

РИОПHE



ST-PETERSBURG

**Research Institute of Hygiene, Occupational
Pathology and Human Ecology,
St.Petersburg, Russia**

**TEST SYSTEM FOR ASSESSMENT IN VITRO
OF EMBRYOTOXIC PROPERTIES OF PHARMACOLOGICAL PREPARATIONS**

WITH PROGNOSIS OF THEIR HAZARD FOR HUMAN EMBRYOGENESIS

V.B.POPOV

LEADING RESEARCHER, Dr. Ph (Biol)

PATHOLOGICAL OUTCOME OF PREGNANCY: PRE- AND POSTNATAL LOSSES, CONGENITAL MALFORMATONS, MENTAL RETARDATION

FACTORS

1-2 %

CHEMICAL

1-2 %

PHYSICAL

BIOLOGICAL

10-25 %

GENETIC

7-8 %

DESEASES

1-2 %

MECHANICAL
DEFECTS

INDUSTRIAL AND AGRICULTURAL TOXICANTS
DRUGS, MYCOTOXINS

RADIATION, ULTRAHIGH FREQUENCY, VIBRATION
HYPO- AND HYPERTERMIA

VIRUSES, BACTERIUM, PARASITES

DOMINANT, GENE,
NUMERICAL, SEX-LINKED MUTATION

METABOLIC AND ENDOCRINE
DESEASES, INFECTION, ALCOHOLISM

AMNIOTIC BANDS, HYDROAMNION
UTERI INFANCY

10 - 12 %

RIHOPHE



ST-PETERSBURG

CRITERIA OF WORLD HEALTH ORGANIZATION FOR SHORT-TERM TEST-SYSTEMS

- inexpensive and come-at-able test-models
- simplicity registration of end effects
- minimization of false positive and false negative results
- repeatability of results in different laboratories
- using maximal quantity of developmental processes
- using systems of metabolic activation
- **prognostic value of experimental data for human embryogenesis**

BASIC GOAL

THE ELABORATION OF IN VITRO TEST SYSTEM FOR EXPRESS EVALUATION OF TERATOGENIC PROPERTIES OF DRUG WITH PROGNOSIS OF THEIR HAZARD FOR HUMAN EMBRYOGENESIS

BASIC TEST- MODELS

THE PRE- AND POSTIMPLANTATION EMBRYOS OF LABORATORY ANIMALS CULTURED IN VITRO

TEST- OBJECTS

PHARMACOLOGICAL PREPARATIONS
INDUSTRIAL AND AGRICULTURAL CHEMICALS
ECOTOXICANTS,
MULTICOMPONENT COMPOUNDS,
BIOLOGICAL LIQUIDS
(BLOOD, AMNIOTIC FLUID, TISSUE FLUID)



IN VITRO TEST SYSTEM BASIC DIRECTIONS AND APPROACHES

• **DIRECT EXPOSURE** – MORPHOFUNCTIONAL DATA

– THRESHOLD DEFINITION

BASIC PRINCIPLE OF TEST SYSTEM

– COMPARISON WITH PHARMACOKINETIC DATA

– EVALUATION OF DRUG

• **BIOTRANSFORMATION** – DIRECTIONS OF METABOLIC CONVERSION

- BIOACTIVATION ; - BIOTRANSFORMATION, NON-METABOLIC INACTIVATION

EMBRYOTOXIC

• **THE TIME OF REALIZATION OF TERATOGENIC EFFECTS** – TIME OF FORMATION OF IRREVERSIBLE PATHOLOGICAL CHANGES

PROPERTIES

• **REVEALING AND EVALUATION EMBRYOTOXIC FACTORS IN BLOOD OF TREATED ANIMALS** -DYNAMICS OF FACTORS; - PERSISTING TIME

• **REVEALING AND EVALUATION EMBRYOTOXIC FACTORS IN BLOOD OF WOMEN HAVING DEAL WITH CHEMICALS**

- BY DRUGS TREATMENT;
- BY WORKING ON CHEMICAL INDUSTRIES OR FARM
- BY LIVING ON ECOLOGICAL UNFAVORABLE REGIONS

RIHOPHE



ST-PETERSBURG

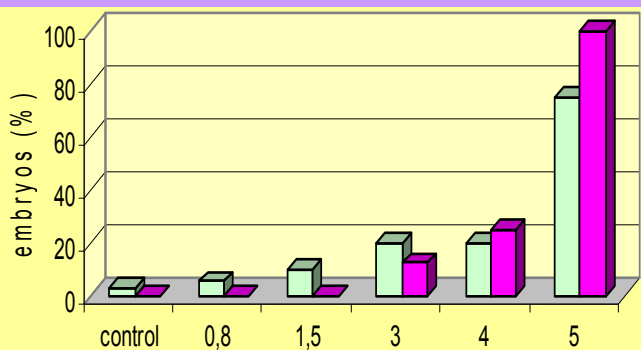


ETHANOL AND ACETALDEHYDE

CONCENTRATIONS OF ETHANOL AND ACETALDEHYDE IN HUMAN BLOOD AND THE SEVERITY OF ALCOHOLIC INTOXICATION

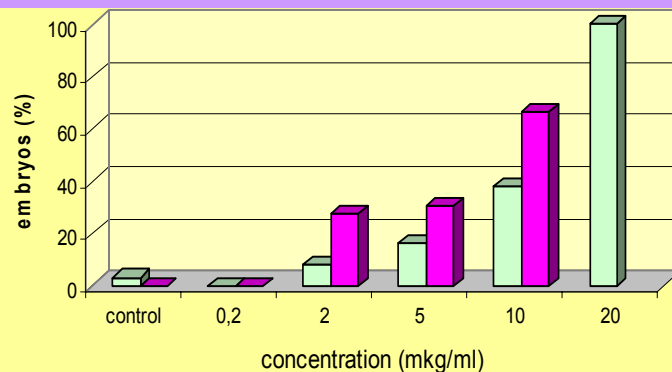
Substance	Concentrations in human blood	Human state
Ethanol	1-3 mg/ml (22-65 mM)	Medium or severe degree of intoxication
	4-5 mg/ml (87-108 mM)	State of coma
	6-9 mg/ml	Death
Acetaldehyde	0.9-1.3 mkg/ml (21-30 μM)	Intoxication of healthy persons
	2 mkg/ml (45 μM)	Chronic intoxication
	5.6 mkg/ml (125 μM)	In women given birth to infants with FAS
	12.4-44 mkg/ml (280-1000 μM)	In case of treating intoxication with AA inhibitors

Direct effects of ethanol



□ emryoethality ■ teratogenicity

Direct effects of acetaldehyde



□ emryoethality ■ teratogenicity

**THE MINIMAL TERATOGENIC CONCENTRATIONS OF DRUGS IN THE CULTURE MEDIUM
AND THERAPEUTIC OR HIGHEST CONCENTRATIONS OF THESE DRUGS IN HUMAN BLOOD**

SUBSTANCES	MINIM. IN VITRO TERATOGENIC CONCENTRATIONS (MKG/ML)	THERAPEUTIC OR MAX CONC. IN HUMAN BLOOD	TERATOGENICITY FOR HUMAN
PHENYTOIN	4-12	2-45	+ Yes
METHOTREXATE	0.4	30	+
FLUOROUROCYL	0.3	0.8 - 50	+
VALPROIC ACID	80 - 160	50 - 150	+
All-trans RETINOIC ACID	3 – 5 ng/ml	3 ng/ml and >	+
DIETHYLSTILBESTROL	1.0	0.04 – 0.7	+
CYCLOPHOSPHAMIDE*	5 - 25	3 - 50	+
ETHANOL	1.5 – 3.0 mg/ml	1.5 – 3.0 mg/ml	+
ACETALDEHYDE	0.3 - 20	0.3 – 12 and >	+/- question-
CdCL 2	0.01	0.002-03	+/- able
SALICYLATES	100 - 150	10 – 50 (300-700)	+/-
CAFFEINE	30	5 - 10	+/-
PHENOBARBITAL*	300	6 - 68	- Not or
MEPBROBAMATE	300	10 - 15	- unlikely
DEXAMETHASON	90	0.02 – 0.13	-
DIAAZEPAM	100	0.5 – 2.5	-
ANTIRETROVIRAL DRUGS	250-500 mkM	1-50 mkM	-

IN VITRO TEST-SYSTEM

THE EXTRAPOLATION OF EXPERIMENTAL DATA TO HUMAN EMBRYOGENESIS

MINIMAL EFFECTIVE CONCENTRATION

! THE EFFECTIVE CONCENTRATIONS (MINIMAL EFFECTIVE CONCENTRATION AND ABOVE) FOUND IN IN VITRO EXPERIMENTS SHOULD BE COMPARED WITH MAXIMAL OR THERAPEUTICAL CONCENTRATIONS IN HUMAN BLOOD

! THE THRESHOLD AND ABOVE CONCENTRATIONS ARE PROGNOSIS AS DANGEROUS FOR HUMAN EMBRYOGENESIS ON CONDITIONS THAT THEY APPEAR IN HUMAN (WOMAN) BLOOD.

! IN CASE OF REAL PRESENCE OF THESE CONCENTRATIONS IN HUMAN BLOOD - THE DRUG SHOULD BE ACCEPTED AS POTENTIAL TERATOGEN

•



TIME OF EXPOSURE NEEDED FOR REALIZATION OF EMBRYOTOXIC EFFECTS PRODUCED BY ACETALDEHYDE AND ETHANOL IN VITRO

CONCENTRATIONS	EXPOSURE TIME (HOURS)				
	1	3	15	24	48
ETHANOL					
17 Mm (0,8 mg/ml)					
33 “ (1,5 mg/ml)					
65 “ (3,0 mg/ml)					
87 “ (4,0 mg/ml)					
108 “ (5,0 mg/ml)					
ACETALDEHYDE					
4,5 mkM (0,2mkg/ml)					
45 “ (2,0 mkg/ml)					
110 “ (5,0mkg/ml)					
225 “ (10 mkg/ml)					
450 “ (20 mkg/ml)					



-EMBRYOLETHAL AND TERATOGENIC EFFECTS

IN VITRO TEST-SYSTEM

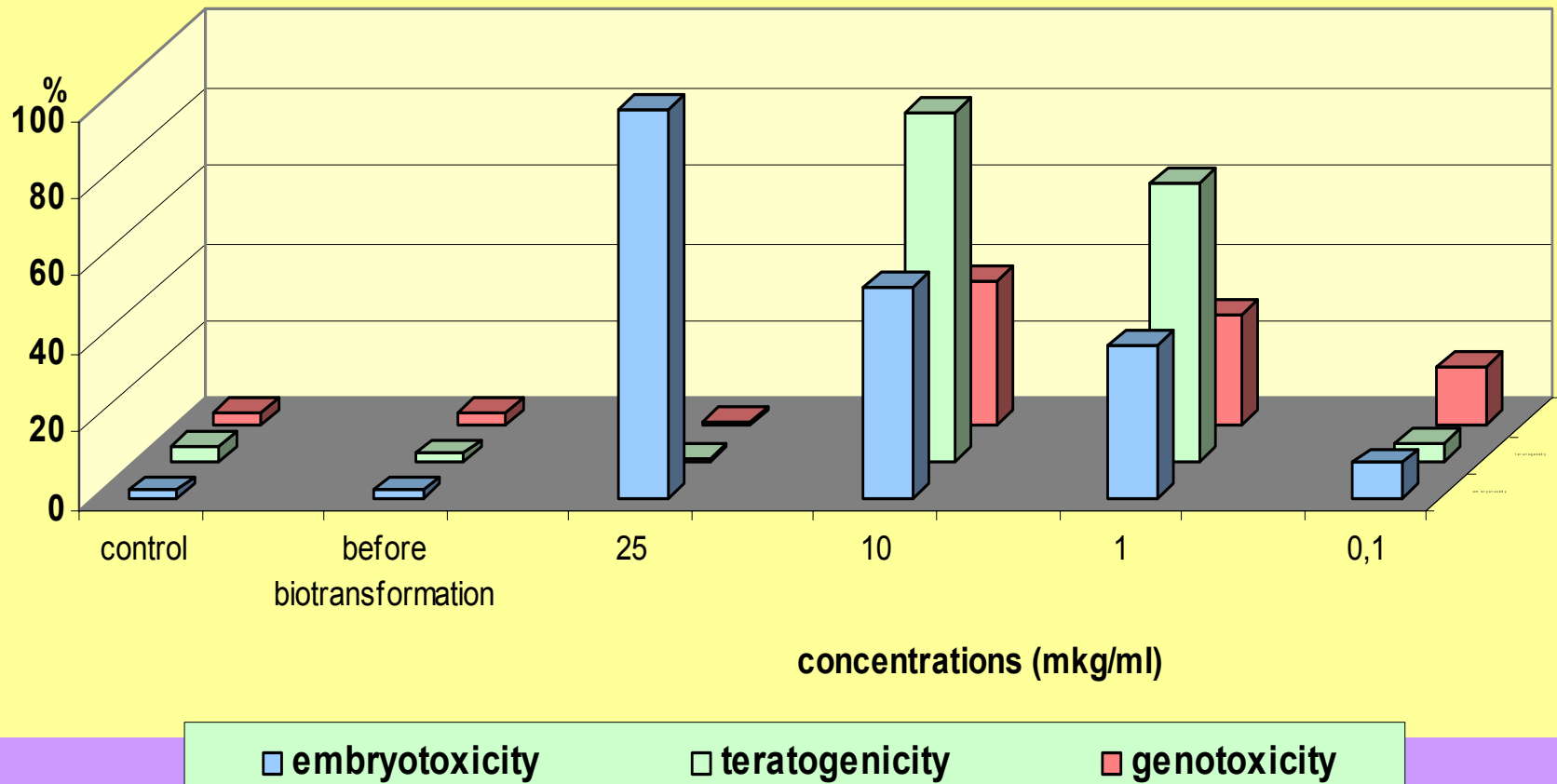
THE EXTRAPOLATION OF EXPERIMENTAL RESULTS TO HUMAN EMBRYOGENESIS

MINIMAL TIME REQUIRED TO PRODUCE A PATHOGENIC EFFECTS

! MINIMAL TIME REQUIRED TO PRODUCE A PATHOGENIC EFFECTS IN VITRO SHOULD BE COMPARED WITH PHARMACOKINETICS DATA – PRESENCE TIME OF HIGHEST DRUG CONCENTRATIONS IN HUMAN BLOOD

! THE LEVELS OF THRESHOLD DRUG CONCENTRATIONS AND MINIMAL TIME REQUIRED TO PRODUCE THEIR PATHOGENIC EFFECTS **ARE KEY PARAMETERS IN EXTRAPOLATION OF EXPERIMENTAL RESULTS TO HUMAN AND PROGNOSIS OF TERATOGENIC HAZARDS FOR HIS EMBRYOGENESIS**

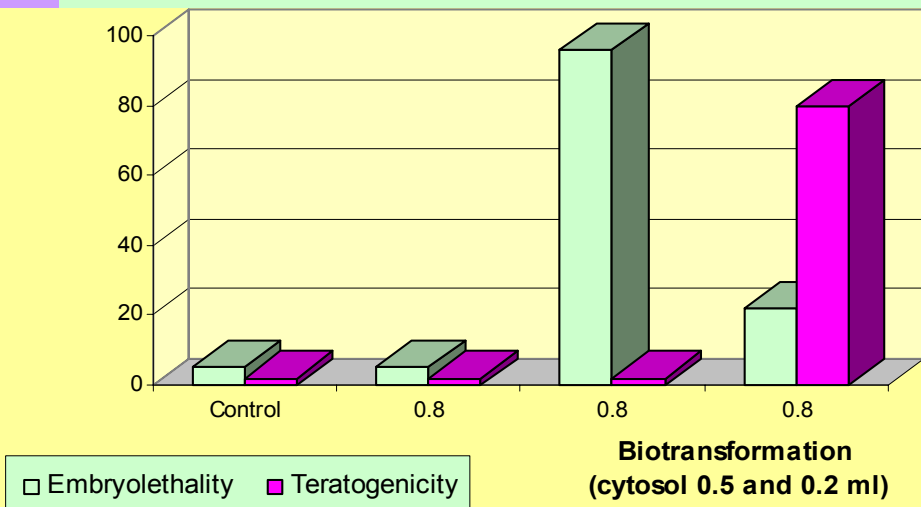
BIOTRANSFORMATION OF CYCLOPHOSPHAMIDE



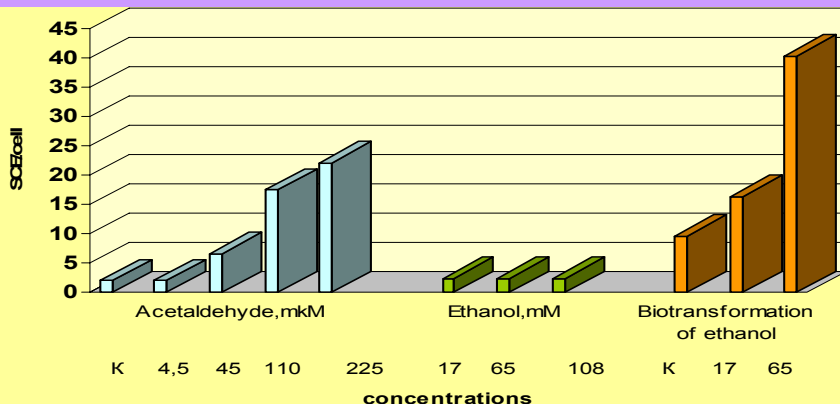
CPHA in the concentration of 50 mkg/ml did not affect the development of rat embryos. Yet, addition into the culture medium EMM (microsomal fraction of rat hepatocytes, NADPH, G6PH) resulted in arising embryo-lethal, teratogenic, and genotoxic properties in CPHA. CPHA induced the reduction of the cerebral hemispheres with pronounced microcephalia, various types of neurulation, decreasing the crown-rump, the total protein, and the number of somites.

BIOTRANSFORMATION OF ETHANOL

Embryotoxic effects of ethanol before and after biotransformation



Incidence of SCE before and after biotransformation of ethanol

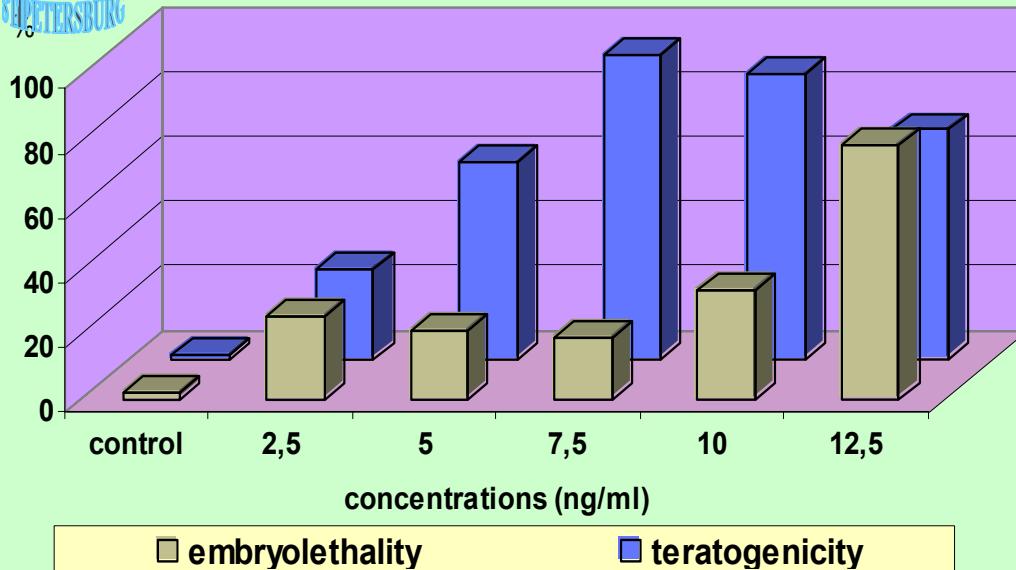


CONCENTRATION OF ETHANOL 0.8 MG/ML IS NOT TOXIC TO CULTURED EMBRYOS. BUT ADDING OF EXOGENIC METABOLIC MIXTURE TO CULTURE MEDIUM (0.2-0.55 MG/ML PROTEIN OF THE S-100 CYTOZOL FRACTION OF RAT HEPATOCYTES, NAD COFACTORS (0.25 MM), AND SODIUM PIRUVATE (2.3 MM0) RESULTED IN SIGNIFICANT INTENSIFICATION OF TERATOGENIC EFFECTS AND IN MARKED INCREASING OF SCE IN EMBRYONIC CELLS. THIS IS THE EVIDENCE OF AA FORMATION AND ITS HIGH TERATOGENIC ACTIVITY



CHITERSBURG

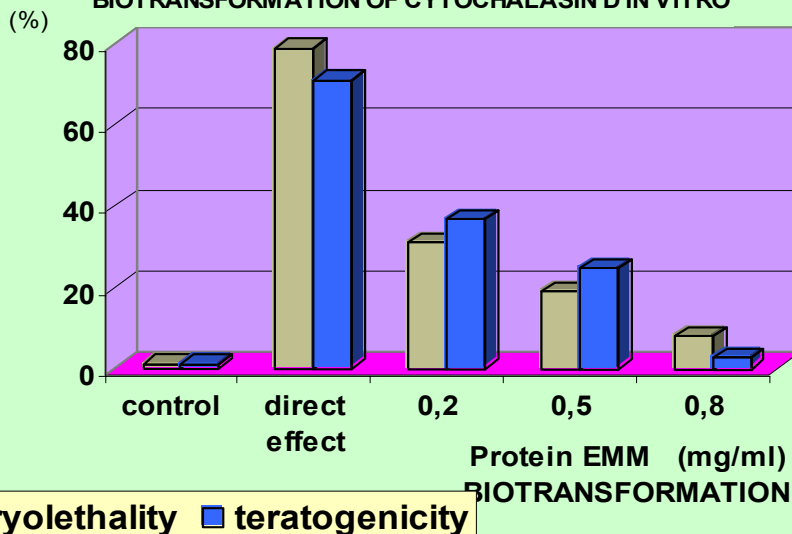
Embryolethality and teratogenicity of cytochalasin D in vitro



Administration of cytochalasin D to rats at the stages of early organogenesis at doses exerting toxic effects on the maternal organism does not produce significant embryotoxic effect.

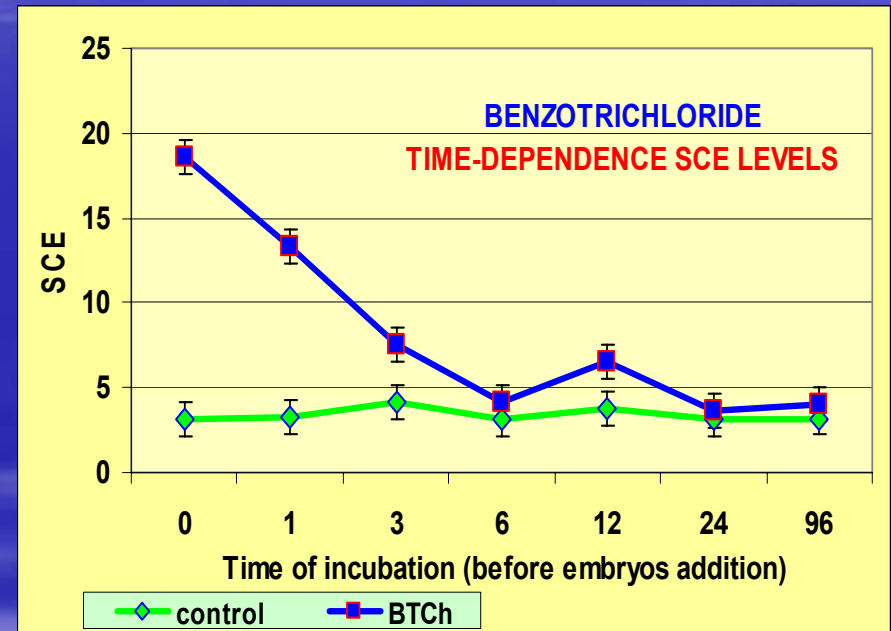
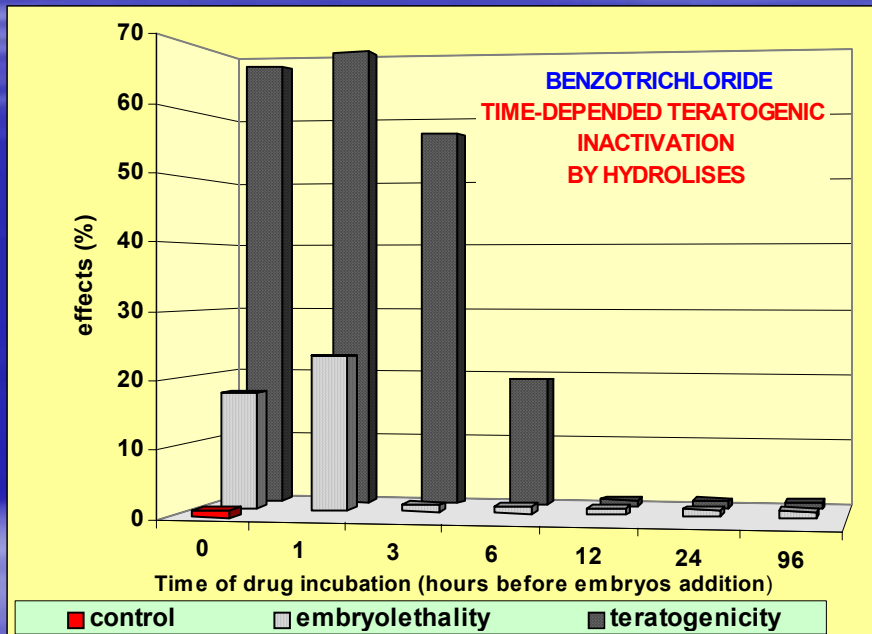
Direct addition of cytochalasin D into the culture medium in concentrations 2.5-15 ng/ml results in the pronounced dose-dependent embryolethal and teratogenic effects.

BIOTRANSFORMATION OF CYTOCHALASIN D IN VITRO

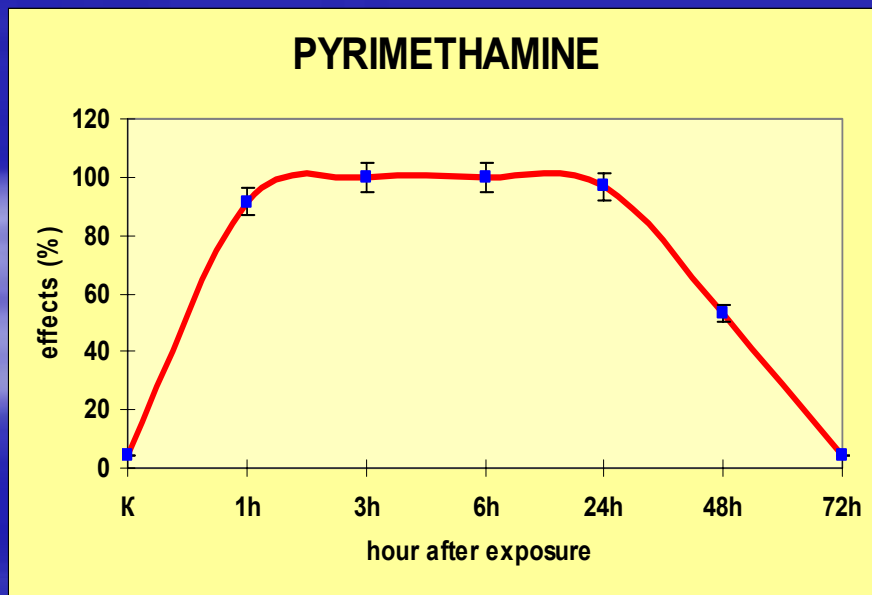
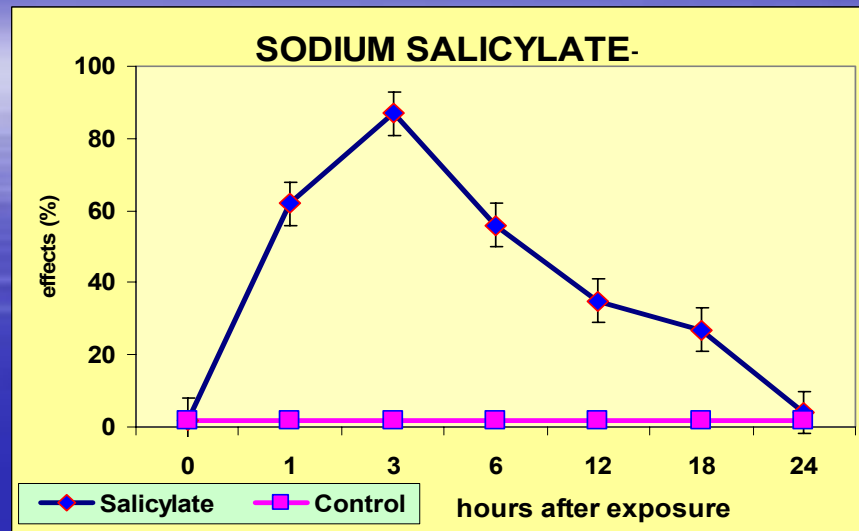
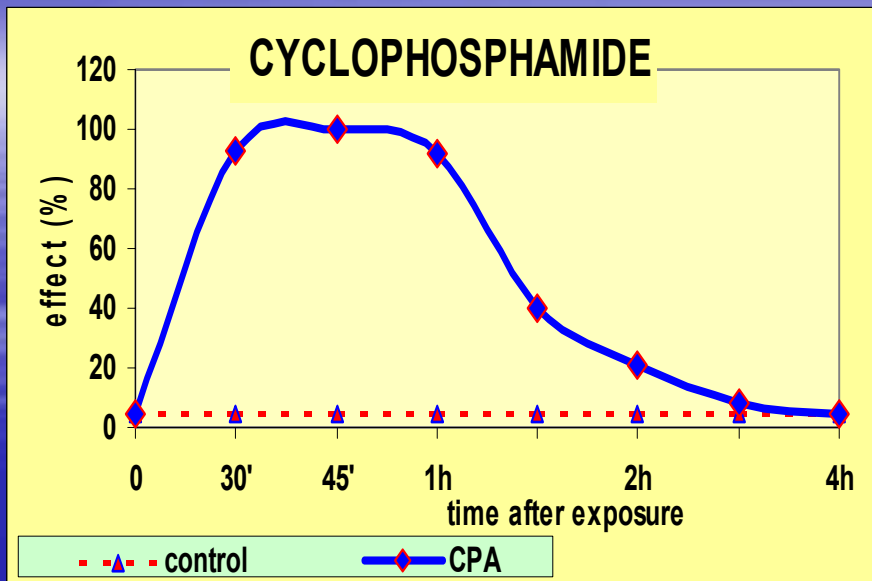


- Addition of S-9 fraction of rat hepatocytes and cofactors result in decreasing the embryolethal and teratogenic effects of cytochalasin D depending upon quantity of the S-9 protein added.
- This suggests the bioinactivation of teratogenic properties of the drug in the animals depending upon the level of activity of the liver microsomal enzymes

BENZOTRICHLORIDE – DIRECT EXPOSURE



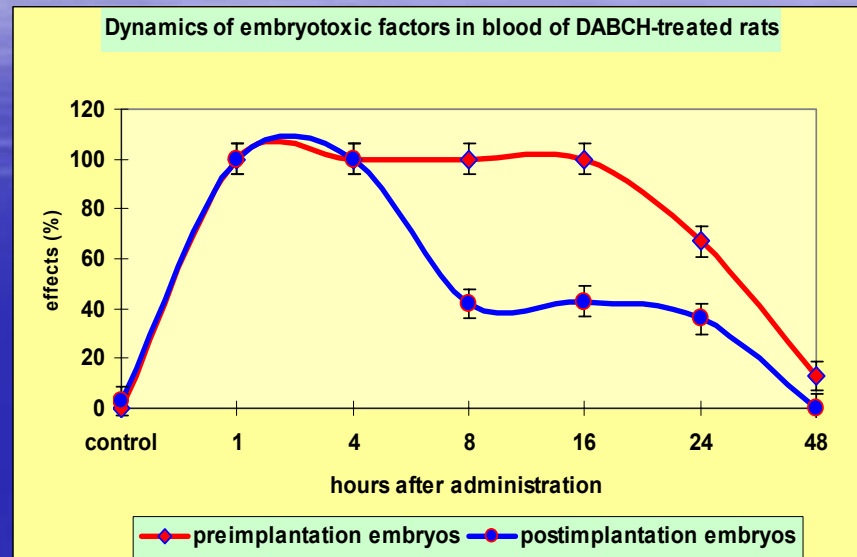
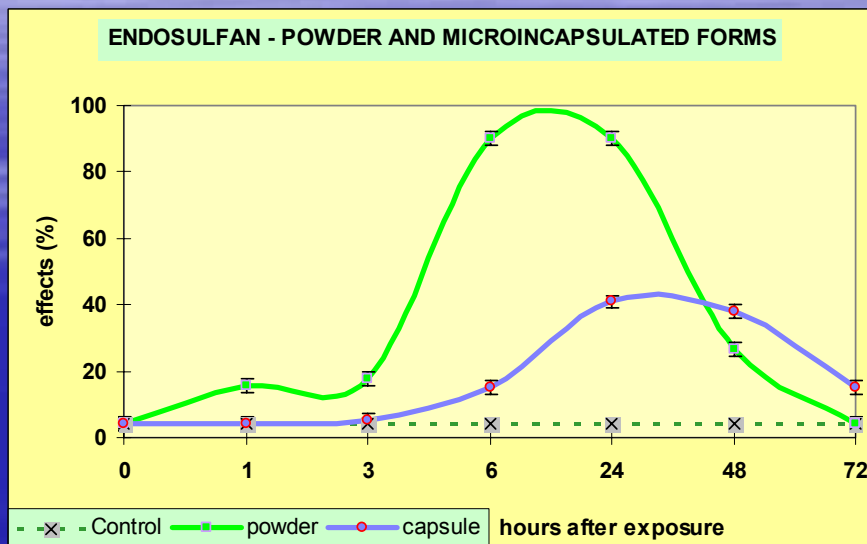
DYNAMICS OF EMBRYOTOXIC FACTORS IN SERA OF TREATED ANIMALS



Test-substance is introduced to animals in maximal tolerant single dose. After different time (1-72 h) take blood and prepare serum, which use as culture medium for development of pre or/and postimplantation embryos

After definition of embryilethality or teratogenicity can be created curve, expressing dynamics of embryotoxicity of sera

SENSITIVITY OF METHODS OF REVEALING EMBRYOTOXIC FACTORS IN SERA OF TREATED RATS



Endosulfan – powder quick absorbs into blood; maximum of embryotoxic effects is marked on 6 h. High toxicity is retaining until 24 h after that decreases rapidly.

Endosulfan – microcapsulated form is slowly increasing into blood; maximum of effects is marked to 24-48 h and slowly decreases to 72 h.

DABCH – dynamics of embryotoxicity is depended on the sensitivity of a test-model to substance. This drug has pronounced cytotoxic effect inducing dismorphogenesis on preimplantation mouse embryos in doses smallest than for postimplantation rat embryos.

PROGNOSTIC ASSESSMENT OF PATHOGENIC FACTORS IN BLOOD OF WOMEN FROM ALTAJ ENDEMIC REGIONS

NO. PATTERNS OF BLOOD SERA					
PREGNANT WOMEN				NON-PREGNANT WOMEN	
ENDEMIC REGION		CONTROL REGION		DIFFERENT REGION	
43		9		17	
OUR PROGNOSIS : + PRESENCE; - ABSENCE					
+	-	+	-	+	-
17	26	2	7	6	11
REALLY: NEWBORN WITH HEMOLYTIC DISEASES AND/OR ENCEPHALOPATHOLOGY					
14	24	2	7		
PROGNOSIS WERE NOT CONFIRMED					
3	2	-	-		
TOTAL					
CORRECT DIAGNOSIS - 47 OR 90 %				PRESENCE OF PATHOLOGIC FACTORS IN BLOOD	
ERRONEOUS DIAGNOSIS - 5 OR 10 %					
PATHOLOGIC DELIVERY				6 OF 17 - 35 %	
16 OF 52 - 31 %					

THE IN VITRO APPROACHES FOR PROGNOSIS OF TERATOGENIC HAZARD OF CHEMICALS

- ! THE REVEALING OF **EMBRYOTOXIC PROPERTIES** OF CHEMICALS
- ! THE COMPARISON OF **MINIMAL EFFECTIVE CONCENTRATIONS OF DRUG** WITH THOSE REALLY ARISE IN HUMAN BLOOD AFTER TREATMENT
- ! THE COMPARISON OF **MINIMAL TIME REQUIRED TO PRODUCE A PATHOGENIC EFFECTS IN VITRO** WITH REAL PRESENCE TIME OF SIMILAR DRUG CONCENTRATIONS IN HUMAN BLOOD
- ! THE EXPERIMENTAL USING OF **HUMAN DRUG PHARMACOKINETIC DATA**
- ! THE EXPERIMENTAL USING OF **HUMAN TISSUE FLUIDS** (BLOOD, AMNIOTIC FLUID, LIQUOR ETC)
- ! THE REVEALING OF EMBRYOTOXIC FACTORS IN **WOMAN BLOOD** WITH THE PURPOSE OF PROGNOSIS OF POSSIBLE PATHOLOGICAL OUTCOMES OF PREGNANCY
- ! **THE MONITORING** OF POSSIBLE PRESENCE OF TERATOGENIC FACTORS IN BLOOD OF WOMEN DEAL WITH CHEMICALS OR LIVING IN ECOLOGICAL UNFAVORABLE REGIONS

THE CAPABILITIES OF IN VITRO TEST-SYSTEM APPLICATION

! THE SCREENING OF POSSIBLE EMBRYOTOXIC PROPERTIES OF CHEMICALS AND PROGNOSIS THEIR TERATOGENIC HAZARD FOR HUMAN EMBRYOGENESIS

!!THE INVESTIGATIONS OF MECANISMS OF TERATOGENIC DRUG EFFECTS

!!! THE JOINT TESTING OF TERATOGENIC DANGEROUS OF CHEMICAL COMPOUNDS WITH ROUTINE SYSTEM AIMED ON IMPROVEMENT OF PROGNOSTIC VALUE OF EXPERIMENTAL DATA

!!!! THE MONITORING OF POSSIBLE PRESENCE OF TERATOGENIC FACTORS IN BLOOD OF WOMEN DEAL WITH CHEMICALS OR LIVING IN ECOLOGICAL UNFAVORABLE REGIONS

IN VITRO TEST-SYSTEM THE DIRECTION OF FURTHER INVESTIGATIONS

! full adaptation of test-system for preclinical assessment

of drug teratogenic properties

! провести работу в области лабораторно-научных исследований
прогнозирование потенциальной опасности эмбриотоксических

! Исследовать in vitro генетические различия в реакциях ранних
зародышей на воздействие молекул на генетических основ

! протератогенного действия лекарственных препаратов, эпилепсия
препараты (железные соли, диклофенак, тканевые жидкости и т.д.)
органогенез, экспрессии генов, регулирующих раннее

! исследование в области протератогенных воздействий
биофармацевтики протератогенов

RIHOPHE



ST-PETERSBURG

THANK YOU FOR ATTENTION

РИНОРНЕ



ST-PETERSBURG

E-MAIL GIPEH@LENS.SPB.RU

VPOPOV@CTINET.RU

