

Metabolism and Toxicology

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

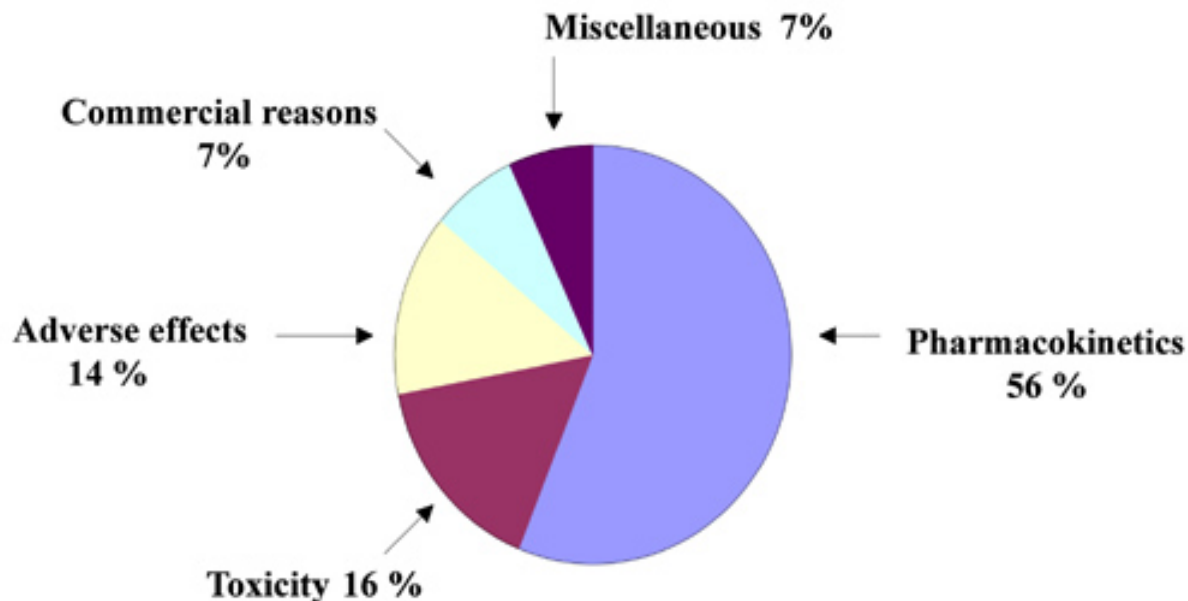
The vast majority of chemical substances undergo biochemical transformations inside the body (metabolisms).

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

It is therefore important to recognize 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 withdrawal 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)

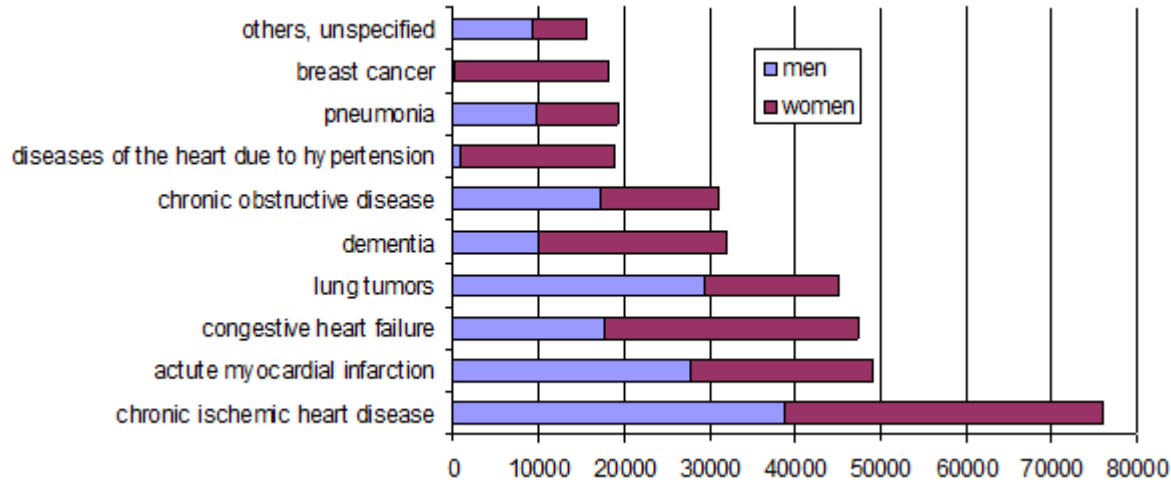
Most frequent (natural) cause: cardio-vascular complications

List of withdrawn drugs (not comprehensive, see wikipedia)

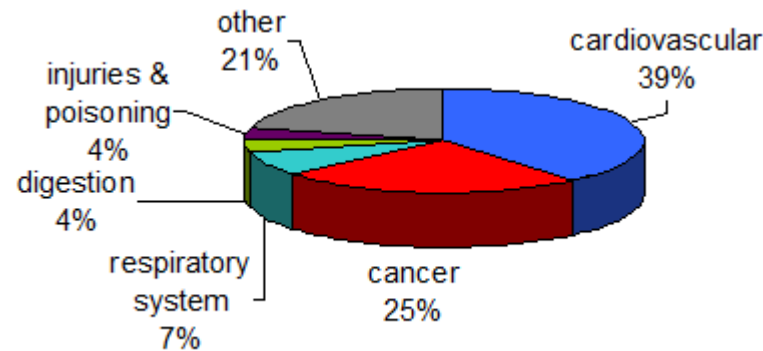
trade name	adverse effect	manufacturer	time
propoxyphene	cardiac arrhythmia	generic	2010
flupirtine	liver toxicity	generic	Mar 2008
clobutinol	QT-prolongation	gerenic	Aug 2007
alatrofloxacin	liver toxicity	Pfizer	Jun 2006
rofecoxib	thrombosis,stroke	Merck(USA)	Sep 2004
cerivastatin	rhabdomyolysis	Bayer	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



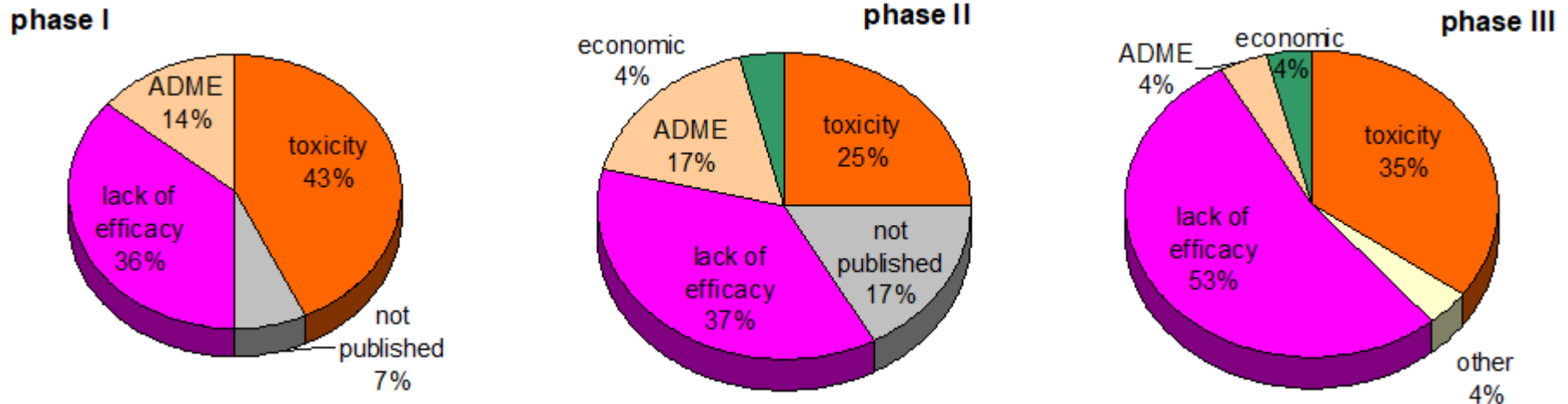
Data sources: Statistisches Bundesamt www.destis.de

Why drugs fail

90% of market withdrawals caused by drug toxicity, from that $\frac{2}{3}$ are due to hepatotoxicity and cardiovascular 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)

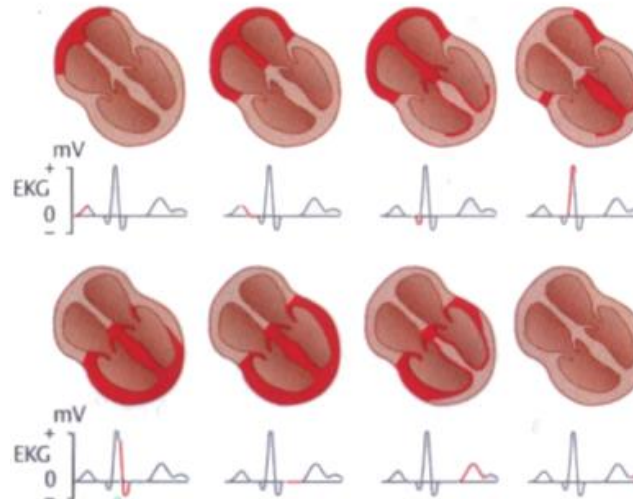
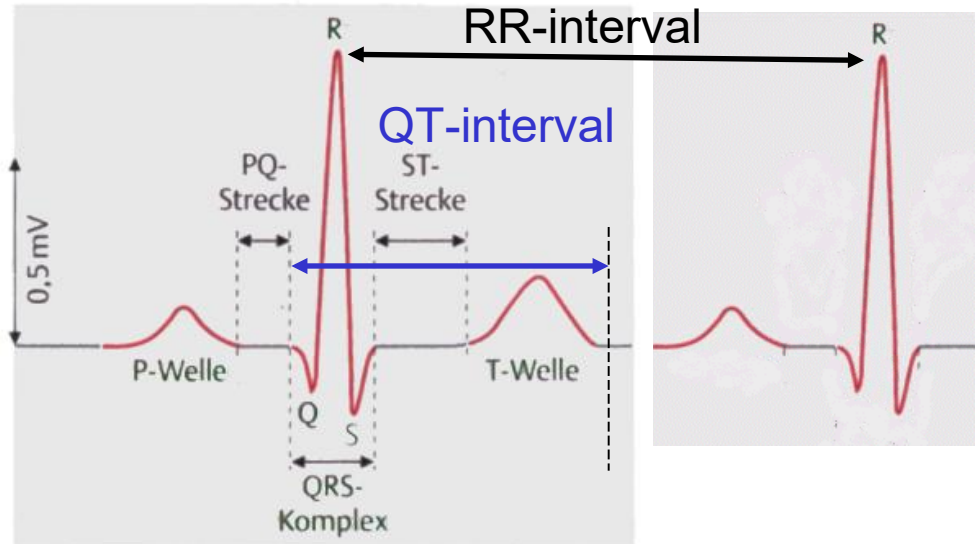
Cardiac arrhythmias are among the most frequent adverse effects that lead to the failure of drugs (frequently as late as in clinical phases III or IV).

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 preceding RR interval (Bazett correction):

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

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

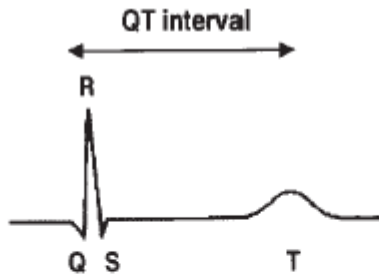
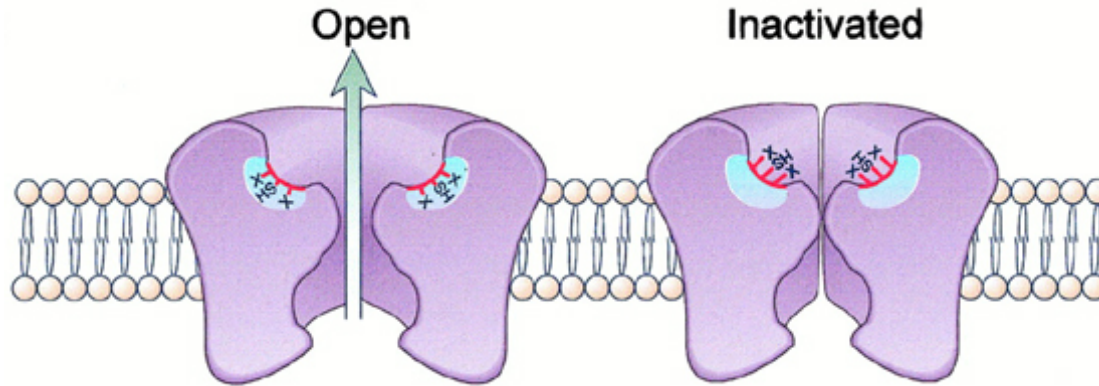
The observed current in the ECG during the QT-time is mainly due to the delayed activity of the cardiac potassium channel (*outward repolarizing current* I_{Kr}).

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

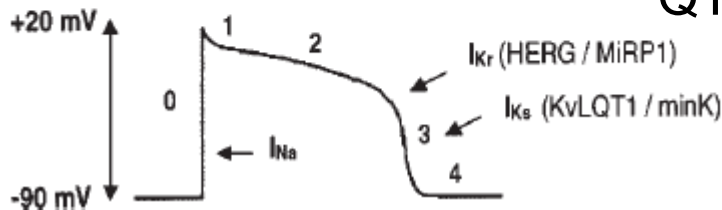
This effect is frequently used by anti-arrhythmic drugs of class III. On the other hand, too long QT-times can lead to fatal distortions of the cardiac rhythm itself.

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

The *hERG* potassium channel (I)

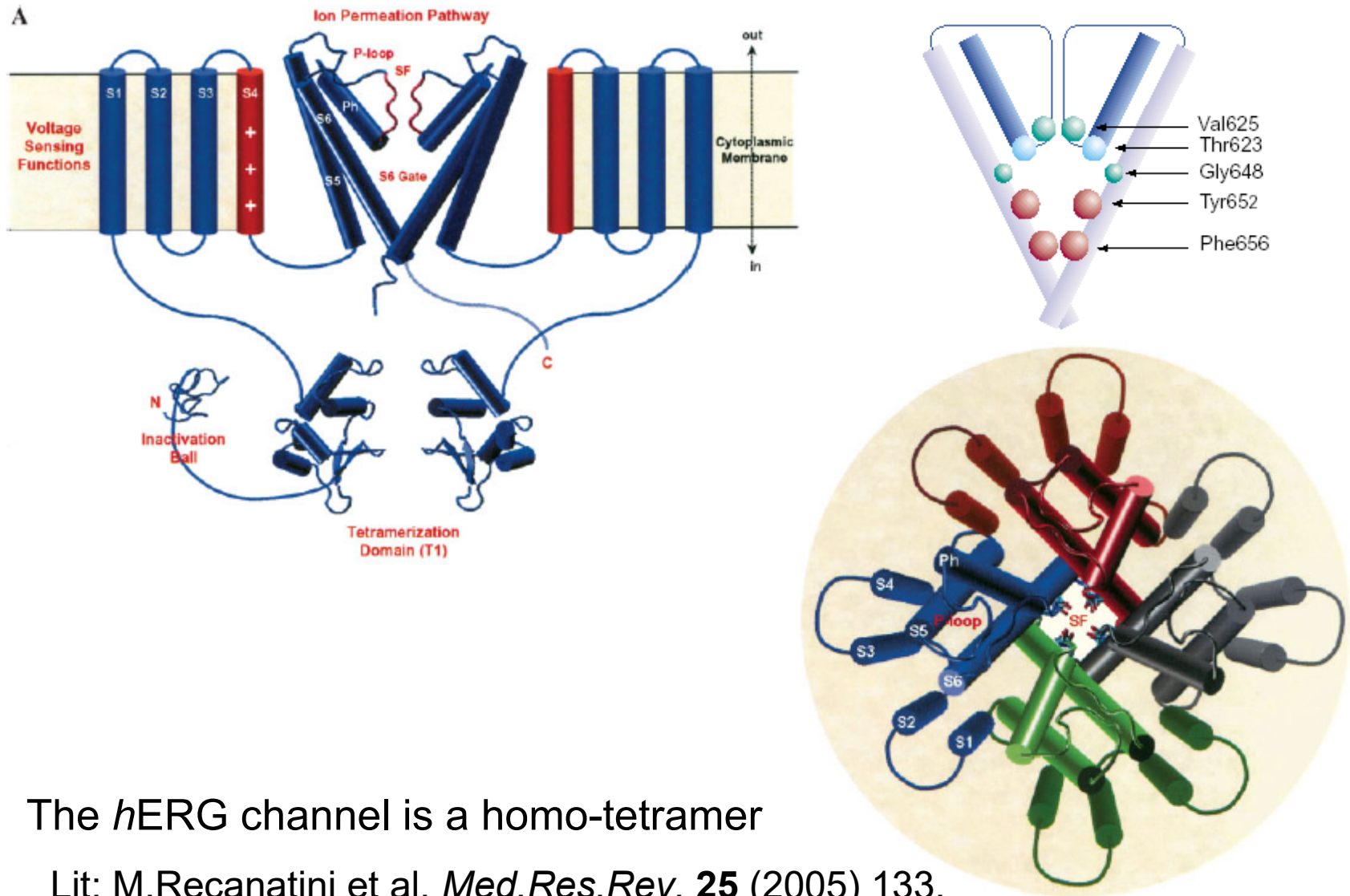


The activity of the *hERG* channel accounts for the rapid potassium component (I_{Kr} , rapid) of the outward repolarizing current I during the QT-interval



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

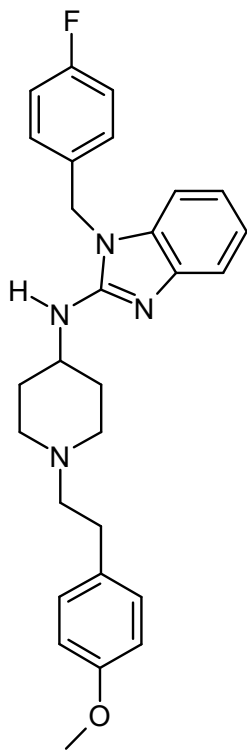
The *h*ERG potassium channel (II)



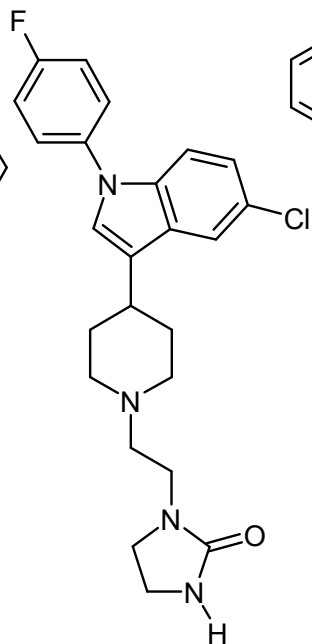
The *h*ERG channel is a homo-tetramer

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

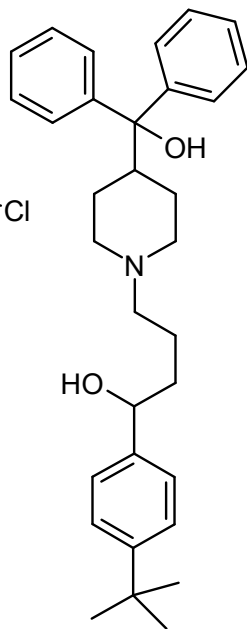
*h*ERG channel blocking drugs



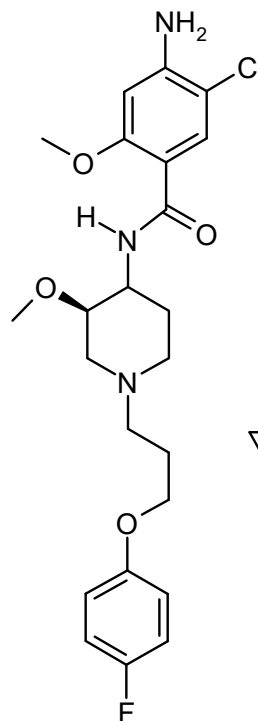
Astemizole
Antihistaminic



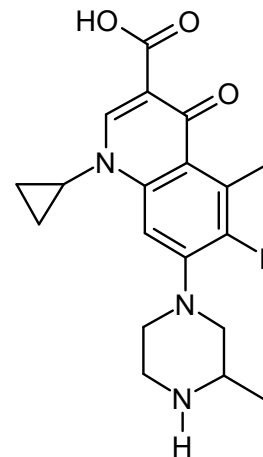
Sertindole
Antipsychotic



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.

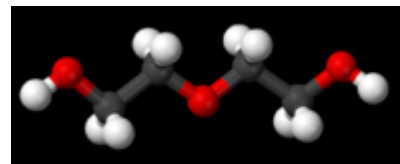
Historical development in the USA

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 approval of substances by the Food and Drug Administration (FDA).

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

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

Lethal dose LD50 \approx 1ml/kg body weight



Historical development in Germany

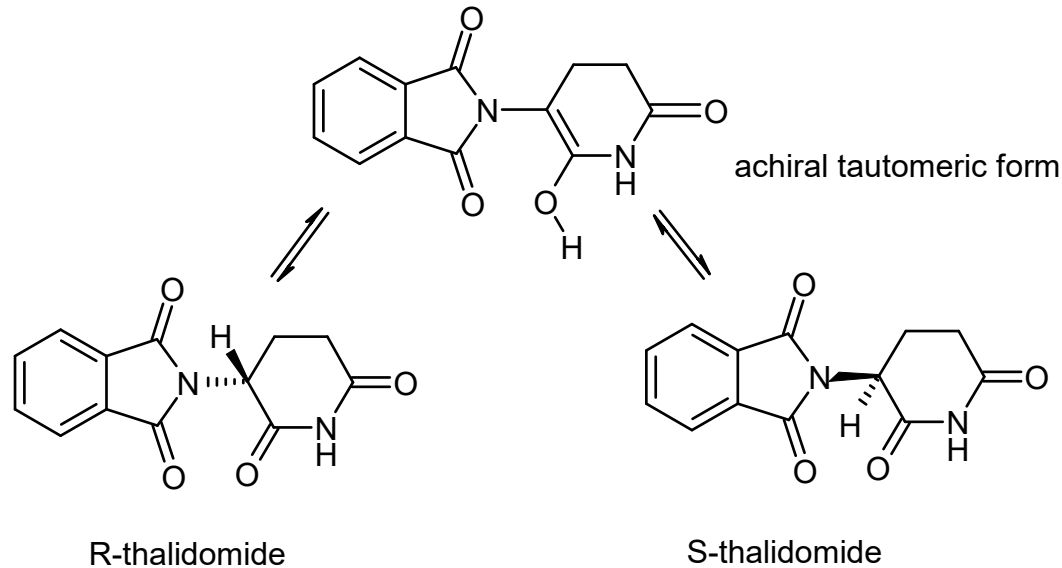
Until 1961 there was no comprehensive legislation regarding marketing of medical drugs in the former Federal Republic of Germany.

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-existent 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 transferred to human. Deriving dosage guidelines (10-50 test persons, „healthy male“, no risk group)

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

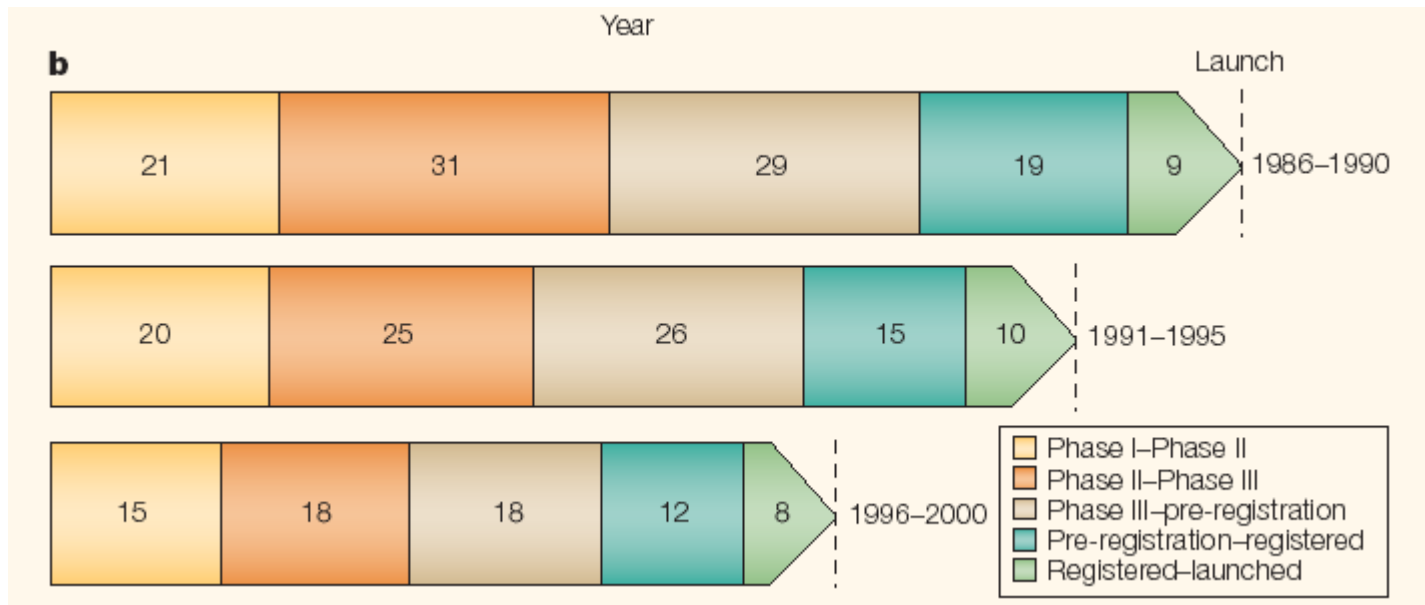
Phase III: Validation of efficacy and relative harmlessness on a larger number of patients. (as well as adverse effects upon co-administration with other medications)

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.

Improvement and launch (I)

The improvement 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 commission.

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 improvement process is more and more decisive for the financial future of the manufacturer.

Approvement and launch (II)

A new medication is also referred 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

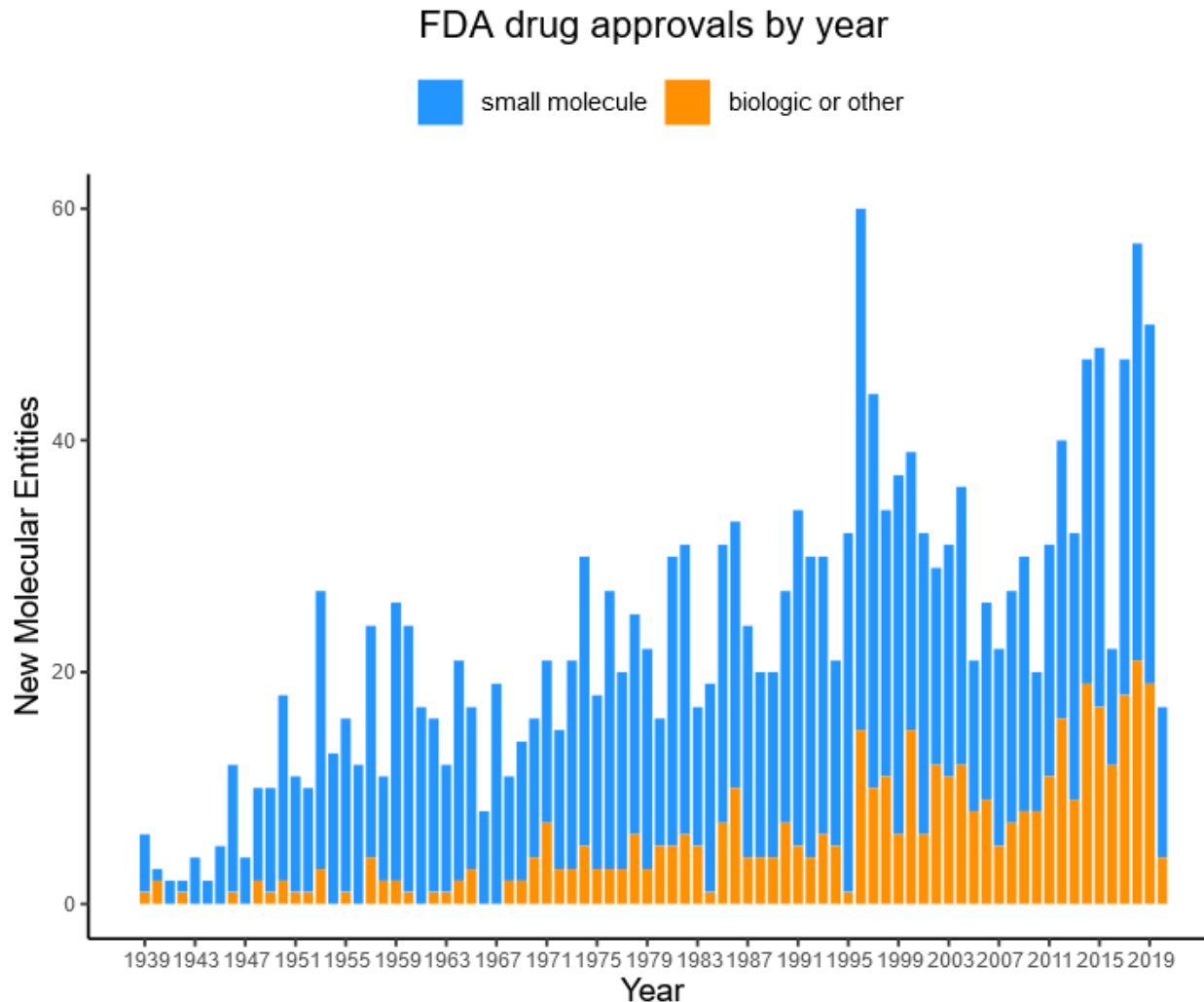
Drugs approved by
the FDA

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\$

Approval and launch (III)

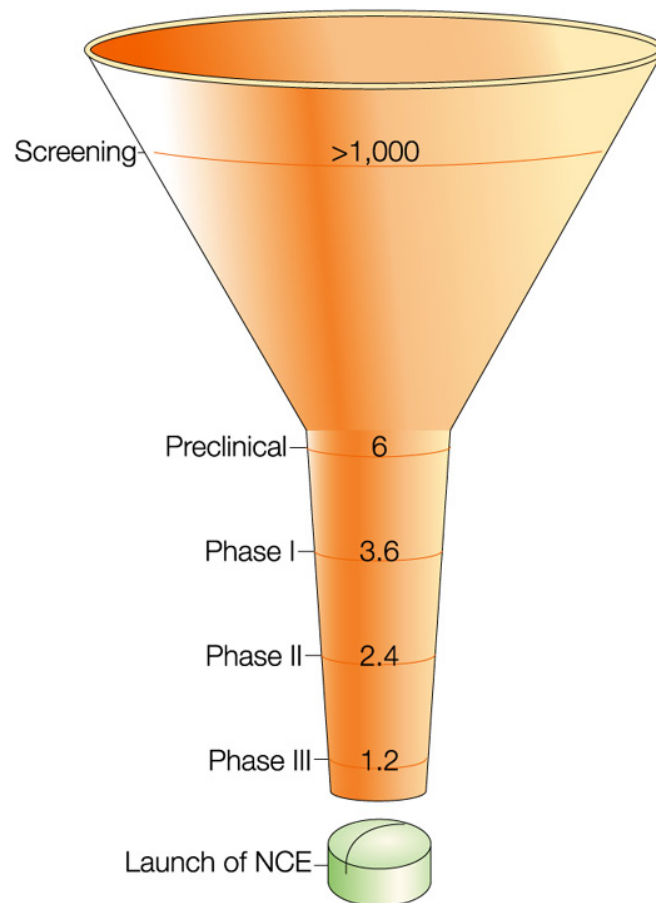
Trend in approval of new chemical entities



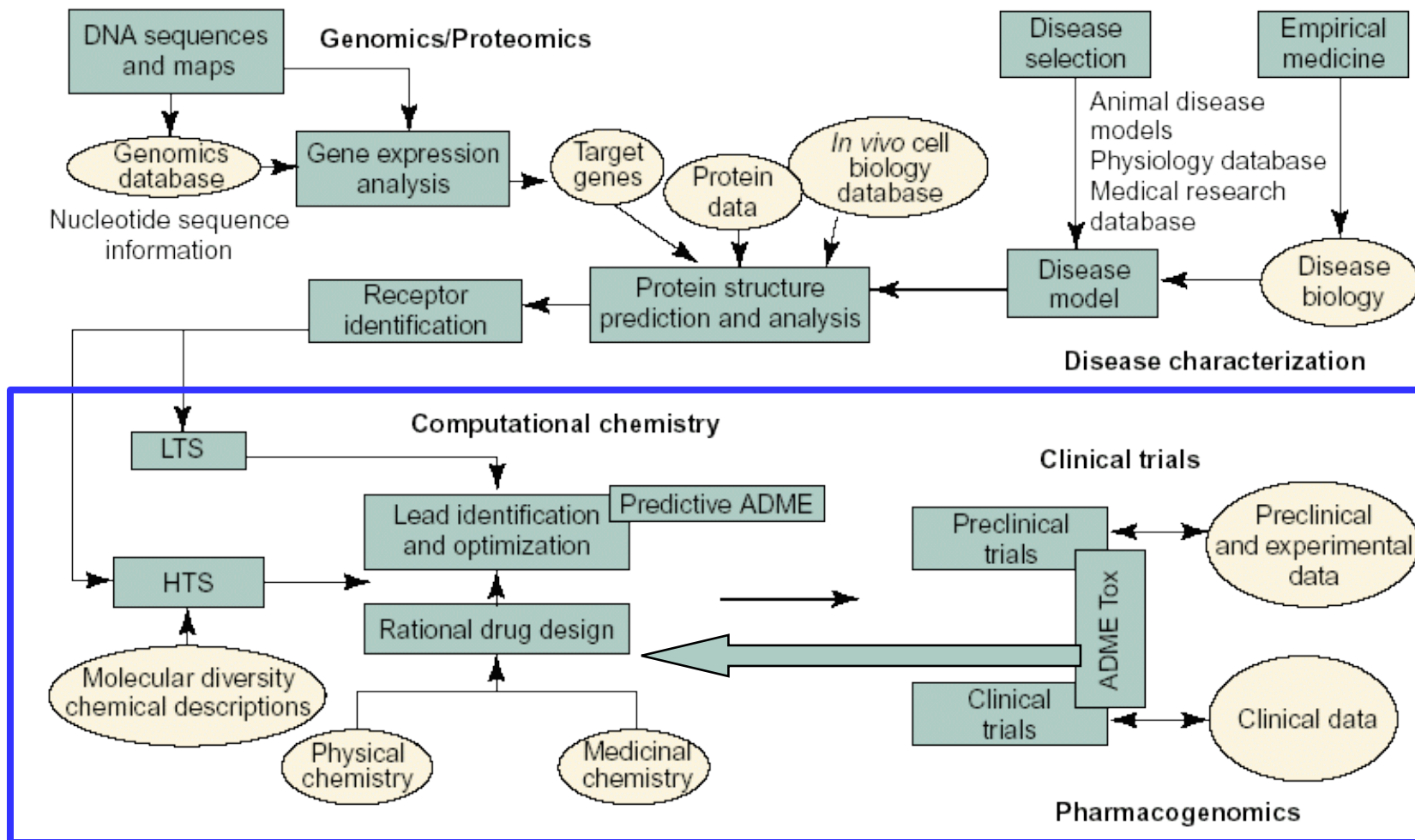
From the *pipeline* to the market launch

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

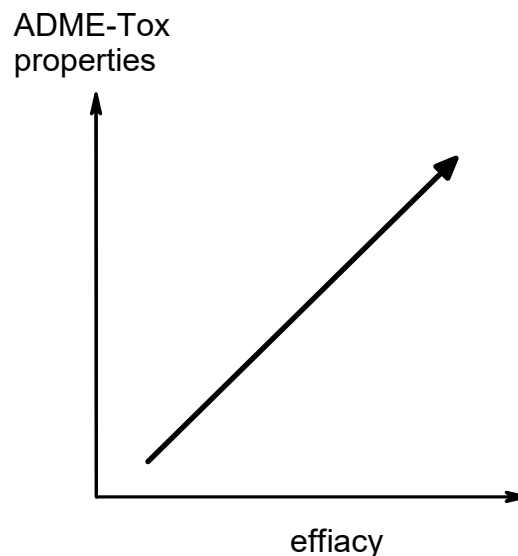
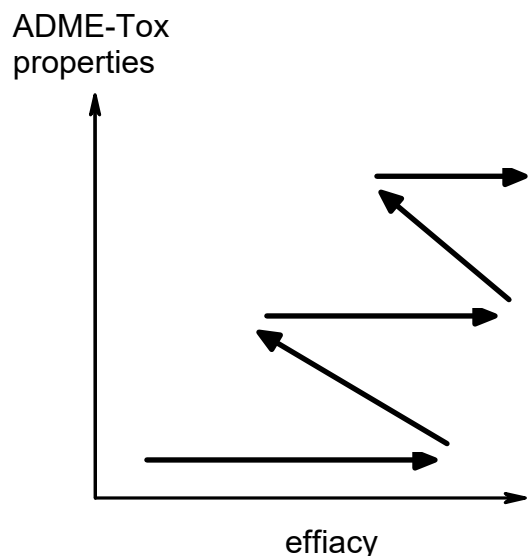
Without the available computer-aided ADMET filters, this number would be even larger.



Flow of information in a *drug discovery pipeline*



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



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

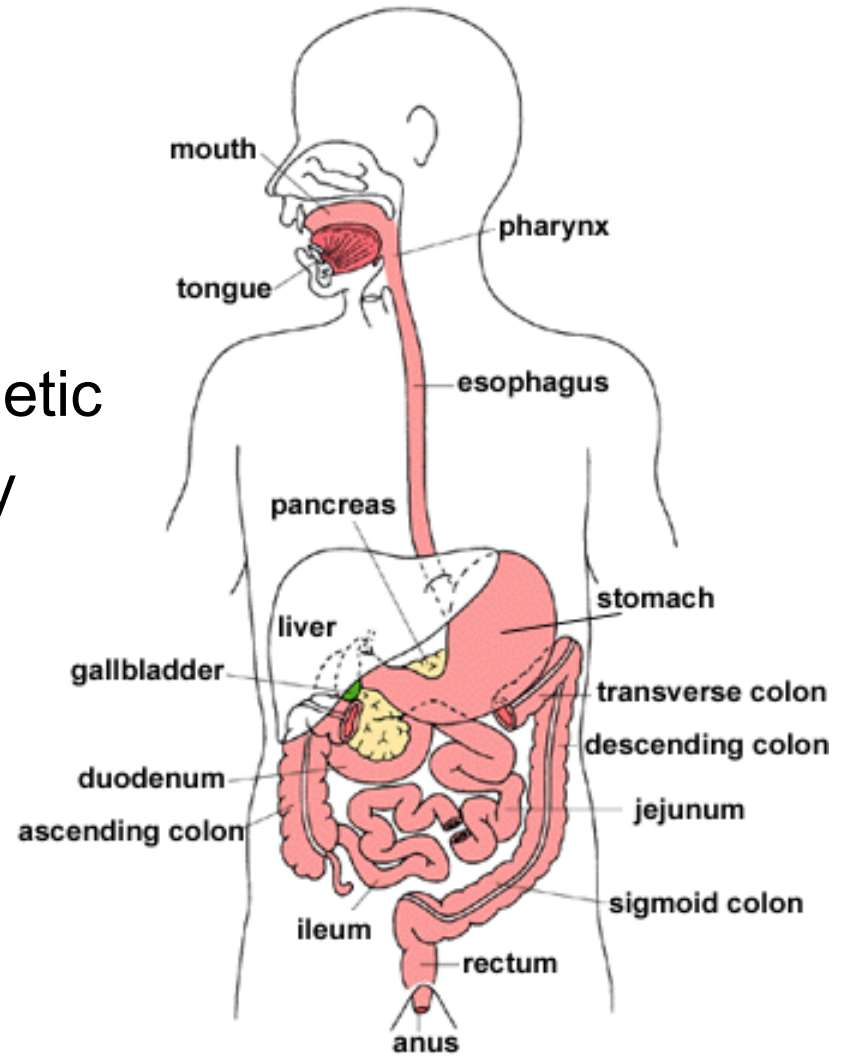
Today: simultaneous optimization of efficacy and ADME-Tox properties (requires *in silico* AMDT models)

eADMET Prediction

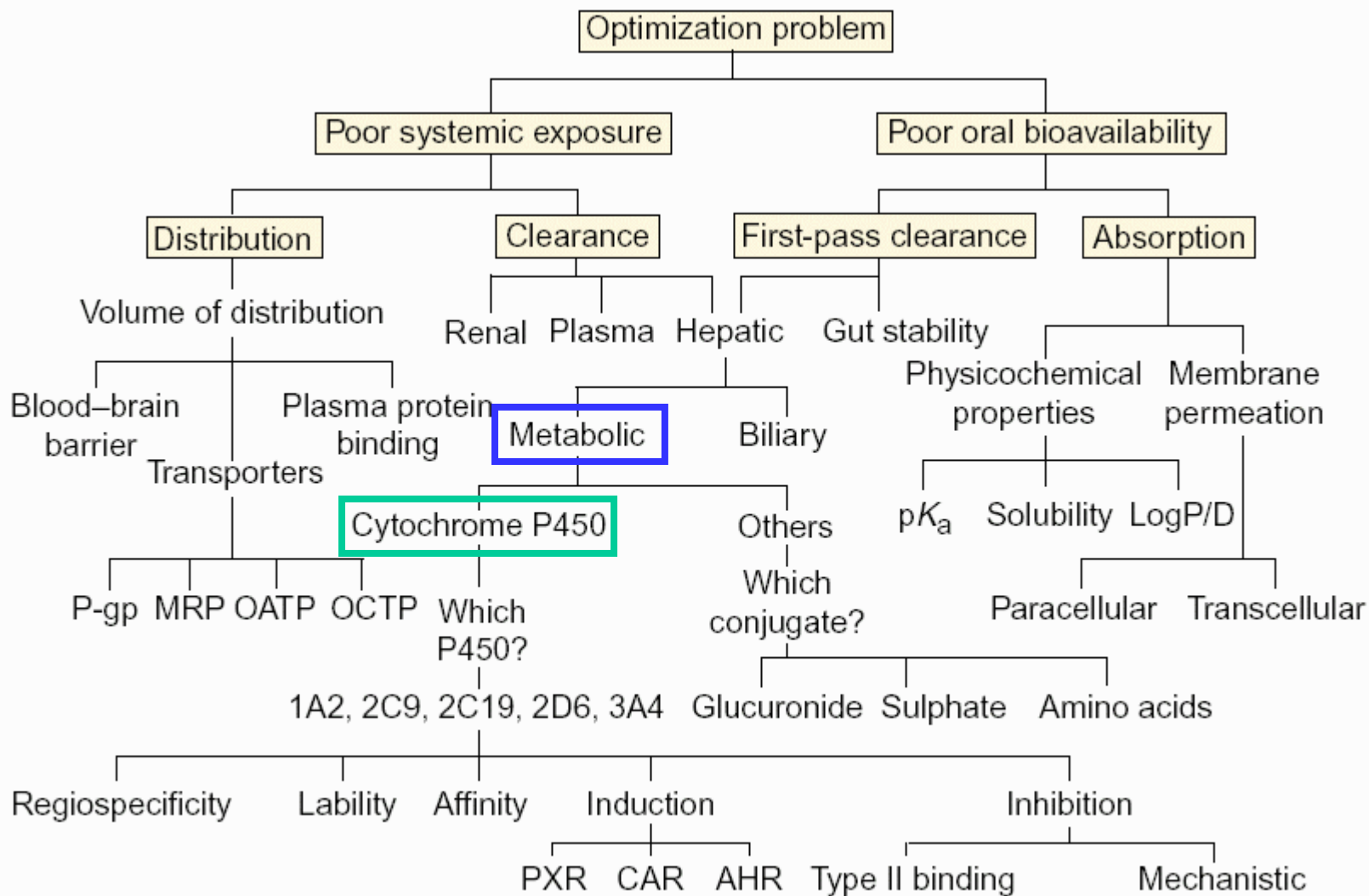
early
Absorption
Distribution
Metabolism
Elimination
Toxicology



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, reduction and hydrolysis

cytochrome P450 enzymes (see lecture 10)

dihydropyrimidin-, alcohol-, and aldehyde dehydrogenases

epoxide hydrolases, esterases and aminases

flavine monooxygenases

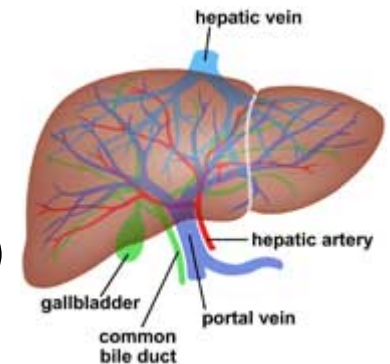
Phase II:

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

N-acetyltransferase, glutathione S-transferase

uridinediphosphate-glucuronosyltransferases

sulfotransferases, methyltransferases



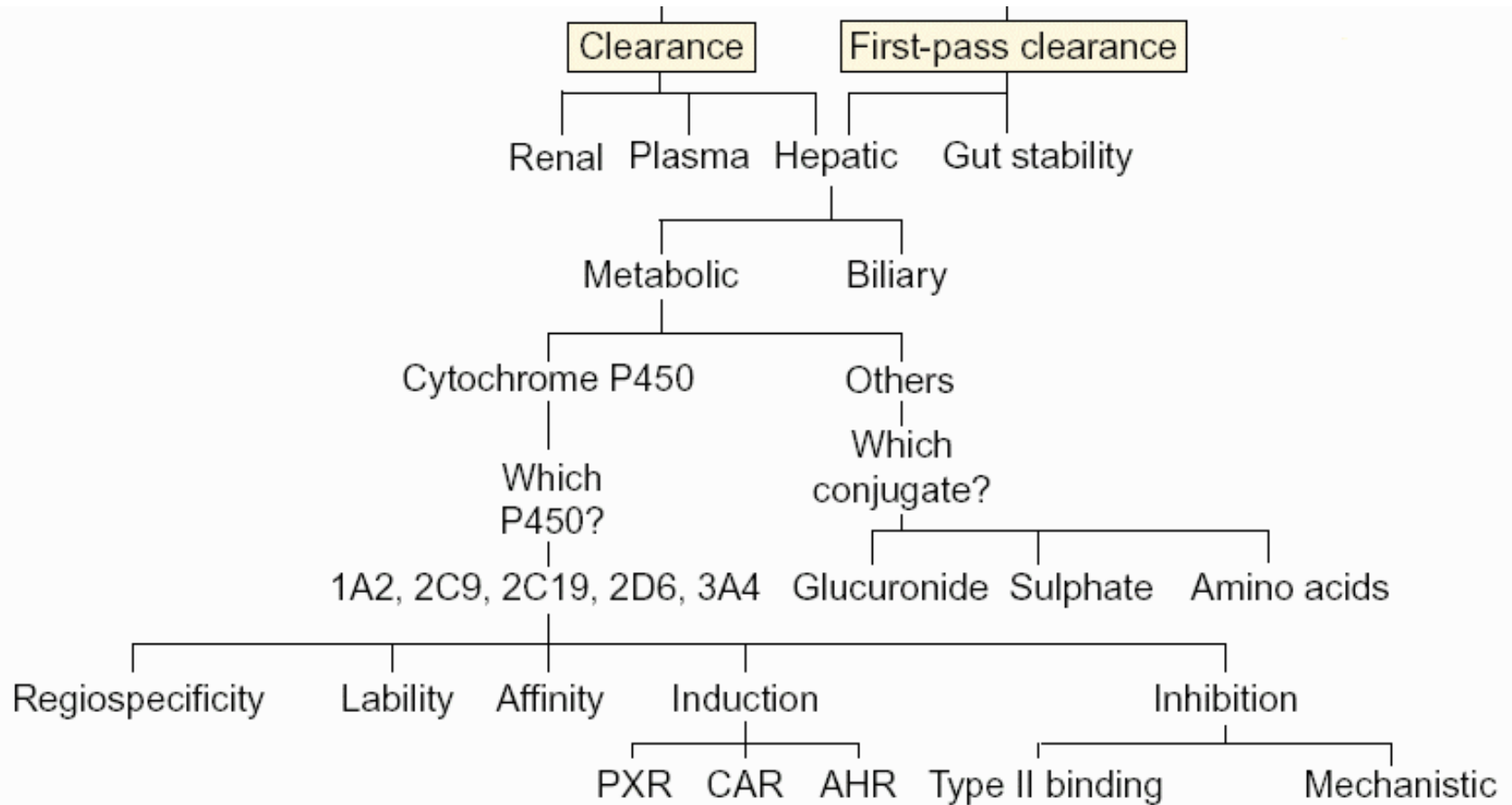
Phase III:

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*)

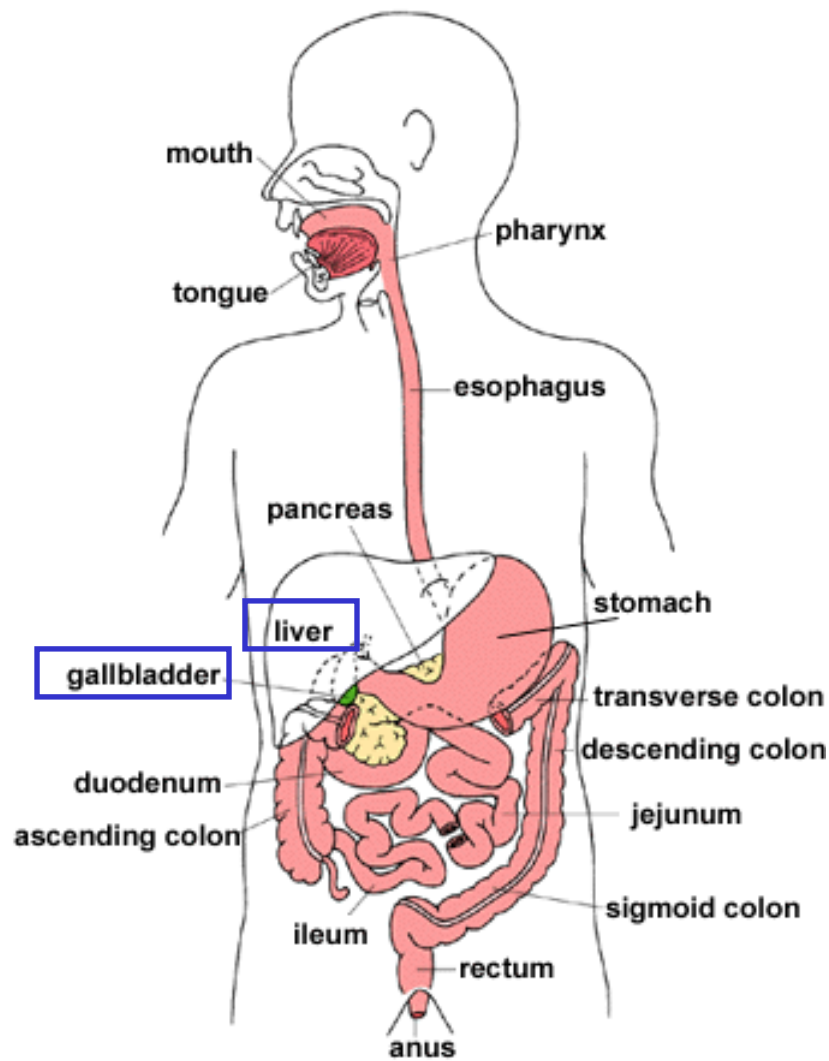
Elimination / Excretion

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

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

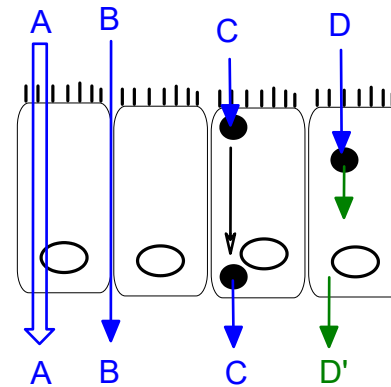
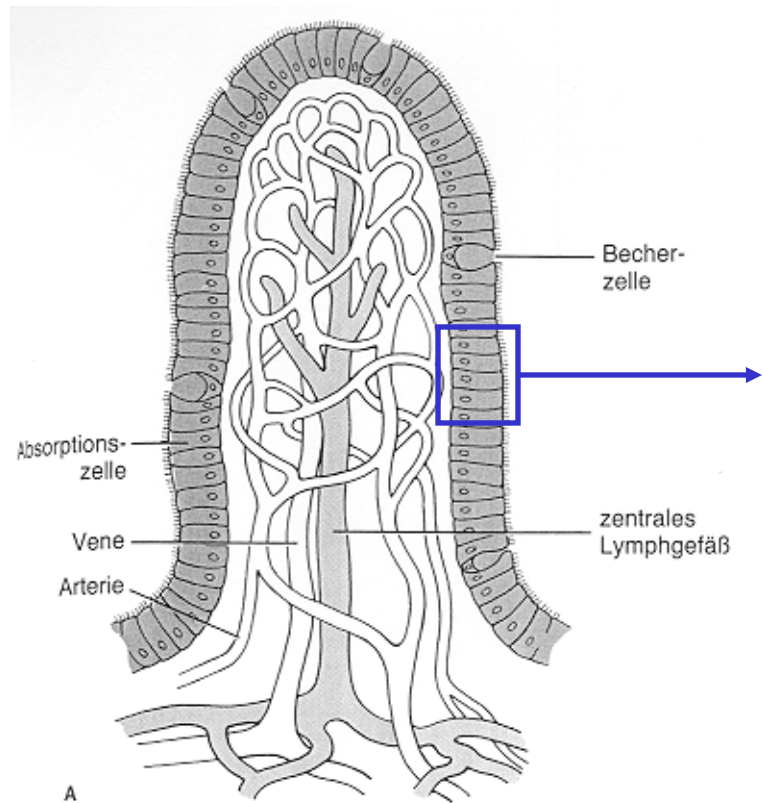
In general:

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



Metabolismus during absorption (I)

Transcytosis (see route of D)

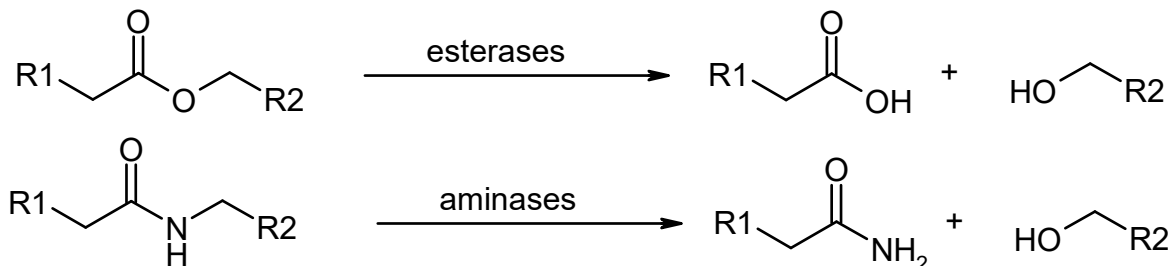


- A transcellular (passive diffusion)
- B paracellular
- C active transport
- D transcytosis

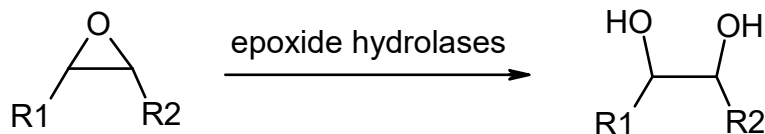
Cross-section from the
colon wall

Phase I processes (I)

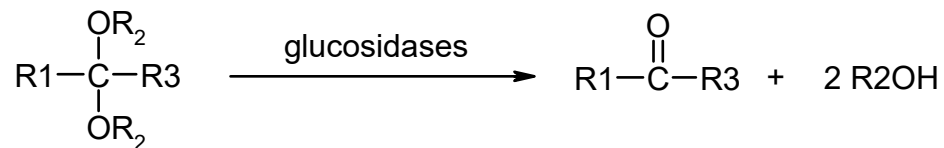
hydrolysis (formal addition of H₂O) of esters and amides by esterases and aminases



epoxides by epoxide hydrolases



acetals 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

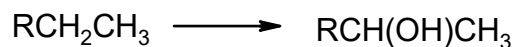
reductive dehalogenation (replacing halogens by hydrogen) of
aliphatic compounds

Phase I processes (III)

Oxidative reactions of
alcohols and aldehydes to carboxylates



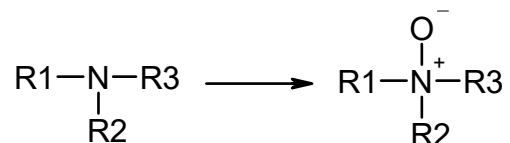
aliphatic chains



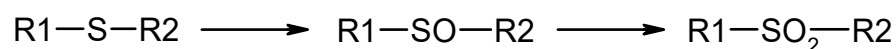
aromatic amines



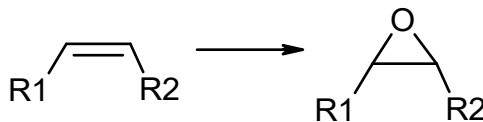
tertiary amines



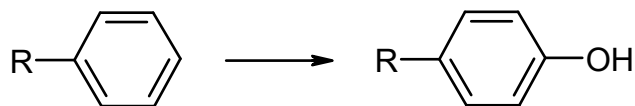
sulfides



alkenes to epoxides

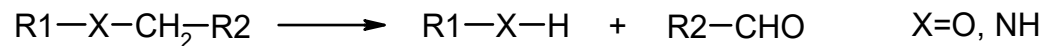


phenyl groups to phenol (in para position)



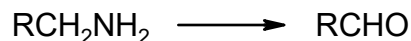
Phase I processes (IV)

Oxidative O- and N-dealkylation

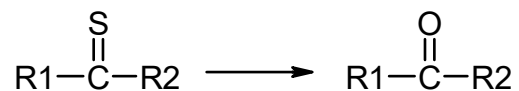


Oxidative deamination

by the monoamine dehydrogenase (MAO)



Oxidative desulfuration



Further oxidases are

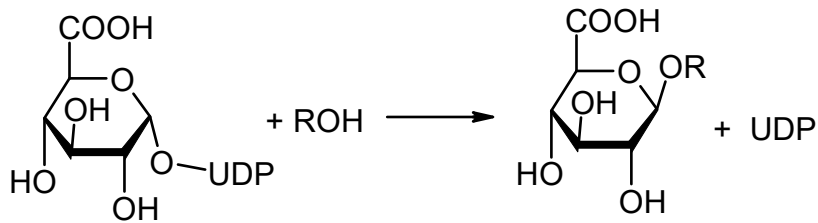
flavine monooxygenase isoenzyme

aldehyde oxidase

superfamily of cytochrome P450 enzymes

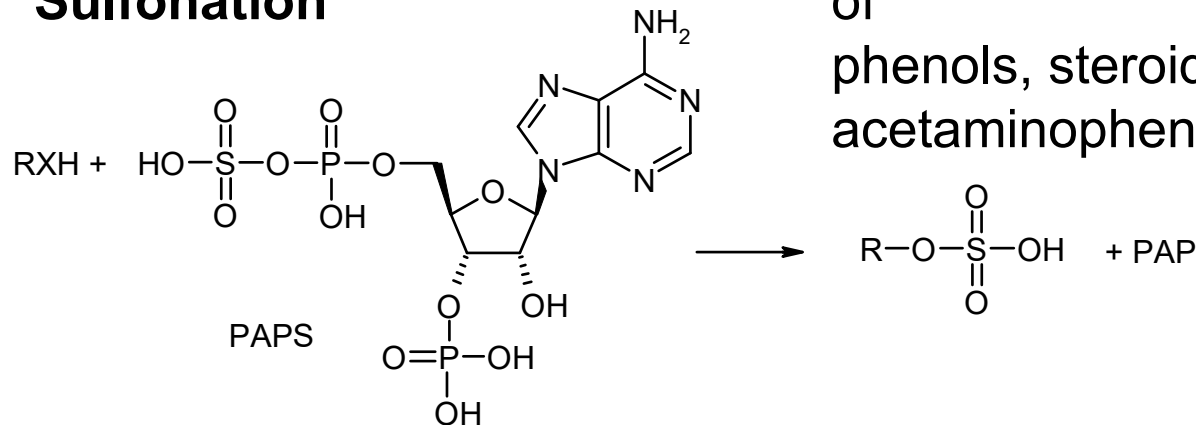
Phase II processes (I)

Glucuronidation



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

Sulfonation

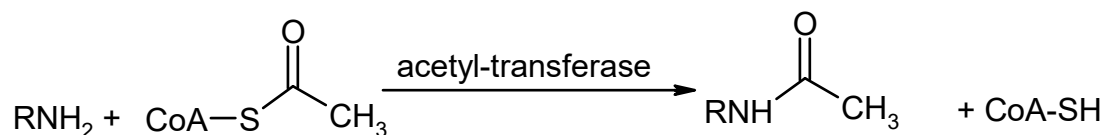


of
phenols, steroids,
acetaminophen, methyldopa

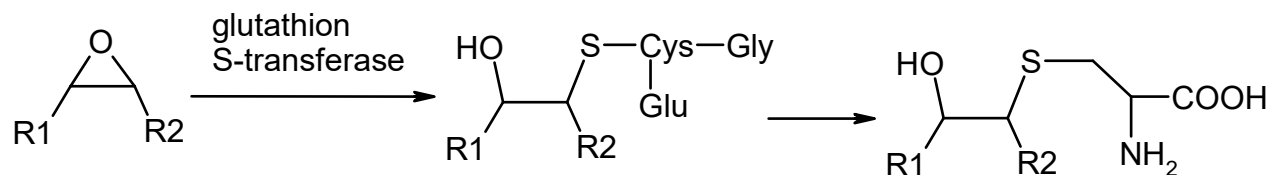
Phase II processes (II)

acetylation

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

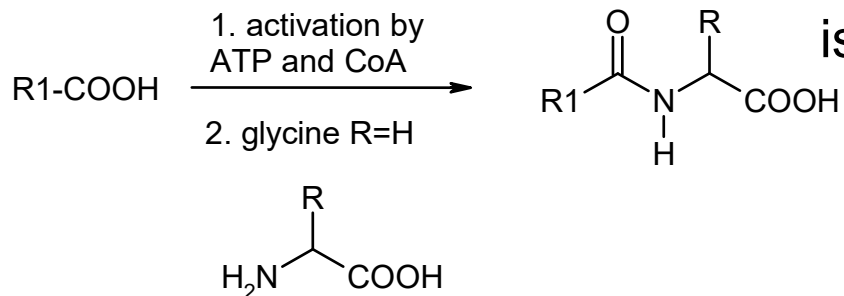


formation of mercapto acids



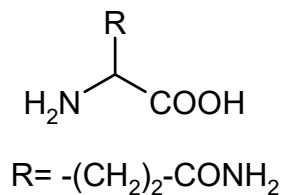
Phase II processes (III)

conjugation with glycine



e.g. of
benzoic acid,
isonicotinic acid

conjugation with glutamine

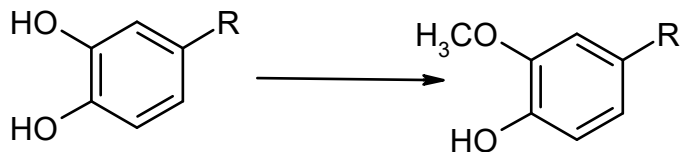
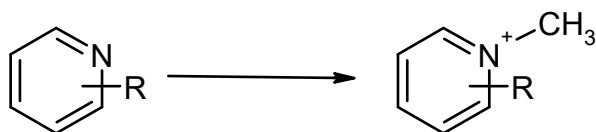
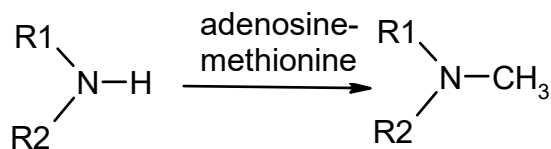


e.g. of
indolyl acetic acid,
phenyl acetic acid

Phase II processes (IV)

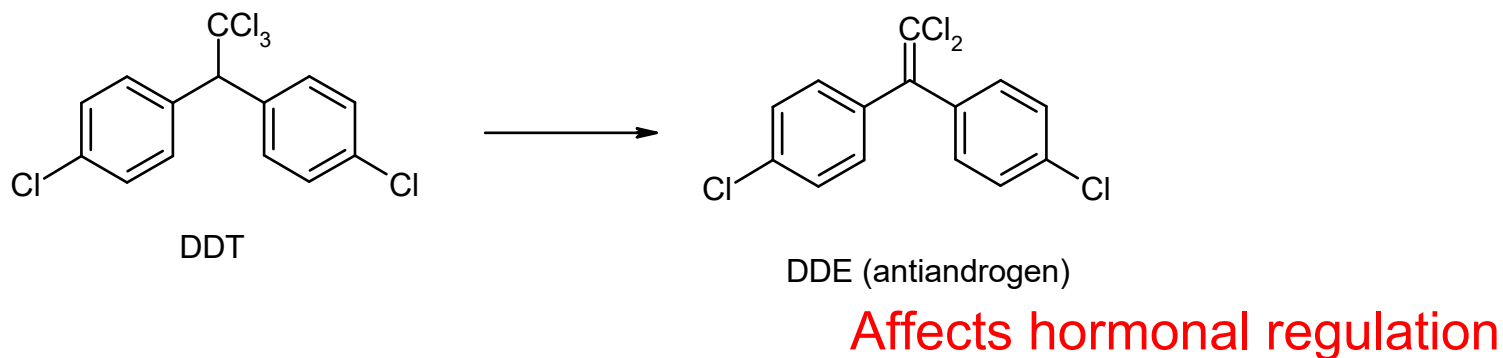
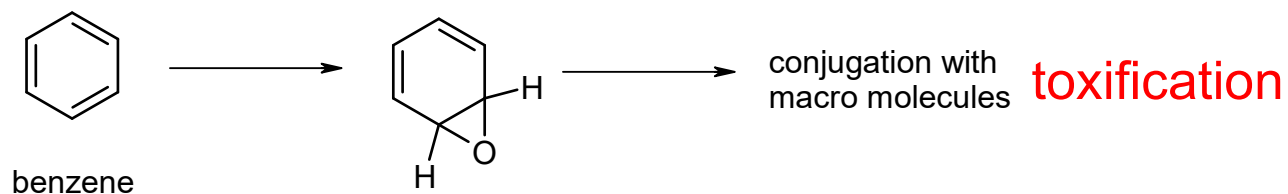
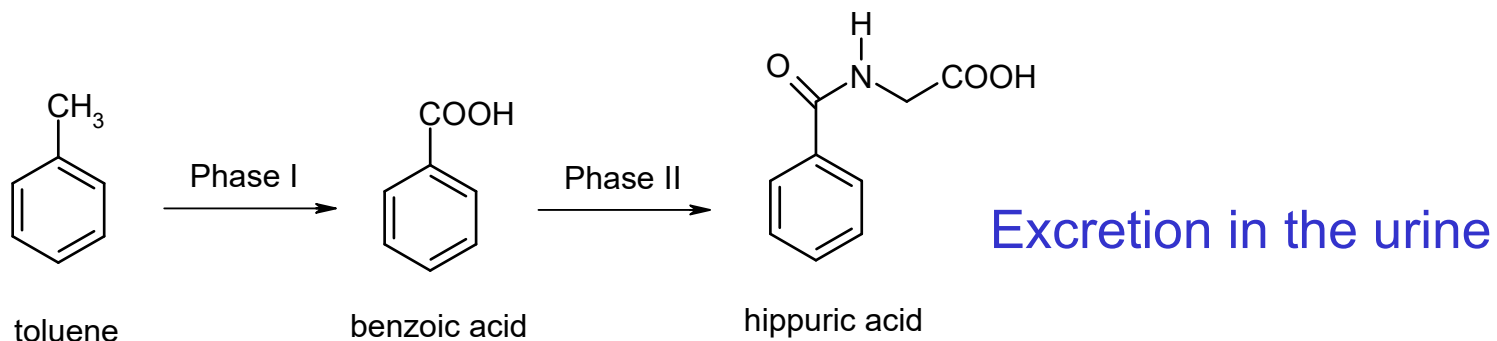
O-, N-, and S-methylation

e.g. of
methadon, nicotinamide,
norepinephrine

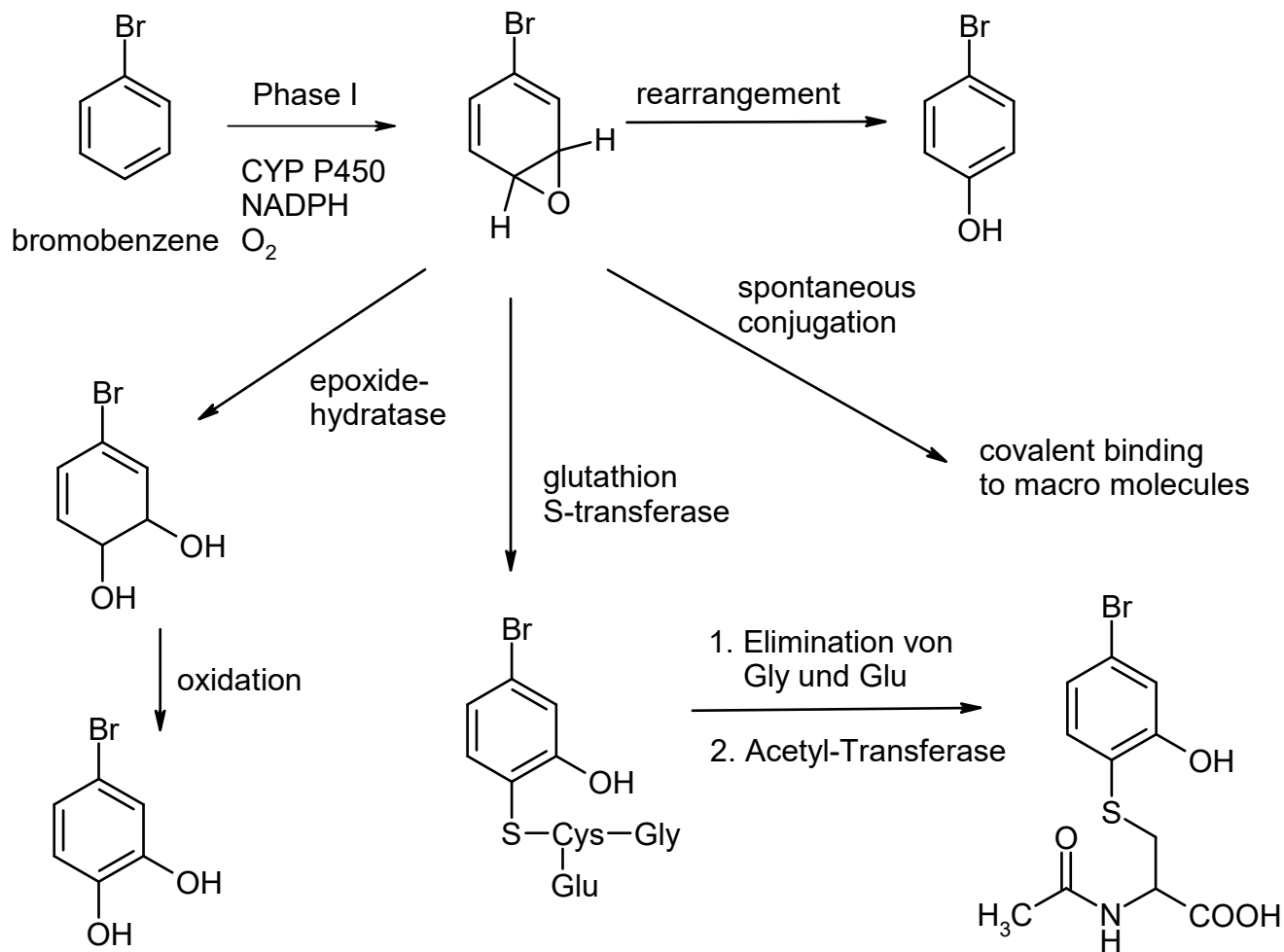


catecholamine (by
catechol-O-methyl transferase)

Metabolization of Xenobiotica (I)

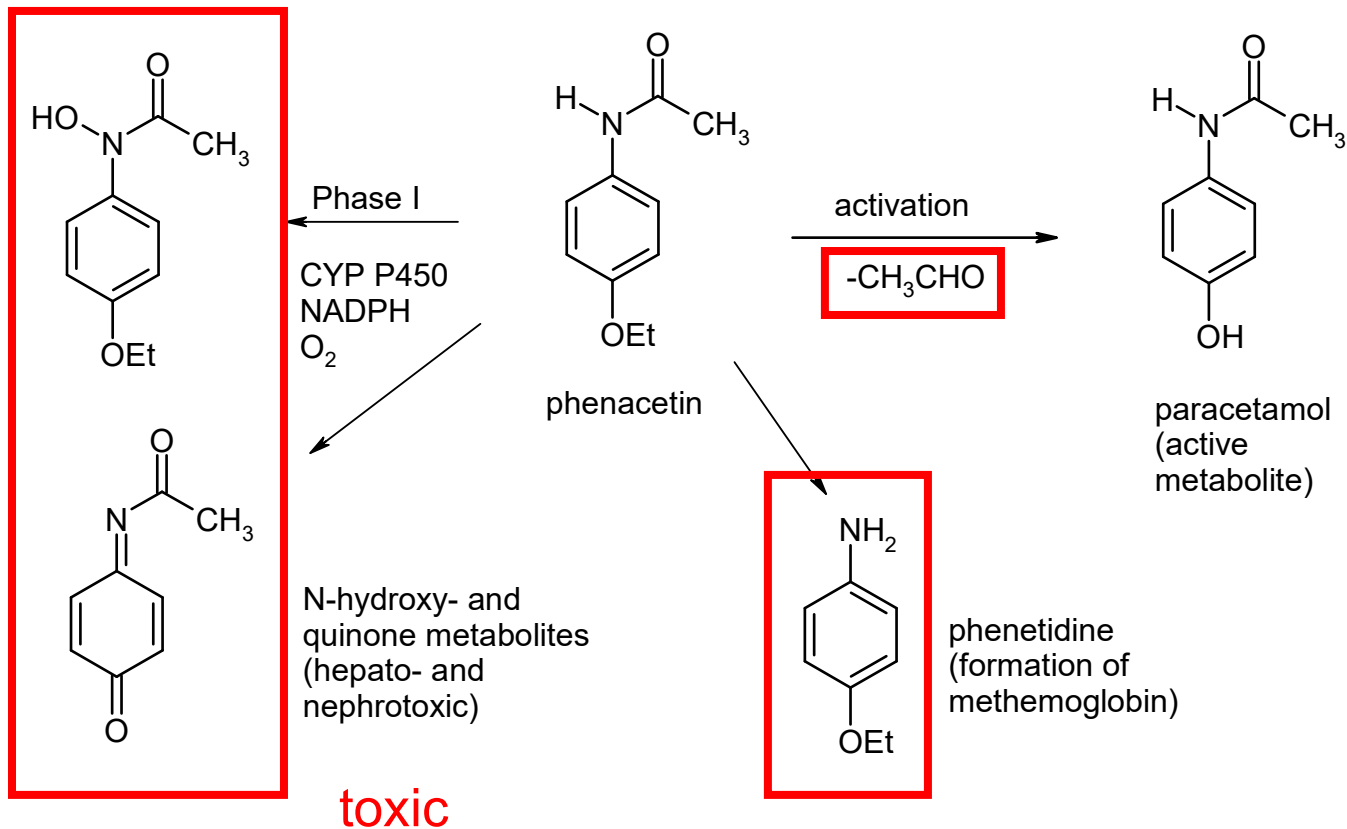


Metabolization of Xenobiotica (II)



Metabolization of Xenobiotica (III)

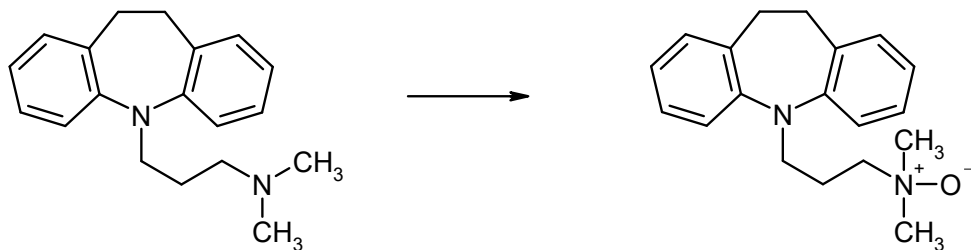
Example for particularly awkward metabolites



Therefore phenacetin is discontinued

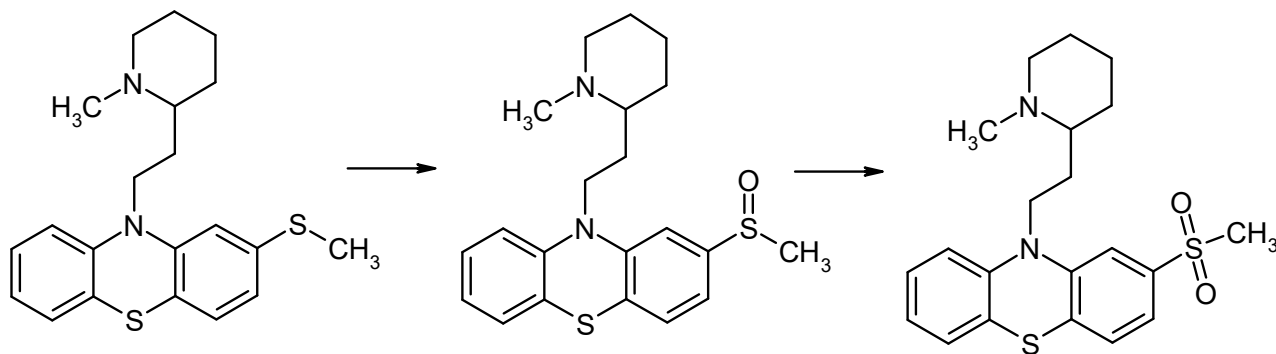
Metabolization of Xenobiotica (IV)

Examples where metabolites of drugs are also pharmacologically active



Imipramine

Imipramine N-Oxide



Thioridazine

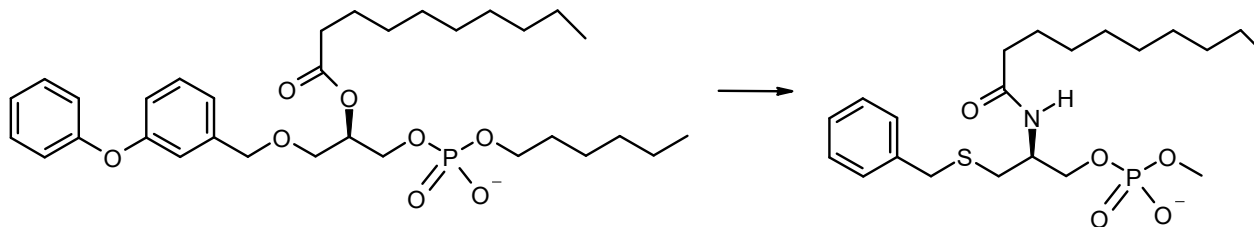
Mesoridazine

Sulforidazine

Improved metabolic stability

Increasing the bioavailability through:

Replacing esters by amides



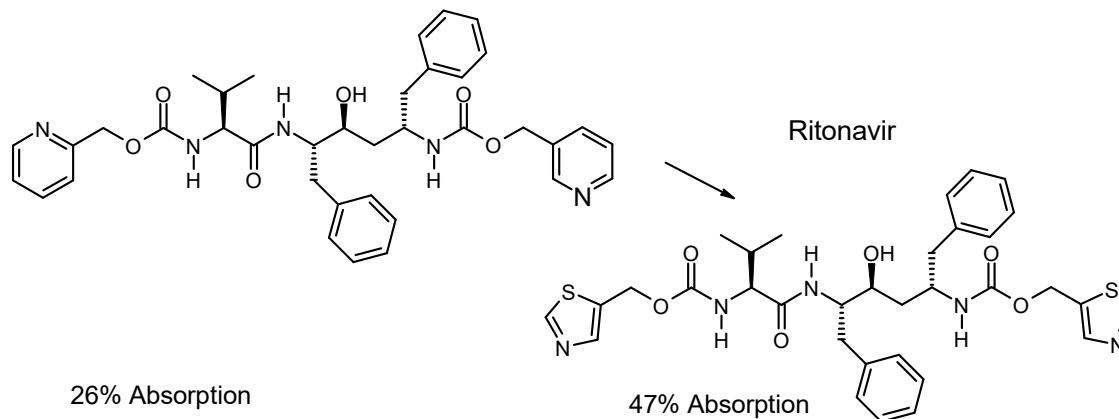
$C_{\max} = 465 \text{ ng ml}^{-1}$

4% Absorption

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

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 summarized in databases.

Commercial 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*

physico-chemical properties

binding to enzymes

esp. to human serum albumin (HSA), cytochrome P450 enzymes (see lecture 10)

catalytic reactions

reaction mechanism
turn over rate

drug-drug interaction

inhibition or induction (see lecture 10)

ADMET models (IV)

Reappearing descriptors in QSAR equations

$$\log(T) = a(H) + b(E) + c(S) + \text{constant}$$

- 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:

Tetrahymena pyriformis: growth inhibition

Vibrio fischeri: bioluminescence

mutagenicity (AMES):

Salmonella typhimurium + S9
(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

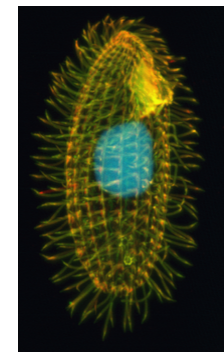
Currently all available machine learning algorithms including QSAR, decision trees, random forest, Naive Bayes, support vector machines, neural networks, deep learning, etc. are applied.

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-administration 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.