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FOOD OCCURRENCE

Diethylstilbestrol has been used in veterinary medicine and as a growth promoter (as a feed supplement or subcutaneous implant) in cattle, sheep, and poultry (IARC 1979). Its use as a growth promoter was banned in 1979 (Raun and Preston 2002). Diethylstilbestrol residues were detected in beef and sheep livers in 1972 and 1973. When diethylstilbestrol was used as a growth promoter for sheep and cattle, people could have been exposed to it at concentrations of up to 10 ppb in beef and mutton (IARC 1979).

IARC In the US formerly, DES was implanted (usually in the neck) in male chickens to caponize them chemically and to improve fattening and finishing. The FDA banned such implants in 1959 when residues of DES were found in edible poultry tissue (US Congress, 1973). It was also widely used in the US as a feed additive and as an ear implant to fatten, promote growth and to increase the feed efficiency of beef cattle and sheep. Because of concern about the appearance of residues of DES in food derived from the animals and about the observed carcinogenic properties of DES in test animals, the FDA banned the use of DES in animal feed in August 1972 (US Environmental Protection Agency, 1972b) and banned the use of DES implants on April 27, 1973 (Anon., 1973).

Veterinary uses of DES include replacement therapy for underdeveloped females, incontinence, vaginitis of spayed bitches, hypertrophy of prostate in dogs and other applications (Merck & Co., 1968). Although no data are available on the quantity of DES consumed in these ways, it is believed that the largest volume used was in feed additives and implants.

In some European countries the use of such oestrogenic hormones as growth promoters in animals is forbidden; in others they are still used.

1 A US Court of Appeal, in January 1974, ruled that the FDA had not met all legal requirements in establishing its position. Thus, the legality of the ban has not as yet been resolved.

LEGISLATION

ASSESSMENT OF POTENTIAL RISKS TO HUMAN HEALTH FROM HORMONE RESIDUES IN BOVINE MEAT AND MEAT PRODUCTS (1999)

Methods of sampling and analysis

On UV irradiation, diethylstilboestrol (DES) is converted specifically to a yellow product with an absorbance at 418 nm, and this forms the basis of many methods of analysis. Tablets are analyzed after extraction with ethanol-water, and oily solutions after partition between iso-octane and sodium hydroxide (US Pharmacopeia, 1965; Hussey et al., 1973). The reaction was used in the official first-action method of the Association of Official Analytical Chemists for measuring DES in feed pre-mixes and supplements after Soxhlet extraction and solvent partition involving alkali (Horwitz, 1970). A later modification included chromatography on a tripotassium phosphate-celite column and made possible the measurement of 0.55-44 mg DES per kg of feed mix (Jeffus & Kenner, 1972).

Schuller (1967) utilized this method for measuring DES in bovine urine. The urine was subjected to hydrolysis and the phenolic fraction was separated by ether extraction and solvent partition with alkali and purified by thin-layer chromatography (TLC) on silica gel. After chromatography the plate was irradiated, and the yellow product was subjected to further chromatography and identified by examination under UV light for absorbance at 254 nm and with fluorescence at 366 nm. The limit of detection was 0.1 µg per sample or 40 µg/L urine.

GLC has also been applied to the measurement of DES in animal foods, using either the free compound or its silyl ethers after simple solvent extraction, with or without further purification (Rutherford, 1970). A method sensitive enough for the measurement of DES residues in various tissues of beef, chicken and lamb was described by Coffin & Pilon (1973). The method involved acetone extraction, acid hydrolysis, solvent partition with alkali, formation of the trifluoro-acetates and gas chromatography using electron capture detection; 2-10 µg/kg could be measured reliably. Two peaks occurred in the chromatograms due to the presence of the cis and trans-isomers, and this added specificity to the method.

The classical method for detecting DES or other oestrogen additives in food samples specified by the US Code of Federal Regulations (1973) was that of Umberger et al. (1958), based on feeding the samples, mixed with normal feed, to immature female mice and measuring the increase in weight of the uterus; positive reaction indicated that excessive amounts of oestrogens were present in the meat.

The method detected 0.8 µg DES/kg. It could also be applied to the detection of oestradiol-17β, with 10 times less sensitivity, and to other oestrogens. Oestrogen feeding could also be detected by examination of prostates of male calves and the genital tracts of heifers receiving the drug (Ruitenber, 1969; Kroes et al., 1970). Radioimmunoassay (RIA) methods for DES have recently been developed, and these are likely to be the preferred methods for detecting specific oestrogens in animal tissues in the future (Abraham et al., 1972). RIA methods are also applicable to oestradiol-17β and its esters occurring in meat (Huis in’t Veld et al., 1973).

CARCINOGENICITY (RoC)

Diethylstilbestrol is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. The strongest evidence comes from epidemiological studies of women exposed to diethylstilbestrol in utero (“diethylstilbestrol daughters”), which found that diethylstilbestrol caused clear-cell adenocarcinoma of the vagina and cervix. This type
of cancer, which typically develops in elderly women, developed in diethylstilbestrol daughters between the ages of 10 and 30 years. Most (though not all) case-control studies found that in utero exposure to diethylstilbestrol increased the risk of testicular cancer in males (“diethylstilbestrol sons”). Several follow-up studies (including cohort studies and randomized clinical trials) found that women who took diethylstilbestrol at high doses during pregnancy were at increased risk for breast cancer. Some studies suggest that diethylstilbestrol-induced breast cancer may have a long latency period (15 to 20 years), but the evidence is inconclusive. As has been found for other estrogens, diethylstilbestrol taken to relieve the symptoms of menopause increases the risk of endometrial cancer (IARC 1974, 1979, 1987). Since diethylstilbestrol was reviewed for listing in the First Annual Report on Carcinogens and by the International Agency for Research on Cancer (IARC), additional studies on diethylstilbestrol daughters and sons have been published. A study of a large cohort of diethylstilbestrol daughters first identified in the mid 1970s confirmed a 40-fold increase in the risk of clear-cell adenocarcinoma of the vagina or cervix and estimated a cumulative incidence rate of 1.5 per 1,000 exposed women (Hatch et al. 1998). The evidence for increased risk of breast cancer in diethylstilbestrol daughters is inconclusive because of the young age of the cohort (Hatch et al. 1998, Palmer et al. 2002). Another cohort study reported an increased risk of testicular cancer among diethylstilbestrol sons, supporting the findings from earlier case-control studies; however, this result was not statistically significant (Strohsnitter et al. 2001).

The findings in humans are supported by studies in experimental animals showing that administration of diethylstilbestrol by various routes causes cancer in multiple species (mice, rats, hamsters, frogs, dogs, and monkeys) and at multiple tissue sites (primarily estrogen-sensitive organs and tissues). As in humans, prenatal exposure to diethylstilbestrol caused cervical and vaginal tumors in female mice and hamsters and testicular tumors in male hamsters. Prenatal exposure also caused uterine tumors in mice and hamsters and ovarian, mammary-gland, and lung tumors in mice. Tumors of the genital tract were observed in rats exposed to diethylstilbestrol (administered by injection) in utero or three weeks postpartum. Mice developed cervical and vaginal tumors after receiving a single subcutaneous (s.c.) injection of diethylstilbestrol on the first day of life, and male rats developed reproductive-tract tumors after receiving daily s.c. injections for the first month of life. Diethylstilbestrol also caused cancer in animals exposed as adults. When administered orally, diethylstilbestrol caused mammary-gland, cervical, vaginal, endometrial, uterine, and bone tumors in mice and pituitary-gland, liver, and mammary-gland tumors in rats. Subcutaneous injections or implants of diethylstilbestrol increased the incidences of leukemia and testicular, lymphoid, and mammary-gland tumors in mice, mammary-gland tumors in rats, kidney tumors in hamsters, ovarian tumors in mice and dogs, and uterine tumors in squirrel monkeys. Diethylstilbestrol dipropionate caused tumors of the liver and the hematopoietic system (organs and tissues involved in production of blood) in male and female frogs and pituitary-gland tumors in rats (IARC 1974, 1979, 1987).

Since diethylstilbestrol was reviewed for listing in the First Annual Report on Carcinogens and by IARC, multigenerational studies in mice and additional prenatal-exposure studies in rats have been published. In the multigenerational studies, mice were exposed to diethylstilbestrol in utero, either during the period of major organogenesis or just before
birth, or on the first five days of life. Female mice from each exposure regimen (the F1 generation) were raised to maturity and bred with unexposed male mice. Both male and female offspring of these mice (the F2 generation) had increased incidences of reproductive-tract tumors, including uterine adenocarcinoma and other tumors in females and seminal-vesicle tumors and other tumors and lesions in males (Newbold et al. 1998, 2000). As in hamsters and mice, prenatal exposure to diethylstilbestrol caused uterine tumors in Donryu rats (a carcinogen-sensitive strain with an increased estrogen-to-progesterone ratio) (Kitamura et al. 1999).

Properties
Diethylstilbestrol is a synthetic nonsteroidal estrogen (female sex hormone). It has a molecular weight of 268.4 and occurs as small white plates from benzene or as a white crystalline powder. It has a melting point of 169°C to 172°C and a log octanol-water partition coefficient of 5.07. Diethylstilbestrol is practically insoluble in water and soluble in ethanol, chloroform, diethyl ether, acetone, dioxane, ethyl acetate, methyl alcohol, vegetable oils, and aqueous solutions of alkaline hydroxides. It emits acrid smoke and fumes when heated to decomposition (HSDB 2003). Diethylstilbestrol dipropionate has a molecular weight of 380.4 and occurs as odorless, tasteless, colorless crystals or a white crystalline powder. It has a melting point of 105°C to 107°C. Diethylstilbestrol dipropionate is soluble in 90% ethanol, diethyl ether, olive oil, fixed oils, acetone, and chloroform, but it is very slightly soluble in water and insoluble in solutions of alkaline hydroxides. Diethylstilbestrol dipropionate differs from diethylstilbestrol in solubility and rate of absorption, but once absorbed into the body diethylstilbestrol dipropionate is converted to diethylstilbestrol.

Use
Diethylstilbestrol was the first synthetic estrogen. It was synthesized in 1938 and was widely prescribed in the United States from the early 1940s until 1971, primarily as a treatment to prevent miscarriages or premature deliveries. The U.S. Food and Drug Administration (FDA) issued a drug bulletin in 1971 advising physicians to stop prescribing diethylstilbestrol to pregnant women because of its link to a rare vaginal cancer (clear-cell adenocarcinoma) in diethylstilbestrol daughters (CDC 2003). Other uses in human medicine continued at least through the 1970s and in some cases into the early 1980s. These uses included hormone replacement therapy, control of menstrual disorders, relief or prevention of postpartum breast engorgement, palliative therapy for cancer of the prostate in men and breast cancer in postmenopausal women, and as a postcoital contraceptive. In 1978, the FDA withdrew approval of any estrogen-containing drug product (including diethylstilbestrol) for the suppression of postpartum breast engorgement (FDA 1998). Diethylstilbestrol sometimes was given in combination with androgens, vitamins, and antibiotics (IARC 1974, 1979). Its use in the treatment of advanced prostate cancer fell out of favor because of its cardiovascular toxicity, the emergence of safer agents, and manufacturers’ economic considerations (Malkowicz 2001). Nevertheless, diethylstilbestrol continues to be used in clinical trials for treatment of prostate and breast cancer (Smith et al. 1998, Peethambaram et al. 1999) and in biochemical research. Diethylstilbestrol also has been used in veterinary medicine and as a growth promoter (as a feed supplement or subcutaneous implant) in cattle, sheep, and poultry (IARC 1979). Its use
as a growth promoter was banned in 1979 (Raun and Preston 2002).

**Production**

U.S. production of diethylstilbestrol was first reported in 1941, as 227 kg (500 lb), and last reported in 1952, as 1,800 kg (3,970 lb) (IARC 1974). In 1972, 454 kg (1,000 lb) of diethylstilbestrol diphosphate (an ester form) were produced (HSDB 2003). Between the early 1940s and early 1970s, there were three to five U.S. producers of diethylstilbestrol, and in 1976, there was one U.S. producer (IARC 1974, 1979). Diethylstilbestrol is no longer manufactured by pharmaceutical companies in the United States (CDC 2004). Annual U.S. imports ranged from about 3,000 to 7,800 kg (6,700 to 17,000 lb) in the 1970s, but had dropped to 130 kg (290 lb) by 1982 (IARC 1974, 1979; HSDB 2003). No export data were found. In 2003, 13 U.S. suppliers of diethylstilbestrol were identified (ChemSources 2003).

**Exposure**

Most current exposure to diethylstilbestrol is through its oral administration as a drug used in clinical trials for the treatment of prostate and breast cancer. Exposure also occurred through the past use of diethylstilbestrol to prevent miscarriages, as hormone replacement therapy, to treat prostate cancer, and in other medical therapies. It has been estimated that between 5 and 10 million Americans received diethylstilbestrol during pregnancy or were exposed to the drug in utero (NIH 1999). In one large cohort of diethylstilbestrol daughters, the median total doses administered to their mothers at five study sites ranged from 1,625 to 10,424 mg (Giusti et al. 1995). Many different forms of diethylstilbestrol, including oral tablets (0.1, 0.25, 0.5, 1, and 5 mg), injectable solutions (0.2, 0.5, 1, and 5 mg/mL), and a vaginal suppository (0.1 and 0.5 mg) were approved by the FDA prior to withdrawal of diethylstilbestrol (FDA 2003). Diethylstilbestrol diphosphate also was available as oral tablets (50 mg) and an injectable solution (250 mg/50 mL).

The National Occupational Exposure Survey (1981–1983) estimated that 1,492 workers, including 934 women, potentially were exposed to diethylstilbestrol during its manufacture or during product formulation (NIOSH 1984). The concentration of diethylstilbestrol in ambient air-samples from plants that manufactured diethylstilbestrol ranged from 0.02 to 24 µg/m³ (IARC 1979).

**OTHER RELEVANT INFORMATIONS**

**The role of hormones in tumour induction**

Steroid hormones and those synthetic steroidal or non-steroidal compounds with hormonal activity interact closely with the secretions of the anterior pituitary and other endocrine secretions to modify the growth and secretory activity of many tissues. In the absence of the requisite hormonal environment and, thus, with incomplete or absent proliferation, other carcinogenic agents may be unable to act. It is, therefore, difficult in experimental tumour induction to dissociate the effects of the hormones themselves from the effects of other agents, such as chemicals, viruses or radiation, to which the test animals may be exposed.
It should be borne in mind that not only may hormones be synergistic for the action of other carcinogens, but, alternatively, that other cancer-inducing agents may act by distributing the hormonal balance, so that hormonal factors are the effective cause of neoplasia. An example of this type of action is the effect of DMBA (7,12-dimethylbenz(a)anthracene) on the mouse ovary, where it probably acts to eliminate the ova, thus producing a state of hyperstimulation by the pituitary gonadotrophins; the resulting granulosa-cell tumours are thus a consequence of the excessive gonadotrophin stimulation (see review by Jull, 1973).

In assessing the effects of hormones on the induction of tumours of the breast and of the haemopoietic system in mice, the interaction of viral factors has been shown to be of decisive importance. In the case of mammary tumourigenesis, the presence of a milk-borne virus (the mammary tumour virus, or MTV) has been established for many years, but there is good reason to suppose that at least one other viral factor, the nodule-inducing virus (or NIV), may also play a vital role.

The NIV is not transmitted via the mother’s milk, and therefore NIV-free strains of mice cannot be obtained by foster-nursing (see review by Nandi, 1965).

Hormones, including the steroids, may affect the action of chemical carcinogens by modifying their metabolism. The production of proximate carcinogenic metabolites might possibly be reduced or eliminated (Weisburger, 1968). Similar effects of hormonal treatment on the activity of environmental carcinogens must therefore be considered before attributing the carcinogenic activity of administered hormones to a direct action by the compounds themselves.

Hormones may precipitate neoplasia:
(a) by a direct carcinogenic action;
(b) by stimulating the production of other hormonal factors which, in excess, cause cancer;
(c) by acting synergistically to promote growth in tissues affected by a physical, chemical or viral carcinogen;
(d) by modifying the metabolism of chemical agents so that they become active carcinogens; or
(e) by modifying immune responses.

CHEMICAL AND PHYSICAL DATA

Synonyms and trade names*
*Chem. Abstr. No.: 56-53-1
DEB; DES; 4,4´-(1,2-diethyl-1,2-ethenediyl)bis-phenol; a,a´-diethylstilbenediol; a,a´-diethyl-4,4´-stilbenediol; trans-a,a´-diethyl-4,4´-stilbenediol; diethylstilbesterol; diethylstilboesterol; trans-diethylstilbesterol; trans-diethylstilboesterol; diethylstilbestrol; trans-diethylstilbestrol; trans-diethylstilbestrol; 4,4´-dihydroxy-α,β-diethylstilbene; 3,4-(4,4´-dihydroxyphenyl)hex-3-ene; 3,4-bis(p-hydroxyphenyl)-3-hexene; stilbesterol; stilboesterol; stilbestrol
Bio-des; Climaterine; Comestrol estrobene; Cyren; Cyren A; Cyren B; Di-Estryl; Distilbene; D Oestromon; Domestrol; Estilben; Estilbin MCO; Estril; Estrobin; Estrosyn; Follidiene; Fonatol; Grafestrol; Hi-Bestrol; Idroestril; Microest; Milestrol; Neodistilbene, Neo-Oestranol, New-Oestranol
I; Oestrogenine; Oestromenin; Oestromensil; Oestromensyl; Oestronienin; Oestromon; Palestrol; Percutatrine Oestrogénique Iscovesco; Protectona; Sedestran; Serral; Sexocretin; Sibol; Sintestrol; Stil; Stilbetin; Stilboefral; Stilboestroform; Stilkap; Stil-Rol; Synerstrin; Synerestrin; Synthoestrin; Syntofolin

*Trade names include mixtures containing diethylstilboestrol

**Chemical and physical properties of the pure substance**

(a) **Description**: White platelets (from benzene)

(b) **Melting-point**: 169-172°C

(c) **Solubility**: Practically insoluble in water; soluble at 25°C in 95% ethanol (1 in 5), chloroform (1 in 200), ether (1 in 3); soluble in acetone, dioxane, ethyl acetate, methyl alcohol, vegetable oils and aqueous solutions of alkali hydroxides.

**COMMENTS ON DATA REPORTED AND EVALUATION**

**Animal data**

Diethylstilboestrol (DES) was tested in mice by oral administration, local application and subcutaneous injection, in mice, rats, hamsters and squirrel monkeys by subcutaneous implantation and in hamsters by subcutaneous injection.

Its administration to mice resulted in an increased incidence of mammary and lymphoid tumours in both males and females, and of interstitial-cell tumours of the testis in males and cervical and vaginal tumours in females, including those exposed only on the first day of life. In rats, increased incidences of pituitary, mammary and bladder tumours were observed. In hamsters, a high incidence of renal tumours was observed in castrated males and females and in intact males, but not in intact females. In squirrel monkeys, malignant mesotheliomas of the uterine serosa were observed.

DES treatment in most cases increased the incidence of mammary tumours in strains of mice having a spontaneous incidence of these tumours, which may be related to the presence of a virus; testicular tumours occurred in strains having a particular genetic susceptibility to such tumours. No evidence of a possible role of a virus has been shown in rats. Bladder tumours occurred only in rats in which bladder calculi were present.

In most cases, an accurate assessment of the effective carcinogenic dose in implantation studies is not possible. However, in oral administration studies, the lowest statistically significant dose (P≤0.01) producing mammary carcinomas in mice was about 0.15 µg/day (6 µg/kg bw/day). This dose is similar to that used in humans in the control of menopausal symptoms by DES (10 µg/kg bw/day) and 30 times less than the dose given for the control of mammary or prostatic cancer (300 µg/kg bw/day).

**Human data**

The administration of diethylstilboestrol to women during pregnancy is associated with an increased risk of vaginal or cervical adenocarcinoma in their exposed female offspring. There may also be an increased risk of endometrial carcinoma in women with gonadal dysgenesis treated with this drug. It is possible that the administration of the drug therapeutically to men with carcinoma of the prostate increases the risk of breast cancer.