

Clinical and forensic toxicology

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Toxicology

- biological science that cover disorders to living organisms caused by toxic substances on account of molecular interaction

Toxic substance

- 1537 – Paracelsus: „All things are poisons, merely dosage can make a thing atoxic“

Philippus Aureolus Theophrastus Bombastus von Hohenheim
(1493-1541)



- nowadays: „substance which application in small amount leads to pathological changes in organism or may even cause its death (all substances that are qualitatively or quantitatively „strange“ for organism)

Sorting of toxic substances on base of LD₅₀

- non-toxic (>15 g/kg)
- mild toxic (5-10 g/kg)
- medium toxic (0,5-5 g/kg)
- intensively toxic (0,05-0,5 g/kg)
- extremely toxic (5-50 mg/kg)
- supertoxic (<5 mg/kg)



Chemical subst.	LD ₅₀ (mg/kg)
ethanol	7000
NaCl	3000
morphine	900
phenobarbital	150
strychnine	2
nicotine	1
dioxine (TCDD)	0,01
batrachotoxine	0,005
botulotoxine	0,00001

Division of toxicology

- general toxicology
- experimental toxicology
- toxicology of foodstuffs
- veterinary toxicology
- military toxicology
- environmental toxicology
- clinical toxicology
- forensic toxicology
- etc.

Sorting of toxic substances - Gonzales's classification of poisons

1. Gaseous poisons: - hydrogen cyanide, carbon monoxide, carbon dioxide, methane, nitrogen oxides, sulphane, etc.

2. Inorganic poisons: - corrosive compounds - acids (sulphuric, nitric, hydrochloric, ...)

- bases (hydroxides of alkalic metals)

- halogenes (fluorine, chlorine, bromine, iodine)

- metals (eg. barium, lithium, „heavy metals“ - cadmium, lead, mercury, ...)

- non-metalic compounds (phosphorus, arsenic, cyanides, ...)



3. Organic compounds: - volatile compounds (ethanol, ketones, aldehydes, chlorinated hydrocarbons,

aromatic compounds (toluene, benzene, ...)

- corrosive compounds (organic acids, phenols)

- alkaloids (morphine, codeine, atropine, scopolamine, strychnine, ...)

- medicaments - hypnotics (barbiturates, some benzodiazepines)

- analgetics (salicylates, paracetamol, tramadol, ...)

- psychopharmaceutics (benzodiazepines, TCA, ...)

- local anaesthetics (mesocaine, lidocaine, ...)

- other medicaments (myorelaxans, antihypertenzives, ...)



- agrochemicals (pesticides, herbicides, insecticides, ...)

- vegetal poisons (mushrooms, ...)

- animal poisons (eg. snakes, wasp, bee, spiders, ...)

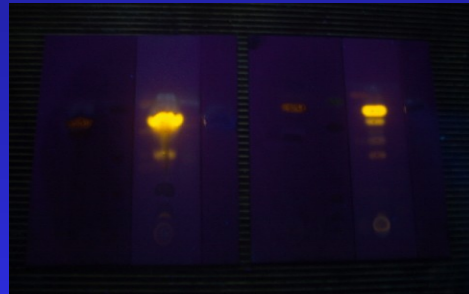
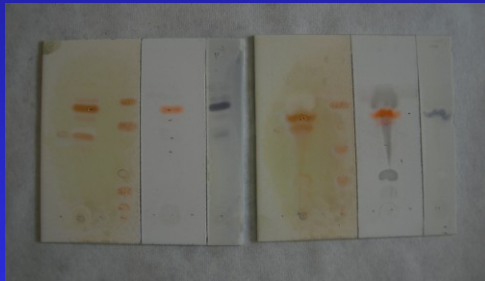


GENERAL PROCEDURE OF THE SYSTEMATIC TOXICOLOGICAL ANALYSIS

Qualitative analysis

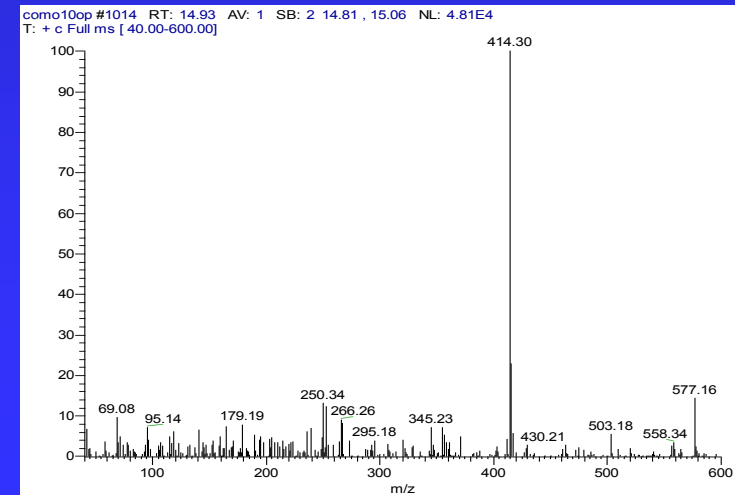
SCREENING

- **immunochemical methods** (RIA, EIA, FPIA,...) - based on the non-specific reaction of antibody-antigen type
- **chromatographic methods** (GC, LC, especially TLC)



IDENTIFICATION = CONFIRMATION

- **chromatographic methods** (2D-TLC, HPLC- vs. GC-MS)
- **spectral methods** (UV-VIS or IR – spectrometry)



GENERAL PROCEDURE OF THE SYSTEMATIC TOXICOLOGICAL ANALYSIS

- continuing

Quantitative analysis

DETERMINATION

- **chromatographic methods**

- GC-FID (determination of volatile compounds)
- GC-NPD (det. of medicaments and abused drugs containing N,P)
- GC-ECD (det. of medicaments and abused drugs containing halogenes)
- GC-TCD (det. of gaseous compounds with a low relative molecular weight)
- HPLC-DAD (FD)(det. of more polar and thermolabile compounds)



INTERPRETATION OF THE RESULTS



Volatile compounds - ethanol



- resorption of ethanol from stomach and GIT is influenced by stomach content and fullness
- affects CNS - results in vasodilatation in skin (feeling warmth), depression of motor ability (difficult coordination of moving), non-articulated speech, drowsiness
- Metabolism:

metabolised through three systems:

1. Alcohol dehydrogenase (ADH) - enzymatic system engaged at consumption of small amounts of EtOH
2. Microsomal oxidation - metabolizes 25-30% of absorbed EtOH
3. Catalase - metabolizes only 2%



chemically double-step oxidation:

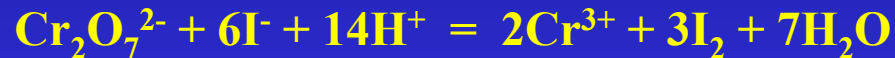


- autopsy findings in lethal poisoning: non-specific - besides the characteristic smell of organs pulmonary oedema, nearly invisible haemorrhages in oesophagus and gastric mucose; the only one really reliable method is laboratory test

Forensic determination of ethanol in blood

- carried out with 2 independent methods:

1. **Widmark's proof - volumetric (iodometric) determination of sum of reducing compounds (including EtOH) present in blood on the base of oxidative-reducing reactions:**



2. **Enzymatic method – non-specific (KIMS – kinetic enzymatic method – oxidative determination of ethanol – measured rate of difference in absorbance of elevating NADH at 340nm):**



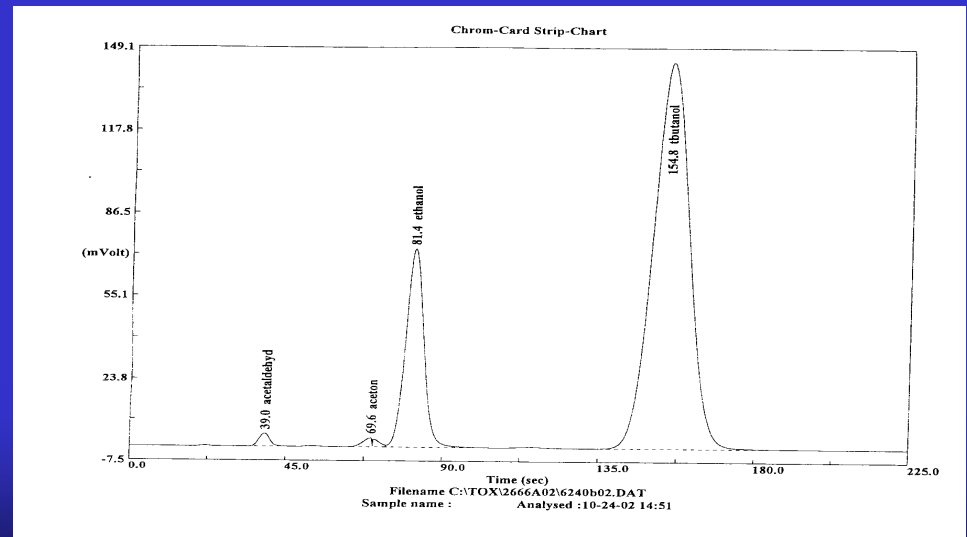
3. **Osmometry – non-specific method of the least precision (broad range of interferences), used rather in clinical examinations**

Forensic determination of ethanol in blood - continuing

2. Gas chromatographic method - specific determination based on separation of respective volatile compounds present in blood sample and their consecutive detection by FID as the elution proceeds



Typical record (chromatogram) of blood alcohol analysis by gas chromatography



GENERAL INTERPRETATION OF ALCOHOL LEVELS

BAC (g/kg)	INFLUENCE LEVEL OF A MAN
up to 0,015	endogenous alcohol
up to 0,3	first limit of positive traffic control
0,3-0,5	a man drank some alcohol, but without influence
0,5-1,5	light drunkenness
1,5-2,0	intermediate drunkenness
2,0-3,0	strong drunkenness
3,0-4,0	alcohol poisoning
over 4,0	risk of life



INTERPRETATION OF ALCOHOL LEVELS IN TRAFFIC CONTROL

(valid in the Czech Republic from 1.1. 2010)

BAC (g/kg)		penalty	interception of driving licence	„points“	imprisonment
(Dräger, Lion)		(CZK)	(years)		(years)
up to 0,24	without sanction				
0,25-0,54	penal offence	10.000-20.000	0,5-1	3	-
0,55-1,24	penal offence	10.000-20.000	0,5-1	6	-
>1,24	criminal offence	25.000-50.000	1-2	7	-
	or		1-10	7	up to 3





Carbon monoxide

- insidious substance - colourless, without any odour, density a bit lower in comparison with the air, together with the air (12%) it makes explosive mixture, about 200-times higher affinity to Hb than oxygen
- product of imperfection burning with lack of oxygen
- threatened groups: firemen, occupants of flats equipped with gas water-heater and/or gas- or solid-fuel local heating, users of propane-butane cylinder sources, asphyxiated victims of fire + attempts of suicide in cars
- symptoms of intoxication with CO proceed with cumulative saturation Hb with CO: difficult breathing, feeling of tiredness, lassitude, headache, sickness, nausea, vomiting, tachycardia, some neuropsychiatric symptoms, >50% unconsciousness, coma, circulatory failure, breathing arrest, death
- autopsy findings: characteristic pink-bright red spots on skin, internal organs of the same colouring (seen only at high COHb-saturation, but not in every case)



Cyanides



- sources: plating of steel, gold mining, some insecticides, chemical laboratories, burning of plastics, bitter almonds or peach kernels (amygdaline)
- principle of intoxication:
cytochromoxidase- $\text{Fe}^{3+} + \text{CN}^- \rightarrow$ stable complex
blocked reduction to cytochromoxidase- Fe^{2+} leading to insufficient delivering of oxygen around the organism (internal asphyxia)
- symptoms of intoxication: pink colouring of skin, nausea, spasmodic stages
- lethal dose: 100-250 mg, about 70 pcs of bitter almonds (for adult)
- autopsy findings: bright red spots on skin, pink colour of blood, in case of peroral consumption sharp line on stomach mucose - border between damaged and non-damaged parts of tissue

CLASSIFICATION OF TOXICOLOGICALLY SIGNIFICANT MEDICAMENTS

1. HYPNOTICS

- a) of barbiturate type
- b) of non-barbiturate type - benzodiazepines with hypnotical effect (flunitrazepam, triazolam, nitrazepam)
- hypnotics of new generation (zolpidem, zopiclone)

2. ANALGETICS

- a) antipyretics - of first generation (containing aminophenazone, phenacetine)
- of second generation (containing acetylsalicylic acid, paracetamol, codeine, etc.)
- b) anodynes (pethidine, pentazocine, tilidine)
- c) spasmolytics (containing metamizole, pitophenone and fentanyl)

3. PSYCHOPHARMACOLOGICAL AGENTS

- a) anxiolytics (benzodiazepines)
- b) antidepressants (thymoleptics of first, second and third generation)
- c) neuroleptics - of phenothiazine type
- of thioxanthene type
- d) psychic stimulants
- e) nootropics

Hypnotics of barbiturate-type

- in the past used as sedatives, hypnotics and antiepileptics, nowadays above all as antiepileptics (depression of CNS)
- on the basis of lasting of effect we differentiate barbiturates with:
 - ultrashort effect (thiopental)
 - short effect (hexobarbital)
 - medium lasting effect (amobarbital)
 - longlasting effect (phenobarbital)
- markers of poisoning: non-specific - drowsiness, headache, vomiting, disturbances of consciousness with possible progression to coma, miosis, hypertermia, cyanosis
- autopsy findings: non-specific - dermal erythema, oedema of brain, hyperaemia of internal organs

Benzodiazepines

- medicaments with anxiolytic, sedative, hypnotic and anticonvulsive effect
- on the basis of half-time of elimination we differentiate benzodiazepines to ones with:
 - ultrashort effect (midazolam, triazolam)
 - short effect (alprazolam, oxazepam)
 - longlasting effect (diazepam, chlordiazepoxide)
- symptoms of intoxication: mild in contrast to the other hypnotics and include drowsiness, sedation, ataxia, diplopia, dysarthria and intellect attenuation
- abused by addicts for depression of unpleasant symptoms of amphetamine withdrawal

DRUGS OF ABUSE

- include legally used substances (alcohol, tobacco), some medicaments (analgetics, hypnotics), organic solvents and illicit drugs

1. *SYNTHETIC*

- **amphetamine, methamphetamine**
- **MDMA, MDEA, MDA, PMA,...**

2. *SEMISYNTHETIC*

- **heroin, lysergic acid diethylamide (LSD)**

3. *NATURAL*

- **cannabinoides** (cannabinol (THC), cannabidiol,...)
- **opiates** (morphine, codeine, papaverine, narcotine, thebaine,..)
- **cocaine**
- **hallucinogens** (mescaline, psilocybine)

Synthetic drugs of abuse – derivatives of amphetamine

- strong psychostimulating effect

- on the basis of chemical structure sorted to:

1. Derivatives of phenethylamine (ephedrine, amphetamine, methamphetamine)
2. Ring-substituted phenethyl derivatives (MDA, MDEA, MDMA, PMA, DOM, DOB, 4-MTA, 2-CB,..)

- amphetamine (preparation Psychoton) used to be prescribed for treatment of obesity and pathological somnolence – tolerance and strong dependence lead to be labeled as an illicit drug

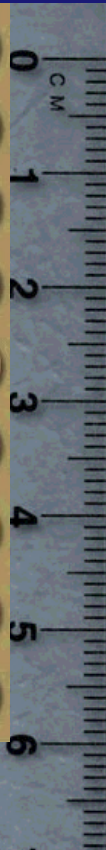
- dosage:	usual dose	lethal dose
Amphetamine	5-50 mg	about 200 mg
Methamphetamine	15-30 mg	up to 1g and more (depends on developed tolerance)

- the most usual ways of application: intravenous injection, snorting (resorption through the nasal mucose)

- symptoms of intoxication: hyperactivity, restlessness, insomnia, euphoria, increased pulse rate

- tolerance and psychological dependence is developing subsequently at chronic abusers

- ring-substituted derivatives of phenethylamine applied usually orally in tablet form; tablets usually bear some sign – eg. three diamonds („Mitsubishi“), „Gate of the East“, etc. – typical for the producer
- MDMA (XTC, Ecstasy) – the most available substance of this group
 - psychostimulative and hallucinogenic properties
 - content of effective compound is variable: 50-200 mg
 - lethal dose: 0,5 g and more
 - manifestations of intoxication: excitation, absence of feeling hungry and tiredness, in contrast to amphetamine and methamphetamine intoxication leads to hypertermia and dehydration of organism, the need of repeated rhythmic moving (usually dancing) – so called „dancing drug“



LSD microdot, 2.3mm x 1.5mm, 7.5 mg pill weight

2C-B tablet, 5.1mm x 1.5mm, 45 mg pill weight

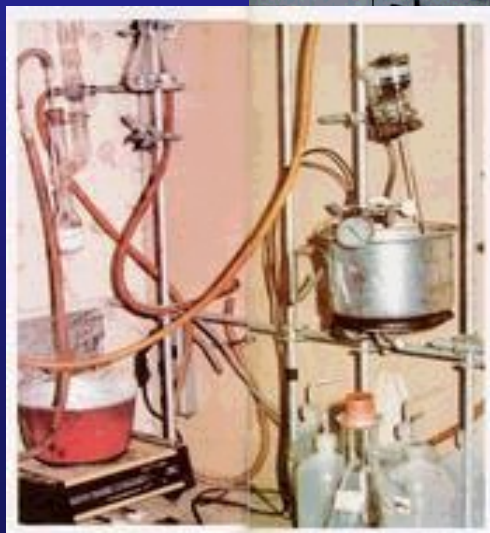
LSD blotter, 6.0mm x 0.2mm, 6 mg total weight

2C-T-2 tablet, 6.9mm x 3.3mm, 166 mg pill weight

MDMA tablet, 8.9mm x 3.5mm, 260 mg pill weight

U.S. Dime, 17.7mm x 1.1mm, 2280 mg total weight

Tablets of Ecstasy



Clandestine lab for illicit methamphetamine production

Logos of selected Ecstasy tablets

Lichtbild der Vorderseite										
Bezeichnung: Rückseite:	ADAM	EVA 130 mg	Amor Bruchrille	Love Herz	Herz Bruchrille	Drops Bruchrille	Sonne Bruchrille	Halbmond Bruchrille	Herzfeil Bruchrille	VW Bruchrille
Käfer	Mercedes	Triple Five	V.I.P.	CAL Bruchrille	PT Bruchrille	Schlitzauge Bruchrille	ANADIN	Boomerang	Bulls Bruchrille	Delphin Bruchrille
Elephant Bruchrille	Hund Bruchrille	Pigs Ringelschwanz	Pelikan Bruchrille	Tauben Bruchrille	Friedens-taube	Spatz Bruchrille	Vogel	Kermit Bruchrille	Feuerstein Bruchrille	Batman
Superman	Popeye Bruchrille	Chiemsee Bruchrille	Fido Bruchrille	Häuptling Bruchrille	Sonic Sonic/Bruchrille	Smiley SMILE	Playboy Bruchrille	Schwalbe	Dino Bruchrille	Anker Bruchrille
Pilz Bruchrille	Olympics	Hammer&Sichel	Gorbys CCCP	Kleeblatt Kleeblatt	Kleeblatt Bruchrille	Liebessymbol Bruchrille	Yellow Sunshine	Pink Panther	Snowball	Ying Yang

(Fotomaterial: Bundeskriminalamt Wiesbaden)

Semisynthetic drugs of abuse – heroine, LSD

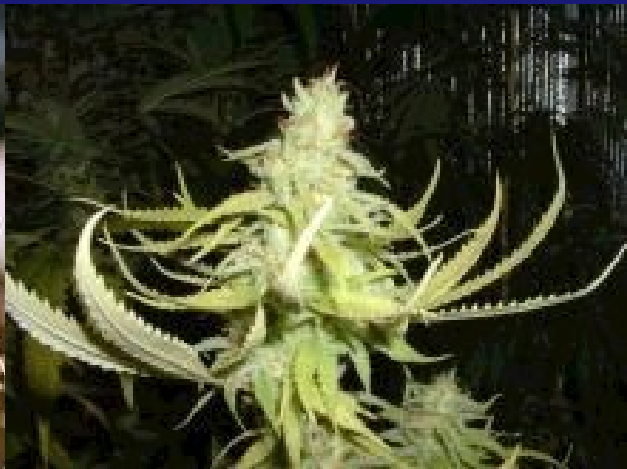
Heroine - semisynthetic opiate (diacetylmorphine)

- white-brown powder (depends on its purity)
- the most usual way of application is intravenous injection of melted powder
- usual dose: 50-250 mg, but already 200 mg may be lethal (depends on developed tolerance), chronic abusers may use doses up to 1g without risk of life
- symptoms of intoxication: extreme constriction of pupils, decrease of pulse rate and blood pressure, drowsiness, lethargy, inarticulate speech and difficult concentration
- chronic abusers are psychically and physically dependent on the drug
- withdrawal syndromes: dilatation of pupils, increased pulse and breathing rate, nausea, headache, pain in muscles, joints and bones, cold sweat; psychological syndromes are anxiety, restlessness, aggressivity, depression and disturbances of sleep
- overdose is manifesting as a progressive depression of breathing centre leading to breathing arrest and circulation failure
- substitution treatment – methadone, buprenorphine (Subutex)

Drugs of abuse of natural origin (cannabinoides, opiates, cocaine, hallucinations generating compounds)

Cannabinoides

- sources: plants of *cannabis sativa* and *cannabis indica*, esp. flowering tops and the seeds of female plant; of the best quality are plants coming from area called „Golden corner“- Barma, Laos and Thailand
- the highest psychotropic effect is that of Δ^9 -tetrahydrocannabinol (THC)
- main products:
 - marihuana
 - hashish
 - hashish oil
- ways of application:
 - smoking of hashish or marihuana
 - oral application (pieces of plant in meal)
- effective dose: 10-15 mg of THC (= 1-3 joints)
- symptoms of intoxication: hyperaemia of conjunctivas, dilatation of pupils, groundless amusement and euphoria; sometimes temporal loss of orientation and hallucinations
- in chronic abusers developing of psychical dependence
- cases of fatal intoxication not known



Cultivated cannabis

„Harvest 2003“



Clandestine cultivation of cannabis

Opiates

- source of opiates: opium (dried juice from green poppy-heads) from plant *Papaver somniferum*: morphine (2,7-20%), narcotine (6-10%), papaverine (0,8-1%), codeine (0,3-4%) and thebaine (0,1-0,6%)
- the biggest producers are countries of „Golden triangle“: Afghanistan, Pakistan and Iran
- Morphine:
 - usually applied intravenously
 - usual dose: 5-20 mg
 - lethal dose: about 200 mg; addicts with well developed tolerance may use doses up to 2 g without risk of life
 - symptoms of intoxication are the same as mentioned in heroine

Papaveraceae
Papaveraceae



Papaver somniferum L.
Papaver somniferum L.



Cocaine

- source: leaves of bush *Erythroxylon coca* (South America – Colombia)
- available as:
 - hydrochloride salt
 - free base („crack“)
- ways of application:
 - hydrochloride salt: snorting or intravenous application
 - free base: smoking
- usual dose: 30-200 mg
- lethal dose: about 1,2 g (in sensitive people already 30 mg may be lethal); addicts with well developed tolerance may use up to 5 g/day without risk of life
- chronic abusing leads to psychical dependence
- manifestations of intoxication: similar to those of amphetamine-derivatives:
 - hyperactivity, insomnia, euphoria, dilatation of pupils, increased pulse rate and blood pressure
- symptoms of overdose: paralysis of respiratory system and cardiac arrhythmia („cocaine shock“)



Erythroxylum coca L.f.
Erythroxylum coca



Hallucinogens (psychedelics)

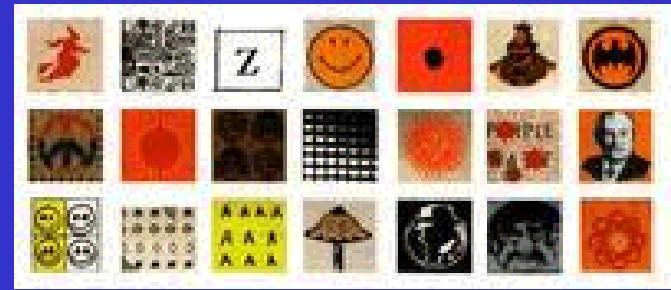
Mescaline – source: some cactuses (peyotl) grown in the south of the North America; Mexican tequila

Psilocybine – source: esp. mushrooms of genus *Psilocybe* (Asia, the Middle Europe)

- application: usually oral (dried or raw form)
- dosage: about 10 mg (equal to about 1g dried mushrooms)
- symptoms of intoxication: hallucinations (visual and acoustic), typical symptom in psilocybin is feeling of ability to fly



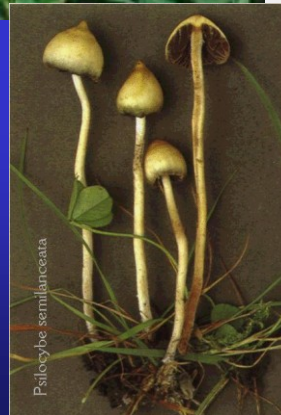
Claviceps purpurea – ergot



LSD trips



Psilocybe bohemica



Psilocybe semilanceata



Psilocybe semilanceata