

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 7

Hazard ID--Types of Studies
Endocrine System Toxicity
Part 1: Overview of Endocrine System

*Types of Experiments for
Assessing Hazard*

- In vitro
- In vivo
- Epidemiological

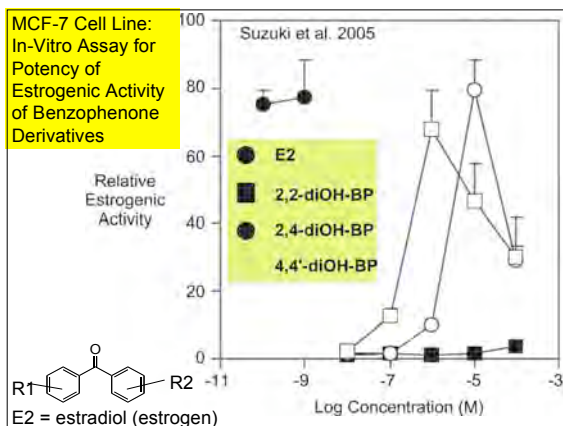
For risk assessment, the in vitro and in vivo experiments give us a NOEL (or NOAEL) and LOEL (LOAEL).

In Vitro Experiments

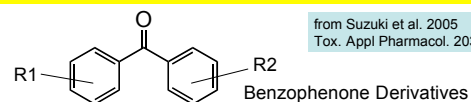
- Use of biological material isolated from the whole organism but kept functional
 - Cell cultures
 - Tissue cultures
 - Enzymes
 - Receptors
- Measure specific response in relationship to increasing dose or concentration of toxicant

In Vitro Testing

- Can estimate an LD50, LC50, ED50, EC50 if measuring a specific endpoint like death or cell numbers, etc.
- If measuring enzyme function or receptor binding and function can measure
 - Km (Michaelis Menton affinity constant); concentration at which velocity is 0.5 max
 - Kd (Equilibrium constant for binding)
 - I₅₀ (Concentration inhibiting activity by 50%)
- Can estimate a NOAEL or NOAEC



In-Vitro Assays for Potency of Estrogenic & Antiandrogenic Activity of Sunscreen Additives

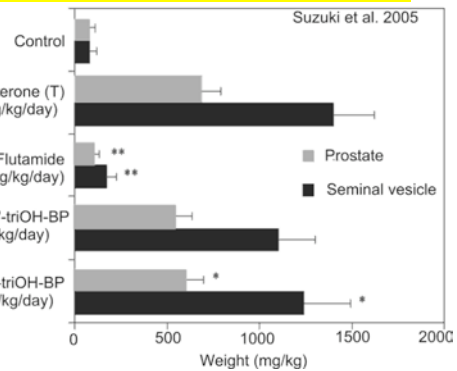


R1	R2	Estrogenic EC50 (μM)	Antiandrogenic EC50 (μM)
E2	(estradiol)	<0.001	NA
2,4-OH	4'-OH	0.10	0.30
4-OH	4'-OH	0.14	4.78
2,4-OH	2',4'-OH	0.30	1.53
H	H	8.13	11.90
2,3,4-OH	4'-OH	31.3	26.50
2-OH	2'-OH	>100	3.20

In Vivo Testing

- Use whole animals
- Determine a NOAEL or NOAEC
- Measure death or sub-lethal responses
- Use a range of doses
 - Dose range limited to control and 1 - 3 doses
 - Doses tend to be high relative to environmental exposures

Hershberger Assay: In-Vivo Test (with rats) for Anti-Androgenic Activity

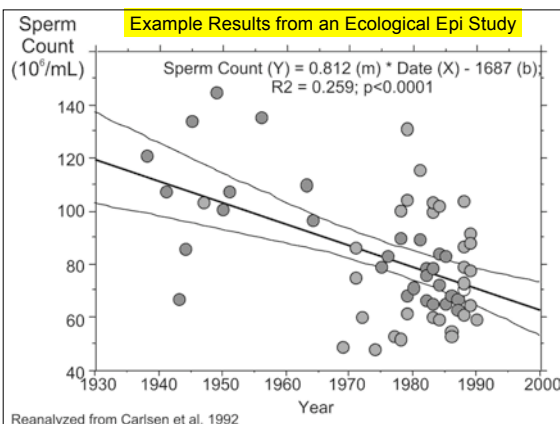


Epidemiological Studies

- Objective: Relate the incidence of a disease or condition with exposure to some agent (microbiological, chemical) or activity (lifestyle, behavior, product use, place of residence, etc.)
- Epidemiological studies of chemical effects are normally applied to chronic exposures and maladies classified as chronic toxicity

Epidemiological Studies

- Controversial when applied to chemicals
 - Confounding factors (variables that may affect or influence the outcome of the results but not controllable)
 - ✓ For example, if wanted to examine the effect of low residues of insecticide on a population of song birds in an area with urbanization, the confounding factor would be habitat fragmentation
 - Epi studies can only make associations, not conclusions about causation
 - For human applications, doses hardly ever measured
- Epi studies good for pathogen caused diseases (follow Koch's postulates)



General Mechanisms of Toxic Action

- Interference with normal receptor-ligand interactions
 - Can result in neurotoxicity, endocrine toxicity, developmental & reproductive toxicity
- Interference with membrane functions
- Interference with cellular energy production
- Binding to biomolecules
- Perturbations in calcium homeostasis
- Toxicity from selective cell loss
- Nonlethal genetic alterations in somatic cells

Endocrine System Toxicity

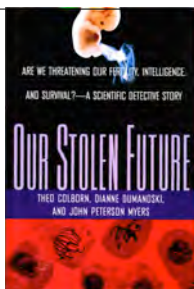
- Over the last 10 years, new hypothesis born (but there were earlier “warnings”)
 - Numerous chemicals, both synthetic and naturally occurring can affect the normal functioning of the endocrine system
 - Contaminants called endocrine disrupters
 - ✓ National Academy of Sciences (NRC) prefers the terms hormonally active agents (HAAs) because mechanism of action of the various syndromes seen in wildlife are not entirely clear as to cause and not necessarily caused through endocrine system action

Endocrine System Toxicity

- Effects on endocrine system observed at doses that are sub-lethal and not associated with cellular toxicity
- Adverse effects observed:
 - Physiological
 - Developmental
 - Reproductive
 - Morphological
 - Behavioral
- Individuals shown affected, but unclear if larger scale effects can be shown

Popularization of Endocrine System Effects

- Theo Colborn et al (1996)
 - Our Stolen Future
 - Premise: Synthetic chemicals released into the environment (and some natural ones, too) mimic hormones of the endocrine system (mainly estrogen and testosterone), and because of their wide ranging effects, essentially threaten human intelligence, fertility, and survival
 - Wildlife is a sentinel for future effects on humans

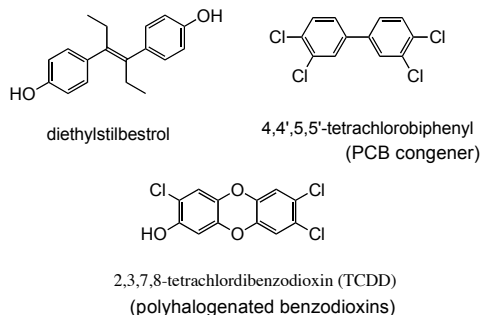


Scientific Roots

- The idea that synthetic chemicals could mimic estrogen dates back over 50 years
 - Phytoestrogens in clover affect sheep ('40s)
 - Organochlorine pesticides affect uterine wet weight (an estrogenic effect) ('50's)
 - DES (diethylstilbestrol and effects on male children)
- “Chemically Induced Alterations in Sexual Development--the Human/Wildlife Connection” --1992 conference in Racine, WI

Consensus Statement from the Racine, WI Conference

- “A large number of man-made chemicals that have been released into the environment, as well as a few natural ones, have the potential to disrupt the endocrine system of animals, including humans”
 - Note the the evidence for effects in humans was based solely on the experience of women using the drug DES;
 - Conference attendees believes wildlife populations were already affected



Presence of chlorine atoms is not a prerequisite for potential to affect endocrine physiology

National Academy of Science National Research Council Rept.

• *Hormonally Active Agents in the Environment* (1999)

- Link to effects on humans is weak
 - ✓ Except for known effects of the drug, DES
- Possible that wildlife populations in some instances and in some environments have shown effects from hormone mimic exposures
 - ✓ Hypothesis has more plausibility than that of effects on humans

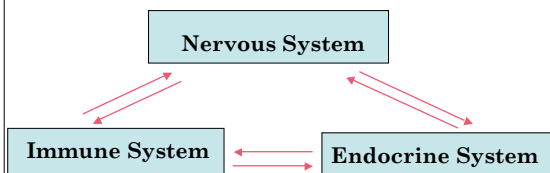


Hormonally Active Agents (Endocrine Disruptors)

- The new paradigm in hazard id essentially links all adverse effects (rightly or wrongly) to adverse effects on the endocrine system
- Endocrine system, nervous system, and immune system linked with another (like a computer network)
 - This biological "internet" either controls or integrates all growth and physiological functions
- Communicate via chemical transmitters
 - Hormones, neurotransmitters, cytokines

What Is the Endocrine System?

- One of three interdependent communication systems of the body that regulate growth, development, physiological processes, and behavior

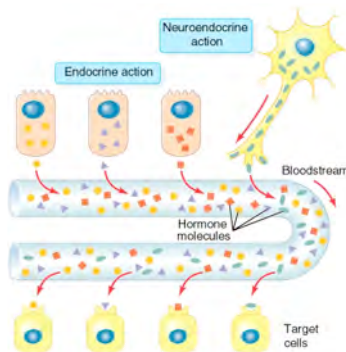


The Body's Internet

- Each system is organized as specialized tissues, glands, and organs
- Communication between systems by secretion of chemical messengers
- Messengers bind to receptors in target cells, causing a response
- Built-in controls called feed-back loops regulate over or under secretion of messengers

Chemical Messengers

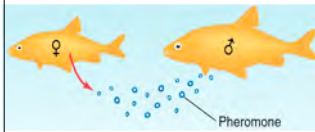
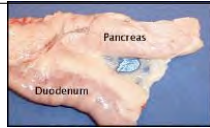
- Endocrine System
 - Hormones
- Nervous System
 - Neurotransmitters
- Immune System
 - Cytokines



Regulatory Functions of Neurotransmitters & Hormones (Integration of Physiological and Metabolic Functions)

- **Neurotransmitters**
 - Mediate rapid signaling over short distances
 - Signaling for regulatory functions required on short time scale
 - Released in response to action potential (or change in membrane potential)
 - Neurotransmitters can also function as hormones when they signal endocrine tissue as opposed to nerve tissue
- **Hormones**
 - Communicate over longer distances & on a longer time scale
 - Signaling for regulatory functions sustained for minutes, hours, or days
 - ✓ Maintenance of blood osmolarity (ADH)
 - ✓ Uptake of glucose by cells (insulin)
 - ✓ Metabolic rates (growth hormone, thyroxine)
 - ✓ Sexual activity/reproductive cycles (sex hormones)
 - ✓ Modification of behavior (various hormones)

Conventionally think of endocrine glands and secretions of hormones affecting other tissues after transport through the blood

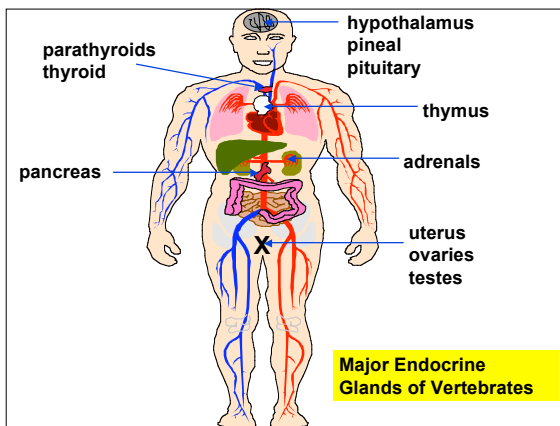


However, in an ecological context should also consider exocrine glands that secrete signaling compounds (like pheromones) outside of the body and effect behavior (e.g. reproduction) of other individuals

Hormones & Receptors

- Hormones: natural, secretory products of endocrine glands (ductless glands that discharge directly into the bloodstream).
 - Hormones travel in the blood in very small concentrations and bind to specific cell sites called receptors in distant target tissues and organs
 - Exert their effects in the target tissues by affecting development, growth, and reproduction
- Endocrine receptors for steroid sex hormones are abundant in many tissues including gonads, brain, pituitary

Adapted from EPA 1997



Main Glands & Hormones of the Endocrine Systems

Gland	Hormone	Major Function
Pituitary	Adrenocorticotrophic hormone (ACTH)	Stimulates adrenal cortex
	Thyrotrophic hormone (TSH)	Stimulates thyroid
	Follicle-stimulating hormone	Ovarian follicle development; seminiferous tubule development
	Luteinizing hormone (LH)	Conversion of ovarian follicle to corpus luteum; progesterone & testosterone production

Main Glands & Hormones of the Endocrine Systems

Gland	Hormone	Major Function
Pituitary	Prolactin	Milk production; osmoregulation in fish
Hypothalamus	Releasing hormones acting on the pituitary	Hormones delivered via portal circulation to the pituitary to stimulate or inhibit release of hormones
Adrenal cortex	Glucocorticoids	Regulate carbohydrate metabolism; inhibit ACTH release
	Mineralocorticoids	Regulate sodium metabolism & excretion
	Androgens; progesterone	Stimulate secondary sexual characteristics; mainly male

Main Glands & Hormones of the Endocrine Systems

Gland	Hormone	Major Function
Ovary	Estrogens (estradiol)	Female secondary sex characteristics-female; thickening of uterine mucosa; inhibit release of FSH
	Progesterone	Interaction with estrogens in female sex characteristic; uterine lining support; inhibits release of LH & FSH
Testis	Testosterone	Initiate & maintain secondary male sexual characters
Thyroid	Thyroxine, triiodothyronine	Stimulate oxidative metabolism; amphibian metamorphosis; inhibit release of TSH; role in brain development

Summary of Major Functions

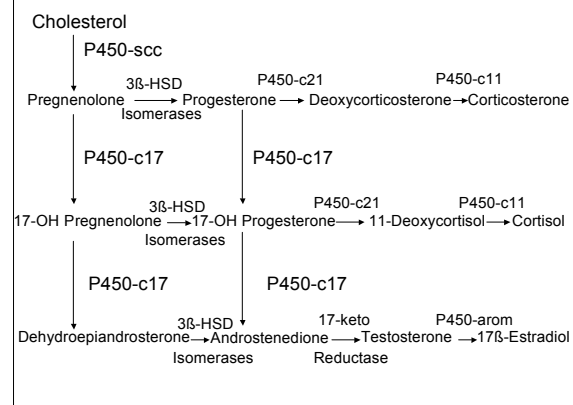
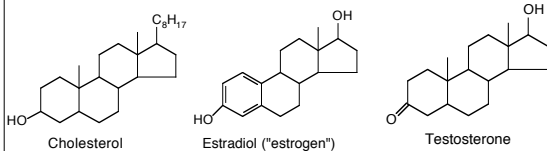
- Digestion & related metabolic functions
- Osmoregulation, excretion, water & salt metabolism
- Calcium metabolism
- Growth & Morphological changes
- **Reproductive organs and reproductive behavior**
- **Brain development**

Hormones--Biochemical Types

- Steroid hormones
 - Glucocorticoids
 - Sex steroids (estrogen; testosterone)
- Peptides & proteins
 - Releasing and release-inhibiting hormones from hypothalamus
 - Gonadotropins (FSH; LH)
 - Pituitary gland hormones
- Tyrosine derivatives
 - Thyroid hormone
 - Adrenaline; noradrenaline

Steroid Hormones

- All derived from cholesterol
- P-450 enzyme isoforms important in transformations
- Small changes in molecule can mean big change in biological activity



Where Are The Receptors?

Estrogen

- Gonads
- Uterus
- Vagina
- Breasts
- Heart
- Pituitary
- Brain

Testosterone

- Gonads
- Secondary Sex Organs
 - prostate
 - seminal vesicles
 - penis
- Pituitary
- Brain

Sex Steroids & the Immune System

- Immune system cells with gonadal steroid receptors
 - Sex differences in immune response
 - bone marrow structure
 - antibody levels
 - disease susceptibility
 - menstrual cycle variations

Gonadal Steroid Hormones

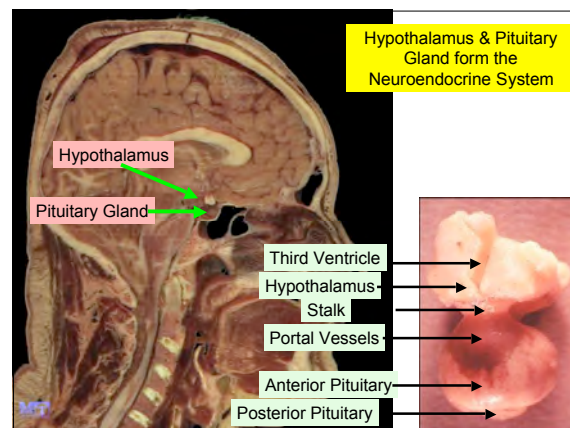
- Androgens
 - Produced by the Leydig cells in the testes
 - Testosterone, dihydrotestosterone
 - functions:
 - masculinization of fetus
 - control of sperm production
 - male secondary sexual characteristics (puberty)
 - activation of sexual, aggressive and other behaviors (adulthood)

Gonadal Steroid Hormones

- Estrogens
 - Produced by the ovaries
 - Estradiol, estrone, estriol
 - Female secondary sex characteristics
 - Influence:
 - metabolic rate, body temperature, skin texture, fat distribution
 - many enzyme, circulatory, and immune functions
 - sexual, parental, and other female behaviors

Gonadal Steroid Hormones

- Progestins
 - Produced by ovaries
 - progesterone
 - Functions:
 - Uterine, vaginal, & mammary gland growth
 - Stimulates enlargement of breast & uterus at puberty & during menstruation
 - Maintains pregnancy



Pituitary Control

- Follicle Stimulating Hormone (FSH)
 - Female: stimulates growth of ovary follicle cells, development of egg, & promotes secretion of estrogen
 - Male: stimulates sperm production and secretion of hormone inhibin (MIS)
- Luteinizing Hormone (LH)
 - Female: stimulates ovulation and formation of progesterone secreting cells in ovary
 - Male: stimulate Leydig cells in testes to secrete testosterone

Sex Hormones & Fetal Development

- Male and female gonads start as undifferentiated cells
- Y chromosome carries a gene that makes Testis Determining Factor (TDF) (6th wk of gestation)
 - TDF starts masculinization process
- All other aspects of masculine and feminine development are mediated through differential exposure of males and females to the gonadal hormones

Sex Hormones & Fetal Development

- Shortly after differentiation of gonads into testes, Leydig cells secrete testosterone & Sertoli cells secrete Mullerian inhibiting substance (MIS)
- Initiates masculinization
 - Defeminization by atrophy of Mullerian duct
- Female organs develop only in the absence of secretion of high levels of testosterone and MIS
 - Ovaries secrete estrogen during sexual differentiation

Sex Hormones & Fetal Development

- High levels of estradiol found circulating in both female and male fetal blood; ditto for testosterone
- Gonadal steroids cause permanent organizational effects in brain
 - Sensitization of brain to later sex hormone exposures

Sex Hormones & Fetal Development

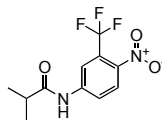
- Naturally occurring variations in the levels of sex hormones in female mice, rats, and gerbils leads to marked differences in a wide range of reproductive traits
- Genital morphology
 - timing of puberty
 - length of estrous cycles
 - age at cessation of fertility
 - aggressive & sexual behavior

Ironically...

- In fetal and neonatal males, high levels of testosterone is converted to estradiol in the brain by the aromatase enzyme;
 - Results in masculinization and defeminization of the brain
- Normal female differentiation occurs when intracellular estrogen formation is low due to lower circulating testosterone levels and aromatase enzyme levels

However...

- Some aspects of sexual differentiation of behavior require androgen binding to receptors
 - Inhibition of androgen binding by a blocker (flutamide) at the time of birth interferes with masculinization and defeminization of sexual behavior in male rats

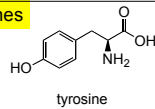


flutamide

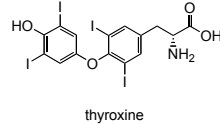
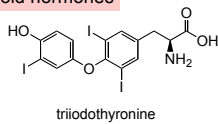
But What Protects Females from Masculinization?

- Females have a high estradiol concentration in fetal blood
- They may be protected from the effects of receptor binding in the brain by the binding of estradiol to proteins in the blood, which effectively lowers its concentration
- However, this hypothesis has not been adequately tested and contradictory results have been reported

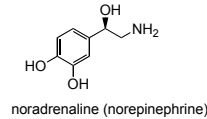
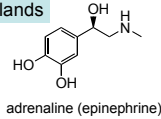
Tyrosine-Derived Hormones



Thyroid hormones

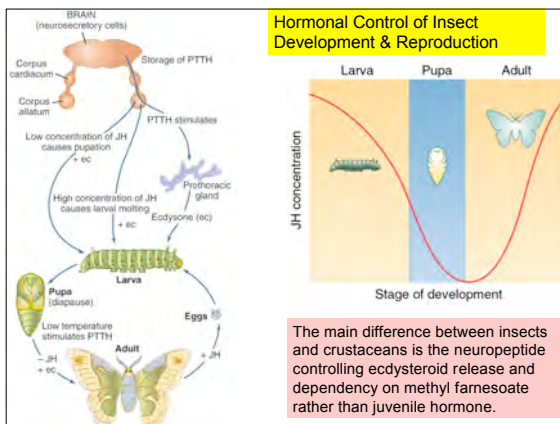


Adrenal glands



Thyroid Hormone

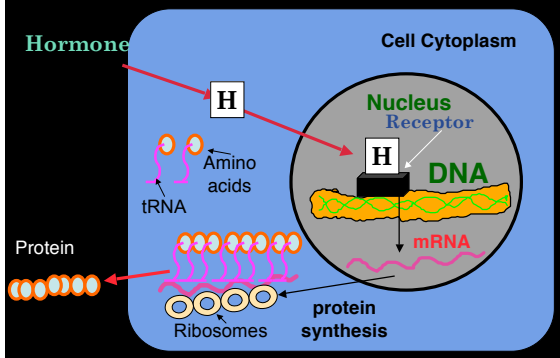
- **Receptors:**
 - pituitary, liver, kidney, heart, brain
 - Influence metabolic rate, respiration rate, oxygen consumption, metabolism of food, synthesis of some enzymes
- **Role in Growth & Differentiation**
 - Maturation of brain, heart, and lungs during prenatal and early postnatal development
 - Neural growth, synapses, normal behavior



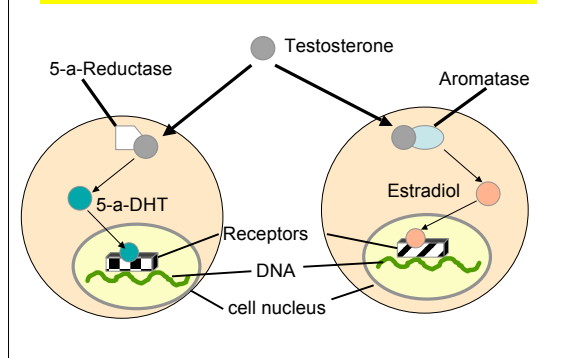
What Happens After Hormone Release

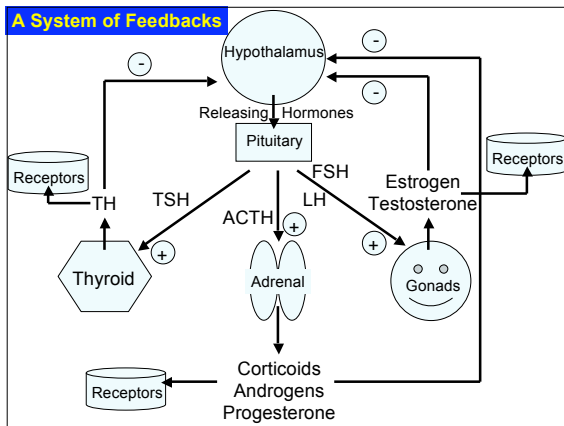
- Hormones released directly into blood
- Diffuse through cell membrane
- Hormone moves through nuclear membrane and attaches to receptor
- Receptor stimulates synthesis of DNA and mRNA
- End product: Protein synthesis

From DNA to Proteins



Steroid Hormones-Cell Interactions





Phenomena Attributed to EDCs

- Reproductive disorders & morphological abnormalities in wildlife
- Lowered human sperm counts, prostate cancer, and dysfunctional or abnormal genitalia
- Breast cancer
- Behavioral problems in children
- Immune system dysfunction

