Minnesota Department of Health

Health Based Guidance for Water
Health Risk Assessment Unit, Environmental Health Division
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Toxicological Summary for: Mestranol

CAS: **72-33-3**

Synonyms: 3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol; (17alpha)-3-Methoxy-19-norpregna-1,3,5(10)-trien-20-yn-17-ol; Ethinylestradiol 3-methyl ether; 17alpha-Ethinylestradiol 3-methyl ether, EEME, MeEE

Mestranol (MeEE) is the 3-methyl ether of 17α-ethinylestradiol (EE2). MeEE is not initially biologically active as a contraceptive hormone but it is rapidly demethylated in the liver to form the biologically active EE2. A dose of 50 µg of MeEE is considered to be bioequivalent to 35 µg of EE2 as a result of a 70% conversion efficiency in the liver (e.g., 35 µg/0.70 = 50 µg) (Brody et al. 1989). The relative bioequivalence is the basis for the different contraceptive dose levels for MeEE and EE2 used to achieve the same effect in adult humans.

HBVs are available for 17α-ethinylestradiol (EE2). The Minnesota Department of Health (MDH) recommends adjusting the reference doses (RfDs) derived for EE2 by a factor of 0.7 to account for relative bioequivalence and then deriving Risk Assessment Advice (RAAs) for MeEE based on the adjusted RfDs.

The following are MeEE RfDs, based on RfDs for EE2 adjusted for relative bioequivalence according to the following equation:

$RfD_{MeEE} = RfD_{EE2}/0.7$

Acute RfD_{MeEE} = Not derived. Insufficient data.

Short-term RfD_{MeEE} = Short-term $RfD_{EE2}/0.7 = 1.7 \times 10^{-7}/0.7 = 2.4 \times 10^{-7}$ mg/kg-d

Subchronic RfD_{MeEE} = Subchronic $RfD_{EE2}/0.7 = 1.4 \times 10^{-8}/0.7 = 2.0 \times 10^{-8}$ mg/kg-d

Chronic RfD_{MeEE} = Chronic $RfD_{EE2}/0.7 = 1.4 \times 10^{-8}/0.7 = 2.0 \times 10^{-8}$ mg/kg-d

The following recommendation represents Risk Assessment Advice (RAA) for mestranol using adjusted RfDs and the same duration-based algorithms applied to derive 17α-ethinylestradiol HBVs:

Acute $nRAA_{MeEE}$ = Not derived. Insufficient data.

Short-term $nRAA_{MeEE}$ = 0.0007 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Short-term Intake Rate, L/kg-d)}}$$

$$= \frac{(2.4 \times 10^{-7} \text{ mg/kg-d}) \times (0.8^*) \times (1000 \text{ µg/mg})}{(0.285 \text{ L/kg-d})}$$

= 0.00067 rounded to **0.0007 µg/L**

Additivity endpoints: Developmental (E), Female reproductive system (E), Male reproductive system (E)*

Subchronic $nRAA_{MeEE}$ = 0.0002 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Subchronic Intake Rate, L/kg-d)}}$$

$$= \frac{(2.0 \times 10^{-8} \text{ mg/kg-d}) \times (0.8^*) \times (1000 \text{ µg/mg})}{(0.070 \text{ L/kg-d})}$$

= 0.00023 rounded to **0.0002 µg/L**

Additivity endpoints: Developmental*

Chronic $nRAA_{MeEE}$ = subchronic $nRAA_{MeEE}$ = 0.0002 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Chronic Intake Rate, L/kg-d)}}$$

$$= \frac{(2.0 \times 10^{-8} \text{ mg/kg-d}) \times (0.8^*) \times (1000 \text{ µg/mg})}{(0.044 \text{ L/kg-d})}$$

= 0.00036, rounded to 0.0004 µg/L

Additivity endpoints: Developmental*

The Chronic nRAA must be protective of the acute, short-term, and subchronic exposures that occur within the chronic period and therefore, the Chronic nRAA is set equal to the Subchronic nRAA of 0.0002 µg/L. Additivity endpoints: Developmental

Cancer $cRAA_{MeEE}$ – Not derived. Non-cancer HBVs are considered protective.

*For additional information on the derivation of RfDs, RSC and HBVs for 17α-ethinylestradiol, critical/co-critical effects, and relevant additivity endpoints see: Toxicological Summary for: 17α-Ethinylestradiol (http://www.health.state.mn.us/divs/eh/risk/guidance/gw/ethinylestsumm.pdf)

Volatile: No

Summary of Guidance Value History:

The RAA values for mestranol are new. No previous values exist.

Summary of toxicity testing for health effects identified in the Health Standards Statute **(144.0751):**

Even if testing for a specific health effect was not conducted for this chemical, information about that effect might be available from studies conducted for other purposes. MDH has considered the following information in developing health protective guidance.

	Endocrine	Immunotoxicity	Development	Reproductive	Neurotoxicity
Tested for specific effect?	Yes	Yes	Yes	Yes	Yes
Effects observed?	Yes[1]	Yes[1]	Yes[1]	Yes[1]	Yes[1]

Comments on extent of testing or effects:

[1]The pharmacological activity of mestranol is due to the conversion to 17α ethinylestradiol in the liver. See the Summary Sheet for 17α ethinylestradiol for information on the endocrine, immunotoxicity, developmental, reproductive and neurotoxicity effects at Toxicological Summary for: 17α-Ethinylestradiol (http://www.health.state.mn.us/divs/eh/risk/guidance/gw/17aethinyl.pdf)

Resources Consulted During Review:

Actavis Pharma Inc. (2014). FDA-Approved Drug Label for Norinyl 1+50 - norethindrone and mestranol.

Australian Natural Resource Management Ministerial Council; Environmental Protection and Heritage Council; and National Health and Medical Research Council. (2008). Australian Guidelines for Water Recycling. Augmentation of Drinking Water Supplies. from http://www.environment.gov.au/system/files/resources/9e4c2a10-fcee-48ab-a655-c4c045a615d0/files/water-recycling-guidelines-augmentation-drinking-22.pdf

Borgert, C. J., LaKind, J. S., & Witorsch, R. J. (2003). A critical review of methods for comparing estrogenic activity of endogenous and exogenous chemicals in human milk and infant formula. *Environ Health Perspect* 111(8): 1020-1036.

Brody, S. A., Turkes, A., & Goldzieher, J. W. (1989). Pharmacokinetics of three bioequivalent norethindrone/mestranol-50 micrograms and three norethindrone/ethinyl estradiol-35 micrograms OC formulations: are "low-dose" pills really lower? *Contraception* 40(3): 269-284.

Canadian Drug Products Monograph. (2011). *Product Monograph. FEMHRT and FEMHRT LO. Estrogen-progestin combination. Warner Chilcott Canada Co.,*. Toronto, Ontario.

Cao, J., Rebuli, M. E., Rogers, J., Todd, K. L., Leyrer, S. M., Ferguson, S. A., & Patisaul, H. B. (2013). Prenatal bisphenol A exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. *Toxicol Sci* 133(1): 157-173.

Capel-Edwards, K., D.E. Hall, A.G. Sansom, (1971). Hematological changes observed in female beagle dogs given ethynylestradiol. *Toxicology and Applied Pharmacology* 20: 319-326.

Curtis, E. M. (1964). Oral-Contraceptive Feminization of a Normal Male Infant: Report of a Case. *Obstet Gynecol* 23: 295-296.

Delclos, K. B., Camacho, L., Lewis, S. M., Vanlandingham, M. M., Latendresse, J. R., Olson, G. R., . . . Thorn, B. T. (2014). Toxicity evaluation of bisphenol A administered by gavage to Sprague Dawley rats from gestation day 6 through postnatal day 90. *Toxicol Sci* 139(1): 174-197.

Delclos, K. B., Weis, C. C., Bucci, T. J., Olson, G., Mellick, P., Sadovova, N., . . . Newbold, R. R. (2009). Overlapping but distinct effects of genistein and ethinyl estradiol (EE(2)) in female Sprague-Dawley rats in multigenerational reproductive and chronic toxicity studies. *Reprod Toxicol* 27(2): 117-132.

Ferguson, S. A., Delclos, K. B., Newbold, R. R., & Flynn, K. M. (2003). Dietary ethinyl estradiol exposure during development causes increased voluntary sodium intake and mild maternal and offspring toxicity in rats. *Neurotoxicol Teratol* 25(4): 491-501.

Ferguson, S. A., Law, C. D., & Abshire, J. S. (2012). Developmental treatment with bisphenol A causes few alterations on measures of postweaning activity and learning. *Neurotoxicol Teratol* 34(6): 598-606.

Ferguson, S. A., Law, C. D., Jr., & Abshire, J. S. (2011). Developmental treatment with bisphenol A or ethinyl estradiol causes few alterations on early preweaning measures. *Toxicol Sci* 124(1): 149-160.

Ferguson, S. A., Law, C. D., & Kissling, G. E. (2014). Developmental treatment with ethinyl estradiol, but not bisphenol A, causes alterations in sexually dimorphic behaviors in male and female Sprague Dawley rats. *Toxicol Sci* 140(2): 374-392.

Guo, T. L., Germolec, D. R., Musgrove, D. L., Delclos, K. B., Newbold, R. R., Weis, C., & White, K. L., Jr. (2005). Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague-Dawley rats following developmental and adult exposures. *Toxicology* 211(3): 207-219.

He, Z., Paule, M. G., & Ferguson, S. A. (2012). Low oral doses of bisphenol A increase volume of the sexually dimorphic nucleus of the preoptic area in male, but not female, rats at postnatal day 21. *Neurotoxicol Teratol* 34(3): 331-337.

Hines, R. N. (2007). Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* 21(4): 169-175.

Hines, R. N. (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther* 118(2): 250-267.

Hotchkiss, C. E., Weis, C., Blaydes, B., Newbold, R., & Delclos, K. B. (2008). Multigenerational exposure to ethinyl estradiol affects bone geometry, but not bone mineral density in rats. *Bone* 43(1): 110-118.

Howdeshell, K. L., Furr, J., Lambright, C. R., Wilson, V. S., Ryan, B. C., & Gray, L. E., Jr. (2008). Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. *Toxicol Sci* 102(2): 371-382.

HSDB. (2011). Hazardous Substances Database. U.S. National Library of Medicine, TOXNET. Mestranol. Retrieved February 2016, from http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~Ys45bR:1

HSDB. (2012). Hazardous Substances Database. U.S. National Library of Medicine, TOXNET. Ethinylestradiol. Retrieved December, 2014, from http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~ecUdyv:1

International Agency for Research on Cancer (IARC). (1979). IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Sex Hormones (II). Ethinyloestradiol. (Vol. Vol. 21). Lyon, France.

International Agency for Research on Cancer (IARC) (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Postmenopausal Estrogen Therapy. Lyon, France, IARC. Vol. 72.

International Agency for Research on Cancer (IARC). (2007). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Combined Estrogen−Progestogen Contraceptives and Combined Estrogen−Progestogen Menopausal Therapy (Vol. Vol. 91). Lyon, France: IARC.

International Agency for Research on Cancer (IARC). (2011a). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Part A: Pharmaceuticals. Estrogen-Only Menopausal Therapy (Vol. Vol. 100). Lyon, France: IARC.

International Agency for Research on Cancer (IARC) (2011b). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Part A: Combined Estrogen-Progestogen Contraceptives. Lyon, France, IARC. Vol. 100.

JECFA. (2000). Toxicological Evaluation of Certain Veterinary Drug Residues in Food: WHO Food Additives Series 43: Production Aids: Estradiol-17beta, Progesterone, and Testosterone. In Prepared by the fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Ed.).

Kendig, E. L., Buesing, D. R., Christie, S. M., Cookman, C. J., Gear, R. B., Hugo, E. R., . . . Belcher, S. M. (2012). Estrogen-like disruptive effects of dietary exposure to bisphenol A or 17alpha-ethinyl estradiol in CD1 mice. *Int J Toxicol* 31(6): 537-550.

Koukouritaki, S. B., Manro, J. R., Marsh, S. A., Stevens, J. C., Rettie, A. E., McCarver, D. G., & Hines, R. N. (2004). Developmental expression of human hepatic CYP2C9 and CYP2C19. *J Pharmacol Exp Ther* 308(3): 965-974.

Latendresse, J. R., Bucci, T. J., Olson, G., Mellick, P., Weis, C. C., Thorn, B., . . . Delclos, K. B. (2009). Genistein and ethinyl estradiol dietary exposure in multigenerational and chronic studies induce similar proliferative lesions in mammary gland of male Sprague-Dawley rats. *Reprod Toxicol* 28(3): 342-353.

Laurenzana, E. M., Weis, C. C., Bryant, C. W., Newbold, R., & Delclos, K. B. (2002). Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor alpha expression. *Food Chem Toxicol* 40(1): 53-63.

Madhavapeddi, R., & Ramachandran, P. (1985). Side effects of oral contraceptive use in lactating women--enlargement of breast in a breast-fed child. *Contraception* 32(5): 437-443.

Mandrup, K. R., Hass, U., Christiansen, S., & Boberg, J. (2012). Perinatal ethinyl oestradiol alters mammary gland development in male and female Wistar rats. *Int J Androl* 35(3): 385-396.

Mandrup, K. R., Jacobsen, P. R., Isling, L. K., Axelstad, M., Dreisig, K., Hadrup, N., . . . Boberg, J. (2013). Effects of perinatal ethinyl estradiol exposure in male and female Wistar rats. *Reprod Toxicol* 42: 180-191.

Marriq, P., & Oddo, G. (1974, Nov 30-Dec 14). [Letter: Gynecomastia in the newborn induced by maternal milk? An unusual complication of oral contraceptives]; article in French. *Nouv Presse Med.* from as cited by Drugs.com, last updated 5/5/2015; http://www.drugs.com/breastfeeding/contraceptives-oral-combined.html

Mashchak, C. A., Lobo, R. A., Dozono-Takano, R., Eggena, P., Nakamura, R. M., Brenner, P. F., & Mishell, D. R., Jr. (1982). Comparison of pharmacodynamic properties of various estrogen formulations. *Am J Obstet Gynecol* 144(5): 511-518.

Minnesota Department of Health (MDH). (2011). MDH Health Risk Assessment Methods to Incorporate Human Equivalent Dose Calculations into Derivation of Oral Reference Doses. from http://www.health.state.mn.us/divs/eh/risk/guidance/hedrefguide.pdf

National Toxicology Program (NTP). (2004). *Final Report. Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage*. TherImmune Research Corporation No. 7244-600.

National Toxicology Program (NTP). (2010a). *Multigenerational Reproductive Toxicology Study Of Ethinyl Estradiol (Cas No. 57-63-6) In Sprague-Dawley Rats* Retrieved from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/TR547.pdf.

National Toxicology Program (NTP). (2010b). *NTP Technical Report on the Toxicology and Carcinogenesis Study of Ethinyl estradiol (CAS No. 57-63-6) in Sprague-Dawley Rats.* . Retrieved from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/TR548.pdf/. .

National Toxicology Program (NTP). (2011). *Report on Carcinogens, Twelfth Edition. Estrogens, Steroidal*. Retrieved from http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/EstrogensSteroidal.pdf.

Nilsson, S., Mellbin, T., Hofvander, Y., Sundelin, C., Valentin, J., & Nygren, K. G. (1986). Long-term follow-up of children breast-fed by mothers using oral contraceptives (reviewed abstract only). *Contraception* 34(5): 443-457.

Nilsson, S., Nygren, K. G., & Johansson, E. D. (1978). Ethinyl estradiol in human milk and plasma after oral administration. *Contraception* 17(2): 131-139.

Norgaard, M., Wogelius, P., Pedersen, L., Rothman, K. J., & Sorensen, H. T. (2009). Maternal use of oral contraceptives during early pregnancy and risk of hypospadias in male offspring (reviewed abstract only). *Urology* 74(3): 583-587.

OEHHA. (1992). *Expedited Cancer Potency Values and Proposed Regulatory Levels for Concern for Certain Proposition 65 Carcinogens*.

OEHHA. (2001). No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen Di(2-ethylhexyl)phthalate. from http://www.oehha.ca.gov/prop65/law/pdf_zip/dehpnsrl.pdf

Pillon, D., Cadiou, V., Angulo, L., & Duittoz, A. H. (2012). Maternal exposure to 17-alpha-ethinylestradiol alters embryonic development of GnRH-1 neurons in mouse. *Brain Res* 1433: 29-37.

Rebuli, M. E., Cao, J., Sluzas, E., Delclos, K. B., Camacho, L., Lewis, S. M., . . . Patisaul, H. B. (2014). Investigation of the effects of subchronic low dose oral exposure to bisphenol A (BPA) and ethinyl estradiol (EE) on estrogen receptor expression in the juvenile and adult female rat hypothalamus. *Toxicol Sci* 140(1): 190-203.

Ryan, B. C., Hotchkiss, A. K., Crofton, K. M., & Gray, L. E., Jr. (2010). In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. *Toxicol Sci* 114(1): 133-148.

Sandoz Inc. (2014). *FDA-Approved Drug Label for Altavera - levonorgestrel and ethinyl estradiol*.

Sawaki, M., Noda, S., Muroi, T., Mitoma, H., Takakura, S., Sakamoto, S., & Yamasaki, K. (2003). In utero through lactational exposure to ethinyl estradiol induces cleft phallus and delayed ovarian dysfunction in the offspring. *Toxicol Sci* 75(2): 402-411.

Schardein, J. L. (1980). Studies of the components of an oral contraceptive agent in albino rats. I. Estrogenic component. *J Toxicol Environ Health* 6(4): 885-894.

Schmider, J., Greenblatt, D. J., von Moltke, L. L., Karsov, D., Vena, R., Friedman, H. L., & Shader, R. I. (1997). Biotransformation of mestranol to ethinyl estradiol in vitro: the role of cytochrome P-450 2C9 and metabolic inhibitors. *J Clin Pharmacol* 37(3): 193-200.

Siddique, Y. H., Beg, T., & Afzal, M. (2005). Genotoxic potential of ethinylestradiol in cultured mammalian cells. *Chem Biol Interact* 151(2): 133-141.

Snyder, S., RA Trenholm, EM Snyder, GM Bruce, RC Pleus, and JDC Hemming,. (2008). Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water. In AWWA Research Foundation (Ed.).

Tavassoli, F. A., Casey, H. W., & Norris, H. J. (1988). The morphologic effects of synthetic reproductive steroids on the mammary gland of rhesus monkeys. Mestranol, ethynerone, mestranol-ethynerone, chloroethynyl norgestrel-mestranol, and anagestone acetate-mestranol combinations. *Am J Pathol* 131(2): 213-234.

Tennant, B. C., Balazs, T., Baldwin, B. H., Hornbuckle, W. E., Castleman, W. L., Boelsterli, U., & Kallfelz, F. A. (1981). Assessment of hepatic function in rabbits with steroid-induced cholestatic liver injury. *Fundam Appl Toxicol* 1(4): 329-333.

Twaddle, N. C., Churchwell, M. I., Newbold, R. R., Delclos, K. B., & Doerge, D. R. (2003). Determination using liquid-chromatography-electrospray tandem mass spectroscopy of ethinylestradiol serum pharmacokinetics in adult Sprague-Dawley rats. *J Chromatogr B Analyt Technol Biomed Life Sci* 793(2): 309-315.

U. S. Environmental Protection Agency - Office of Water. (2009). Contaminant Information Sheets for the PCCL Chemicals Considered for CCL3. from http://www2.epa.gov/sites/production/files/2014-05/documents/final-pccl-3-contaminant-information-sheets.pdf

U.S. Environmental Protection Agency - Office of Research and Development. (1988). Recommendations for and Documentation of Biological Values for Use in Risk Assessment. from http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855

U.S. Environmental Protection Agency - Office of the Science Advisor. (2011). Recommended Use of Body Weight¾ as the Default Method in Derivation of the Oral Reference Dose. from http://www.epa.gov/raf/publications/pdfs/recommended-use-of-bw34.pdf

U.S. Food and Drug Administration (FDA). (2015). Drugs@FDA: FDA Approved Drug Products database; search for Estinyl, Lynoral, Feminone historical dosage information. Retrieved June 26, 2015, from http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm

Vosges, M., Braguer, J. C., & Combarnous, Y. (2008). Long-term exposure of male rats to low-dose ethinylestradiol (EE2) in drinking water: effects on ponderal growth and on litter size of their progeny. *Reprod Toxicol* 25(2): 161-168.

Warner Chilcott (US), L. (2012). Lo Loestrin Fe, approved drug label. from http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=c33072cf-625d-4b4a-981e-ec049c5d78aa

Wogelius, P., Horvath-Puho, E., Pedersen, L., Norgaard, M., Czeizel, A. E., & Sorensen, H. T. (2006). Maternal use of oral contraceptives and risk of hypospadias - a population-based case-control study (reviewed abstract only). *Eur J Epidemiol* 21(10): 777-781.

Yadav, M., & Volkar, J. (2013). Female Contraception. Mechanism of Action of Hormonal Contraceptives. Cleveland Clinic Center for Continuing Education. Disease Management. Retrieved 3/17/2016, from http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/womens-health/female-contraception/

Yanagimachi, R., & Sato, A. (1968). Effects of a single oral administration of ethinyl estradiol on early pregnancy in the mouse. *Fertil Steril* 19(5): 787-801.

Yasuda, Y., Kihara, T., & Nishimura, H. (1981). Effect of ethinyl estradiol on development of mouse fetuses. *Teratology* 23(2): 233-239.