta is developmentally regulated in reproductive tissues of male and female mice. *Biol Reprod*, 62: 310–317. doi:10.1095/biolreprod62.2.310 PMID:10642567

Jiang YG, Chen JK, Wu ZL (2000). Promotive effect of diethylstilbestrol on urethan-induced mouse lung tumorigenesis. *Chemosphere*, 41: 187–190. doi:10.1016/S0045-6535(99)00410-5 PMID:10819200

Johnstone SR, Stanley MA, Quigley JP (1984). The effect of diethylstilboestrol on the in vitro growth of human ectocervical cells. *Carcinogenesis*, 5: 741–745. doi:10.1093/carcin/5.6.741 PMID:6609784

Kabbarah O, Mallon MA, Pfeifer JD *et al.* (2003). A panel of repeat markers for detection of microsatellite instability in murine tumors. *Mol Carcinog*, 38: 155–159. doi:10.1002/mc.10157 PMID:14639654

Kabbarah O, Sotelo AK, Mallon MA *et al.* (2005). Diethylstilbestrol effects and lymphomagenesis in Mlh1-deficient mice. *Int J Cancer*, 115: 666–669. doi:10.1002/ijc.20918 PMID:15700306

Kalyanaraman B, Sealy RC, Liehr JG (1989). Characterization of semiquinone free radicals formed from stilbene catechol estrogens. An ESR spin stabilization and spin trapping study. *J Biol Chem*, 264: 11014–11019. PMID:2544580

Kaufman RH & Adam E (1978). Genital tract anomalies associated with in utero exposure to diethylstilbestrol. *Isr J Med Sci*, 14: 353–362. PMID:640820

Kaufman RH & Adam E (2002). Findings in female offspring of women exposed in utero to diethylstilbestrol. *Obstet Gynecol*, 99: 197–200. doi:10.1016/S0029-7844(01)01682-9 PMID:11814496

Kaufman RH, Korhonen MO, Strama T *et al.* (1982). Development of clear cell adenocarcinoma in DES-exposed offspring under observation. *Obstet Gynecol*, 59: Suppl68S–72S. PMID:7088431

Kerklaan PR, Bouter S, van Elburg PE, Mohn GR (1986). Evaluation of the DNA repair host-mediated assay. II. Presence of genotoxic factors in various organs of mice treated with chemotherapeutic agents. *Mutat Res*, 164: 19–29. PMID:2419750

Khurana S, Ranmal S, Ben-Jonathan N (2000). Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression. *Endocrinology*, 141: 4512–4517. doi:10.1210/en.141.12.4512 PMID:11108262

Kitamura T, Nishimura S, Sasahara K *et al.* (1999). Transplacental administration of diethylstilbestrol (DES) causes lesions in female reproductive organs of Donryu rats, including endometrial neoplasia. *Cancer Lett*, 141: 219–228. doi:10.1016/S0304-3835(99)00108-1 PMID:10454265

Klip H, Verloop J, van Gool JD *et al.*OMEGA Project Group. (2002). Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet*, 359: 1102–1107. doi:10.1016/S0140-6736(02)08152-7 PMID:11943257

Klotz DM, Hewitt SC, Korach KS, Diaugustine RP (2000). Activation of a uterine insulin-like growth factor I signaling pathway by clinical and environmental estrogens: requirement of estrogen receptor-alpha. *Endocrinology*, 141: 3430–3439. doi:10.1210/en.141.9.3430 PMID:10965916

Konac E, Ekmekci A, Barkar V *et al.* (2005). Effects of diethylstilbestrol in human lymphocytes in vitro: a dose and time-dependent study on genotoxic, cytotoxic and apoptotic effects. *Mol Cell Biochem*, 276: 45–53. doi:10.1007/s11010-005-2815-8 PMID:16132684

Korah RM & Humayun MZ (1993). Mutagenic and recombinagenic effects of diethylstilbestrol quinone. *Mutat Res*, 289: 205–214. PMID:7690889

Labarthe D, Adam E, Noller KL *et al.* (1978). Design and preliminary observations of National Cooperative Diethylstilbestrol Adenosis (DESAD) Project. *Obstet Gynecol*, 51: 453–458. doi:10.1097/00006250-197804000-00014 PMID:662228

Lanier AP, Noller KL, Decker DG *et al.* (1973). Cancer and stilbestrol. A follow-up of 1,719 persons exposed to estrogens in utero and born 1943–1959. *Mayo Clin Proc*, 48: 793–799. PMID:4758151

Larson PS, Ungarelli RA, de Las Morenas A *et al.* (2006). In utero exposure to diethylstilbestrol (DES) does not increase genomic instability in normal or neoplastic breast epithelium. *Cancer*, 107: 2122–2126. doi:10.1002/cncr.22223 PMID:16998936

Li JJ, Gonzalez A, Banerjee S *et al.* (1993). Estrogen carcinogenesis in the hamster kidney: role of cytotoxicity and cell proliferation. *Environ Health Perspect*, 101: Suppl 5259–264. doi:10.2307/3431878 PMID:8013417

Li JJ, Hou X, Banerjee SK *et al.* (1999). Overexpression and amplification of c-myc in the Syrian hamster kidney during estrogen carcinogenesis: a probable critical role in neoplastic transformation. *Cancer Res*, 59: 2340–2346. PMID:10344741

Li JJ, Li SA, Oberley TD, Parsons JA (1995). Carcinogenic activities of various steroidal and nonsteroidal

estrogens in the hamster kidney: relation to hormonal activity and cell proliferation. *Cancer Res*, 55: 4347–4351. PMID:7671246

Li S, Hansman R, Newbold R *et al.* (2003a). Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinog*, 38: 78–84. doi:10.1002/mc.10147 PMID:14502647

Li S, Ma L, Chiang T *et al.* (2001). Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol Carcinog*, 32: 213–219. doi:10.1002/mc.10015 PMID:11746833

Li S, Washburn KA, Moore R *et al.* (1997). Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res*, 57: 4356–4359. PMID:9331098

Liehr JG, Avitts TA, Randerath E, Randerath K (1986). Estrogen-induced endogenous DNA adduction: possible mechanism of hormonal cancer. *Proc Natl Acad Sci USA*, 83: 5301–5305. doi:10.1073/pnas.83.14.5301 PMID:3460092

Liehr JG, DaGue BB, Ballatore AM (1985a). Reactivity of 4′,4″-diethylstilbestrol quinone, a metabolic intermediate of diethylstilbestrol. *Carcinogenesis*, 6: 829–836. doi:10.1093/carcin/6.6.829 PMID:4006069

Liehr JG, DaGue BB, Ballatore AM, Henkin J (1983). Diethylstilbestrol (DES) quinone: a reactive intermediate in DES metabolism. *Biochem Pharmacol*, 32: 3711–3718. doi:10.1016/0006-2952(83)90139-9 PMID:6661246

Liehr JG, Randerath K, Randerath E (1985b). Target organ-specific covalent DNA damage preceding diethylstilbestrol-induced carcinogenesis. *Carcinogenesis*, 6: 1067–1069. doi:10.1093/carcin/6.7.1067 PMID:4017174

Liehr JG, Roy D, Gladek A (1989). Mechanism of inhibition of estrogen-induced renal carcinogenesis in male Syrian hamsters by vitamin C. *Carcinogenesis*, 10: 1983–1988. doi:10.1093/carcin/10.11.1983 PMID:2572356

Liehr JG & Wheeler WJ (1983). Inhibition of estrogen-induced renal carcinoma in Syrian hamsters by vitamin C. *Cancer Res*, 43: 4638–4642. PMID:6883321

Linden G & Henderson BE (1972). Genital-tract cancers in adolescents and young adults. *N Engl J Med*, 286: 760–761. doi:10.1056/NEJM197204062861406 PMID:5025778

Lopez J, Ogren L, Verjan R, Talamantes F (1988). Effects of perinatal exposure to a synthetic estrogen and progestin on mammary tumorigenesis in mice. *Teratology*, 38: 129–134. doi:10.1002/tera.1420380205 PMID:3175946

Lundgren K, Randerath K, Everson RB (1988). Role of metabolism and DNA adduct formation in the induction of sister chromatid exchanges in human lymphocytes by diethylstilbestrol. *Cancer Res*, 48: 335–338. PMID:3335009

Ma L, Benson GV, Lim H *et al.* (1998). Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in müllerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol*, 197: 141–154. doi:10.1006/dbio.1998.8907 PMID:9630742

Maier DB, Newbold RR, McLachlan JA (1985). Prenatal diethylstilbestrol exposure alters murine uterine responses to prepubertal estrogen stimulation. *Endocrinology*, 116: 1878–1886. doi:10.1210/endo-116-5-1878 PMID:3987622

Marselos M & Tomatis L (1992). Diethylstilboestrol: II, pharmacology, toxicology and carcinogenicity in experimental animals. *Eur J Cancer*, 29A: 149–155. PMID:1445734

Martin OV, Shialis T, Lester JN *et al.* (2008). Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative meta-analysis. *Environ Health Perspect*, 116: 149–157. doi:10.1289/ehp.10545 PMID:18288311

Mattingly RF & Stafl A (1976). Cancer risk in diethyl-stilbestrol-exposed offspring. *Am J Obstet Gynecol*, 126: 543–548. PMID:984124

Maydl R, McLachlan JA, Newbold RR, Metzler M (1985). Localization of diethylstilbestrol metabolites in the mouse genital tract. *Biochem Pharmacol*, 34: 710–712. doi:10.1016/0006-2952(85)90270-9 PMID:3977948

McAnulty PA & Skydsgaard M (2005). Diethylstilbestrol (DES): carcinogenic potential in Xpa-/-, Xpa-/- / p53+/-, and wild-type mice during 9 months' dietary exposure. *Toxicol Pathol*, 33: 609–620. doi:10.1080/01926230500261377 PMID:16178126

McEvoy GK, editor (2007) *2007 AHFS Drug Information*, Bethesda, MD, American Society of Health-System Pharmacists, American Hospital Formulary Service.

McMartin KE, Kennedy KA, Greenspan P *et al.* (1978). Diethylstilbestrol: a review of its toxicity and use as a growth promotant in food-producing animals. *J Environ Pathol Toxicol*, 1: 279–313. PMID:363962

Medlock KL, Branham WS, Sheehan DM (1992). Long-term effects of postnatal exposure to diethylstilbestrol on uterine estrogen receptor and growth. *J Steroid Biochem Mol Biol*, 42: 23–28. doi:10.1016/0960-0760(92)90007-6 PMID:1558818

Melnick S, Cole P, Anderson D, Herbst A (1987). Rates and risks of diethylstilbestrol-related clear-cell adenocarcinoma of the vagina and cervix. An update. *N Engl J Med*, 316: 514–516. doi:10.1056/NEJM198702263160905 PMID:3807995

Metzler M (1984). Metabolism of stilbene estrogens and steroidal estrogens in relation to carcinogenicity. *Arch Toxicol*, 55: 104–109. doi:10.1007/BF00346047 PMID:6383273

Metzler M & Fischer LJ (1981). The metabolism of diethylstilbestrol. *CRC Crit Rev Biochem*, 10: 171–212. doi:10.3109/10409238109113599 PMID:6163591

Metzler M & Pfeiffer E (1995). Effects of estrogens on microtubule polymerization in vitro: correlation with estrogenicity. *Environ Health Perspect*, 103: Suppl 721–22. doi:10.2307/3432502 PMID:8593868

Miller C, Degenhardt K, Sassoon DA (1998). Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis. *Nat Genet*, 20: 228–230. doi:10.1038/3027 PMID:9806537

Miyagawa S, Katsu Y, Watanabe H, Iguchi T (2004a). Estrogen-independent activation of erbBs signaling and estrogen receptor alpha in the mouse vagina exposed neonatally to diethylstilbestrol. *Oncogene*, 23: 340–349. doi:10.1038/sj.onc.1207207 PMID:14647453

Miyagawa S, Suzuki A, Katsu Y *et al.* (2004b). Persistent gene expression in mouse vagina exposed neonatally to diethylstilbestrol. *J Mol Endocrinol*, 32: 663–677. doi:10.1677/jme.0.0320663 PMID:15171707

Moss AR, Osmond D, Bacchetti P *et al.* (1986). Hormonal risk factors in testicular cancer. A case-control study. *Am J Epidemiol*, 124: 39–52. PMID:2872797

Nash S, Tilley BC, Kurland LT *et al.* (1983). Identifying and tracing a population at risk: the DESAD Project experience. *Am J Public Health*, 73: 253–259. doi:10.2105/AJPH.73.3.253 PMID:6824111

Newbold RR, Bullock BC, McLachlan JA (1990). Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res*, 50: 7677–7681. PMID:2174729

Newbold RR, Hanson RB, Jefferson WN (1997). Ontogeny of lactoferrin in the developing mouse uterus: a marker of early hormone response. *Biol Reprod*, 56: 1147–1157. doi:10.1095/biolreprod56.5.1147 PMID:9160713

Newbold RR, Hanson RB, Jefferson WN *et al.* (1998). Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, 19: 1655–1663. doi:10.1093/carcin/19.9.1655 PMID:9771938

Newbold RR, Hanson RB, Jefferson WN *et al.* (2000). Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, 21: 1355–1363. doi:10.1093/carcin/21.7.1355 PMID:10874014

Newbold RR, Jefferson WN, Grissom SF *et al.* (2007). Developmental exposure to diethylstilbestrol alters uterine gene expression that may be associated with uterine neoplasia later in life. *Mol Carcinog*, 46: 783–796. doi:10.1002/mc.20308 PMID:17394237

Ninomiya K, Kawaguchi H, Souda M *et al.* (2007). Effects of neonatally administered diethylstilbestrol on induction of mammary carcinomas induced by 7, 12-dimethylbenz(a)anthracene in female rats. *Toxicol Pathol*, 35: 811–818. doi:10.1080/01926230701584205 PMID:17943655

Noller KL, Blair PB, O'Brien PC *et al.* (1988). Increased occurrence of autoimmune disease among women exposed in utero to diethylstilbestrol. *Fertil Steril*, 49: 1080–1082. PMID:3371486

Noller KL, Decker DG, Lanier AP, Kurland LT (1972). Clear-cell adenocarcinoma of the cervix after maternal treatment with synthetic estrogens. *Mayo Clin Proc*, 47: 629–630. PMID:5073941

Noller KL, Townsend DE, Kaufman RH *et al.* (1983). Maturation of vaginal and cervical epithelium in women exposed in utero to diethylstilbestrol (DESAD Project). *Am J Obstet Gynecol*, 146: 279–285. PMID:6859137

O'Brien PC, Noller KL, Robboy SJ *et al.* (1979). Vaginal epithelial changes in young women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) project. *Obstet Gynecol*, 53: 300–308. PMID:424101

Oberley TD, Gonzalez A, Lauchner LJ *et al.* (1991). Characterization of early kidney lesions in estrogen-induced tumors in the Syrian hamster. *Cancer Res*, 51: 1922–1929. PMID:2004377

Ochi T (1999). Induction of multiple microtubule-organizing centers, multipolar spindles and multipolar division in cultured V79 cells exposed to diethylstilbestrol, estradiol-17beta and bisphenol A. *Mutat Res*, 431: 105–121. PMID:10656490

Okada A, Sato T, Ohta Y *et al.* (2001). Effect of diethylstilbestrol on cell proliferation and expression of epidermal growth factor in the developing female rat reproductive tract. *J Endocrinol*, 170: 539–554. doi:10.1677/joe.0.1700539 PMID:11524234

Palmer JR, Anderson D, Helmrich SP, Herbst AL (2000). Risk factors for diethylstilbestrol-associated clear cell adenocarcinoma. *Obstet Gynecol*, 95: 814–820. doi:10.1016/S0029-7844(00)00827-9 PMID:10831973

Palmer JR, Hatch EE, Rao RS *et al.* (2001). Infertility among women exposed prenatally to diethylstilbestrol. *Am J Epidemiol*, 154: 316–321. doi:10.1093/aje/154.4.316 PMID:11495854

Palmer JR, Wise LA, Hatch EE *et al.* (2006). Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*, 15: 1509–1514. doi:10.1158/1055-9965.EPI-06-0109 PMID:16896041

Palmer JR, Wise LA, Robboy SJ *et al.* (2005). Hypospadias in sons of women exposed to diethylstilbestrol in utero. *Epidemiology*, 16: 583–586. doi:10.1097/01.ede.0000164789.59728.6d PMID:15951681

Parry EM, Danford N, Parry JM (1982). Differential staining of chromosomes and spindle and its use as an assay for determining the effect of diethylstilboestrol on cultured mammalian cells. *Mutat Res*, 105: 243–252. doi:10.1016/0165-7992(82)90037-9 PMID:6182462

Perez KM, Titus-Ernstoff L, Hatch EE *et al.*National Cancer Institute's DES Follow-up Study Group. (2005). Reproductive outcomes in men with prenatal exposure to diethylstilbestrol. *Fertil Steril*, 84: 1649–1656. doi:10.1016/j.fertnstert.2005.05.062 PMID:16359959

Pons JC, Papiernik E, Billon A *et al.* (2005). Hypospadias in sons of women exposed to diethylstilbestrol in utero. *Prenat Diagn*, 25: 418–419. doi:10.1002/pd.1136 PMID:15906411

Prins GS, Birch L, Couse JF *et al.* (2001). Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor alpha: studies with alphaERKO and betaERKO mice. *Cancer Res*, 61: 6089–6097. PMID:11507058

Pylkkänen L, Jahnukainen K, Parvinen M, Santti R (1991a). Testicular toxicity and mutagenicity of steroidal and non-steroidal estrogens in the male mouse. *Mutat Res*, 261: 181–191. doi:10.1016/0165-1218(91)90066-U PMID:1719410

Pylkkänen L, Mäkelä S, Valve E *et al.* (1993). Prostatic dysplasia associated with increased expression of c-myc in neonatally estrogenized mice. *J Urol*, 149: 1593–1601. PMID:8501817

Pylkkänen L, Santti R, Newbold R, McLachlan JA (1991b). Regional differences in the prostate of the neonatally estrogenized mouse. *Prostate*, 18: 117–129. doi:10.1002/pros.2990180204 PMID:2006118

Quick EL, Parry EM, Parry JM (2008). Do oestrogens induce chromosome specific aneuploidy in vitro, similar to the pattern of aneuploidy seen in breast cancer? *Mutat Res*, 651: 46–55. PMID:18162433

Rinehart CA, Xu LH, Van Le L, Kaufman DG (1996). Diethylstilbestrol-induced immortalization of human endometrial cells: alterations in p53 and estrogen receptor. *Mol Carcinog*, 15: 115–123. doi:10.1002/(SICI)1098-2744(199602)15:2<115::AID-MC4>3.0.CO;2-I PMID:8599578

Robboy SJ, Kaufman RH, Prat J *et al.* (1979). Pathologic findings in young women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) project. *Obstet Gynecol*, 53: 309–317. PMID:424102

Robboy SJ, Noller KL, O'Brien P *et al.*; Experience of the National Collaborative Diethylstilbestrol Adenosis Project. (1984b). Increased incidence of cervical and vaginal dysplasia in 3,980 diethylstilbestrol-exposed young women. *JAMA*, 252: 2979–2983. doi:10.1001/jama.252.21.2979 PMID:6502858

Robboy SJ, Szyfelbein WM, Goellner JR *et al.* (1981). Dysplasia and cytologic findings in 4,589 young women enrolled in diethylstilbestrol-adenosis (DESAD) project. *Am J Obstet Gynecol*, 140: 579–586. PMID:7195652

Robboy SJ, Young RH, Welch WR *et al.* (1984a). Atypical vaginal adenosis and cervical ectropion. Association with clear cell adenocarcinoma in diethylstilbestrol-exposed offspring. *Cancer*, 54: 869–875. doi:10.1002/1097-0142(19840901)54:5<869::AID-CNCR2820540519>3.0.CO;2-I PMID:6537153

Rogers JM, Kavlock RJ (2008) Chapter 10. Developmental Toxicology. In: Klaassen, C.D., ed., *Casarett and Doull's Toxicology, The Basic Science of Poisons*, 7th Ed., New York, McGraw-Hill Medical Publishing Division, p.418.

Ross D, Mehlhorn RJ, Moldeus P, Smith MT (1985). Metabolism of diethylstilbestrol by horseradish peroxidase and prostaglandin-H synthase. Generation of a free radical intermediate and its interaction with glutathione. *J Biol Chem*, 260: 16210–16214. PMID:2999150

Rothschild TC, Boylan ES, Calhoon RE, Vonderhaar BK (1987). Transplacental effects of diethylstilbestrol on mammary development and tumorigenesis in female ACI rats. *Cancer Res*, 47: 4508–4516. PMID:3607779

Roy D, Bernhardt A, Strobel HW, Liehr JG (1992). Catalysis of the oxidation of steroid and stilbene estrogens to estrogen quinone metabolites by the beta-naphthoflavone-inducible cytochrome P450 IA family. *Arch Biochem Biophys*, 296: 450–456. doi:10.1016/0003-9861(92)90596-O PMID:1632637

Roy D, Floyd RA, Liehr JG (1991c). Elevated 8-hydroxydeoxyguanosine levels in DNA of diethylstilbestrol-treated Syrian hamsters: covalent DNA damage by free radicals generated by redox cycling of diethylstilbestrol. *Cancer Res*, 51: 3882–3885. PMID:1855206

Roy D, Kalyanaraman B, Liehr JG (1991b). Xanthine oxidase-catalyzed reduction of estrogen quinones to semiquinones and hydroquinones. *Biochem Pharmacol*, 42: 1627–1631. doi:10.1016/0006-2952(91)90433-6 PMID:1656992

Roy D & Liehr JG (1988). Characterization of drug metabolism enzymes in estrogen-induced kidney tumors in male Syrian hamsters. *Cancer Res*, 48: 5726–5729. PMID:3048647

Roy D & Liehr JG (1989). Metabolic oxidation of diethylstilbestrol to diethylstilbestrol-4′,4″-quinone in Syrian hamsters. *Carcinogenesis*, 10: 1241–1245. doi:10.1093/carcin/10.7.1241 PMID:2736717

Roy D, Strobel HW, Liehr JG (1991a). Cytochrome b5-mediated redox cycling of estrogen. *Arch Biochem Biophys*, 285: 331–338. doi:10.1016/0003-9861(91)90368-S PMID:1897935

Royal Pharmaceutical Society of Great Britain (2007) *British National Formulary*, No. 54, BMJ Publishing Group Ltd./RPS Publishing, London.

Rumsey TS, Oltjen RR, Daniels FL, Kozak AS (1975a). Depletion patterns of radioactivity and tissue residues in beef cattle after the withdrawal of oral C-diethylstilbestrol. *J Anim Sci*, 40: 539–549. PMID:1116975

Rumsey TS, Oltjen RR, Kozak AS *et al.* (1975b). Fate of radiocarbon in beef steers implanted with 14C-diethylstilbestrol. *J Anim Sci*, 40: 550–560. PMID:1116976

Rupa DS, Schuler M, Eastmond DA (1997). Detection of hyperdiploidy and breakage affecting the 1cen-1q12 region of cultured interphase human lymphocytes treated with various genotoxic agents. *Environ*

Mol Mutagen, 29: 161–167. doi:10.1002/(SICI)1098-2280(1997)29:2<161::AID-EM7>3.0.CO;2-H PMID:9118968

Russo J, Hasan Lareef M, Balogh G *et al.* (2003). Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. *J Steroid Biochem Mol Biol*, 87: 1–25. doi:10.1016/S0960-0760(03)00390-X PMID:14630087

Russo J, Hu YF, Tahin Q *et al.* (2001). Carcinogenicity of estrogens in human breast epithelial cells. *APMIS*, 109: 39–52. doi:10.1111/j.1600-0463.2001.tb00013.x PMID:11297193

Sakakibara Y, Saito I, Ichinoseki K *et al.* (1991). Effects of diethylstilbestrol and its methyl ethers on aneuploidy induction and microtubule distribution in Chinese hamster V79 cells. *Mutat Res*, 263: 269–276. doi:10.1016/0165-7992(91)90012-S PMID:1861692

Salo LK, Mäkelä SI, Stancel GM, Santti RS (1997). Neonatal exposure to diethylstilbestrol permanently alters the basal and 17 beta-estradiol induced expression of c-fos proto-oncogene in mouse urethroprostatic complex. *Mol Cell Endocrinol*, 126: 133–141. doi:10.1016/S0303-7207(96)03978-0 PMID:9089651

Sarma AV, McLaughlin JC, Schottenfeld D In: Schottenfeld D, Fraumeni JF Jr (eds) (2006) Testicular Cancer in Cancer Epidemiology (3rd edition). Oxford Univ Press; ch 60; pp 1151–1165.

Sato K, Fukata H, Kogo Y *et al.* (2006). Neonatal exposure to diethylstilbestrol alters the expression of DNA methyltransferases and methylation of genomic DNA in the epididymis of mice. *Endocr J*, 53: 331–337. doi:10.1507/endocrj.K06-009 PMID:16714842

Sato T, Fukazawa Y, Ohta Y, Iguchi T (2004). Involvement of growth factors in induction of persistent proliferation of vaginal epithelium of mice exposed neonatally to diethylstilbestrol. *Reprod Toxicol*, 19: 43–51. doi:10.1016/j.reprotox.2004.05.004 PMID:15336711

Sato Y, Murai T, Oda T *et al.* (1987). Inhibition of microtubule polymerization by synthetic estrogens: formation of a ribbon structure. *J Biochem*, 101: 1247–1252. PMID:3654591

Schottenfeld D, Warshauer ME, Sherlock S *et al.* (1980). The epidemiology of testicular cancer in young adults. *Am J Epidemiol*, 112: 232–246. PMID:6106385

Schuler M, Hasegawa L, Parks R *et al.* (1998). Dose-response studies of the induction of hyperdiploidy and polyploidy by diethylstilbestrol and 17beta-estradiol in cultured human lymphocytes using multicolor fluorescence in situ hybridization. *Environ Mol Mutagen*, 31: 263–273. doi:10.1002/(SICI)1098-2280(1998)31:3<263::AID-EM8>3.0.CO;2-L PMID:9585265

Segura-Aguilar J, Cortés-Vizcaino V, Llombart-Bosch A *et al.* (1990). The levels of quinone reductases, superoxide dismutase and glutathione-related enzymatic activities in diethylstilbestrol-induced carcinogenesis in the kidney of male Syrian golden hamsters. *Carcinogenesis*, 11: 1727–1732. doi:10.1093/carcin/11.10.1727 PMID:2119905

Shah HC & McLachlan JA (1976). The fate of diethylstilbestrol in the pregnant mouse. *J Pharmacol Exp Ther*, 197: 687–696. PMID:819646

Sharp DC & Parry JM (1985). Diethylstilboestrol: the binding and effects of diethylstilboestrol upon the polymerisation and depolymerisation of purified microtubule protein in vitro. *Carcinogenesis*, 6: 865–871. doi:10.1093/carcin/6.6.865 PMID:4006072

Sharp GB & Cole P (1990). Vaginal bleeding and diethylstilbestrol exposure during pregnancy: relationship to genital tract clear cell adenocarcinoma and vaginal adenosis in daughters. *Am J Obstet Gynecol*, 162: 994–1001. PMID:2327468

Sharp GB & Cole P (1991). Identification of risk factors for diethylstilbestrol-associated clear cell adenocarcinoma of the vagina: similarities to endometrial cancer. *Am J Epidemiol*, 134: 1316–1324. PMID:1755445

Siegfried JM, Nelson KG, Martin JL, Kaufman DG (1984). Promotional effect of diethylstilbestrol on human endometrial stromal cells pretreated with a direct-acting carcinogen. *Carcinogenesis*, 5: 641–646. doi:10.1093/carcin/5.5.641 PMID:6144401

Singh KP & Roy D (2004). Somatic mutations in stilbene estrogen-induced Syrian hamster kidney tumors identified by DNA fingerprinting. *J Carcinog*, 3: 4 doi:10.1186/1477-3163-3-4 PMID:15003126

Singh KP & Roy D (2008). Allelic loss and mutations in a new ETRG-1 gene are early events in diethylstilbestrol-induced renal carcinogenesis in Syrian hamsters. *Gene*, 408: 18–26. doi:10.1016/j.gene.2007.10.022 PMID:18068315

Sofuni T, Honma M, Hayashi M *et al.* (1996). Detection of in vitro clastogens and spindle poisons by the mouse lymphoma assay using the microwell method: interim report of an international collaborative study. *Mutagenesis*, 11: 349–355. doi:10.1093/mutage/11.4.349 PMID:8671759

Stafl A & Mattingly RF (1974). Vaginal adenosis: a precancerous lesion? *Am J Obstet Gynecol*, 120: 666–677. PMID:4422247

Stanley MA, Crowcroft NS, Quigley JP, Parkinson EK (1985). Responses of human cervical keratinocytes in vitro to tumour promoters and diethylstilboestrol. *Carcinogenesis*, 6: 1011–1015. doi:10.1093/carcin/6.7.1011 PMID:2410159

Stopper H, Kirchner S, Schiffmann D, Poot M (1994). Cell cycle disturbance in relation to micronucleus formation induced by the carcinogenic estrogen diethylstilbestrol. *Pathobiology*, 62: 180–185. doi:10.1159/000163908 PMID:7734061

Storer RD, French JE, Haseman J *et al.* (2001). P53+/- hemizygous knockout mouse: overview of available data. *Toxicol Pathol*, 29: Suppl30–50. doi:10.1080/019262301753178465 PMID:11695560

Strecker TE, Spady TJ, Schaffer BS *et al.* (2005). Genetic bases of estrogen-induced pituitary tumorigenesis: identification of genetic loci determining estrogen-induced pituitary growth in reciprocal crosses between the ACI and Copenhagen rat strains. *Genetics*, 169: 2189–2197. doi:10.1534/genetics.104.039370 PMID:15687265

Strohsnitter WC, Noller KL, Hoover RN *et al.* (2001). Cancer risk in men exposed in utero to diethylstilbestrol. *J Natl Cancer Inst*, 93: 545–551. doi:10.1093/jnci/93.7.545 PMID:11287449

Suzuki A, Enari M, Iguchi T (1996). Effect of neonatal exposure to DES in Fas and Bcl-2 expression in the adult mouse vagina and approach to the DES syndrome. *Reprod Toxicol*, 10: 465–470. doi:10.1016/S0890-6238(96)00133-5 PMID:8946560

Suzuki A, Watanabe H, Mizutani T *et al.* (2006). Global gene expression in mouse vaginae exposed to diethylstilbestrol at different ages. *Exp Biol Med (Maywood)*, 231: 632–640. PMID:16636312

Suzuki K, Ishii-Ohba H, Yamanouchi H *et al.* (1994). Susceptibility of lactating rat mammary glands to gamma-ray-irradiation-induced tumorigenesis. *Int J Cancer*, 56: 413–417. doi:10.1002/ijc.2910560321 PMID:8314329

Sweetman SC, editor (2008) *Martindale: The Complete Drug Reference*, London, Pharmaceutical Press, Electronic version, (Edition 2008)

Takahashi T, Eitzman B, Bossert NL *et al.* (1994). Transforming growth factors beta 1, beta 2, and beta 3 messenger RNA and protein expression in mouse uterus and vagina during estrogen-induced growth: a comparison to other estrogen-regulated genes. *Cell Growth Differ*, 5: 919–935. PMID:7819129

Tam NN, Ghatak S, Ho SM (2003). Sex hormone-induced alterations in the activities of antioxidant enzymes and lipid peroxidation status in the prostate of Noble rats. *Prostate*, 55: 1–8. doi:10.1002/pros.10169 PMID:12640655

Tang WY, Newbold R, Mardilovich K *et al.* (2008). Persistent hypomethylation in the promoter of nucleosomal binding protein 1 (Nsbp1) correlates with overexpression of Nsbp1 in mouse uteri neonatally exposed to diethylstilbestrol or genistein. *Endocrinology*, 149: 5922–5931. doi:10.1210/en.2008-0682 PMID:18669593

Tayama S, Nakagawa Y, Tayama K (2008). Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells. *Mutat Res*, 649: 114–125. PMID:17913570

Teras LR, Patel AV, Rodriguez C *et al.* (2005). Parity, other reproductive factors, and risk of pancreatic cancer mortality in a large cohort of U.S. women (United States). *Cancer Causes Control*, 16: 1035–1040. doi:10.1007/s10552-005-0332-4 PMID:16184468

Thomas RD, Green MR, Wilson C, Sadrud-Din S (2004). Diallyl sulfide inhibits the oxidation and reduction reactions of stilbene estrogens catalyzed by microsomes, mitochondria and nuclei isolated from breast tissue of female ACI rats. *Carcinogenesis*, 25: 787–791. doi:10.1093/carcin/bgg161 PMID:12949044

Thomas RD & Roy D (1995). Mitochondrial enzyme-catalyzed oxidation and reduction reactions of stilbene estrogen. *Carcinogenesis*, 16: 891–895. doi:10.1093/carcin/16.4.891 PMID:7728971

Thomas RD & Roy D (2001a). Base sequence-specific attack of stilbene estrogen metabolite(s) on the mitochondrial DNA: implications in the induction of instability in the mitochondrial genome in the kidney of Syrian hamsters. *Int J Mol Med*, 7: 389–395. PMID:11254879

Thomas RD & Roy D (2001b). Stilbene estrogen produces higher levels of mitochondrial DNA adducts than nuclear DNA adducts in the target organ of cancer (liver) of male Sprague Dawley rats. *Oncol Rep*, 8: 1035–1038. PMID:11496312

Titus-Ernstoff L, Hatch EE, Hoover RN *et al.* (2001). Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer*, 84: 126–133. doi:10.1054/bjoc.2000.1521 PMID:11139327

Titus-Ernstoff L, Troisi R, Hatch EE *et al.* (2006b). Menstrual and reproductive characteristics of women whose mothers were exposed in utero to diethylstilbestrol (DES). *Int J Epidemiol*, 35: 862–868. doi:10.1093/ije/dyl106 PMID:16723367

Titus-Ernstoff L, Troisi R, Hatch EE *et al.* (2006a). Mortality in women given diethylstilbestrol during pregnancy. *Br J Cancer*, 95: 107–111. doi:10.1038/sj.bjc.6603221 PMID:16786044

Titus-Ernstoff L, Troisi R, Hatch EE *et al.* (2008). Offspring of women exposed in utero to diethylstilbestrol (DES): a preliminary report of benign and malignant pathology in the third generation. *Epidemiology*, 19: 251–257. doi:10.1097/EDE.0b013e318163152a PMID:18223485

Troisi R, Hatch EE, Titus-Ernstoff L *et al.* (2007). Cancer risk in women prenatally exposed to diethylstilbestrol. *Int J Cancer*, 121: 356–360. doi:10.1002/ijc.22631 PMID:17390375

Tsutsui T & Barrett JC (1997). Neoplastic transformation of cultured mammalian cells by estrogens and estrogenlike chemicals. *Environ Health Perspect*, 105: Suppl 3619–624. doi:10.2307/3433380 PMID:9168005

Tsutsui T, Degen GH, Schiffmann D *et al.* (1984). Dependence on exogenous metabolic activation for induction of unscheduled DNA synthesis in Syrian hamster embryo cells by diethylstilbestrol and related compounds. *Cancer Res*, 44: 184–189. PMID:6317168

Tsutsui T, Suzuki N, Maizumi H, Barrett JC (1990). Aneuploidy induction in human fibroblasts: comparison with results in Syrian hamster fibroblasts. *Mutat Res*, 240: 241–249. doi:10.1016/0165-1218(90)90074-C PMID:2330010

Tsutsui T, Taguchi S, Tanaka Y, Barrett JC (1997). 17beta-estradiol, diethylstilbestrol, tamoxifen, toremifene and ICI 164,384 induce morphological transformation and aneuploidy in cultured Syrian hamster embryo cells.

Int J Cancer, 70: 188–193. doi:10.1002/(SICI)1097-0215(19970117)70:2<188::AID-IJC9>3.0.CO;2-T PMID:9009159

Tucker RW & Barrett JC (1986). Deceased numbers of spindle and cytoplasmic microtubules in hamster embryo cells treated with a carcinogen, diethylstilbestrol. *Cancer Res*, 46: 2088–2095. PMID:3948182

Turusov VS, Trukhanova LS, Lanko NS *et al.* (1997). 1,2-Dimethylhydrazine carcinogenesis in C3HA and CBA female mice prenatally treated with diethylstilbestrol. *Teratog Carcinog Mutagen*, 17: 19–28. doi:10.1002/(SICI)1520-6866(1997)17:1<19::AID-TCM4>3.0.CO;2-I PMID:9249927

Turusov VS, Trukhanova LS, Parfenov YuD , Tomatis L (1992). Occurrence of tumours in the descendants of CBA male mice prenatally treated with diethylstilbestrol. *Int J Cancer*, 50: 131–135. doi:10.1002/ijc.2910500126 PMID:1728603

Usui T, Mutai M, Hisada S *et al.* (2001). CB6F1-rasH2 mouse: overview of available data. *Toxicol Pathol*, 29: Suppl90–108. doi:10.1080/019262301753178500 PMID:11695565

Utsuyama M, Kanno J, Inoue T, Hirokawa K (2002). Age/sex dependent and non-monotonous dose-response effect of diethylstilbestrol on the immune functions in mice. *Toxicol Lett*, 135: 145–153. doi:10.1016/S0378-4274(02)00256-4 PMID:12243873

Verloop J, Rookus MA, van Leeuwen FE (2000). Prevalence of gynecologic cancer in women exposed to diethylstilbestrol in utero. *N Engl J Med*, 342: 1838–1839. doi:10.1056/NEJM200006153422415 PMID:10866558

Vessey MP, Fairweather DV, Norman-Smith B, Buckley J (1983). A randomized double-blind controlled trial of the value of stilboestrol therapy in pregnancy: long-term follow-up of mothers and their offspring. *Br J Obstet Gynaecol*, 90: 1007–1017. doi:10.1111/j.1471-0528.1983.tb06438.x PMID:6357269

Waalkes MP, Liu J, Ward JM *et al.* (2006a). Urogenital carcinogenesis in female CD1 mice induced by in utero arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res*, 66: 1337–1345. doi:10.1158/0008-5472.CAN-05-3530 PMID:16452187

Waalkes MP, Liu J, Ward JM, Diwan BA (2006b). Enhanced urinary bladder and liver carcinogenesis in male CD1 mice exposed to transplacental inorganic arsenic and postnatal diethylstilbestrol or tamoxifen. *Toxicol Appl Pharmacol*, 215: 295–305. doi:10.1016/j.taap.2006.03.010 PMID:16712894

Waggoner SE, Anderson SM, Luce MC *et al.* (1996). p53 protein expression and gene analysis in clear cell adenocarcinoma of the vagina and cervix. *Gynecol Oncol*, 60: 339–344. doi:10.1006/gyno.1996.0052 PMID:8774636

Wakata A, Matsuoka A, Yamakage K *et al.* (2006). SFTG international collaborative study on in vitro micronucleus test IV. Using CHL cells. *Mutat Res*, 607: 88–124. PMID:16782396

Walker BE (1988). Vaginal tumors in mice from methylcholanthrene and prenatal exposure to diethylstilbestrol. *Cancer Lett*, 39: 227–231. doi:10.1016/0304-3835(88)90064-X PMID:3359417

Walker BE (1990). Tumors in female offspring of control and diethylstilbestrol-exposed mice fed high-fat diets. *J Natl Cancer Inst*, 82: 50–54. doi:10.1093/jnci/82.1.50 PMID:2293656

Walker BE & Haven MI (1997). Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis*, 18: 791–793. doi:10.1093/carcin/18.4.791 PMID:9111216

Walker BE & Kurth LA (1993). Pituitary tumors in mice exposed prenatally to diethylstilbestrol. *Cancer Res*, 53: 1546–1549. PMID:8453621

Wang MY & Liehr JG (1994). Identification of fatty acid hydroperoxide cofactors in the cytochrome P450-mediated oxidation of estrogens to quinone metabolites. Role and balance of lipid peroxides during estrogen-induced carcinogenesis. *J Biol Chem*, 269: 284–291. PMID:8276808

Wang MY & Liehr JG (1995a). Induction by estrogens of lipid peroxidation and lipid peroxide-derived malonaldehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis. *Carcinogenesis*, 16: 1941–1945. doi:10.1093/carcin/16.8.1941 PMID:7634425

Wang MY & Liehr JG (1995b). Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis. *Arch Biochem Biophys*, 316: 38–46. doi:10.1006/abbi.1995.1007 PMID:7840640

Ways SC, Mortola JF, Zvaifler NJ *et al.* (1987). Alterations in immune responsiveness in women exposed to diethylstilbestrol in utero. *Fertil Steril*, 48: 193–197. PMID:3609331

Welch WR, Fu YS, Robboy SJ, Herbst AL (1983). Nuclear DNA content of clear cell adenocarcinoma of the vagina and cervix and its relationship to prognosis. *Gynecol Oncol*, 15: 230–238. doi:10.1016/0090-8258(83)90079-3 PMID:6832636

Wendell DL, Herman A, Gorski J (1996). Genetic separation of tumor growth and hemorrhagic phenotypes in an estrogen-induced tumor. *Proc Natl Acad Sci USA*, 93: 8112–8116. doi:10.1073/pnas.93.15.8112 PMID:8755612

Wingard DL & Turiel J (1988). Long-term effects of exposure to diethylstilbestrol. *West J Med*, 149: 551–554. PMID:3250102

Xu LH, Rinehart CA, Kaufman DG (1995). Estrogen-induced anchorage-independence in human endometrial stromal cells. *Int J Cancer*, 62: 772–776. doi:10.1002/ijc.2910620621 PMID:7558429

Yager JD & Chen JQ (2007). Mitochondrial estrogen receptors–new insights into specific functions. *Trends Endocrinol Metab*, 18: 89–91. doi:10.1016/j.tem.2007.02.006 PMID:17324583

Yan ZJ & Roy D (1995). Mutations in DNA polymerase beta mRNA of stilbene estrogen-induced kidney tumors in Syrian hamster. *Biochem Mol Biol Int*, 37: 175–183. PMID:8653081

Yuen MT, Leung LK, Wang J *et al.* (2005). Enhanced induction of prostatic dysplasia and carcinoma in Noble rat model by combination of neonatal estrogen exposure and hormonal treatments at adulthood. *Int J Oncol*, 27: 1685–1695. PMID:16273225

Zijno A, Quaggia S, Pacchierotti F (1989). A cytogenetic approach to evaluate in vivo somatic aneuploidy. Effects of diethylstilboestrol on mouse bone marrow cells. *Mutagenesis*, 4: 62–66. doi:10.1093/mutage/4.1.62 PMID:2654554