Toxicity of transformation products

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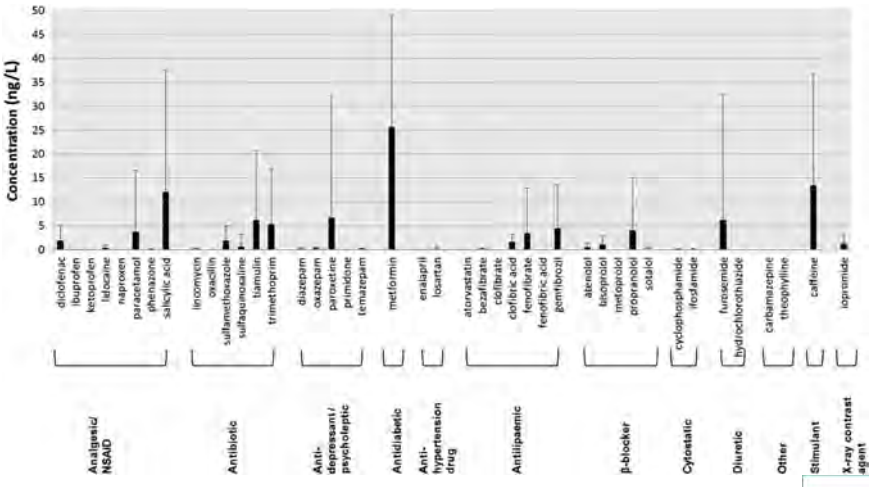
Daily drug cocktail when consuming Rhine-derived drinking water near Amsterdam



Houtman CJ && 2014

Indicators of anthropogenic activity

Pharmaceutical	ATC	DDD	Annual DDD	Predicted consumption
aciclovir	J05AB01	4000mg	4.8 Mio	> 19.2 tons
carbamazepine	N03AF01	1000mg	38.9 Mio	> 38.9 tons
diclofenac	M01AB05	100mg	316.8 Mio	> 31.7 tons
metformin	A10BA02	2000mg	595.2 Mio	> 1190.4 tons
sulfamethoxazole	J01EC01	1600mg	11.2 Mio	> 17.9 tons

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Toxicity of transformation products (= TPs)

- Definitions
- Exposure differences: Eco- vs Human toxicology
- Biological Targets: Identification and Implications
- Biotic and abiotic transformation
- TP Inflation: Example Pharmaceuticals
- TPOS (= "transformation product object space")
- Means for priorisation of TPs
- Outlook

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Definitions

- Exposure scenarios
  - ➔ inner vs outer exposure ; environmental occurrence vs active or passive absorption
- LADME scheme
  - ➔ liberation > absorption > distribution > metabolism > excretion
- Biological targets
  - ➔ functional or non-functional binding site
- Transformation products – biotic vs abiotic
  - ➔ a parent compound derivative formed by an organism or in the environment
- TPOS
  - ➔ transformation product object space
- Genetic variants and polymorphisms
  - ➔ balanced intra-species genetic variants with distinctive biological activity or phenotype

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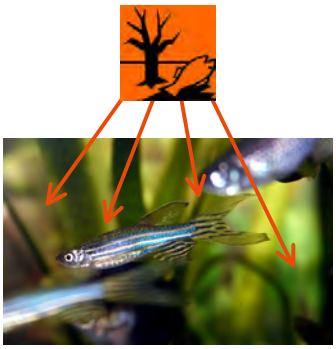
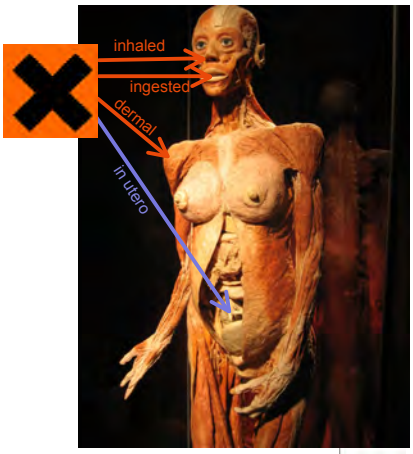
Exposure scenarios

Ecotoxicology



$Eff \sim c_w * t_x$

Human toxicology



$Eff \sim D_i * T_{res}$

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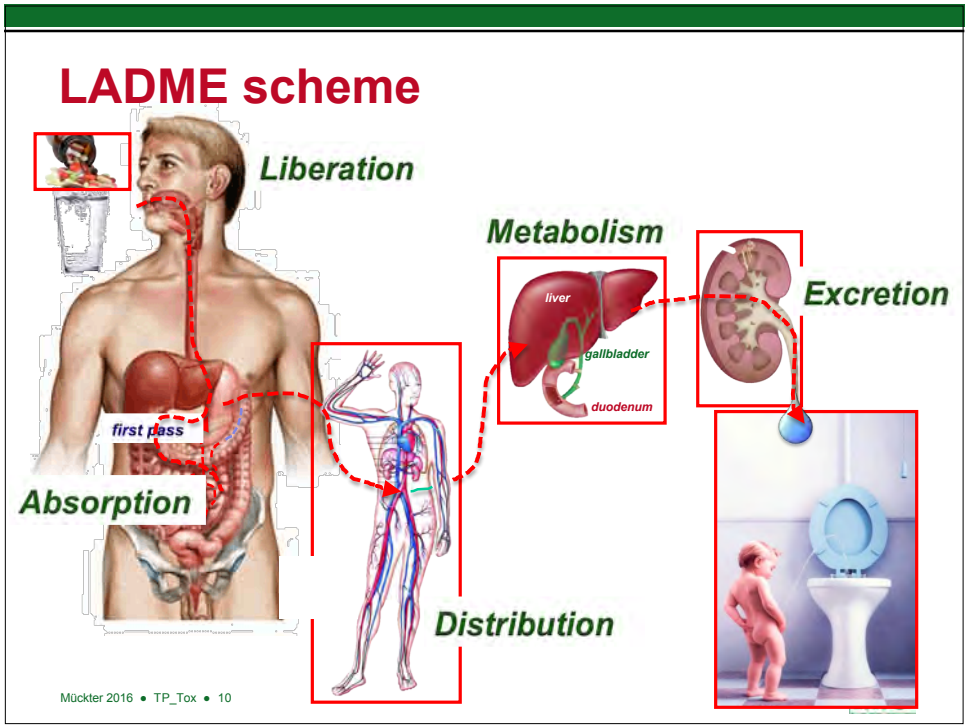
LADME scheme
Liberation
Metabolism
Excretion
liver
gallbladder
duodenum
first pass
Absorption
Distribution
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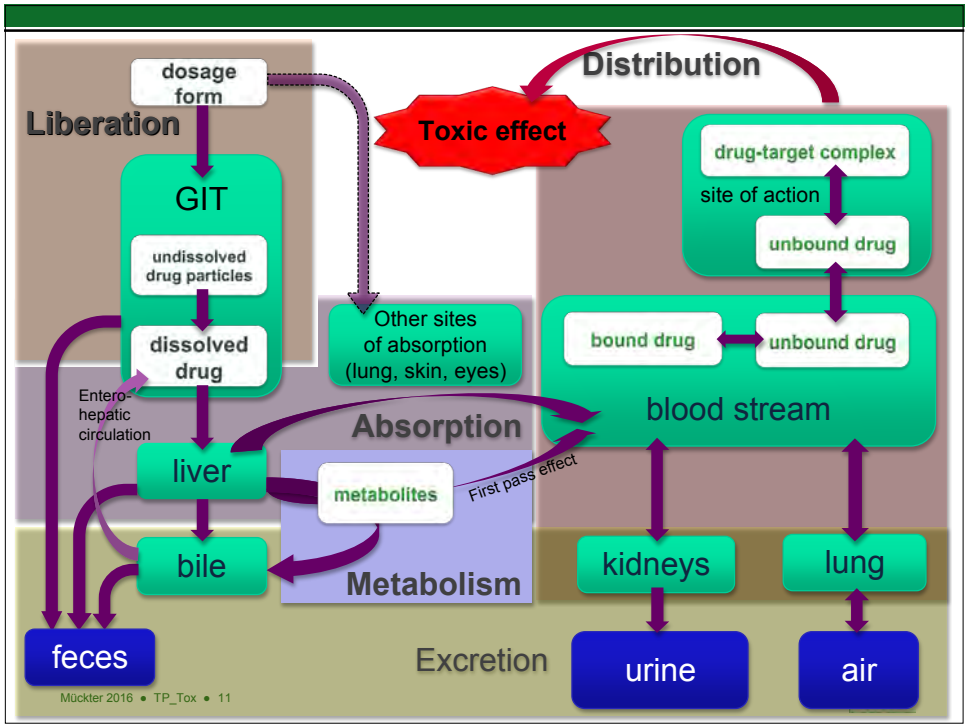
dosage form
Liberation
Distribution
Toxic effect
drug-target complex
site of action
unbound drug
GIT
undissolved drug particles
dissolved drug
Other sites of absorption (lung, skin, eyes)
bound drug
unbound drug
blood stream
Entero-hepatic circulation
Absorption
liver
metabolites
First pass effect
bile
Metabolism
kidneys
lung
feces
Excretion
urine
air
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The "target" concept in human toxicology

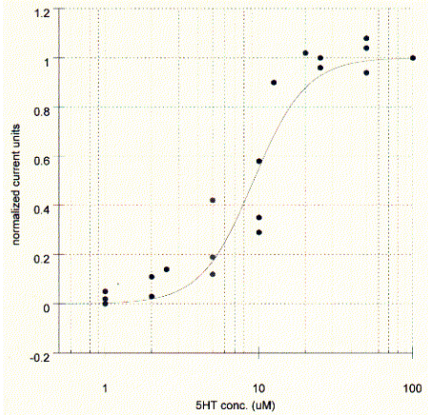
- Typical biological targets :
  - Enzymes
  - Receptors
  - Ion channels
  - Transporters
  - Structural proteins
  - Biomembranes
  - DNA , RNA molecules
  - Signaling & messenger molecules
  - Molecules of the intermediary metabolism

CBZ Targets

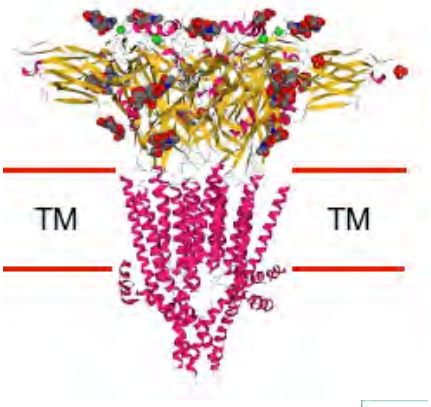
- CYP3A4 (EC 1.14.13.97) : 28 human variants
- CYP2B6 (EC 1.14.14.1) : 17 human variants
- CYP2C9 (EC 1.14.13.49) : 9 human variants
- CYP2C19 (EC 1.14.13.80) : 3+ human variants
- Epoxide hydrolase (EC 3.3.2.9) : 4 human isoenzymes
- NAT1 / NAT2 (EC 2.3.1.5) : 2 isoenzymes plus many polymorphisms
- O-methyl transferases (EC 2.1.1.x) ? ? ?
- UDP glucuronyl transferases (EC 2.4.1.17) : 23 human variants

Concentration Effect Curve

Sigmoid curve



$5HT3_A$ Receptor



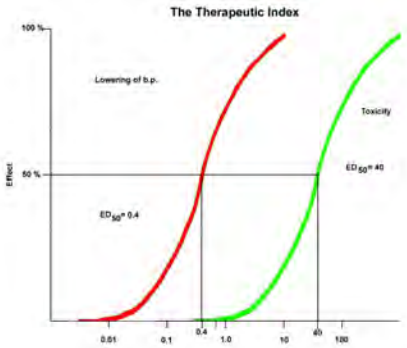
Wanted and Adverse Effects

Paracelsus (*1493 – †1541):
"Sola dosis facit venenum"

Kuschinsky (*1904 – †1922):
"Wenn behauptet wird, dass eine Substanz keine Nebenwirkung zeigt, so besteht der dringende Verdacht, dass sie auch keine Hauptwirkung hat."

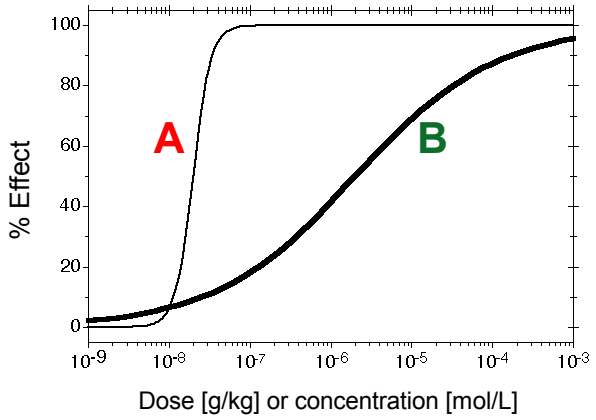
Various endpoints (effects) occur often very close.

The relevant part of any individual dose-response curve hardly spans two orders of magnitude.



Hypersensitivity reactions may be triggered by <1µg.

Toxicological comparisons



Occurrence of transformation products (TPs)

biotic

- Biotransformation
  - Phase I reactions
    - CYP450 monooxygenases
    - NADPH-CYP540 reductase
    - Esterases , amidases
    - Epoxide hydrolase
  - Phase II reactions
    - Glucuronosyl transferases
    - N-acetyl-transferases
    - Glutathione transferases
    - Methyl transferases
    - Sulfo transferases

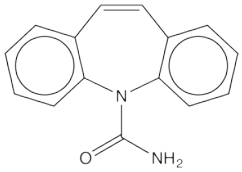
abiotic

- Photoreactions
  - Photooxidation
  - Photolysis
  - Photoactivation
- Advanced oxidation
  - Ozone
  - Chlorine dioxide
- (Partial) combustion / thermal degradation

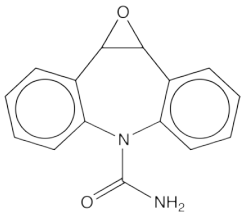
Carbamazepine (CBZ)

Profile

- Synthesis **1953**
  (as tricyclic antidepressant)
  CAS RegNo 298-46-4
- Drug approval **1962**
  (as anticonvulsant)
- PK (oral)
  c_{blood} 4 – 12mg/L (17 – 51µM)
  BA 80%, $t_{1/2}$ **25 – 65h**
  (12 – 17h long term use)
  renal elimination **3%**
- active metabolite : CBZ-10,11-epoxide
- CYP inducer (3A4)

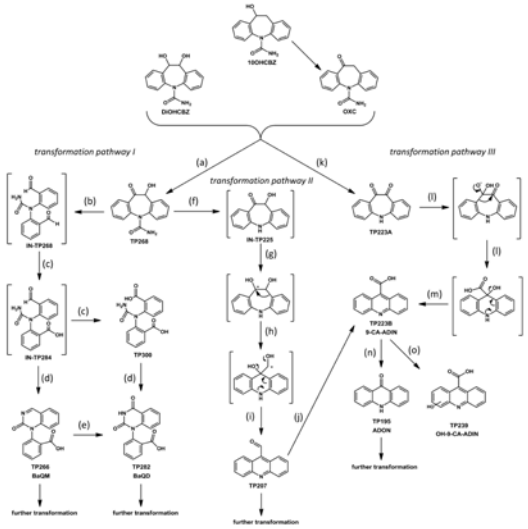


CBZ



CBZ-10,11-epoxide

Carbamazepine TPs and reaction pathways



Kaiser E && (2014) Environ Sci Technol.

TP inflation by biotic & abiotic transformation

	Aciclovir	Carbamazepine	Sulfamethoxazole
DDD	4000mg	1000mg	1600mg
No of identified	**12**	**82**	**30**
Biotic vs abiotic	7 vs 3 vs 2	42 vs 30 vs 10	11 vs 19
No of identified	3		
Prioritized TPs	(0)	≥7	≥4
Epidemiology	unknown	inconclusive	unknown
Organ toxicity	Gastrointestinal, Neurotoxicity, Myelotoxicity	Gastrointestinal, Hepatotoxicity, Myelotoxicity, Fetotoxicity	Gastrointestinal, Myelotoxicity, Nephrotoxicity, Fetotoxicity
Hypersensitivity	Very rare	Rare	Frequent

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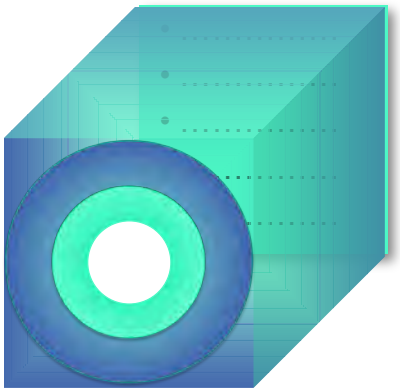
Transformation Product Object Space = TPOS (1)

- Kind = container
- Type = categorial
- Dimensions = 2

target screen



substance screen

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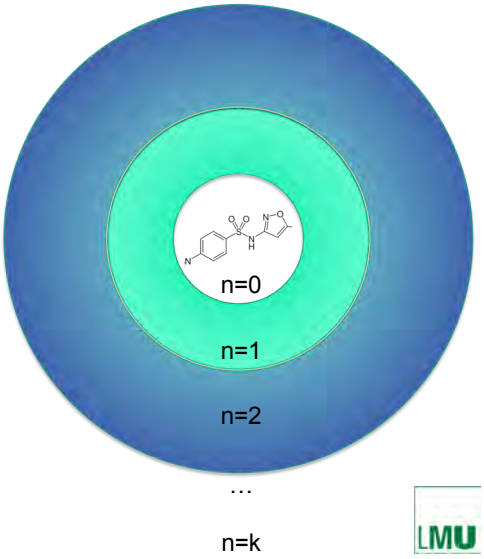
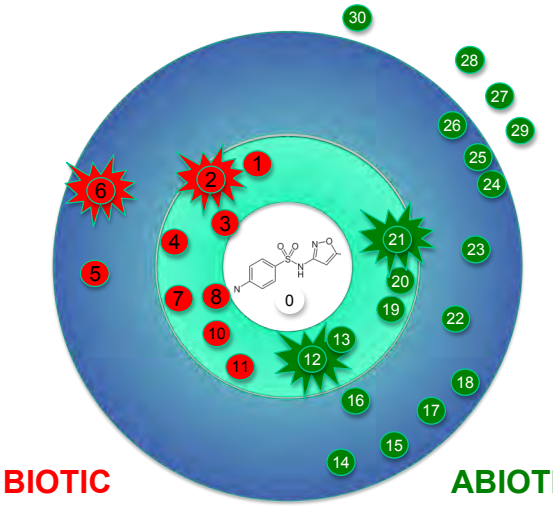
Transformation Product Object Space = TPOS (2)

Criteria:

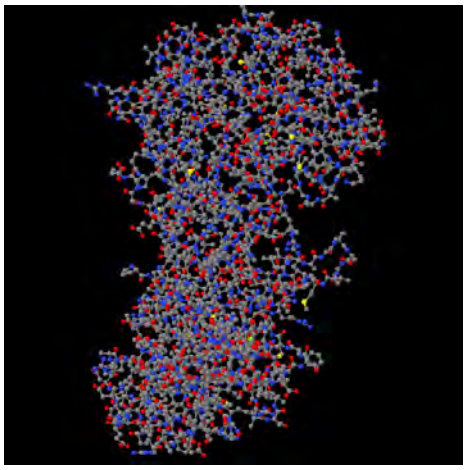
- No of reaction steps
- Occurrence
- cLogP
- Toxicophores
- Molecular weight
- No of conjugation sites



Sulfamethoxazole TPOS



Modelling



Dihydropteroate synthase, EC 2.5.1.15, M. tuberculosis (PDB: 1AD1)
with 6-OH-7,8-dihydropterin-PP : dimer w/ two SMZ binding sites

Ligand binding affinities & free binding energies

Chemical equilibrium between bound and unbound ligands

Gibbs energy ΔG and association constant K_a

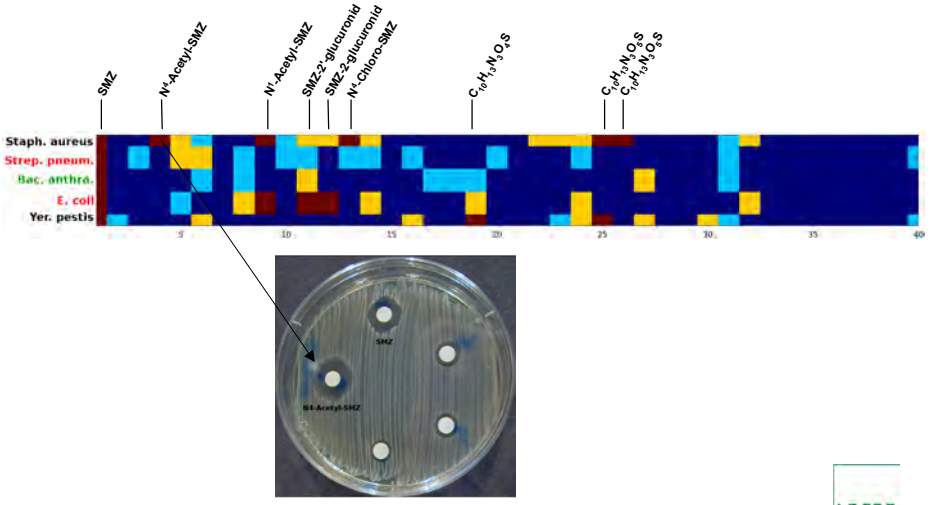
$$\Delta G = \Delta H - T\Delta S = -RT \ln \left(\frac{[RL]}{[R]\,[L]} \right)$$

Simulation of TP **bound/unbound** to estimate (interaction-)energy & entropy **changes** for ΔG

$$\Delta G = x_1 \Delta \langle E^{elec} \rangle + x_2 \Delta \langle E^{vdw} \rangle + x_3 \Delta U - x_4 T \Delta S$$

Enthalpy ΔH Entropy ΔS

Qualitative Predictions : Affinity



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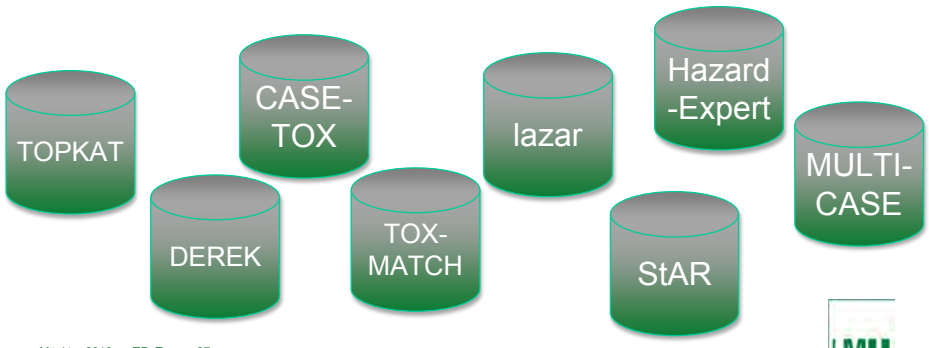
Expert systems

When biological targets are unknown or unavailable as 3D data set *expert systems* may serve as an educated guess for the priorisation of TPs.



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(= Lazy Structure-Activity Relationships)

OpenTox EURO2014
24. – 28. October 2016

- DSSTox Databases of US EPA storing peer-reviewed carciongenicity data
  - DSSTox Carcinogenic Potency DBS SingleCellCall
  - DSSTox Carcinogenic Potency DBS MultiCellCall
  - DSSTox Carcinogenic Potency DBS Mouse
  - DSSTox Carcinogenic Potency DBS Rat
  - DSSTox Carcinogenic Potency DBS Hamster
- DSSTox ISSCAN v3a Canc
- DSSTox Carcinogenic Potency DBS Mutagenicity
- Kazius-Bursi Salmonella Mutagenicity
- EPA v4b Fathead Minnow Acute Toxicity LC50_mM
- FDA v3b Maximum Recommended Daily Dose mmol / 70kg

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Predicted Genotoxicity

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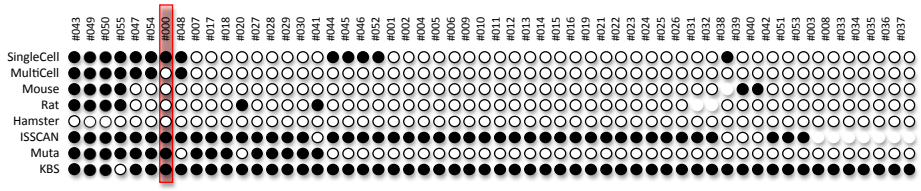
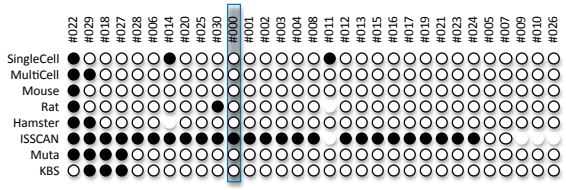
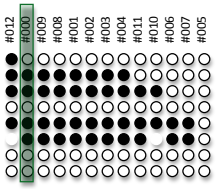
Carbamazepine





Sulfamethoxazole



Aciclovir

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Predicted Ecotoxicity & Consumer Safety

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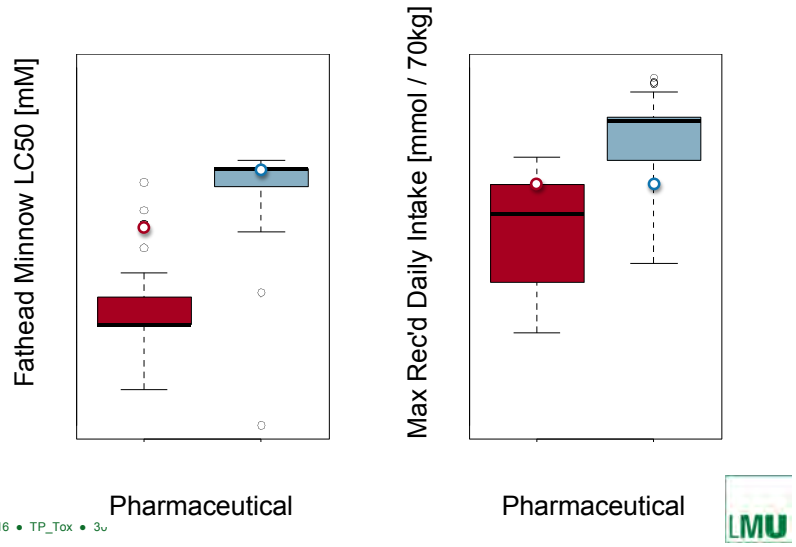

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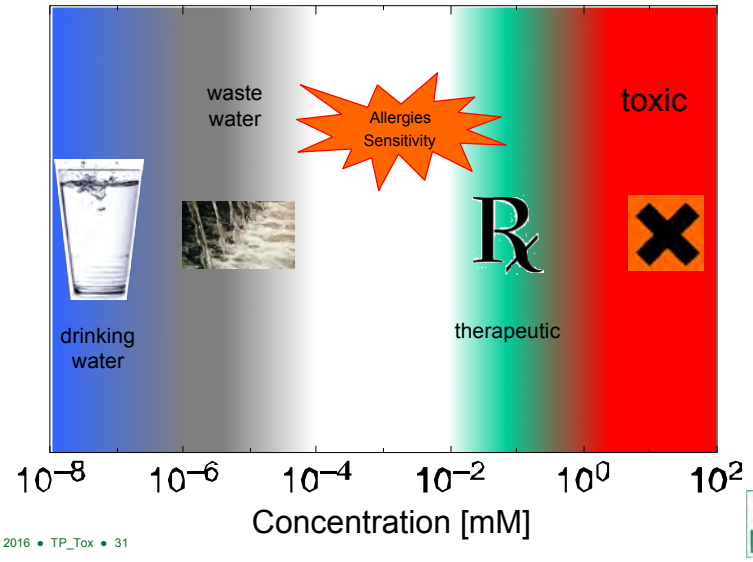
Concentration ranges CBZ



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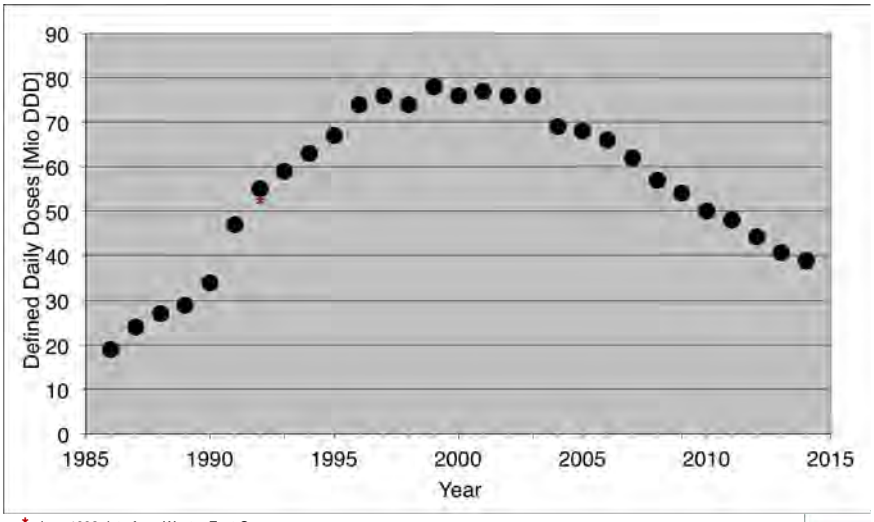
CBZ prescription (German statutory health insurance)

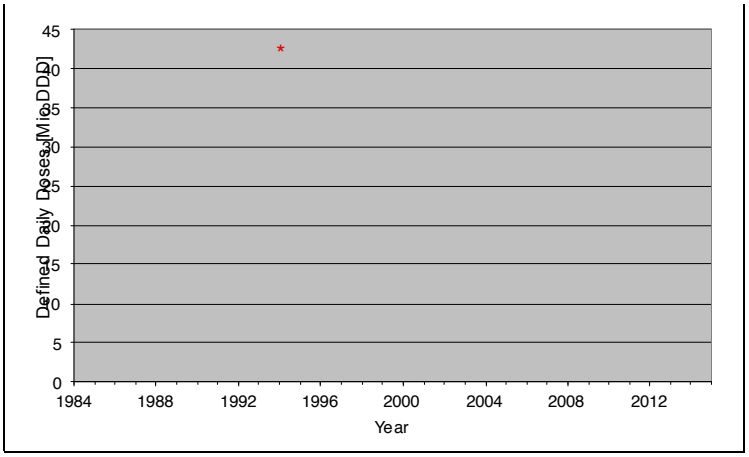


* since 1993 data from West + East Germany

Source : Schwabe U, Paffrath D. Arzneiverordnungsreport 1986-2012

DDD (= defined daily dose) for CBZ: 1000mg Quelle: Schwabe/Paffrath Arzneiverordnungs-Report

SMZ prescription (German statutory health insurance)



* since 1993 data from West + East Germany

Sulfamethoxazole DDD = 1600mg

Source : Schwabe U, Paffrath D. Arzneiverordnungsreport 1986-2012

Outlook

- Compartimentation of the human organism provides several barriers to increase human safety.
- Lifelong water consumption led to pharmaceutical exposure <10% of a daily medical dose (Houtman && 2014)
- Waste water treatment by advanced oxidation processes introduces a plenty of unknown chemicals – with some suspected genotoxic activity.
- Expert systems and molecular modelling may help to prioritize the "new" transformation products.

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