# Metabolism and Toxicology

Finding a substance that shows an effect *in vitro* does not mean that this is a suitable *drug candidate* as well.

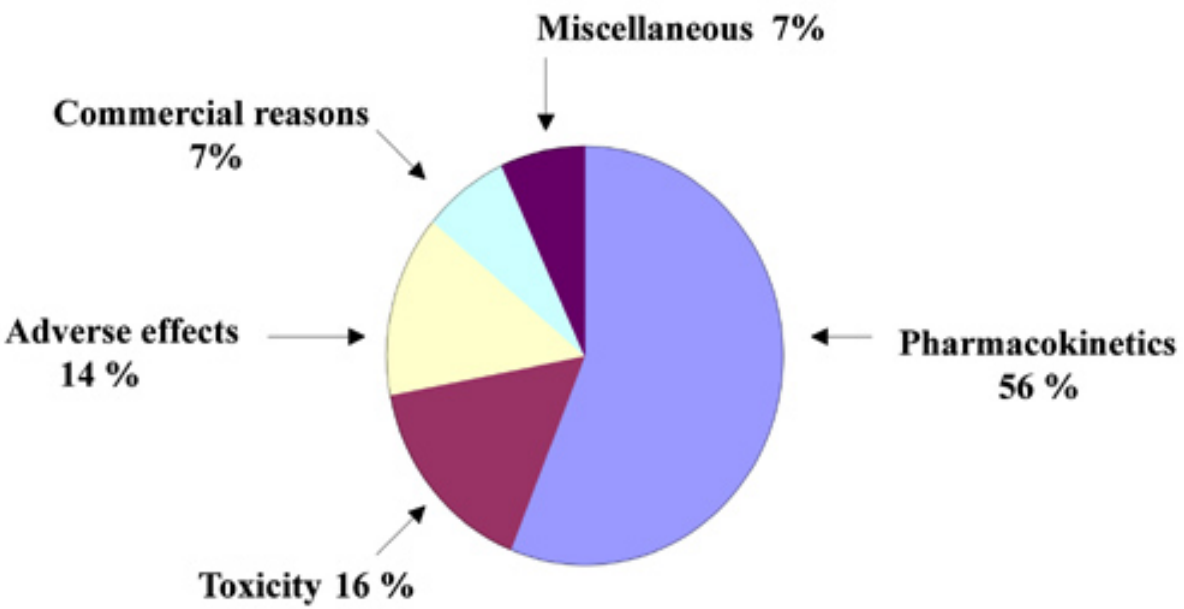
The vast majority of chemical substances undergo biochemical transformations inside the body (metabolisms).

Some of these reactions lead to degradation products (metabolites) that are toxic, mutagenic, etc.

It is therefore important to reckognize unsuitable compounds as early as possible:

„Fail early, fail fast, fail cheap“

# Why is the prediction of ADME parameters that important ?




Reasons that lead to failure or withdrawl of a potential drug around 1995 – 2000

# For risks and side effects...

Adverse effects are assumed to be the 5.-6.most frequent cause of death (USA 1994)

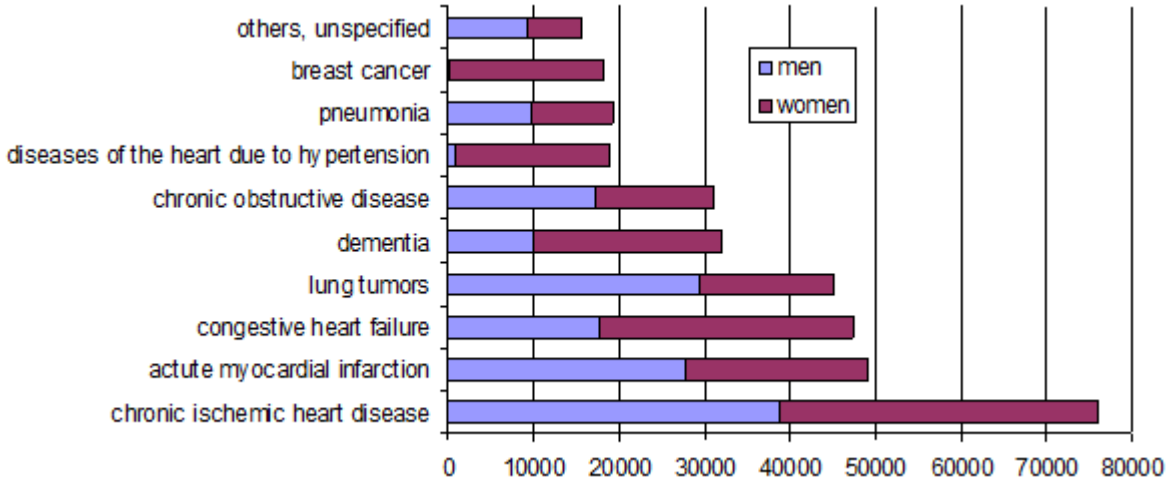
Most frequent (natural) cause: cardio-vasucular complications

List of withdrawn drugs (not comprehensive, see wikipedia)

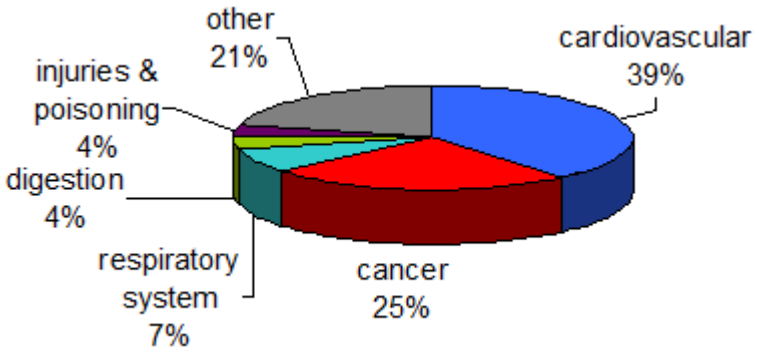
| trade name | adverse effect | manufacturer | time |
|---|---|---|---|
| propoxyphene | cardiac arrhythmia | generic | 2010 |
| flupirtine | liver toxicity | generic | Mar 2008 |
| clobutinol | QT-prolongation | gerenic | Aug 2007 |
| alatrofloxacin | liver toxicity | Pfizer | Jun 2006 |
| rofecoxib | thrombosis,stroke | Merck(USA) | Sep 2004 |
| cerivastatin | rhabdomyolysis | Bayer | Aug 2001 |
| alosetron | ischemic colitis | GSK | Nov 2000 |
| cisapride | cardiac arrhythmia | Janssen | Jun 2000 |
| pemoline | liver toxicity | Warner-Lambert | May 2000 |
| mibefradil | drug/drug Interaction | Roche | Jun 1998 |
| terfenadine | cardiac arrhythmia | Höchst | Dec 1997 |
| fenfluramine | heart valve disease | Wyeth | Sep 1997 |

# Actual causes of death

Most frequent causes of death in Germany (2015)




According to type of disease



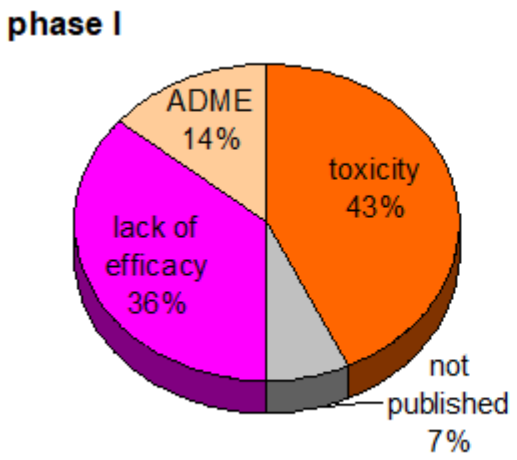

Data sources: Statistisches Bundesamt www.destis.de

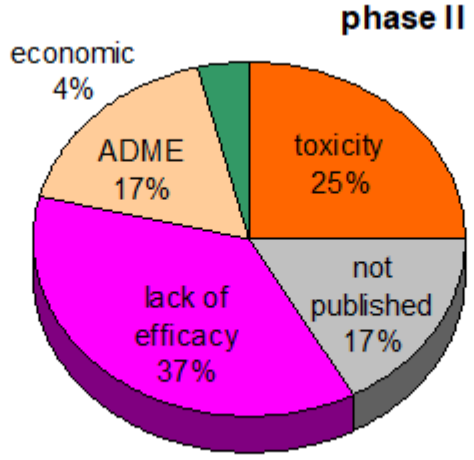
# Why drugs fail

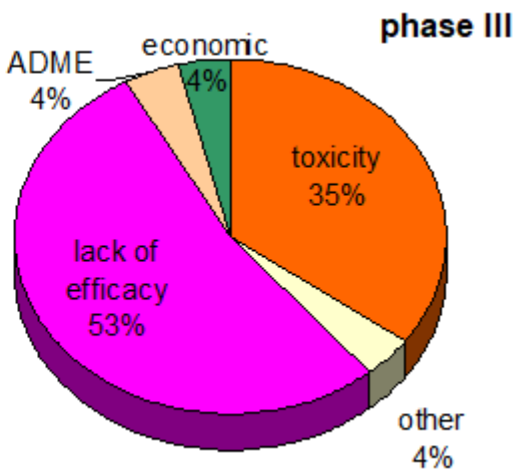
90% of market withdrawals caused by drug toxicity, from that ⅔ are due to hepatotoxicity and cardiovasuclar toxicity

Drugs failing in clinical phases I-III between 1992 to 2002 were mainly due to insufficient efficacy (43%)

→ Drug toxicity must be detected earlier than after market launch



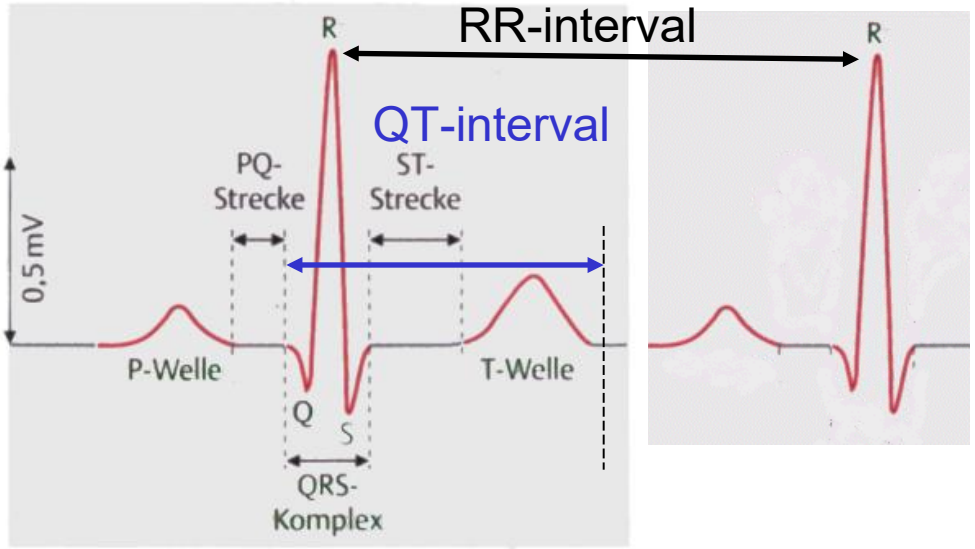



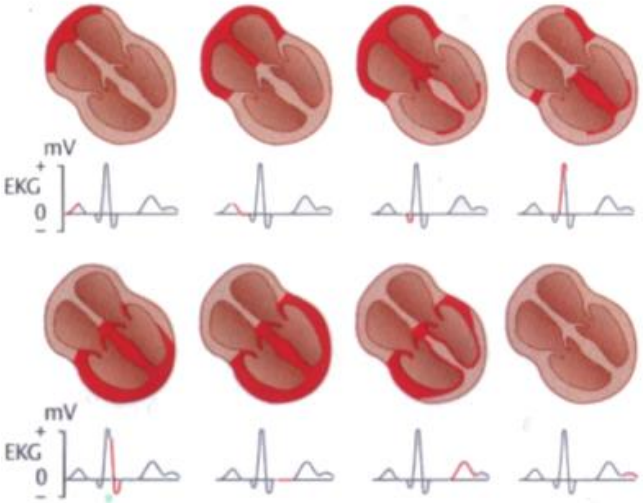

Data source: Schuster, Laggner, Langer, *Curr.Pharm.Des.* **11** (2005) 3545.

# QT interval prolongation (I)

Cardiac arrhythmias are among the most frequent adverse effects that lead to the failure of drugs (frequently as late as in clinical phases III or IV).

Often a prolongation of the so-called QT-interval in the ECG is observed.
The upper limit is usually at 440-470 msec for pulse of 60 beats per minute.




Picture source:
http://medizinus.de/ekg.php

# QT interval prolongation (II)

Since the heart beat rate is subject to change, the QT-time is normalized to the so-called *QTc interval* via division by the root of the preceeding RR interval (Bazett correction):

$$QTc = QT / RR^{1/2}$$

For pulse of 60 the RR-interval is 1 sec long

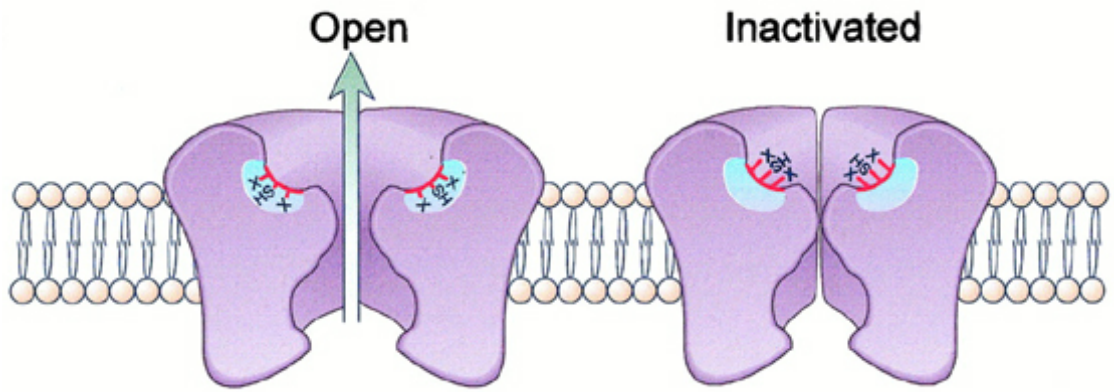
The observed current in the ECG during the QT-time is mainly due to the delayed activity of the cardial potassium channel (*outward repolarizing current* $I_{Kr}$).

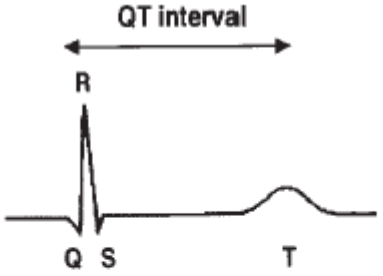
This voltage gated channel is coded by the so-called *human ether-a-gogo related gene* (hERG).

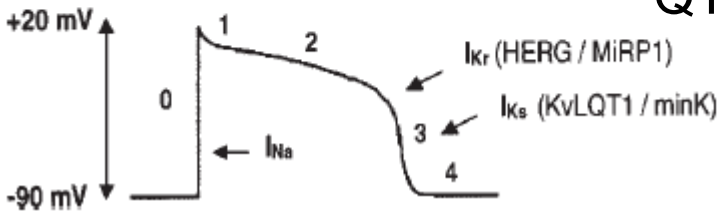
This effect is frequently used by anti-arrhythmic drugs of class III. On the other hand, too long QT-times can lead to fatal distortions of the cardial rhythm itself.

Lit: R.R.Shah *Brit.J.Clin.Pharmacol.* **54** (2002) 188.

# The *h*ERG potassium channel (I)



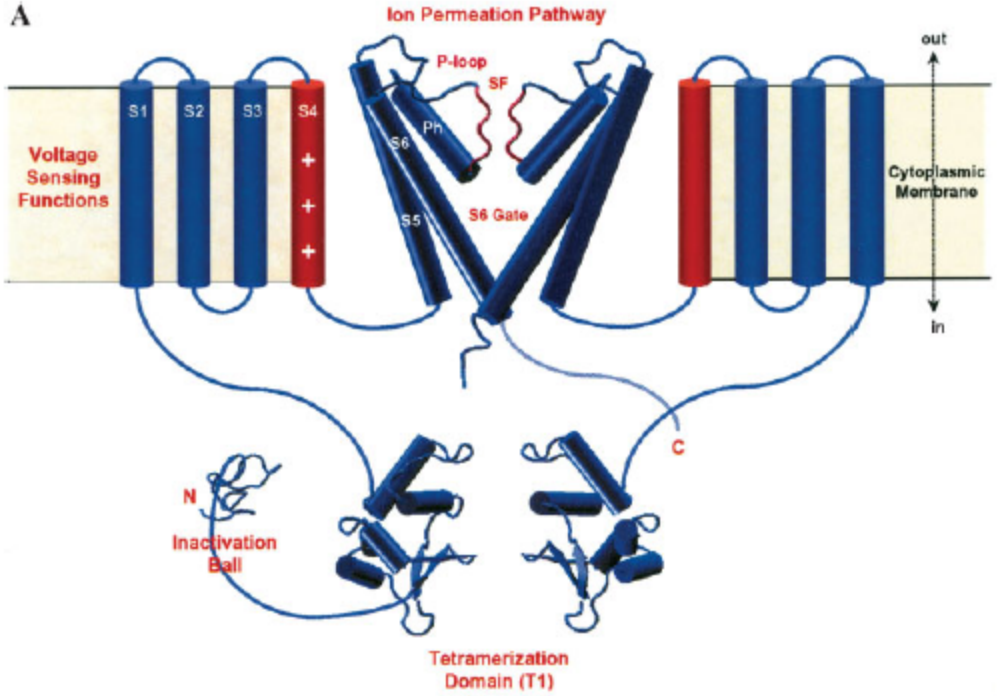



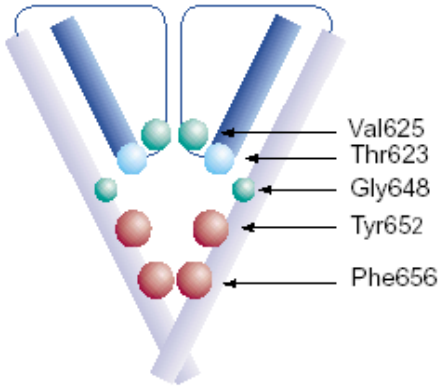



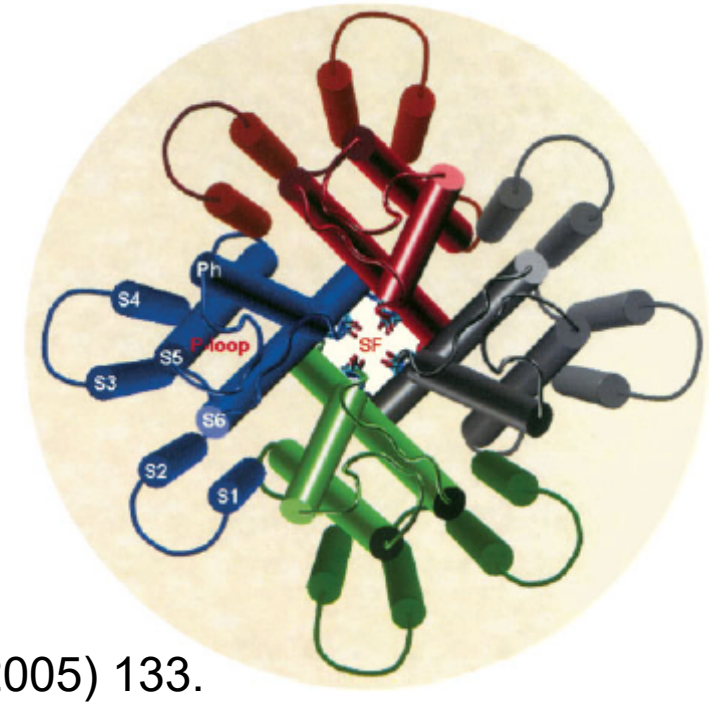

The activity of the *h*ERG channel accounts for the rapid potassium component ($K_r$ rapid) of the outward repolarizing current I during the QT-interval

Lit: M.Recanatini et al. *Med.Res.Rev.* **25** (2005) 133.

# The *h*ERG potassium channel (II)



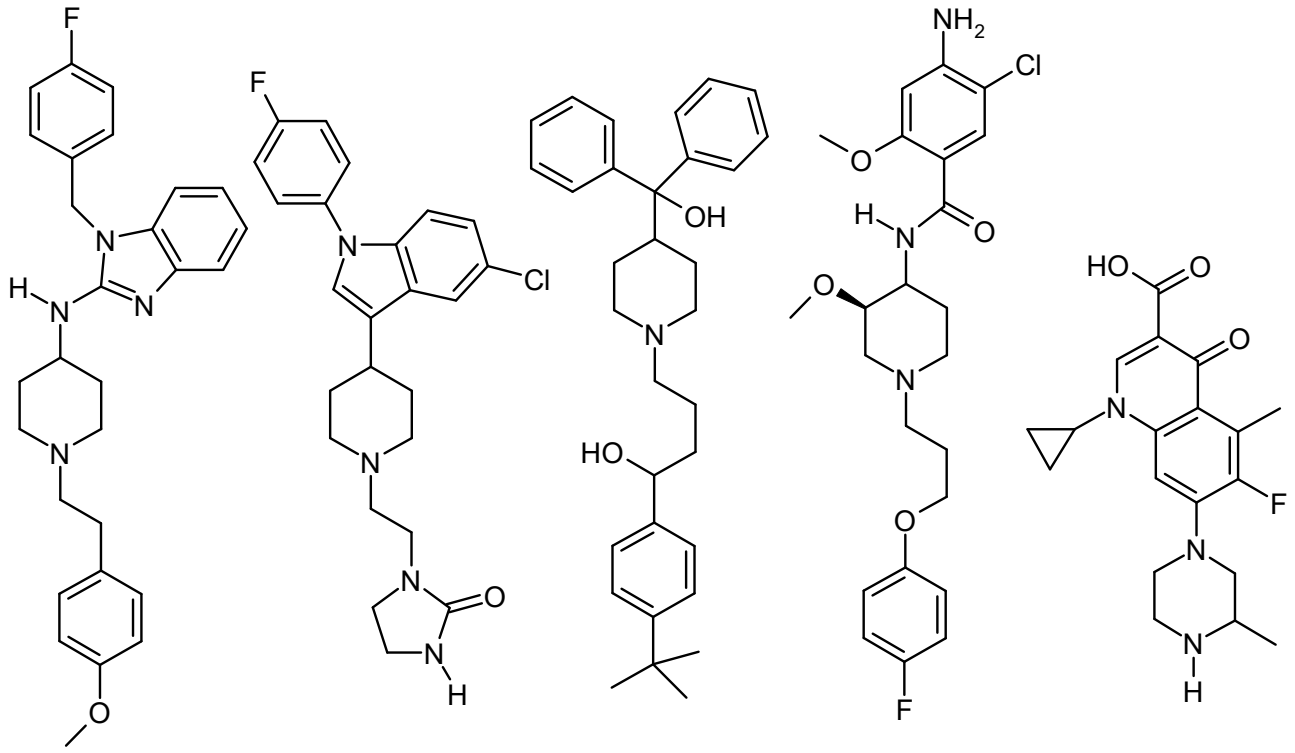




The *h*ERG channel is a homo-tetramer

Lit: M.Recanatini et al. *Med.Res.Rev.* **25** (2005) 133.

# *h*ERG channel blocking drugs




Astemizole
Antihistaminic

Sertindole
Antipsychotic

Terfenadine
Antihistaminic

Cisapride
Gastroprokinetic

Grepafloxacin
Antibiotic

In connection with QT-Interval prolongation withdrawn drugs: all exhibit high binding affinity to the *h*ERG potassium channel.

Lit: A.M.Aronov *Drug Discov. Today* **10** (2005) 149.

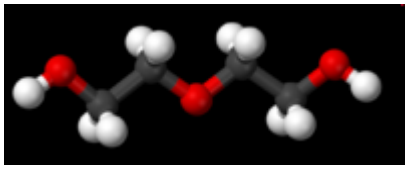
# Historical development in the USA

As a consequence of about 105 deaths caused by poisoning from an elixir of sulphanilamide in 72% diethylene glycole (Massengill incident), the United States Federal Food, Drug and Cosmetic Act of 1938 was passed, that regulates the passive approvement of substances by the Food and Drug Administration (FDA).

According to that, drugs have to be safe (at least) for their indicated use.

The approvement for (chemical) substances that are manufactured in larger quantities is subject to the Environmental Protecting Agency (EPA).

Lethal dose LD50 ≈ 1ml/kg body weight



# Historical development in Germany

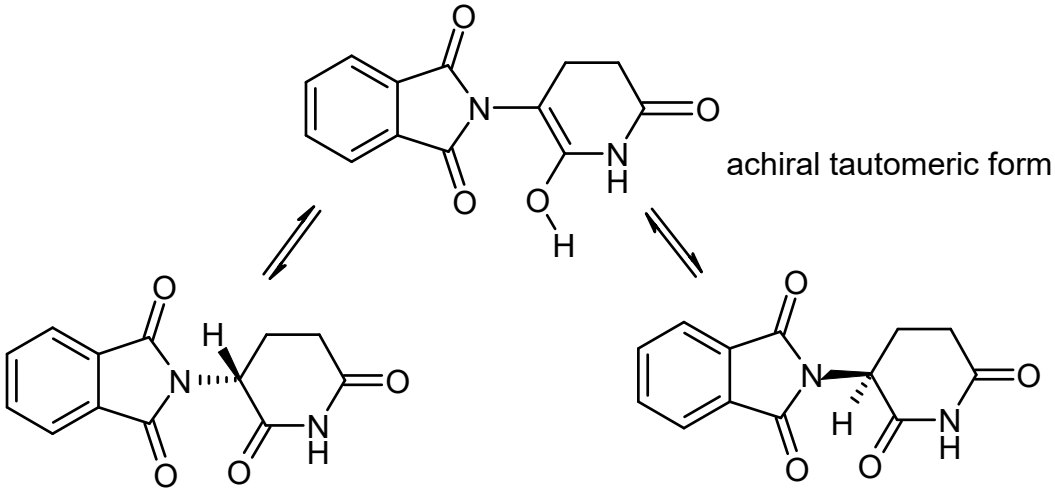
Until 1961 there was no comprehensive legislation regarding marketing of medical drugs in the former Federal Republic of Germany.

Decisive for the new legislation was the so-called Contergan-scandal: The responsible substance thalidomid (a sedative) did not show any indications in the original animal tests (mice), but turned to be teratogen in humans.

The *Arzneimittelgesetz* regulates among other things:

- requirements for clinical studies and tests
- prove of efficacy [Wirksamkeit]
- prove of non-existant toxicity for humans

# Interconversion of thalidomide




Racemizes (interconversion of the two isomers) within few hours.

New indications:

Antineoplastic, antiangiogenetic (both anti-tumor)

# Pre-clinical phase

After completing the *lead optimization* there are studies *in vitro* (model system of single and multiple cells) and *in vivo* (testing on animals) on the *lead candidate(s).*

During this stage filing for patent also occurs, whereby always a series of compounds is claimed in order to

- not stick to one single substance
- reserve similar potential substances
- complicate generic drugs („*me-too*“) [Nachahmungspräparate]

If not already introduced, compounds now receive an United States Adopted Name (USAN) at this stage. Example: cisapride

→ Simplifies scientific publishing and database assignment

# clinical studies / tests (I)

Phase I: Validation if the animal model can be transfered to human. Deriving dosage guidelines
(10-50 test persons, „healthy male“, no risk group)

Phase II: Validation of effiacy and relative harmlessness on some patients

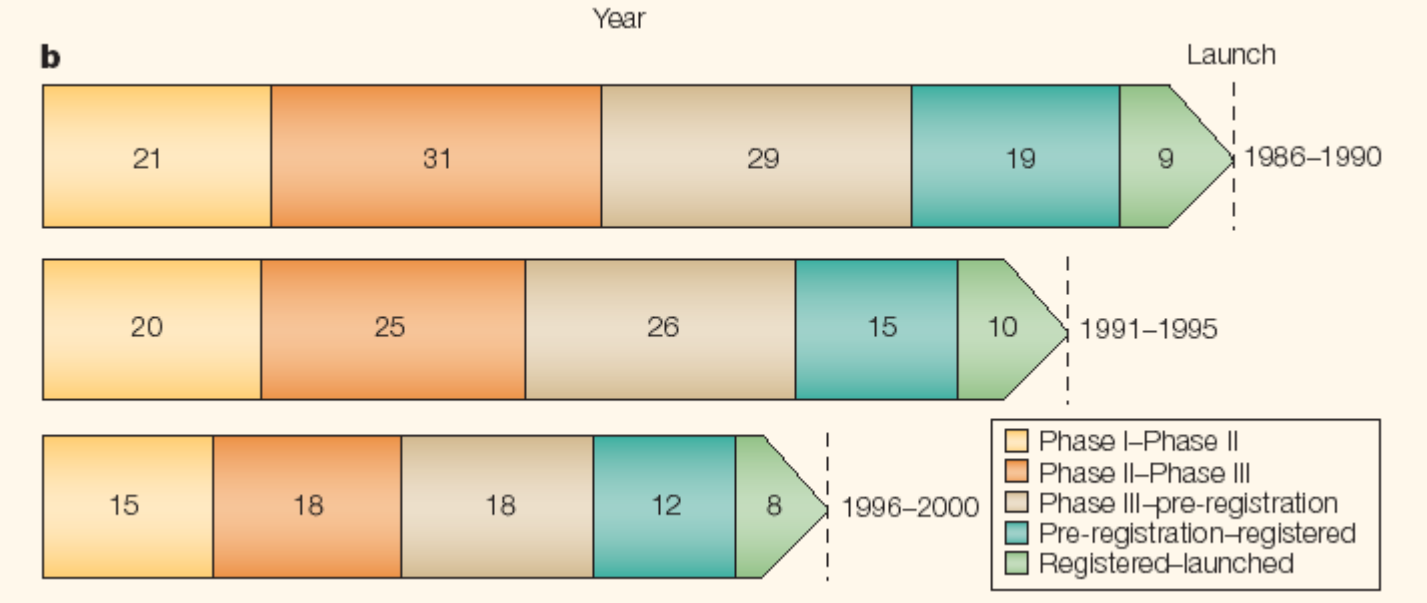
Phase III: Validation of effiacy and relative harmlessness on a larger number of patients. (as well as adverse effects upon co-administration with other medications)

After the market launch

Phase IV: As in phase III, but more comprehensive number of patients, recording of rare side effects, long term studies, validation of cost efficiency

# clinical studies / tests (II)

(Typical) duration (in months) for the pre-clinical development and clinical trials




Source: P.Preziosi *Nature Rev.Drug.Discov.* **3** (2004) 521.

# Approvement and launch (I)

The approvement in the USA is regulated by the Food and Drug Administration (FDA), in the EU now centrally the European Medicines Agency (EMA), respectively the EU comission.

A new medication is only approved if

- the field of application or the mode of action is new
- it shows a better efficacy than existing drugs
- it is better tolerated or shows less adverse effects
- it has a different administration [Darreichungsform] (Galenik)

The result of an approvement process is more and more decisive for the financial future of the manufacturer.

# Approvement and launch (II)

A new medication is also refered to as *new chemical entity* (NCE).

Investment per new chemical entity: >500,000 $
New chemical entities per year: ca. 15

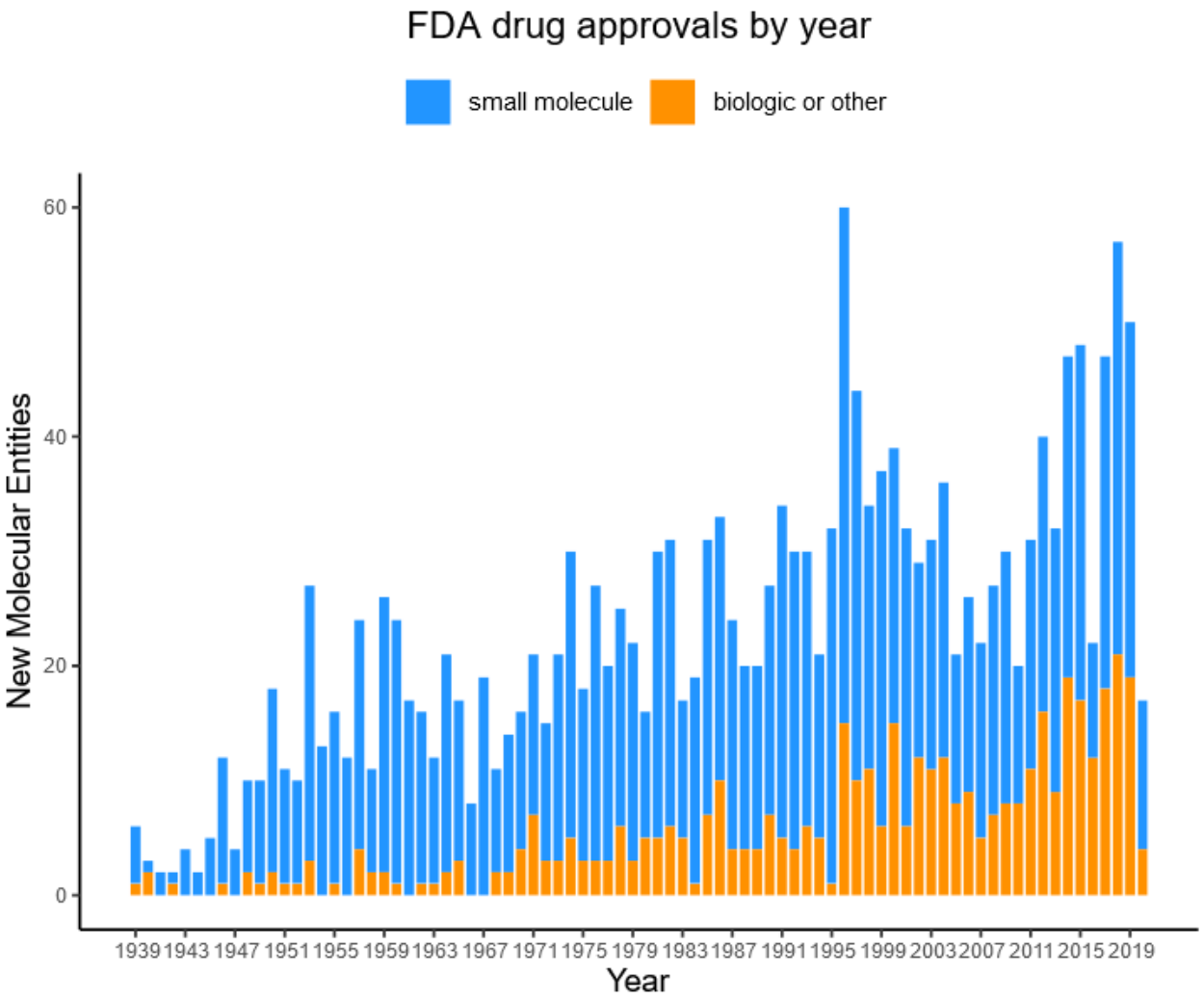
World Drug Index 58,000 marketed drugs

USAN <10,000 substances in clinical trials

| Drugs approved by the FDA | | expenses for research and development (USA) | |
|---|---|---|---|
| 1996 | 53 | 1980 | 2 Mrd US$ |
| 1997 | 39 | 1985 | 4 Mrd US$ |
| 1998 | 30 | 1990 | 8 Mrd US$ |
| 1999 | 35 | 1995 | 15 Mrd US$ |
| 2000 | 27 | 2000 | 26 Mrd US$ |
| 2001 | 24 | 2001 | 30 Mrd US$ |
| 2002 | 17 | 2002 | estimated 32 Mrd US$ |

# Approvement and launch (III)

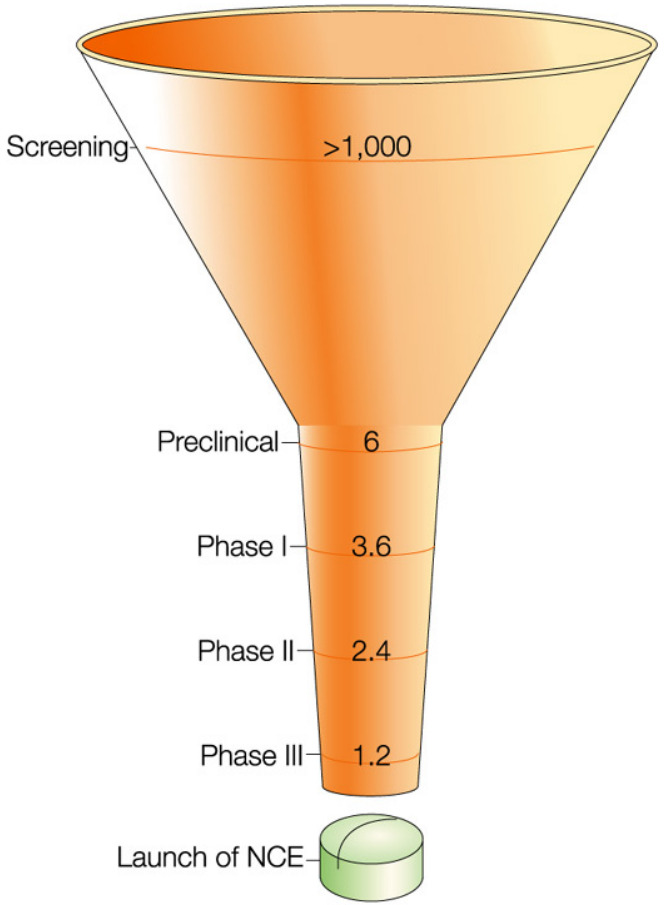
Trend in approval of new chemical entities



# From the *pipeline* to the market launch

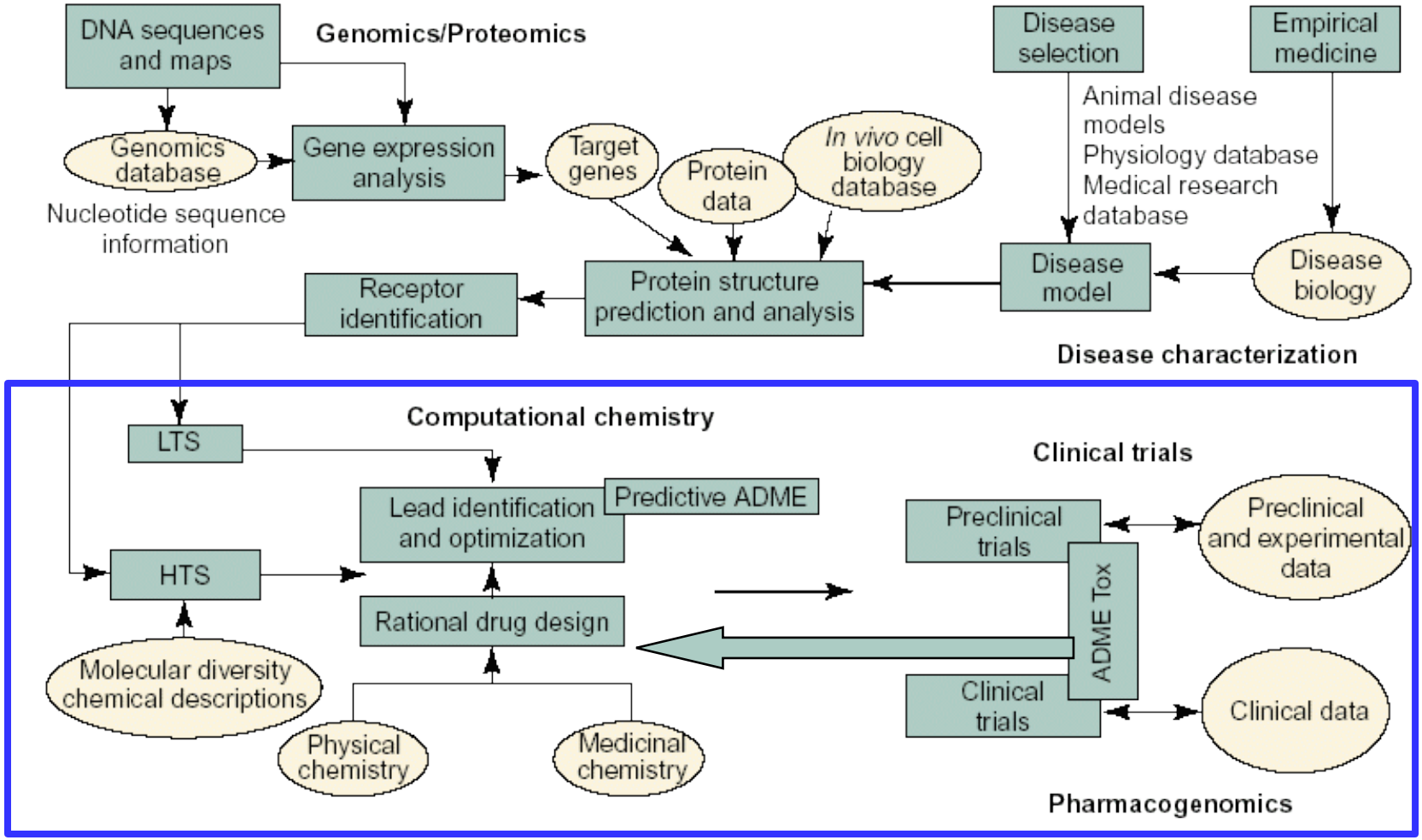
Counting from the number of actually approved drugs (*new chemical enitity, NCE*) back to the number of *in vitro* screened compounds, results in more than 1,000 per new drug.

Without the available computer-aided ADMET filters, this number would be even larger.



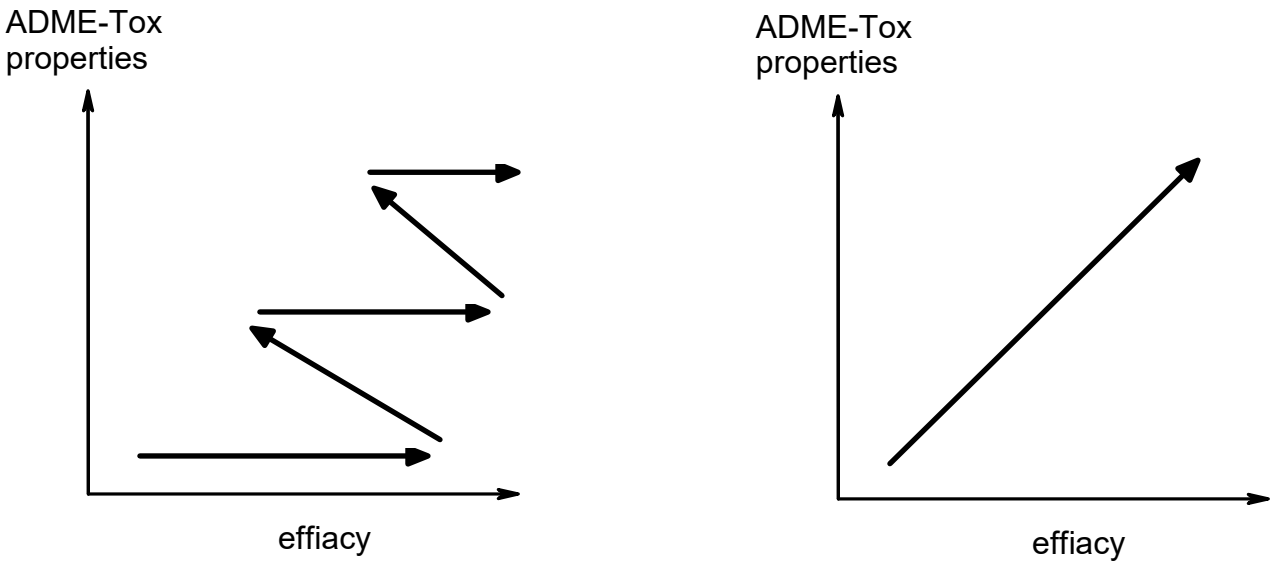

Nature Reviews | Drug Discovery

# Flow of information in a *drug discovery pipeline*




*Drug Discovery Today*

# Process of optimization from the *lead candidate* to the *drug candidate*



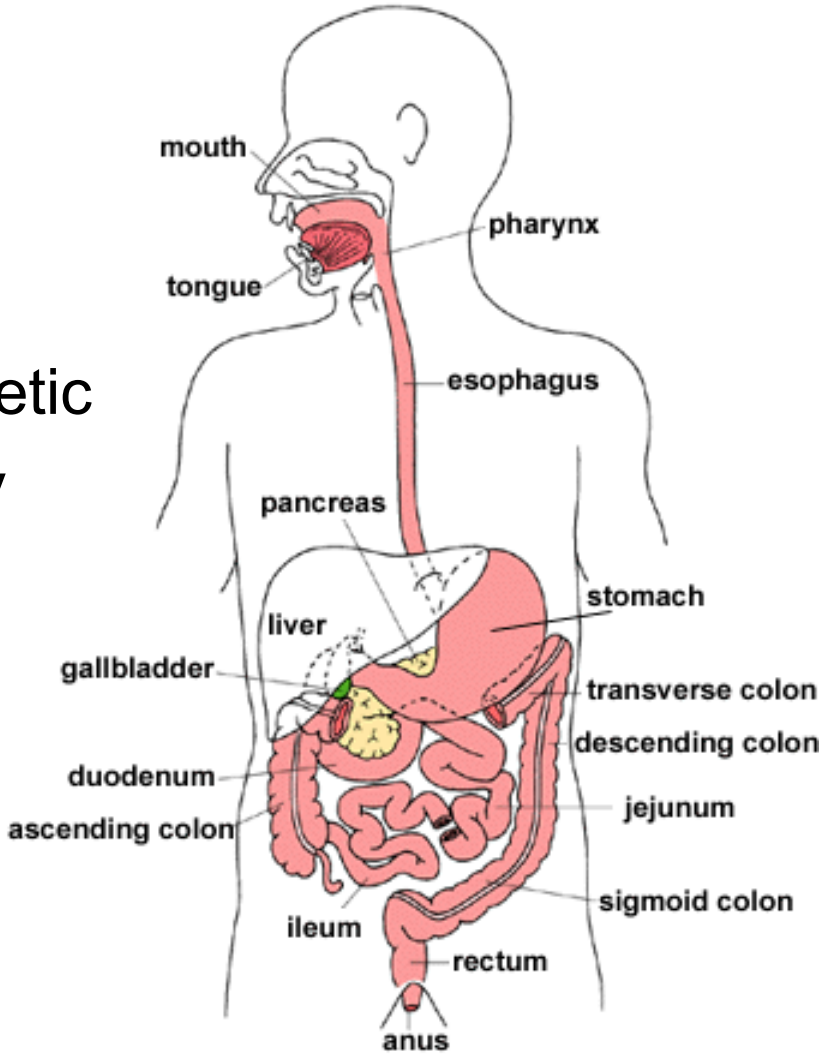

Past: optimization of effiacy first, then improvement of ADME-Tox criteria

Today: simultaneous optimization of effiacy and ADME-Tox properties (requires *in silico* AMDET models)

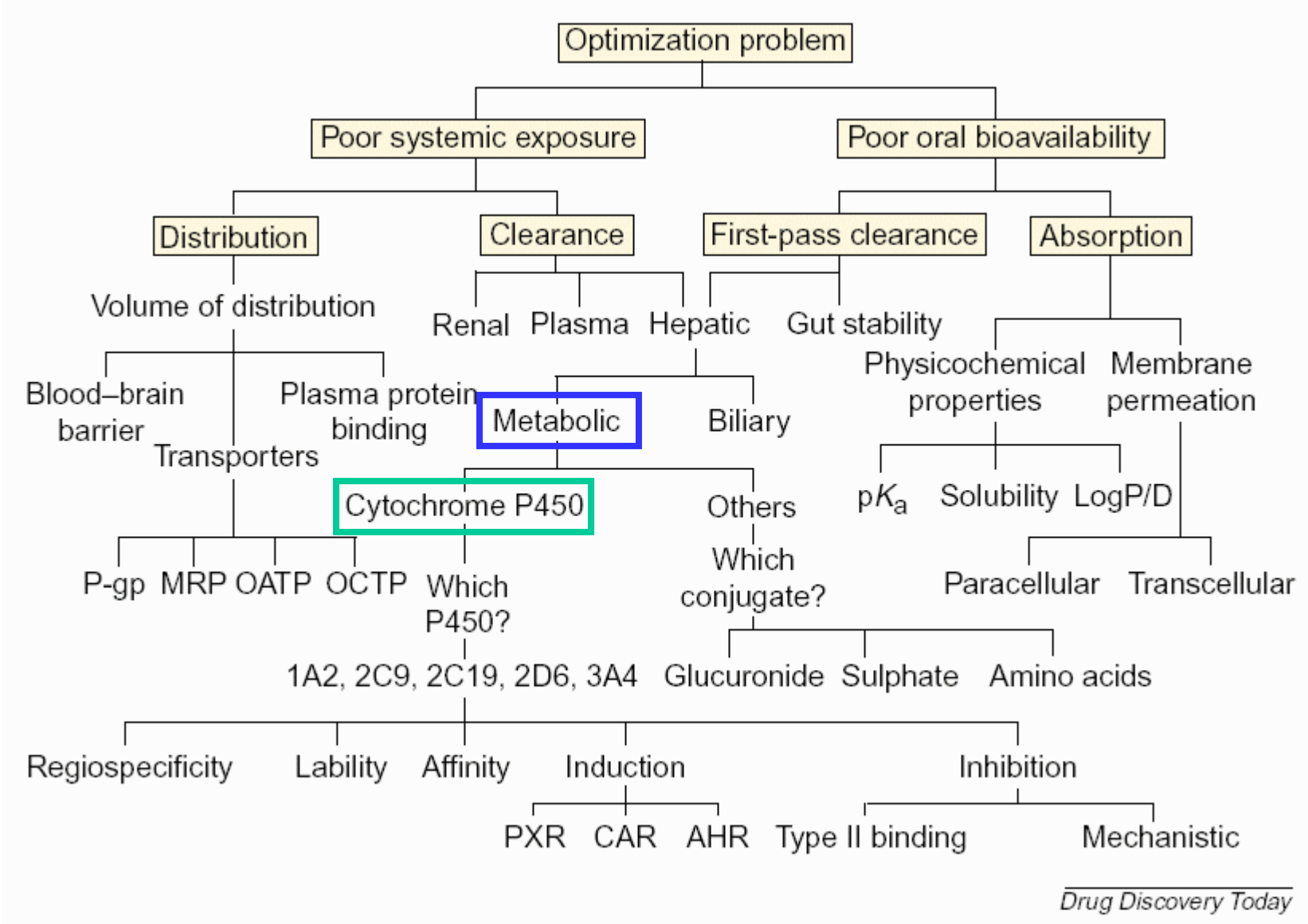
# eADMET Prediction

early
Absorption
Distribution
Metabolism
Elimination
Toxicology

} Pharmacokinetic
Bioavailability



# Scope of ADME-Tox models



# ADMET models

„ ... the modification of organic compounds by the microsomal enzymes can be understood in terms of physico-chemical constants in a quantitative fashion.“

Corwin Hansch (1972)

Lit: H. van de Waterbeemd, E. Gifford „ADMET *in silico* Modelling: Towards Prediction Paradise ?“ *Nature Reviews Drug Discovery* **2** (2003) 192-204

# Metabolism (I)

(bio-)chemical reactions of xenobiotics in the body

*First pass effect:*

Extensive metabolization of mainly lipophilic molecules, such with MW>500, or those that have a specific affinity to certain transporters, during the first passage through the liver

Phase I:

Oxidation, reduction and hydrolysis especially by Cytochrome P450 enzymes

Phase II:

Conjugation with small molecules (e.g. glutamine)

Phase III:

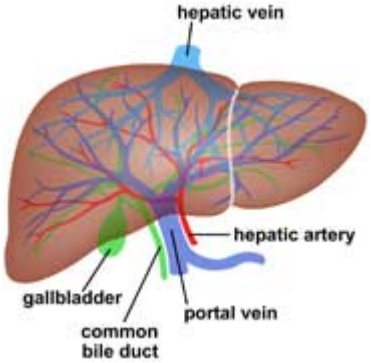
elimination by transporters

# Enzymes contributing to metabolism

Phase I:

oxidation, reduktion and hydrolysis

- cytochrome P450 enzymes (see lecture 10)
- dihydropyrimidin-, alcohol-, and aldehyde dehydrogenases
- epoxide hydrolases, esterases and aminases
- flavine monoxygenases




Phase II:

conjugation with small molecules (e.g. amino acids)

- N-acetyltransferase, glutathione S-transferase
- uridinediphosphate-glucuronosyltransferases
- sulfotransferases, methyltransferases

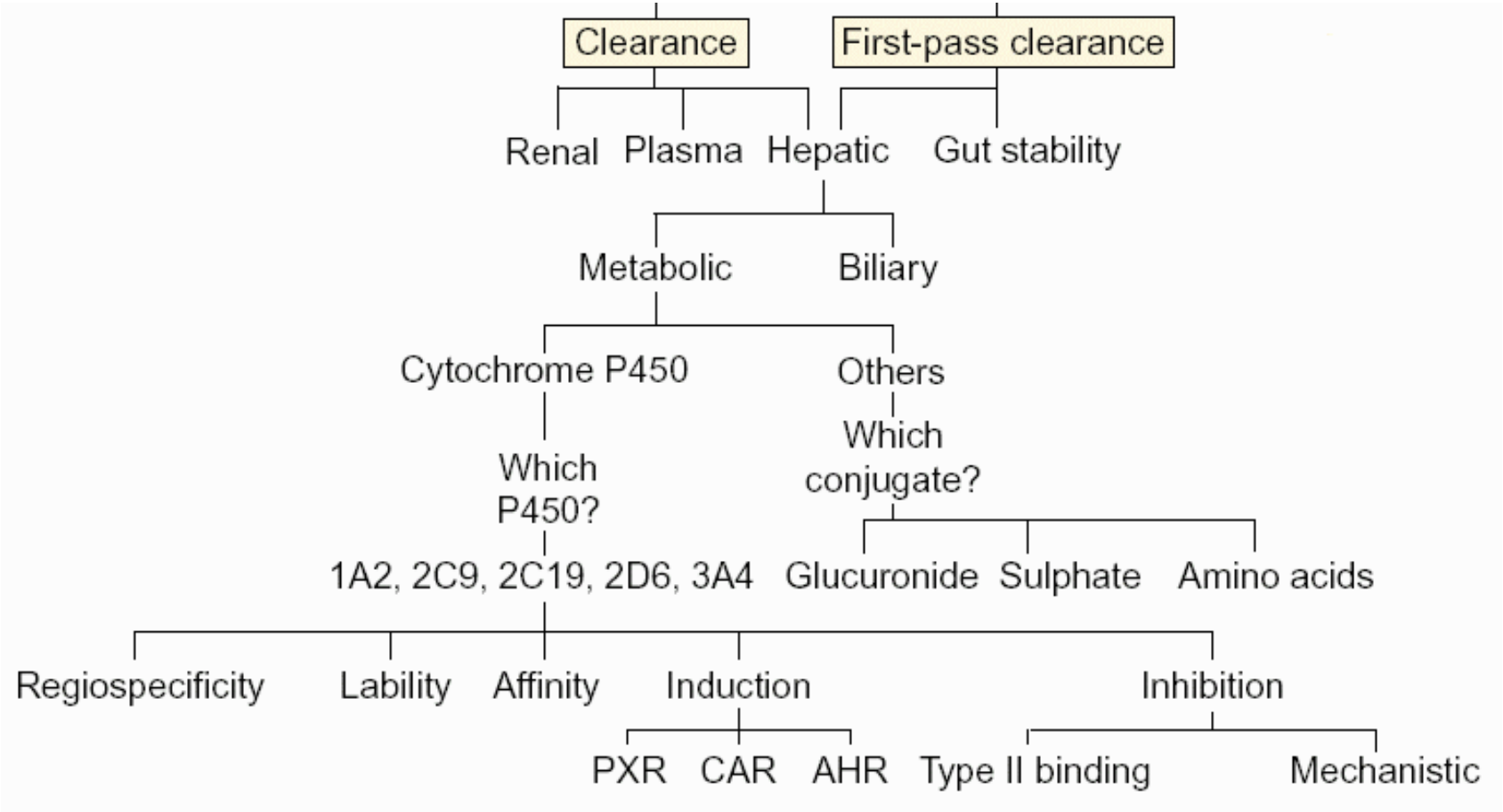
Phase III:

elimination by transporters

- P-glycoprotein (MDR1)

All of these enzymes are subject to individual and sometimes large variations.

# Metabolisms (II)




experimental (*in vitro*) methods:
human liver microsomes, hepatocytes and recombinant P450 enzymes (expressed in *E. coli*)

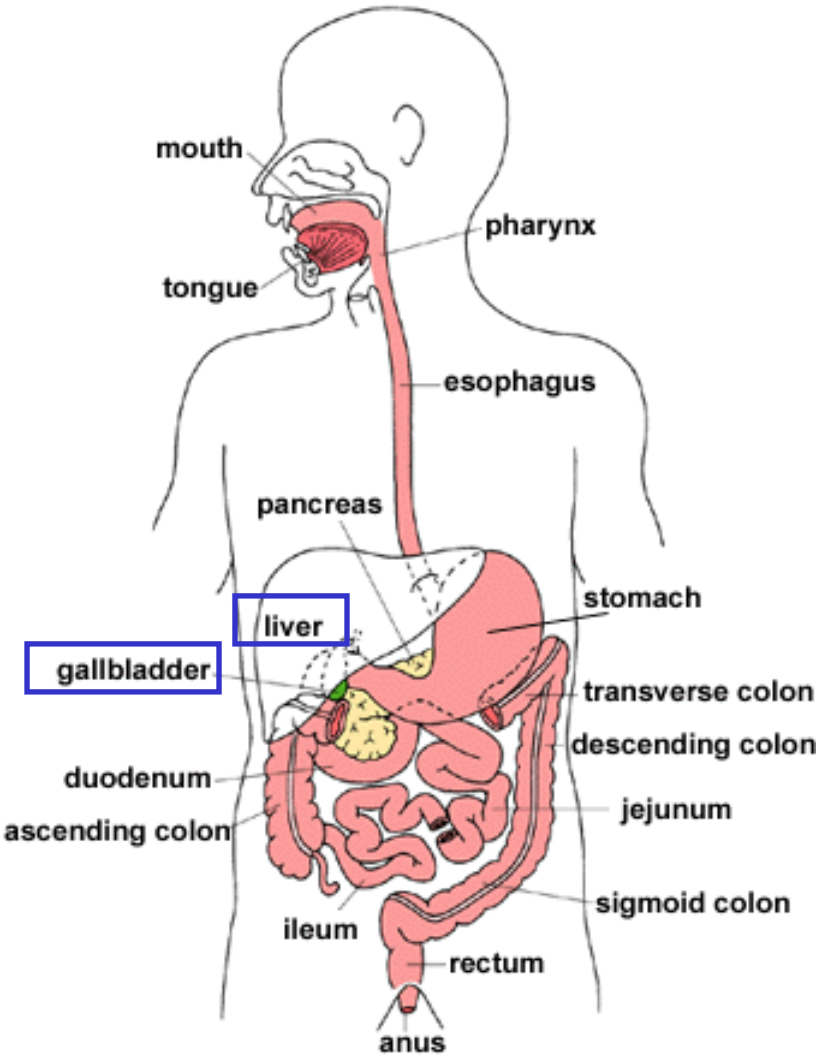
# Elimination / Excretion

Elelimination comprises all processes that lead to removing of a substance from a compartment. These can also of metabolic nature.

Lipophilic substances can be excreted using bile [Gallensaft], hydrophilic compounds via urine.

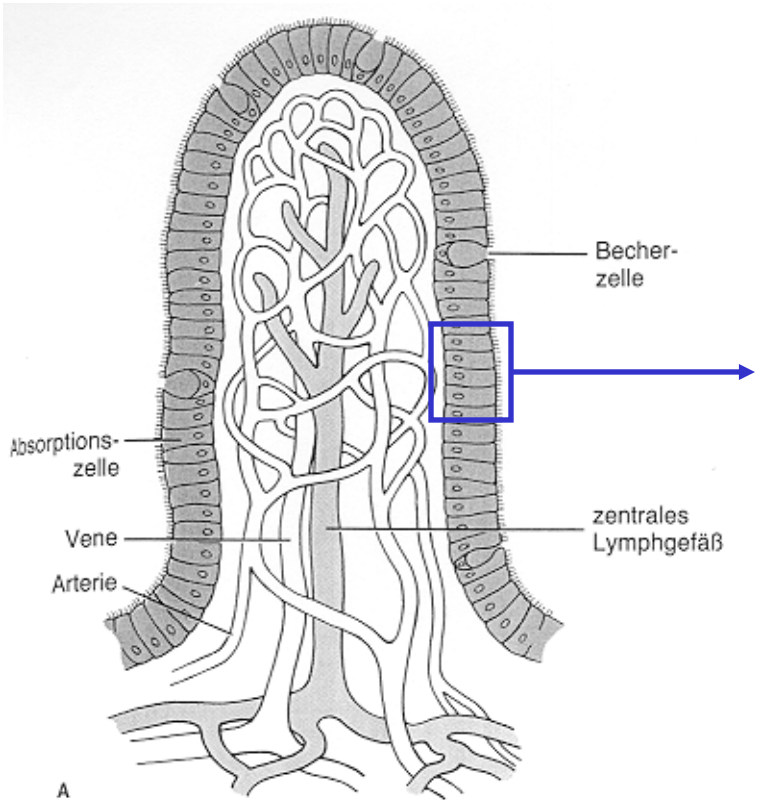
In general:

| MW <300 | 300-500 | >500 |
|---|---|---|
| bile | bile & urine | urine |

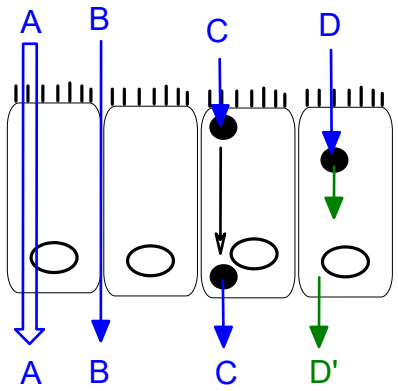


# Metabolismus during absorbtion (I)

Transcytosis (see route of D)




Cross-section from the colon wall




A transcellular (passive diffusion)

B paracellular

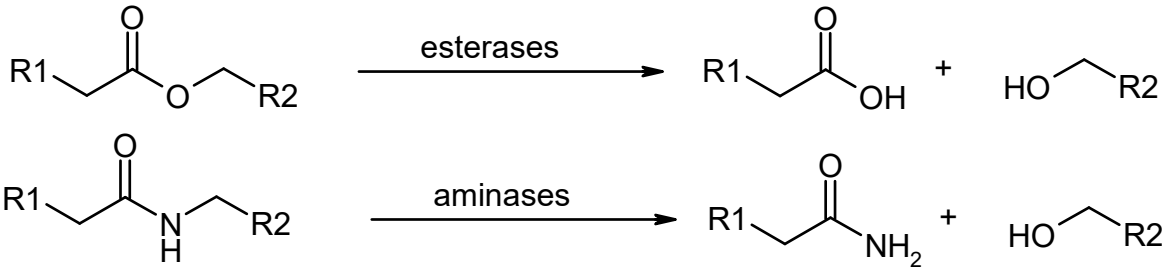
C active transport

D transcytosis

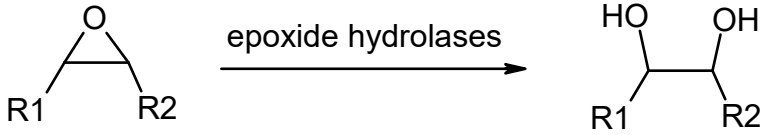
# Phase I processes (I)

**hydrolysis** (formal addition of $H_2O$) of

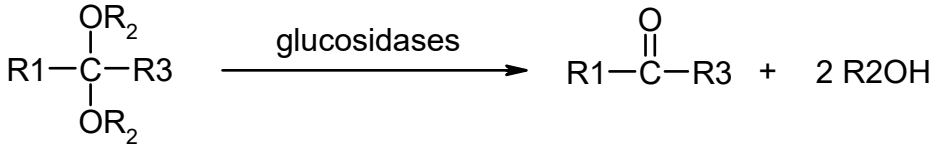
esters and amides by esterases and aminases




epoxides by epoxide hydrolases




acetales by glycosidases



# Phase I processes (II)

**decarboxylation** (release of $CO_2$) of

carboxylate groups of amino acids, etc.

**reduction** (formal addition of $H_2$) of

carbonyl compounds by alcohol dehydrogenases or aldo-keto reductases

azo compounds (via hydrazo compounds to amines) by NADPH-cytochrome *c* reductase and other enzymes

nitro compounds

**reductive dehalogenation** (replacing halogens by hydrogen) of

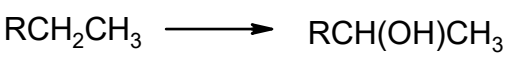
aliphatic compounds

# Phase I processes (III)

**Oxidative reactions** of

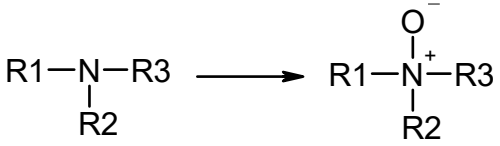
alcoholes and aldehydes to carboxylates


aliphatic chains

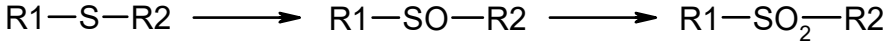



aromatic amines

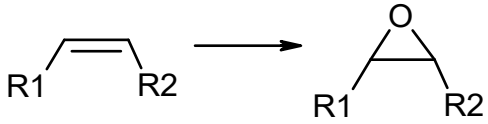

tertiary amines



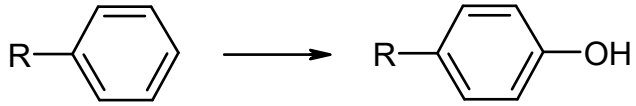

sulfides




alkenes to epoxides

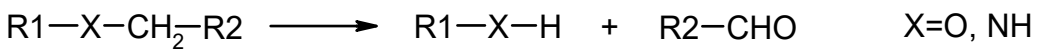



phenyl groups to phenol (in para position)



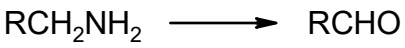
# Phase I processes (IV)

## Oxidative O- and N-dealkylation

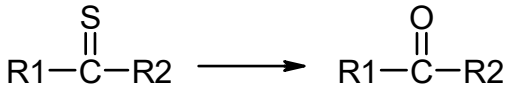



## Oxidative deamination

by the monoamine dehydrogenase (MAO)




## Oxidative desulfuration

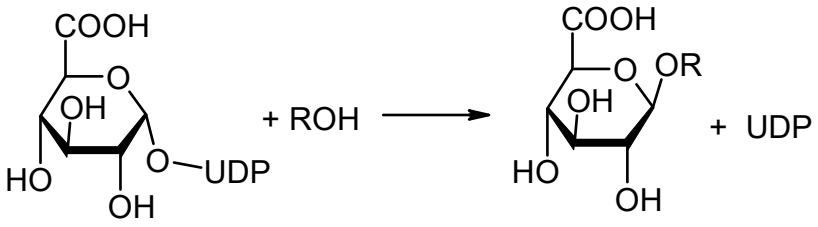



Further oxidases are

flavine monooxygenase isoenzyme
aldehyde oxidase
superfamily of cytochrome P450 enzymes

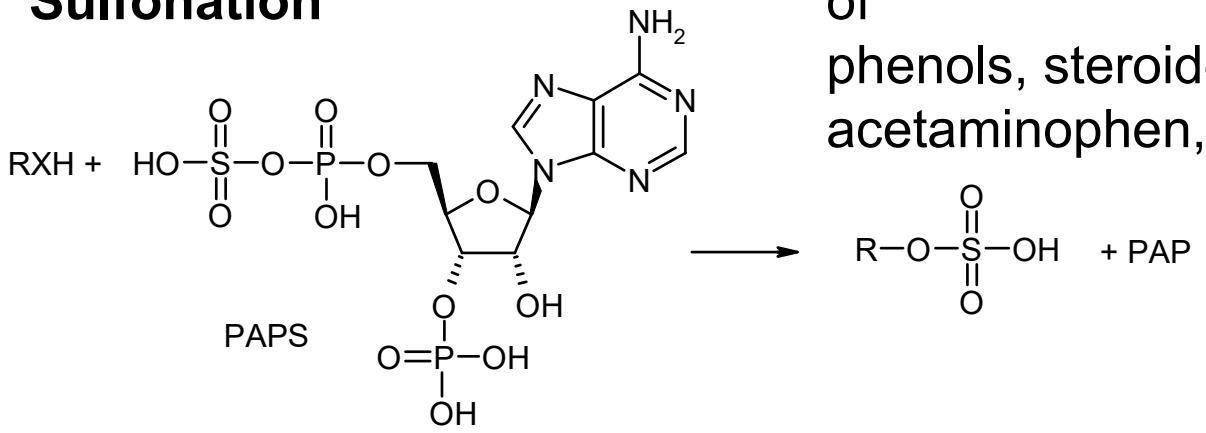
# Phase II processes (I)

## Glucuronidation




e.g. of
acetaminophen, morphium,
diazepam, trichlorethanol
phenol groups in general

## Sulfonation

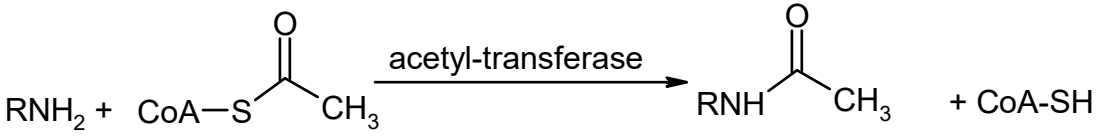



of
phenols, steroides,
acetaminophen, methyldopa

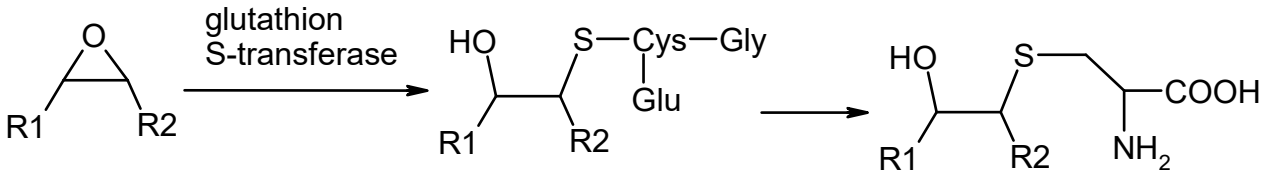
# Phase II processes (II)

**acetylation**

e.g. of
sulfonamides, isoniazid,
dapson, clonazepam

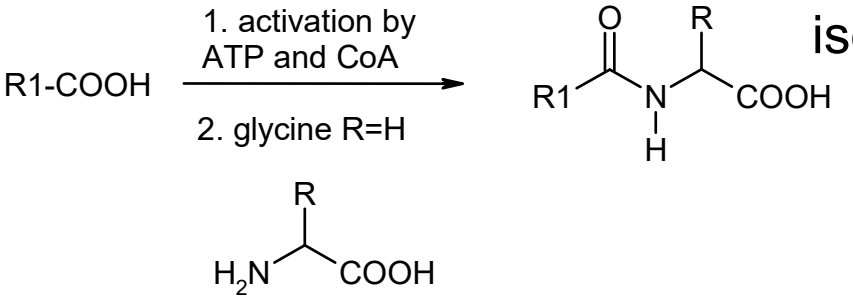



**formation of mercapto acids**



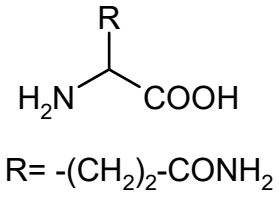
# Phase II processes (III)

**conjugation with glycin**




e.g. of
benzoic acid,
isonicotinic acid

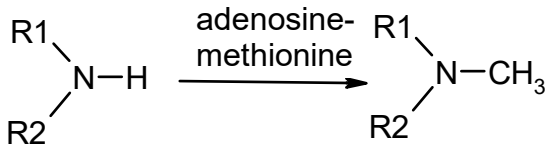
**conjugation with glutamine**

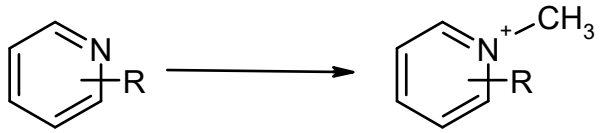


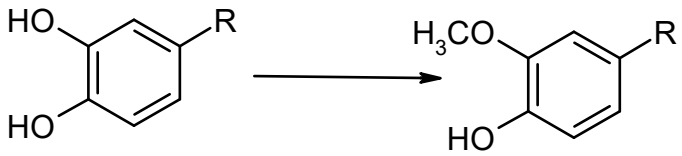

e.g. of
indolyl acetic acid,
phenyl acetic acid

# Phase II processes (IV)

**O-, N-, and S-methylation**



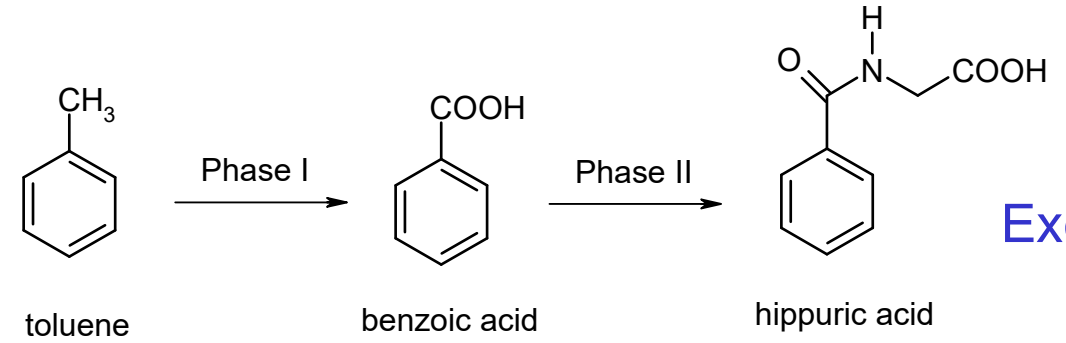




e.g. of
methadon, nicotinamide,
norepinephrine

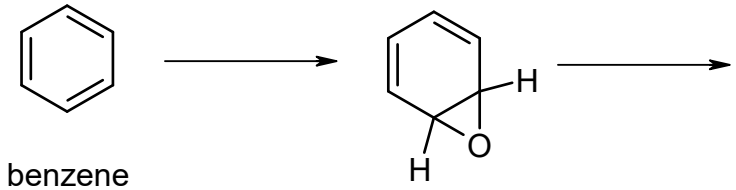
catechloamine (by
catechlol-*O*-methyl transferase)

# Metabolization of Xenobiotica (I)




toluene benzoic acid hippuric acid

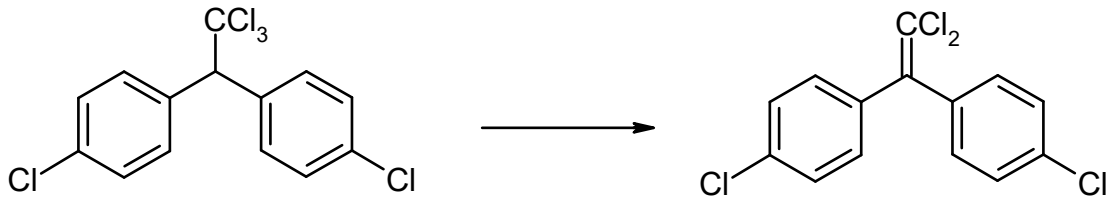
Excretion in the urine




benzene

conjugation with macro molecules

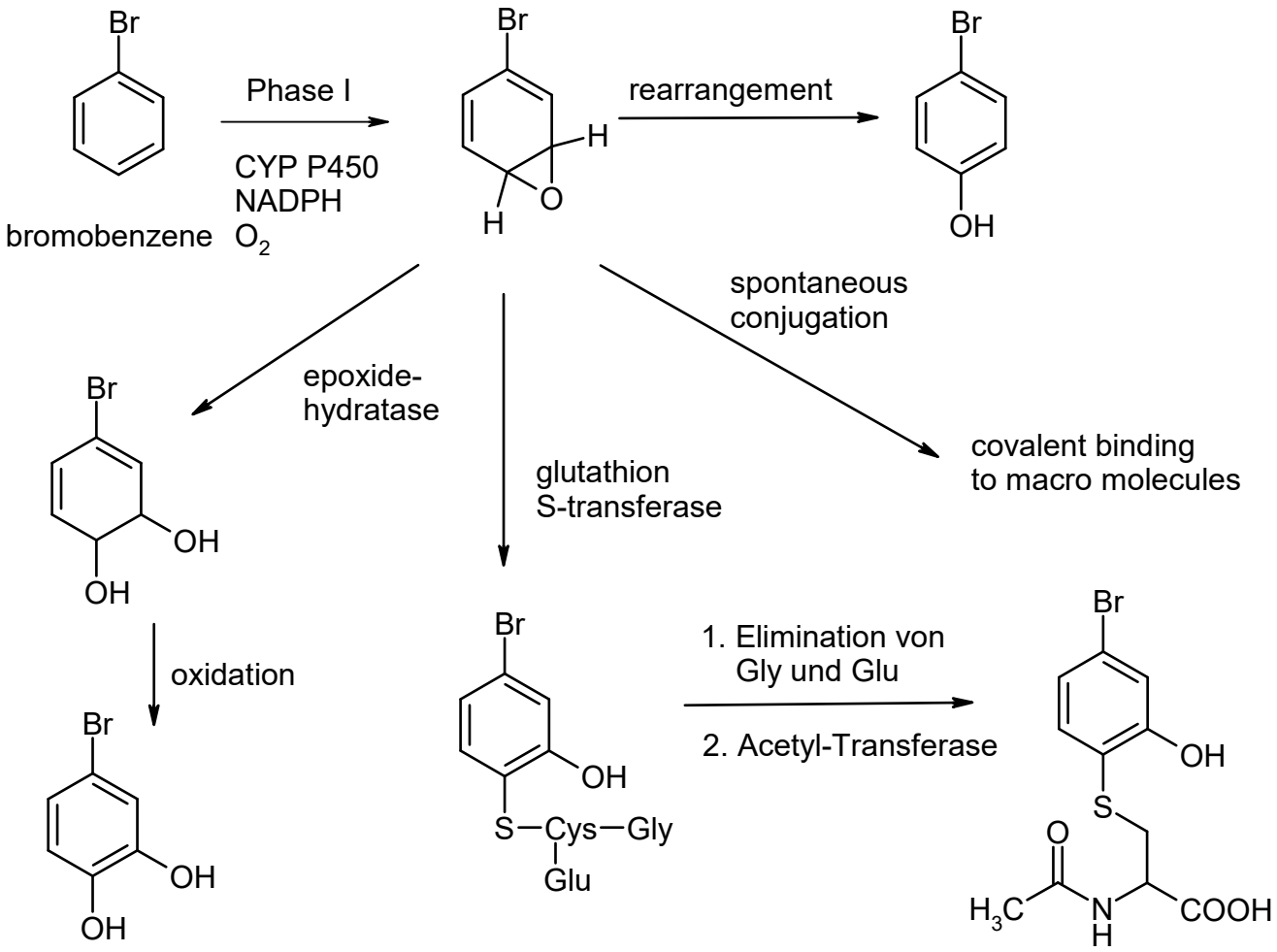
toxification




DDT DDE (antiandrogen)

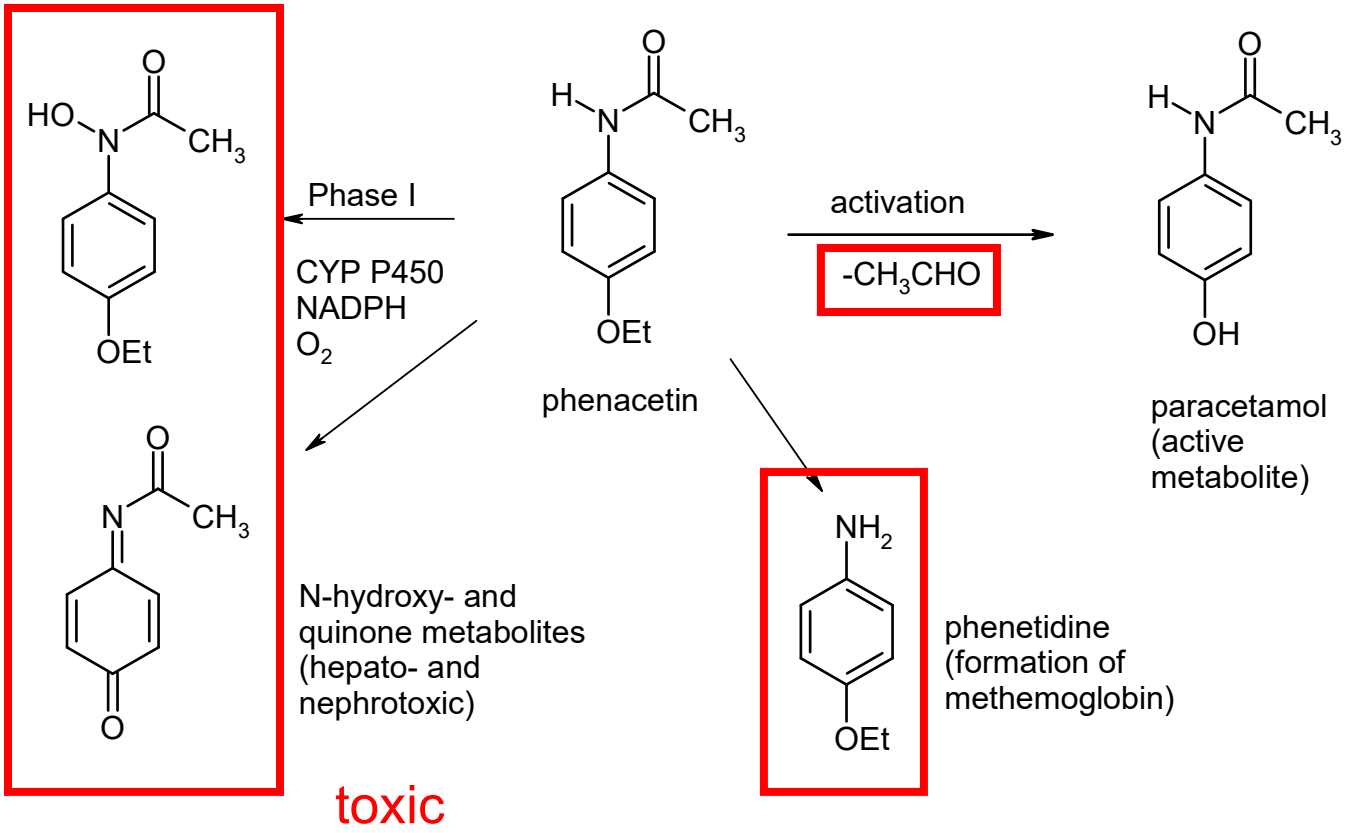
Affects hormonal regulation

# Metabolization of Xenobiotica (II)



# Metabolization of Xenobiotica (III)

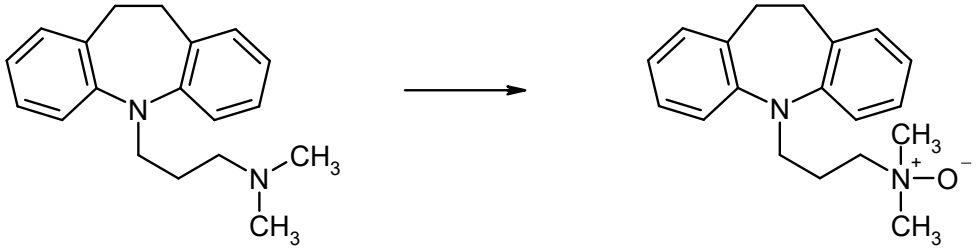
Example for particularly awkward metabolites




Therefore phenacetin is discontinued

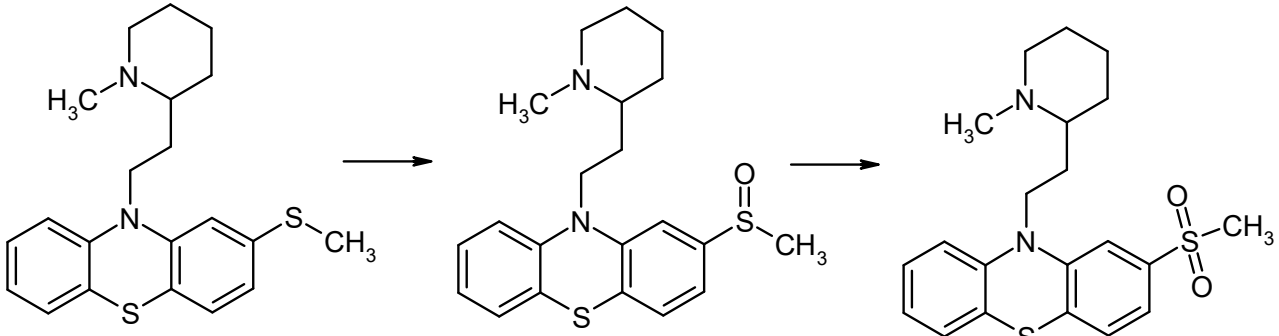
# Metabolization of Xenobiotica (IV)

Examples where metabolites of drugs are also pharmacologically active



Imipramine

Imipramine *N*-Oxide



Thioridazine

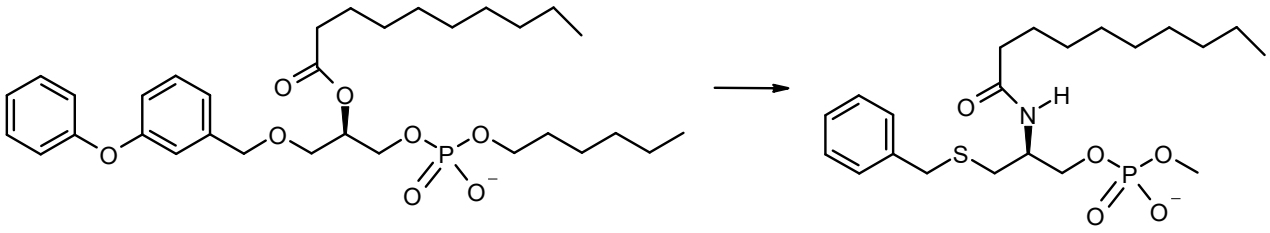
Mesoridazine

Sulforidazine

# Improved metabolic stability

Increasing the bioavailability through:

Replacing esters by amides



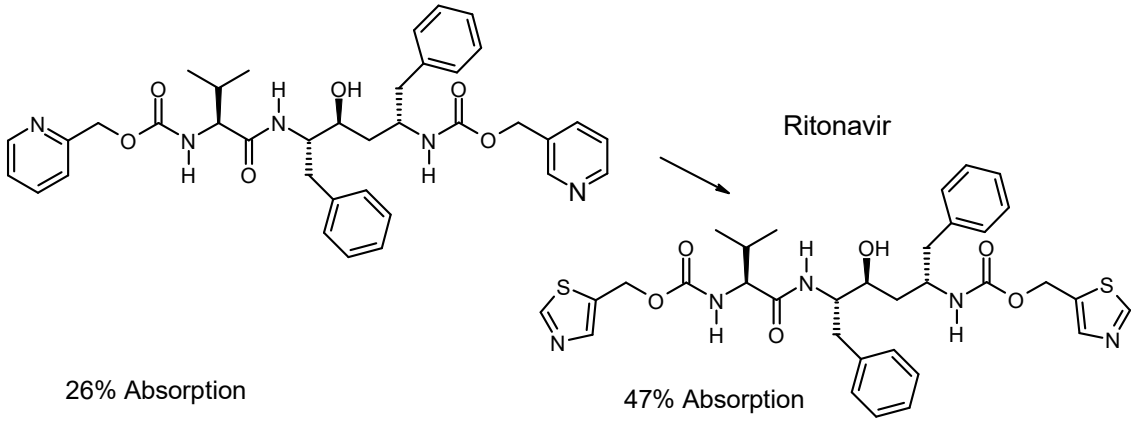
$C_{max}$ = 465 ng ml$^{-1}$

4% Absorption

$C_{max}$ = 3261 ng ml$^{-1}$

90% Absorption

Avoiding *N*-oxidation




26% Absorption

47% Absorption

Lit: A.-E.Nassar et al. *Drug Discov. Today* **9** (2004) 1020

# Toxicological endpoints (I)

effects on the body: Modifications of the

- metabolism (e.g. hormones)
- organs
- behaviour

Common toxicity, acute poisoning, irritation of skin and eyes, acute vs. chronic toxicological endpoints

cytotoxic
cardial toxicity (*h*ERG channel inhibition)
hepatotoxic (PXR, CAR: induction of CYP P450 expression)
nephrotoxic
immunotoxicity (sensibilization, allergens)
neurotoxic (neural receptor binding)
drug-drug interactions (cytochrome P450 induction)
genotoxic, cancerogenic, mutagenic
teratogenic

# Toxicological endpoints (II)

Suggested endpoints in terms of biological assays

AMES mutagenicity (Salmonella typhimurium TA100)
Acute oral toxicity
Carcinogenicity
inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4
substrate of CYP2C9, CYP2D6, CYP3A4
hERG inhibition
P-gp inhibition, P-gp substrate

Lit: L.Guan et al. *Med.Chem.Commun.* **10** (2019) 148.

Additional endpoints used in this publication to derive an ADMET-score for a given compound:

Caco-2 permeability
CYP inhibitory promiscuity
Human intestinal absorption
Organic cation transporter protein 2 (OCT2) inhibitor

# ADMET models (II)

The vast amount of possible reactions makes prediction of metabolic and toxic properties difficult.

Characteristic reactions of specific compounds are summerized in databases.

Commerical expert systems (selection)

| | |
|---|---|
| DEREK, METEOR | http://www.chem.leeds.ac.uk/luk/ |
| HazardExpert | CompuDrug Ltd. |
| TOPKAT | Accelrys |
| M-CASE | Multicase |
| ToxPredict | Idea Consult |
| GastroPlus | Simulations Plus, Inc. |

# ADMET models (III)

| metabolic aspects | descriptors |
|---|---|
| biotransformation | chemical structure of some metabolites to derive a *decision tree*<br>physico-chemical properties |
| binding to enzymes | esp. to human serum albumin (HSA), cytochrome P450 enzymes (see lecture 10) |
| catalytic reactions | reaction mechanism<br>turn over rate |
| drug-drug interaction | inhibition or induction (see lecture 10) |

# ADMET models (IV)

**Reappearing descriptors in QSAR equations**

log(T) = a(H) + b(E) + c(S) + constant

T: (specific) toxicity
H: hydrophobicity $\rightarrow$ logP
E: electronic terms
S: steric terms

C. Hansch et al. *J.Am.Chem.Soc.* **86** (1964) 1616

Over time nothing has changed on this elementary equation!

Dominance of a single term indicates a particular mode of action such as in other QSAR equations

# ADMET models (V)

Experimental assays:

| | |
|---|---|
| aquatic toxicity: | uni-cellular organisms:<br>*Tetrahymena pyrifomis:* growth inhibition<br>*Vibro fischeri:* bioluminescence |
| mutagenicity (AMES): | *Salmonella typhimurium* + S9<br>(liver enzymes) |
| Skin irritation: | guinea pig [Meerschweinchen] |
| Eye irritation: | rabbit eye |
| *in vivo* ADMET: | zebra fish |

Review of QSAR-methods regarding toxicology:

T.W. Schultz et al. *J.Mol.Struct.(THEOCHEM)* **622** (2003) 1

T.W. Schultz et al. *idem* **622** (2003) 23

# Toxicity models

Publicly funded prediction server:

www.opentox.org hosts models for:

- Oral toxicity
- Mutagenicity
- Cytochrome P450 metabolism

www.cosmostox.org for long-term toxicity of cosmetic ingredients

Currently all available machine learning algorithms including QSAR, decision trees, random forest, Naive Bayes, support vector machines, neural networks, deep learning, etc. are applied.

Lit. X.Li et al. *J.Chem.Inf.Model.* **54** (2014) 1061-1069.

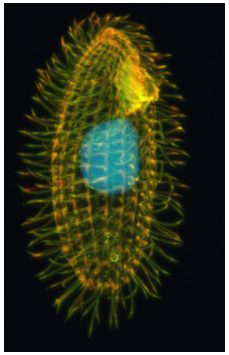
# Environmental Toxicity

Experimental assays/endpoints routinely applied (e.g. for EPA and REACH legislation in the EU):

- 96-hour fathead minnow (*Pimephales promelas*) 50 percent lethal concentration (LC50)

- 48-hour *daphnia magna* LC50 concentration

- *Tetrahymena pyriformis* 50 percent growth inhibition concentration (ICG50)



- Oral rat 50 percent lethal dose (LD50)

- Bioaccumulation factor

Source of pictures: wikipedia

# Drug Safety

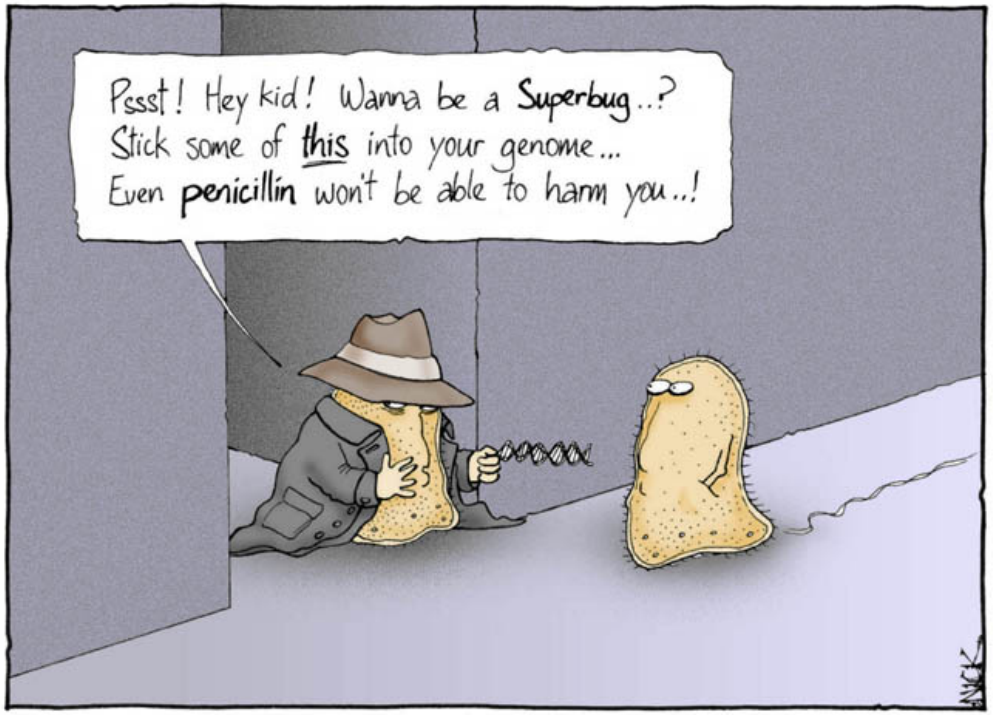
Drug-Drug interactions:

Co-adminstration with other medications
Drug Interaction Database
http://depts.washington.edu/ventures/pfolio/didb.htm

Ecotoxicology:

How do the excreted drugs and their metabolites react in the environment?

→ biodegradability of drugs, e.g. antibiotics, build-up of bacterial resistance




It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.