

Reproductive/developmental toxicity

- Broadest definition: any adverse effect on any aspect of male or female sexual structure or function, or on the conceptus or on lactation, which would interfere with the production of development of normal offspring which could be reared to sexual maturity, capable in turn of reproducing the species.
- Two major classes:
 - Reproductive toxicity -Effects on sexual behavior and fertility in males and non-pregnant females
 - Developmental toxicity-abnormal structure or functional development following exposure of pregnant or lactating females

More definitions

- Teratogenicity-ability to cause gross structural malformations
- Behavioral teratogenicity-ability to cause abnormal mental development, to impair intellectual development or behavior of offspring

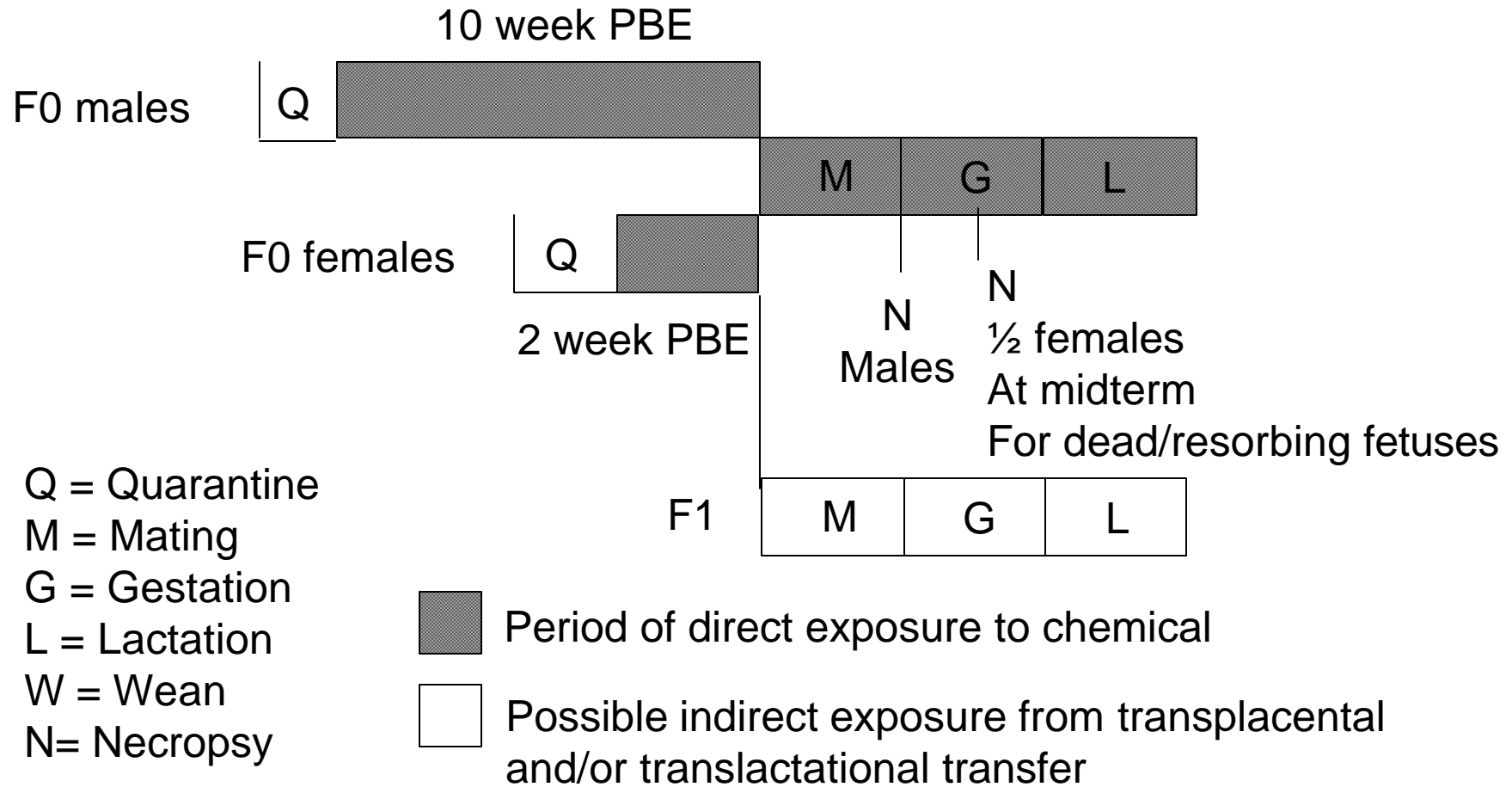
Screening tests in animals for reproductive/developmental toxicity

- Drug testing
 - 1966 FDA Three segment approach

Segment I-Fertility and general reproductive performance segment

- Usually uses rats (20 per group)
 - Young males treated for 60-80 days (spermatogenesis period)
 - Female rats treated for 14 days to cover three estrus cycles
 - Three dose levels (without signs of maternal toxicity)
-
- Information on: breeding, fertility, nidation, parturition, lactation, neonatal effects

Segment I design



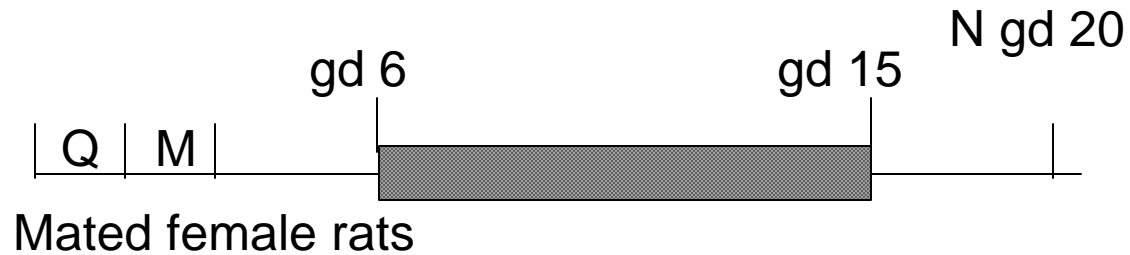
Segment I endpoints

- Fertility index = % matings that result in pregnancy
- gestation index = % pregnancies yielding live litters
- viability index = % animals surviving 4 days
- lactation index = % of animals alive at 4 days that survive the 21 day lactation period
- Pup body weights pnd 4, 7, 14, and 21
- Gross necropsy and histopathology on some parents (both reproductive and non-reproductive organs)
- **Many other possible endpoints**

Segment 2-Teratogenesis segment

- Usually two species: rabbits (12 per group) and rats (20 per group)
- Mated animals are treated during the period of organogenesis (days 6-18 in rabbits, 6-15 in rats)
- Three dose levels
- Pups delivered by Caesarean one day before expected parturition (21 days rat, 31 days rabbit)
- Uterus removed, weighed, and examined for dead or resorbed fetuses
- live pups are weighed, 1/2 examined for skeletal abnormalities, other half for soft tissue abnormalities, histology
- Information provided: embryotoxicity, fetotoxicity, teratogenicity

Segment II design



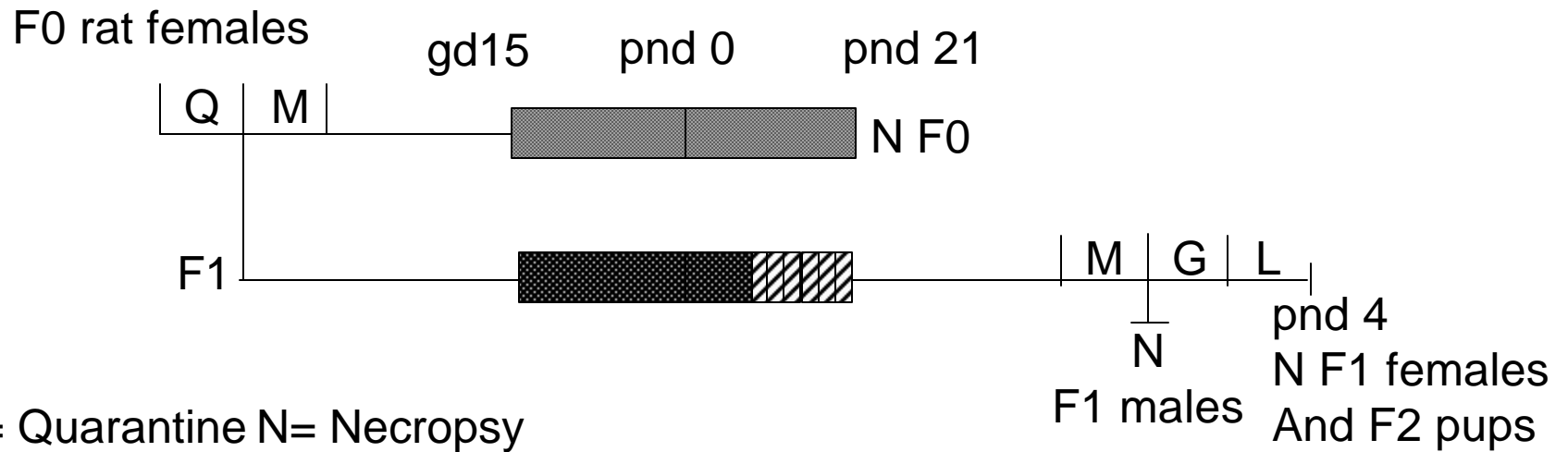
N= Necropsy
gd = gestational day

 Direct exposure to pregnant dams




Segment III – Peri/postnatal segment

- Usually one species (rats)
- Pregnant females (20 per group)
- 2-3 dosages administered from end of organogenesis period through delivery and lactation
- Endpoints: birthweight, survival, growth during first 3 weeks of life, many others

Segment III design



Q = Quarantine N= Necropsy
M = Mating gd = gestational day
L = Lactation

-  Direct exposure to adults
-  Possible indirect exposure from transplacental and/or translactational transfer
-  Direct exposure to offspring if test material is administered via feed or water

Screening tests in animals for reproductive/developmental toxicity

- Drug testing
 - 1966 FDA Three segment approach
 - 1994 International Conference on Harmonization (ICH) guidelines accepted
- Pesticide testing
- Food additives testing
- Industrial chemicals testing

Multigeneration study (EPA, NTP)

- F0 generation: 30 pairs M/F per dose level, at least three dose levels; expose for at least 30-60 days prior to mating, continue exposure through the periods of gestation, birth, and development through the time of weaning, necropsy at pnd 150
- Continue exposure of F1 generation (30 M/F pairs randomly selected) through mating, gestation, birth and postnatal development of F2 generation, necropsy at pnd 150
- Repeat as above for F2 generation leading to F3

Methods for assessing toxic effects on male reproductive system

- Experimental
 - Gross pathology, histology
 - Analysis of sperm-
 - Sperm counts, sperm motility, sperm viability, sperm morphology
- Clinical (infertility clinic)
 - Sperm count, viability, motility, morphology
- Epidemiological

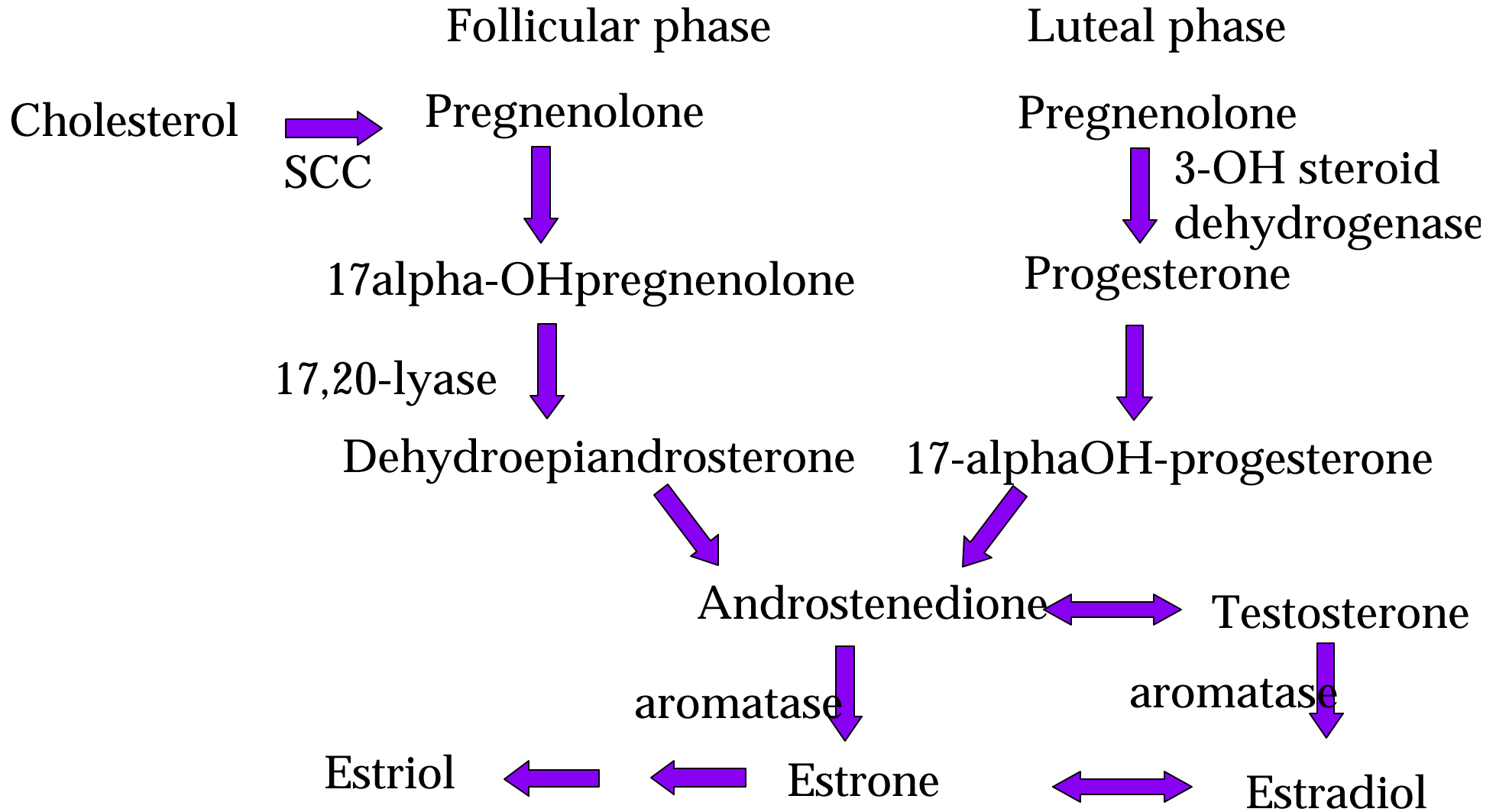
Methods for assessing toxic effects on female reproductive system

- Experimental
 - Gross pathology, histology
 - Analysis of sperm-
 - Sperm counts, sperm motility, sperm viability, sperm morphology
- Clinical (infertility clinic)
 - Sperm count, viability, motility, morphology
- Epidemiological

Classes of reproductive toxicants

- 1. Agents that interfere with the activity of hormones at their receptors
 - Clomiphene and tamoxifen
 - Oral contraceptives
 - Xenoestrogens (genistein and other isoflavones in clover, soybeans, alfalfa, fruits and vegetables)
 - Pesticides (DDT, PCBs, dioxin, kepone)
- 2. Agents that interfere with steroid hormone metabolism
 - Inhibitors: danazol, ketoconazole, metyrapone, aromatase inhibitors
 - Inducers: methoxychlor, heptochlor, chlordane, DDT, and other organochlorine pesticides, dioxin

Pathways of steroid hormone synthesis



Some inhibitors of steroidogenic enzymes

- Cholesterol SCC
 - aminoglutethimide
 - danazol
- Aromatase
 - aminoglutethimide, letrozole
- 17,20-lyase
 - ketoconazole
- 3-OH steroid dehydrogenase
 - danazol

Classes of reproductive toxicants

- 3. Agents that affect Sertoli cells in the testes
 - Dibromochloropropane
 - Monoethylhexylphthalate
 - n-Hexane
 - Tetrahydrocannabinol
- 4. Agents that affect Leydig cell function
 - Cadmium
 - Inhibitors of androgen synthesis

Classes of reproductive toxicants

- 5. Agents that affect germ cell chromosomes/DNA
 - Mercury, lead, cadmium
 - Alkylating agents and other cytotoxic agents (cyclophosphamide, chlorambucil, busulfan, methotrexate, adriamycin, cytosine-arabioside, vincristine, vinblastine)

Drugs that induce male impotence

Class	Agent
Narcotics	Morphine
	Ethanol
Psychotropic	Chlorpromazine
	Diazepam
	Tricyclic antidepressants
	MAO inhibitor
Hypotensives	Methyldopa, clonidine, reserpine
	Guanethidine
Hormones/antagonists	Estrogens
	Cyproteronone

Toxicology of the placenta

- Structure (species differences)
- Serves as lungs, gut, kidney, and exocrine/endocrine glands of developing fetus
- Abundance of drug/xenobiotic metabolizing enzyme systems (qualitative/quantitative differences from maternal liver)
- Ability to concentrate chemicals (protein-rich)
- Ability of chemicals to cross placenta (active transport, lipid diffusion)
- Susceptibility to specific chemical toxicity
 - Examples: heavy metals, organophosphate insecticides, pyrethroids, PCBs, dioxin, drugs of abuse, nicotine, cigarette smoke, opiates, alcohol

Experimental models for placental pharmacology/toxicology studies

- Dually perfused human placental cotyledon
- Models using human trophoblastic tissue
- Placental slices
- Dissected syncytiotrophoblastic tissue
- Cultured human placental villus tissue
- Microvillus membrane vesicles
- Subcellular fractions-microsomes

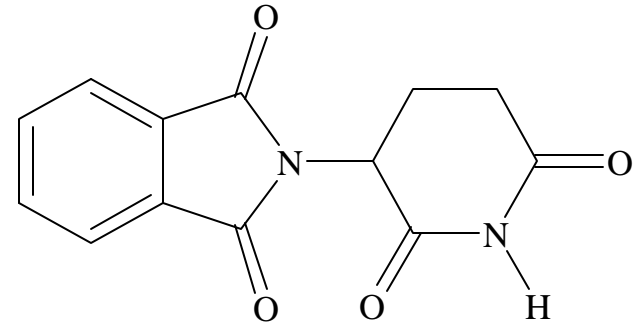
Drugs and pregnancy: a realistic perspective

- Prior to marketing, drugs are almost invariably not tested in pregnant women
- Drug labeling issue: “Do not use this drug unless the potential benefits outweigh risks”
- Exposure to drugs before pregnancy is known: 1 of every 2 pregnancies is unplanned
- Likelihood of drug exposure in early pregnancy is increasing as the age at which women have children increases

Historical perspectives

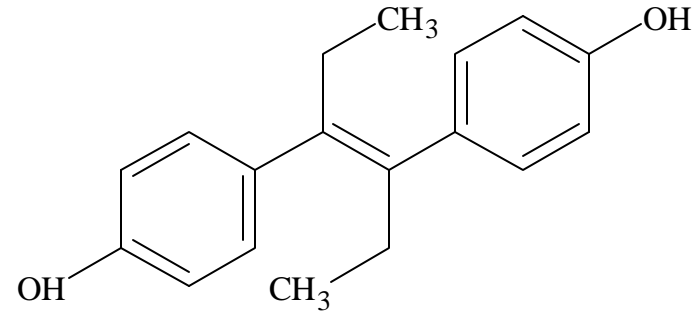
- Early ages- Paracelsus-- the “hybrid theory”
- 1890’s-Darwin-temperature shifts during the incubation of fowl eggs produced malformed chicks
- 1890’s Fere -injection of nicotine, alcohol, and other chemicals into fowl eggs produces malformations
- 1929 Goldstein and Murphy-birth defects and IR exposure
- 1940 Warkany - vitamin A deficiency and chemical exposures produced defects in rat embryos
- 1941 Gregg-relation between Rubella virus exposure during pregnancy and birth defects
- 1961-Lenz and McBride-thalidomide and phocomelia

Thalidomide



- Thalidomide introduced in Europe in 1956 as a sedative-hypnotic and to reduce N/V of pregnancy.
- In 1959, one newborn reported with limb defects; 1960, 30 cases; 1961, 154 cases
- Cases featured phocomelia along with defects of the heart, eye, external ears, intestine, and kidney
- 1961 Lenz and McBride report thalidomide connection: no new cases after mid-1962
- Only other apparent toxicity of thalidomide in humans was peripheral neuritis
- Total number of affected infants in 1950s estimated at 5800
- Risk HIGH: 200 to 300 cases per 1000 exposed pregnancies
- Major outcomes

Diethylstilbestrol (DES)



- Synthetic nonsteroidal estrogen used 1940-1970 in U.S., structural resemblance to estrogen in trans configuration
- Potency comparable to estradiol, orally active, longer half life in body, used to prevent miscarriages by stimulating placental synthesis of estrogen and progesterone
- From 1966-1969, seven cases of clear cell vaginal cancer in young women (ages 15-22) were seen at Massachusetts General Hospital
- Epidemiological study found association with maternal use of DES prior to week 18 of gestation
- Absolute risk very low: 0.14 -1.4 per 1000 exposed but risk of benign growths high (750/1000)

Ethanol

- Biblical references to teratogenic effects of ethanol
- Probably the most common teratogen
- Elicits the “Fetal alcohol syndrome”
- Occurrence of FAS: fullblown 1-2 per 1000 live births, partial 3-5 per 1000
- High risk in newborns of alcoholics (depends on dose, 6oz is high risk)
- Smallest quantity associated with full blown FAS: 75 ml, 2.5 oz daily (5 glasses of wine) but risk of isolated FAS components at intermediate doses
- There is currently no evidence that 15 ml of alcohol daily (0.5 oz = 1 glass of wine) has any adverse effect
- Complicating factors: poor nutrition, cigarette smoking

Molecular and pharmacogenetic aspects of ethanol metabolism

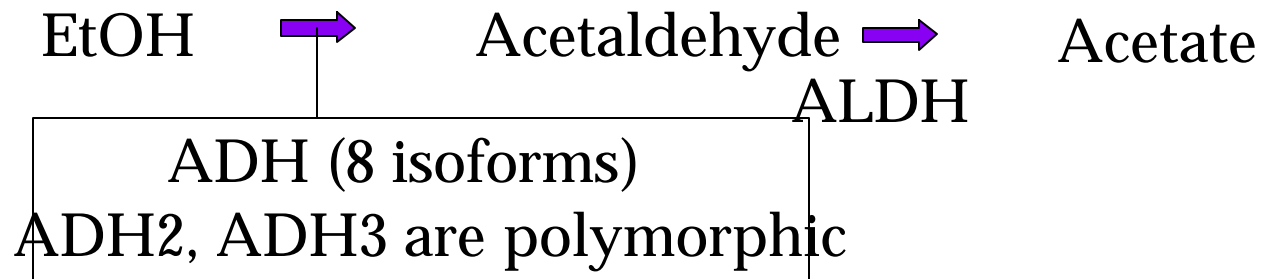
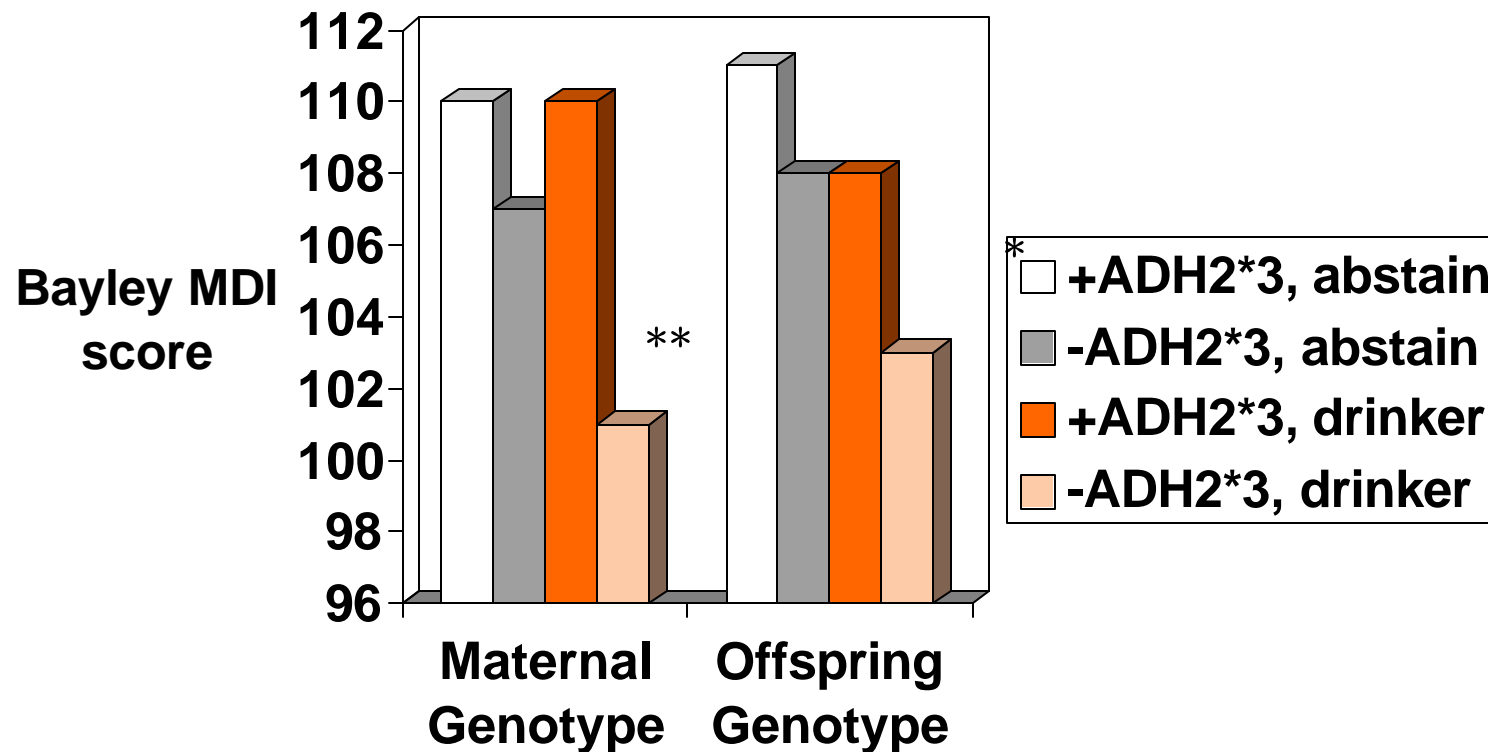


Table 1

Kinetic characteristics of two ADH2 alleles

ADH genotype	ADH isozyme	K _m (mM ethanol)	V _{max} (min ⁻¹)
ADH2*1	β ₁ β ₁	0.05	9
ADH2*3	β ₃ β ₃	36	300

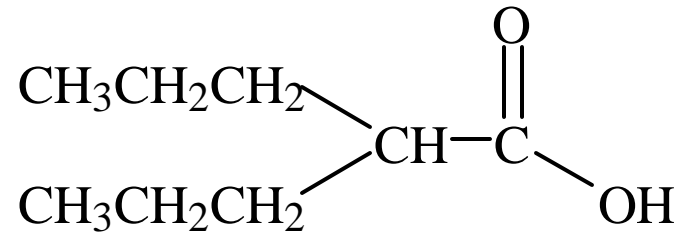
ADH2 genotype and child development after ethanol-exposed pregnancies



*p<0.05

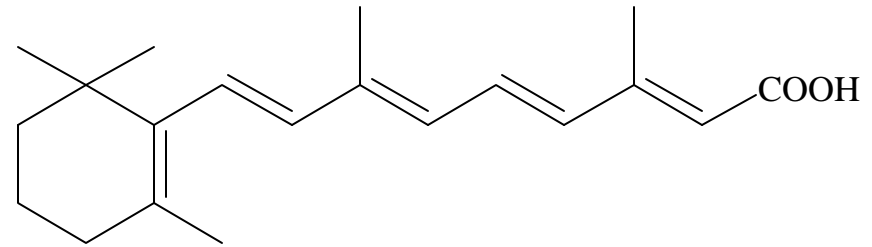
**p<0.01

Valproic acid



- Anticonvulsant marketed in US 1978
- Debate about whether teratogenicity is caused by the disease (epilepsy)
- Teratogenicity first revealed by an epidemiological case control study linking spina bifida cases with use of VPA
- 146 cases, 9 had used VPA
- Risk: Low, 12 per 1000 exposed pregnancies
- “Neural tube defects”

Isotretinoin



- Derivative of vitamin A (Accutane)
- Was marketed in the US in 80's for severe cystic acne
- Labelling gave strong warnings against use during pregnancy restrictive requirements for prescription to women of child bearing age.
- Cases began to be reported with characteristic teratogenic manifestations of retinoids: “retinoid embryopathy”
 - defects of ears, heart, brain, thymus
- Risk: HIGH 18 out of 115 exposed pregnancies were spontaneously aborted, 32 had at least 1 malformation

Bendectin

- Mixture of doxylamine + pyridoxine prescribed for nausea and vomiting of early pregnancy
- Used by a very high percentage of pregnant women (40%)
- Individuals who gave birth to baby's with birth defects filed lawsuits in 1970s against manufacturer claiming it was teratogenic
- Epidemiological studies indicated that it was not
- Withdrawn in 1982

Drugs with proven teratogenic effects in humans when used at clinically recommended doses

- Aminopterin, methotrexate
- Angiotensin-converting enzyme inhibitors
- Antithyroid drugs (propylthiouracil, methimazole)
- Carbamazepine
- Cyclophosphamide
- Danazol, other androgenic drugs
- Diethylstilbestrol
- Hypoglycemic drugs
- Lithium
- Misoprostol
- Nonsteroidal antiinflammatory drugs
- Paramethadione, trimethadione
- Phenytoin
- Psychoactive drugs (barbiturates, opioids, and benzodiazpines)
- Systemic retinoids (isotretinoin, etretinate)
- Tetracycline
- Thalidomide
- Valproic acid
- Warfarin

Briggs et al. 1994

Animal studies

- Segment 2 teratology study
 - Groups of animals are exposed to a range of doses during the period of organ development and effects are compared to untreated animals
- 24 of the 25 known human drugs or drug classes that are known to be teratogenic tested positive in one or more animal species
- 3300 chemicals tested in animals
- 63% non teratogenic, 7% positive in 1 species, 21% positive in multiple species

Alternative testing methodologies: examples

- Mouse ovarian tumor (inhibition of cell attachment to disks)
 - Concordance:
 - Sensitivity (19/31)
 - Specificity (7/13)
- Micromass culture (midbrain and limb bud cells dissociated from gd13 rat embryos, grown in culture for 5 days, cell proliferation and markers of differentiation)
 - Concordance
 - Sensitivity 25/27
 - Specificity 17/19

Post-marketing drug surveillance

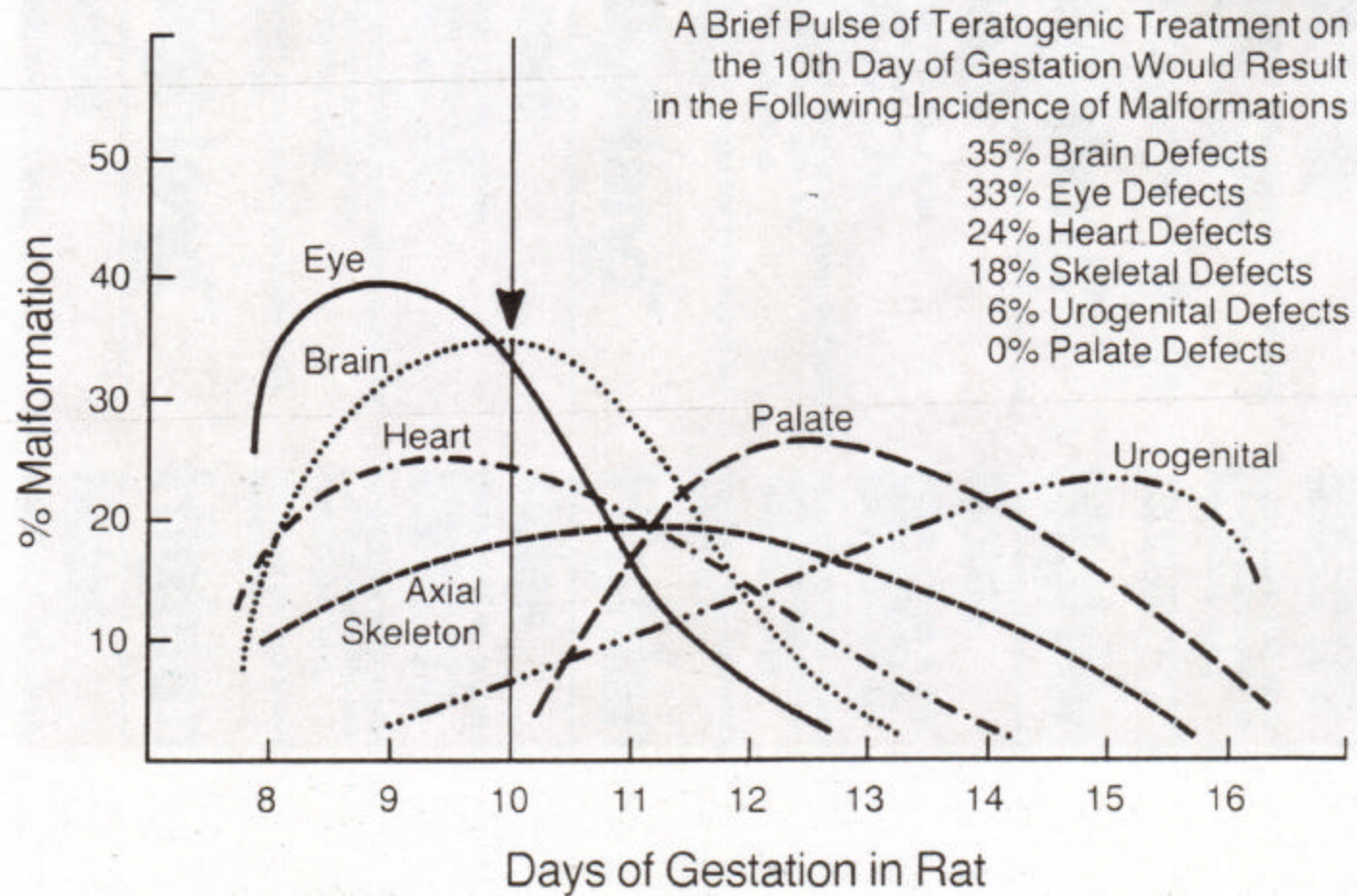
- Individual case reports
- Epidemiological studies (retrospective)
 - Cohort study (compare groups of unexposed and drug-exposed patients for incidence of birth)
 - Examples: bendectin, fluoxetine, and acyclovir
 - Case-control-(compare birth defect cases with control cases for reported drug exposures_
 - Example: VPA

Factors complicating the identification of teratogens by epidemiological approaches

- Low risk of exposure
- Influence of disease state
- Multiple drug therapy including other known teratogens (phenobarbital, carbamazepine, phenytoin)

Six principles of teratogenicity (Wilson, 1973)

- 1. Teratogenic susceptibility is determined by the genotype of the conceptus and the interaction of this genotype with the environment
- 2: Susceptibility to teratogenic agents depends on the developmental stage of the embryo or fetus at the time of exposure
- 3: Teratogenic agents work by specific mechanisms on developing cells and tissues to initiate pathogenesis
- 4: Perturbations of developmental processes can result in death, malformation, growth retardation, and/or functional disorders
- 5: The nature of the influence (or agent) determines the extent of the interaction between the environmental agent and the conceptus
- 6: A dose response relationship exists in the occurrence of birth defects induced by a chemical or physical agent, from the no effect level to the totally lethal level



References

- Ruddon, R. W. (1990) Chemical Teratogenesis in Principles of Drug Action-The Basis of Pharmacology, Third Ed. Chapter 13, p. 775-796 Eds. W.B. Pratt and P. Taylor, Churchill Livingstone
- Eaton, D. L., and Klaassen, C.D., (1996) Section on Developmental and Reproductive Toxicity, p. 31 in Chapter 2, Principles of Toxicology, in Cassarett and Doull's Toxicology, Fifth Ed., McGraw Hill
- Koren, G., Pastuszak, A., and Ito, S. (1998) Drugs in Pregnancy N. Engl. J. Med. 338: 1128-1137.
- Collins, M.D., and Mao, M.D. (1999) Teratology of retinoids. Ann. Rev. Pharmacol. Toxicol. 39: 399-430.
- Section R. Environmental Agents of Chapter 1 of Smith's Recognizable Patterns of Human Malformations Ed. K.L. Jones, Fifth edition, Saunders
- Chapin et al. (1999) The effects of 4-nonylphenol in rats: a multigeneration reproduction study Toxicol. Sci. 52: 80-91.
- Schardein, J.L. (1976) Drugs as Teratogens, CRC Press