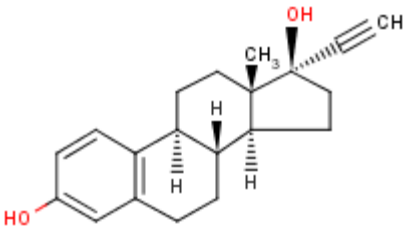
Bijlage 5 van het advies 29-2009 : Fiche Ethinylœstradiol

ETHINYLŒSTRADIOL

CAS no: 57-63-6

(17 α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol
Structural modification of the estradiol molecule by insertion of an ethinyl group at carbon 17 yields ethinylœstradiol, which is considerably more potent than estradiol and has high activity following oral administration. This compound is used frequently as the estrogenic component of oral contraceptives (IARC, 2007).

Chemical form



Source:
http://chem.sis.nlm.nih.gov/chemidplus/jsp/common/ChemInfo.jsp?calledFrom=lite&type=names

Contamination source, origin, use

17 α-Ethinylœstradiol (EE2) is a semisynthetic estrogen. 17 α-EE2 was originally synthesized by Inhoffen and Hohlweg (1938) as an orally active estrogen (Cited in Salman *et al*., 1999).
Estrogens are some of the most commonly prescribed pharmacological agents, being used most frequently for hormone replacement therapy of postmenopausal women and as components of combination oral contraceptives, although they are also used for a variety of other purposes (Salman *et al*., 1999) such as for peri-menopausal symptoms, hormonal therapy for hypogonadal women, treatment of post-partum breast engorgement and dysfunctional uterine bleeding and therapy for carcinoma of the breast and prostate (IARC, 1999). 17 α-EE2 is one of most widely prescribed estrogens.

Oral contraceptives have been used since the early 1960s and are now used by about 90 million women worldwide (IARC, 1979, 1999 and related references). 'The pill' is given as a combination of an estrogen and progestagen or, formerly, as sequential therapy. Ethinylœstradiol is the most estrogen used in oral contraceptives in combination with a progestagen. Oral contraceptive preparations containing 50 µg of ethinylœstradiol contain 1, 2.5, 3, or 4 mg of norethisterone, while preparations containing 30 µg of ethinylœstradiol contain 150 or 250 µg of levonorgestrel (WHO, 2000). Ethinylœstradiol is also used in conjunction with a progestagen as a post-coital contraceptive (IARC, 1999).

Mode of action (toxicological data: major biologic effect, active dose)

Carcinogenicity

- **Epidemiological studies**

A lot of case-control studies have been reported on risk of cancer related to the use of combined oral contraceptives.
Combined oral estrogen-progestagen contraceptives are *carcinogenic to humans (Group 1)*. There is also convincing evidence in humans that these agents confer a protective effect against cancer of the endometrium and ovary (IARC, 2007).

Endocrine disrupting activity

Most of the pharmacological actions of estrogens are thought to result from interactions with the classic nuclear estrogen receptor (ER), which is a ligand-activated transcription factor. The ER-ligand complex interacts with DNA binding sites, termed estrogen response elements (EREs), in target genes, and this complex recruits coactivators (or corepressors) and other regulatory proteins that form the active transcription complex (Cited in Salman *et al.*, 1999).

Ethinylœstradiol binds to the estrogen receptor complex and enters the nucleus, activating DNA transcription of genes involved in estrogenic cellular responses. This agent also inhibits 5-α-reductase in epididymal tissue, which lowers testosterone levels and may delay progression of prostatic cancer. In addition to its antineoplastic effects, ethinylœstradiol protects against osteoporosis (http://www.cancer.gov/Templates/drugdictionary.aspx?CdrID=39238).

The 17 α-substituent renders the D-ring of the steroid resistant to 16 α-hydroxylation in the liver, and it thus has a higher oral bioavailability and slower elimination than endogenous ovarian estrogens such as 17 β-E2 (Cited in Salman *et al*., 1999).

The major pathway of ethinylœstradiol metabolism in the liver of humans and animals is 2-hydroxylation, which is presumably catalysed by CYP 3A4 (IARC, 2007).

Combined oral contraceptives tend to have progestagenic, estrogenic, and anti-estrogenic effects which vary according to the target organ and the hormonal content of the formulation (WHO, 1998).

- **Epidemiological studies**

A NOEL (No Observed Effect Level) of 0.3 mg/day was established on the basis of changes in the serum concentrations of corticosteroid-binding globulin (CBG). These results indicate that there is a threshold concentration of estrogen administered orally, below which there is no increase in serum concentrations of CBG. Thus, 0.3 mg/day, equivalent to 5 µg/kg bw/day, was the NOEL for these hormonal effects of estradiol. A further indication that this is the NOEL is that 0.3 mg/day of estradiol administered orally to women did not relieve symptoms of the menopause (WHO, 2000).

Several new studies have investigated the relationship between combined oral contraceptives and hormonal parameters. In four of these, serum levels of SHBG and free testosterone were measured. Levels of SHBG were increased by two- to fourfold and free testosterone levels were reduced by 40–80%, regardless of the combined oral contraceptive regimen used (cited in IARC, 2007). These data suggest the possible involvement of reduced androgenic and estrogenic stimulation of responsive tissue, e.g. the breast. However, all combined oral contraceptives contain estrogens and several of the progestogens used, such as levonorgestrel and norethisterone, have androgenic activity. These studies highlight the possibility of complex interactions with other hormonal systems (cited in IARC, 2007).

- ***in vivo studies***

In animal models, short-term therapy with ethinylœstradiol has been shown to provide long-term protection against breast cancer, mimicking the antitumor effects of pregnancy (http://www.cancer.gov/Templates/drugdictionary.aspx?CdrID=39238).

In vivo studies have established that administration of 17 α-EE2 leads to occupancy of nuclear estrogen receptors (ERs) in both the uterus and liver of rats (Cited in Salman *et al*., 1999).

Most of the attention currently focused on endocrine-active chemicals is directed to their effects on the development of offspring exposed to them in utero or during the neonatal period. Pregnant Crj:CD(SD)IGS rats were given ethinylœstradiol (EE2) orally in doses of 0.5–50 µg/kg/day from gestational day 7 to postnatal day 18, and their offspring were examined for its effects. At 50 µg/kg cleft phallus was observed in almost all of the female offspring, and slight retardation of body weight gain was detected in both sexes. At 15–17 weeks of age the animals with cleft phallus could copulate and had fertility comparable to the control group. At 6 months of age, on the other hand, 6/8 of the female offspring at 50 µg/kg exhibited abnormal cyclicity, including persistent estrus, and histological examination revealed follicular cysts and absence of corpora lutea in the ovaries of the rats with persistent estrus. The results suggest that observation of cyclicity at 6 months old is able to detect possible delayed ovarian dysfunction induced by perinatal exposure to chemicals (Sawaki *et al.,* 2003).

17 α-Ethinylœstradiol (EE2) is thought to contribute to the feminisation of fish exposed to wastewater effluents. During laboratory exposure studies of fish to EE2, it was observed that the estrogen in the aquarium water was rapidly transformed (within 10 min) to mono- and di-brominated A-ring products. Exposure of roach (*Rutilis rutilus*) to 30 ng/L EE2 resulted in accumulation of dibrominated EE2 in ovaries (apparent bioconcentration factor, BCF 130) and liver (apparent BCF 7894) at concentrations which were 18-67-fold greater than the test EE2 compound. The estrogenic activities of brominated EE2 compounds were assayed in an *in vitro* yeast recombinant estrogen receptor transcription screening test (YES). All the brominated products of EE2 were estrogenic, however monobrominated isomers of EE2 were 18-105-fold less estrogenic, and dibrominated EE2 2058-fold less active in the YES than EE2 itself (Flores *et al*., 2008).

A study of Shved *et al.* (2008) evaluated whether effects of environmental estrogens on fish growth and reproduction may be mediated via modulating the growth hormone (GH)/insulin-like growth factor I (IGF-I) system. To this end, developing male and female monosex populations of tilapia were exposed to 17 α-ethinylœstradiol (EE2) at 5 and 25 ng EE2/L water from 10-day postfertilization (DPF) until 100 DPF. Results provide evidence that EE2 at environmentally relevant concentrations is able to interfere with the GH/IGF-I system in bony fish and that the impairing effects of estrogens reported on fish growth and reproductive functions may rather result from a cross talk between the sex steroid and the IGF-I system than being purely toxicological (Shved *et al*., 2008).

Estradiol- or diethylstilbestrol-induced growth of cultured proximal renal tubular cells could be inhibited by ethinylœstradiol. Expression of estrogen-responsive protooncogene (c- myc, c- fos, and c- jun) RNA and protein in kidneys was reduced in animals treated concomitantly relative to that found in animals treated with estradiol or diethylstilbestrol. The authors concluded that ethinylœstradiol interferes with estrogen receptor-mediated mitogenic pathways, preventing gene dysregulation and tumour development. This effect does not appear to be due to differential binding to estrogen receptors by estrogenic substances (Li *et al*., 1998).

Treatment of male Syrian hamsters with estradiol or ethinylœstradiol for two or four weeks altered the intensity, distribution, and subcellular location of immunoreactivity to catechol-O-methyltransferase (WHO, 2000).

The effects, on the phenotypic changes induced in the endocrine/reproductive system by perinatal exposure to an estrogen agonist during a critical period for brain sexual differentiation in rats, of two diets, differing in phytoestrogen content, was investigated. Ethinylœstradiol (EE2) was mixed at a concentration of 0.5 ppm into two diets: CRF-1, a standard rodent diet containing soybean-derived phytoestrogens; and a soy-free (SF) diet. These diets were provided to maternal Sprague-Dawley rats during gestational day 15 to postnatal day 10. Growth suppression of offspring was evident with EE2 especially during the exposure period and was slightly enhanced with the SF diet. On the other hand, most of the female offspring exposed to EE2 with CRF-1 showed early onset of vaginal opening, strong irregularity in estrous cycle (persistent estrus) and profound histopathological alterations, such as multifollicular ovaries, endometrial hypertrophy, and diffuse hyperplasia of the anterior pituitary. These EE2-induced changes were much less pronounced with the SF diet. The

results thus demonstrated differential effects of perinatal EE2 depending on the basal diet used, with enhancement of typical estrogenic responses in females by potential soybean-derived factor(s) (Masutomi *et al*., 2004).

In a transgenic zebrafish model which use a luciferase reporter gene stably introduced, EE2 was the most potent (xeno)estrogen tested and was 100 times more potent than E2 (Legler *et al.*, 2002).

- ***in vitro* studies**

17α-EE2 display the same potency than 17 β-E2 to induce vitellogenin from cultured carp or trout hepatocytes (Segner *et al.,* 2003).
In an *in vitro* estrogen receptor (ER)-mediated chemically activated luciferase gene expression (ER-CALUX) assay, which uses T47D human breast cancer cells stably transfected with an ER-mediated luciferase gene construct, EE2 and E2 were equipotent to induce luciferase expression (Legler *et al*, 2002). The same observations were reported by other authors with a similar reporter gene assay (Wilson *et al.*, 2004).

In cell-free systems with ER from rats, humans, and other sources, it has been established that 17α-EE2 and 17β-E2 have essentially identical affinities for the receptor (Scippo *et al.,* 2002; Segner *et al.,* 2003), and more recently it has been shown that the DNA binding properties of the ER liganded with the two compounds are also very similar (Cited in Salman *et al*., 1999).

Exposure source

17α-Ethinylœstradiol (EE_2) is a synthetic estrogen identified in sewage effluents, surface water and groundwater (Streck, 2009).

The analysis of river water in the Netherlands showed that they contained 0.3 ng/L ethinylœstradiol. A study in Germany of the river water showed that they contained 1- 4 ng/L ethinylœstradiol (Miyamoto and Klein, 1998).

Kolpin *et al.* (2002) reported median and maximal concentration of 17 α–ethinylœstradiol in streams sampled in US of 73 ng/L and 831 ng/L, respectively.

The effluent of four sewage treatment plants (STP) and eight surface water samples from the river Rhine in Germany and two smaller rivers were monitored for the hormones estradiol (E2) and ethinylœstradiol (EE2). Median concentrations for E2 and EE2 in effluent samples were 12 and 1.8 ng/L, respectively. Median concentrations for E2 and EE2 in surface water were 4.0 and 0.7 ng/L, respectively. The highest oestrogen concentrations were found in the effluent of the lagoon, equipped with very basic means of wastewater treatment (Hintemann *et al.,* 2006).

In an influent sample of the Ebro river (in Spain), EE2 was detected at a concentration of 160 ng/L (pedrouzo *et al.*, 2009).

Miège *et al.* (2009) reported concentration of 17 α–ethinylœstradiol in influent and effluent of the dissolved phase of wastewater-treatment plants with activated sludge processes. Concentrations in influent range from 0,4 ng/L to 70 ng/L with a mean of 4,2 ng/L. Concentrations in effluent range from 0,2 ng/L to 5 ng/L with a mean of 0,9 ng/L (Miège *et al.,* 2009).

Level of exposure

A consortium of Austrian scientists (ARCEM) carried out a multidisciplinary environmental study on Austrian surface and ground waters including chemical monitoring, bioindication, risk assessment and risk management for selected endocrine disrupters: 17 β-estradiol, estriol, estrone, 17 α-ethinylœstradiol, 4-nonylphenol, 4-nonylphenol ethoxylates (4-NP1EO, 4-NP2EO) and their degradation products, octylphenol, octylphenol ethoxylates (OP1EO, OP2EO) as well as bisphenol

A. For humans, exposure via either drinking water abstraction (ground water) or fish consumption was considered. The exposure levels of the compounds under study were below those considered to result in human health risks. Likewise, for bisphenol A and octylphenols, there was no indication for risk posed upon the aquatic environment (fish). However, nonylphenol or 17 α-ethinylœstradiol exposure along with results of bioindication suggest a borderline estrogenic activity in a considerable number of surface waters. Consequently the emissions of these substances into the surface waters affected have to be reduced (Bursch *et al.,* 2004).

TDI

NOEL = 0.3 mg/day (WHO, 2000). With a safety factor of 100, this can leads to an hypothetical TDI of 0.003 mg/day.

Estrogenic potency

- Relative binding affinity

Relative binding activity of EE2 is similar (100 %) compared to E2

- Uterotrophic activity

Ethinylœstradiol at 0.3 or 1 µg/kg /day significantly increase both uterine wet and blotted weights in uterotrophic assay using Sprague-Dawley immature female rats (Kim *et al.,* 2005).

%TDI

The hypothetical TDI of 3 µg/day is chosen as toxicological endpoint.
Two scenarios are considered as exposure endpoint

1) Exposure via contraceptive pill is estimated to be 50 µg/day (Oral contraceptive preparations containing 50 µg of ethinylœstradiol (WHO, 2000).
2) Exposure via consumption of water is estimated to be 2.7 ng/day (1,8 ng/L X 1,5 L) on basis of concentrations found in surface water in Europe with the following assumption extrapolation of concentrations of ethinylœstradiol found in surface water to consumption water.

In scenario 1, %TDI is 1666%
In scenario 2, %TDI is 0.09%.

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