## Moleculaire toxicologie: uitzicht op inzicht?

Vermeulen, Nico P.E.

2018

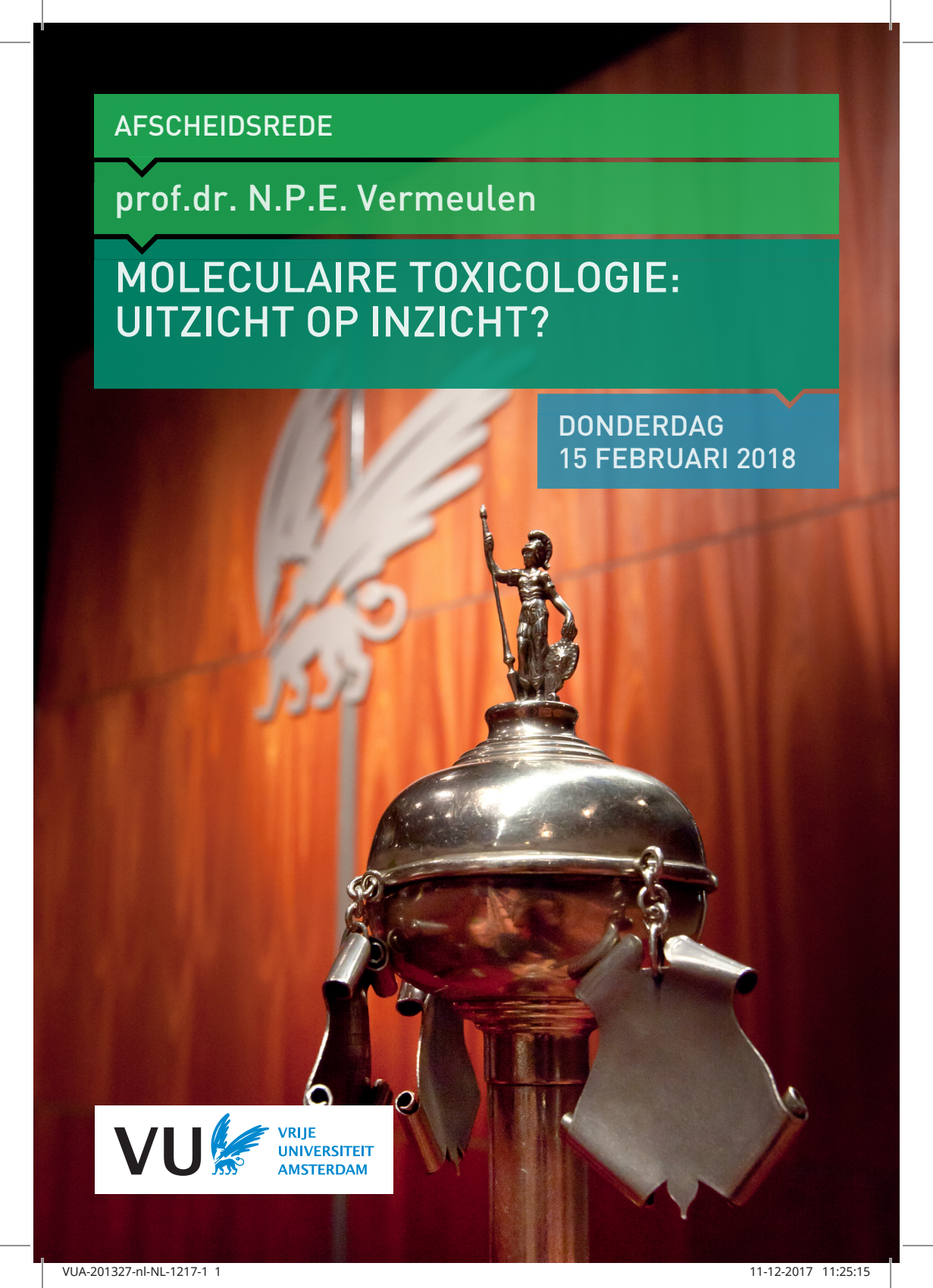
***document version***
Publisher's PDF, also known as Version of record

AFSCHEIDSREDE

prof.dr. N.P.E. Vermeulen

# MOLECULAIRE TOXICOLOGIE: UITZICHT OP INZICHT?

DONDERDAG
15 FEBRUARI 2018



VU VRIJE UNIVERSITEIT AMSTERDAM

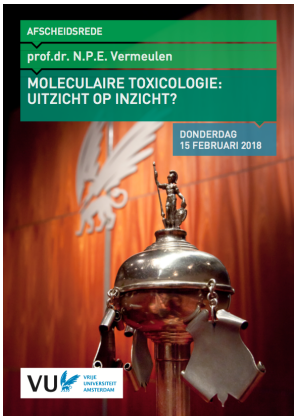
prof.dr. N.P.E. Vermeulen

# MOLECULAIRE TOXICOLOGIE: UITZICHT OP INZICHT?

Rede uitgesproken bij zijn afscheid als hoogleraar Moleculaire Toxicologie aan de Faculteit der Betawetenschappen van de Vrije Universiteit Amsterdam op 15 Februari 2018.

VU VRIJE UNIVERSITEIT AMSTERDAM

# Molecular Toxicology: Outlook on Insight?




**Prof. Nico P.E. Vermeulen**

**AIMMS - Molecular Toxicology**
**Department of Chemistry & Pharmaceutical Sciences**
**Vrije Universiteit**
**Amsterdam**
**The Netherlands**

**n.p.e.vermeulen@vu.nl**
**www.moltox.chem.vu.nl**

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM



# Scope and Content


- Back to the beginning
- Some general principles
- Drug discovery & development process and challenges
- Drug safety & adverse drug reactions (ADRs)
- Paracetamol liver toxicity and modulation
- Computational enzyme modeling & prediction
- Variability in liver enzymes & ADRs
- Final considerations
- Thanks

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

# 1975: Chemistry degree Nijmegen & start PhD programme in Leiden

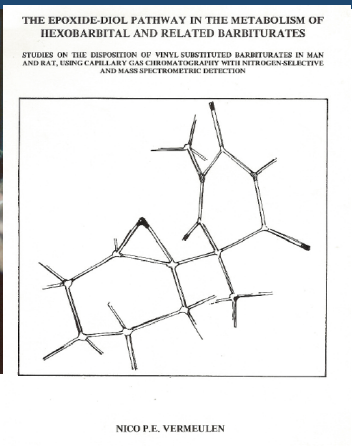
Chemistry degree (Universities Nijmegen + Amsterdam)

(Organic + Pharmacochemistry + Mass Spectrometry)

Start PhD programme at University of Leiden

(profs Douwe D. Breimer & Henk J. de Jong

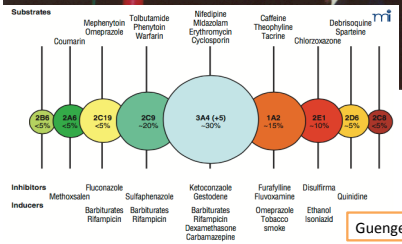
# 1980: PhD-degree from Faculty of Pharmacy University of Leiden




- Hexobarbital, Antipyrine, Nifedipine
- Model substrates for drug metabolism capacity
- GSH-conjugation, urinary mercapturic acids
  - Bioactivation and toxification
  - Biomonitoring agro- / industrial chemicals

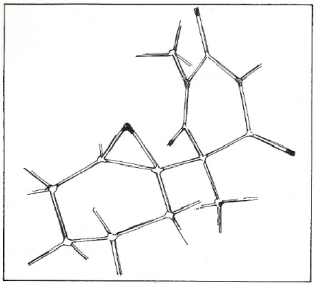
VU UNIVERSITY AMSTERDAM

# 1980: PhD-degree from Faculty of Pharmacy University of Leiden




THE EPOXIDE-DIOL PATHWAY IN THE METABOLISM OF HEXOBARBITAL AND RELATED BARBITURATES

STUDIES ON THE DISPOSITION OF VINYL SUBSTITUTED BARBITURATES IN MAN AND RAT, USING CAPILLARY GAS CHROMATOGRAPHY WITH NITROGEN-SELECTIVE AND MASS SPECTROMETRIC DETECTION



NICO P.E. VERMEULEN

Guengerich, Rendic, Molec. Interv. 2003

VU UNIVERSITY AMSTERDAM

# Molecular Toxicology Amsterdam (1985 - > )

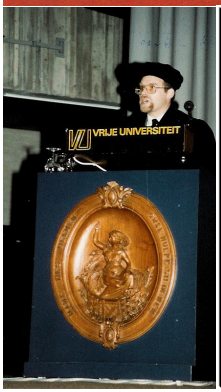
Dr. J.P.E. Vermeulen.

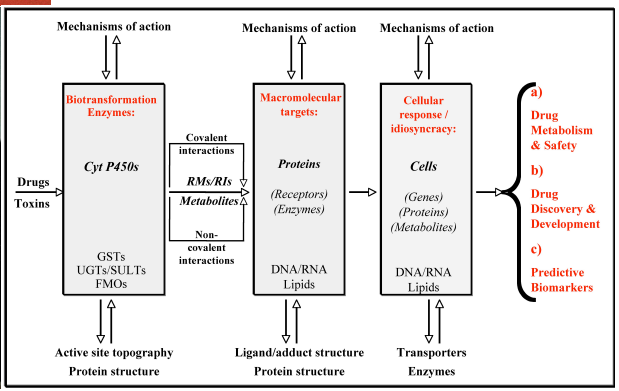
Moleculaire Toxicologie: Uitzicht op Inzicht

**1985: appointed professor at VU University**

**1987: Inaugural lecture**

**Molecular Toxicology, a new sub-discipline**

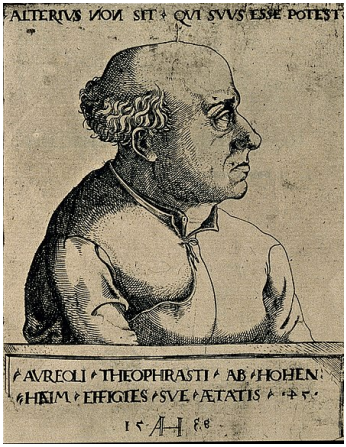


# Intermezzo 1: begin van loopbaan

- 1975: Doctoraal Scheikunde KU Nijmegen en Univ. van Amsterdam
  - (Farmacochemie/Van Rossum, Organische chemie/Ottenheijm, Massa sprectrometrie/ Nibbering, UVA)
- 1980: Promotie Universiteit Leiden, toen Faculteit Farmacie, bij prof. D.D. Breimer en prof. H.J. de Jong
  - Onderwerp: Metabolisme studies aan Hexobarbital en andere slaapmiddelen. En het gebruik van Hexobarbital als modelgeneesmiddel om de metabole capaciteit in de lever te meten.
- 1980: Staf-medewerker op de Afdeling Farmacologie van prof. Breimer
  - Onderzoek aan Hexobarbital en andere modelgeneesmiddelen. Begin van toxicologisch onderzoek. Docent en begeleider van studenten en promovendi.
- 1985: Benoeming als Hoogleraar *Moleculaire Toxicologie* op de Afdeling Farmacochemie/Faculteit Scheikunde van prof. Henk Timmerman
- 1987: Oratie *'Moleculaire Toxicologie: Uitzicht op Inzicht'*.
  - *MolTox is een nieuwe discipline:* onderverdeling in toxicokinetiek (ADME), toxische / ontgiftingsprocessen, reversibele/irreversibele interacties met macromoleculen, biochemische beschermings- & reparatiemechanismen en uiteindelijk de gevolgen voor toxisch effecten.
  - *Doelen van MolTox zijn:* reductie van toxische effecten tot basisprincipes, afleiding van structuur-activiteits relaties, betrouwbaardere en efficiëntere risicobeoordeling, het ontwerpen van preventieve en therapeutische strategieën tegen toxiciteiten, en het ontwerpen van efficiëntere, veiligere geneesmiddelen en (agro-) chemicaliën

# Paracelsus: Dose and Toxicity




**Paracelsus (1493 – 1541):**

- All substances are poisons, it is the dose that determines if a substance is not toxic

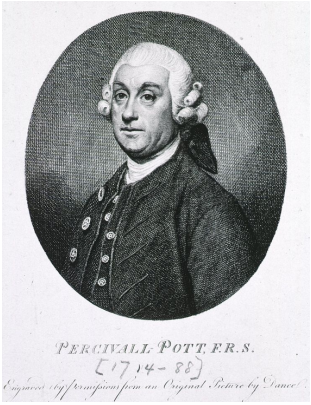
- Dose determines whether a substance is a a toxin, a medicine or food: e.g. Ethanol:
  - ✓ food (< 0.1%, in cellular energy)
  - ✓ analgesic (once / high)
  - ✓ toxin: chronic / high

- Note, it is in fact **exposure** that determines if a substance is not toxic

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

# Polycyclic Aromatic Hydrocarbons (PAHs)



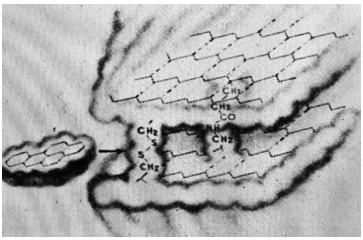

**History:**

- 1775, Percival Pott: relation soot ~ scrotum cancer in chimney sweepers (often children)
- 1788: Chimney sweepers act: Stop child labor
- 1933: PAKs isolated from soot, idenfied and linked to mouse skin tumors
- 1950's: Tabacco smoke linked to lung cancer
- 1960's: B(a)P is metabolized to metabolic products
- 1970's: link between B(a)P and cancer
- 1980's: B(a)P metabolites react with DNA
- 1990's: link between B(a)P in tabacco smoke and lung cancer, via DNA adducts
- 2000's: multiple cancers caused by B(a)P

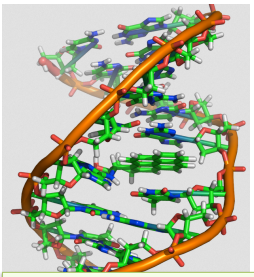
Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

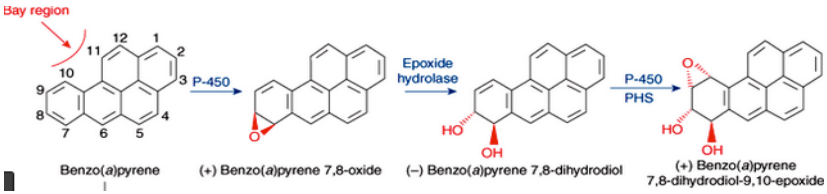
# Benzo[a]pyrene carcinogenicity: Mechanisms



1963: Arcos & Arcos: charge-transfer reactions between layers of poly-peptide chains

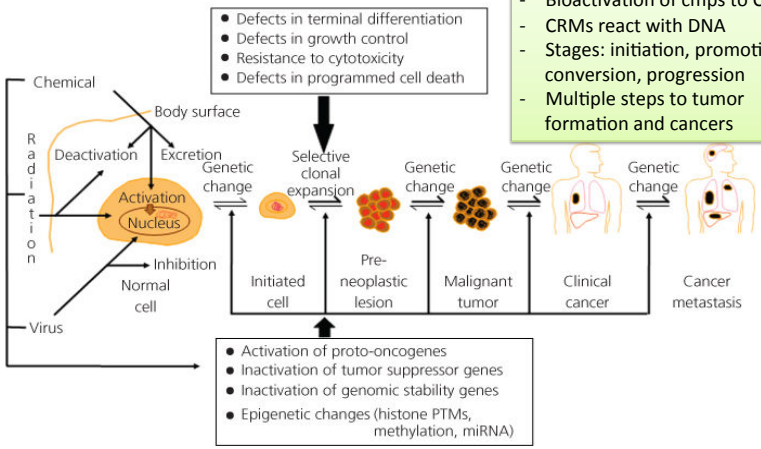


1983: Intercalation in DNA, covalently bound to DNA-guanines



# Multistage carcinogenesis

**Multiple steps:**
- Bioactivation of cmps to CRMs
- CRMs react with DNA
- Stages: initiation, promotion, conversion, progression
- Multiple steps to tumor formation and cancers




Amsterdam Institute for Molecules, Medicines and Systems

Weston et al., Cancer Meds., 2003

VU UNIVERSITY AMSTERDAM

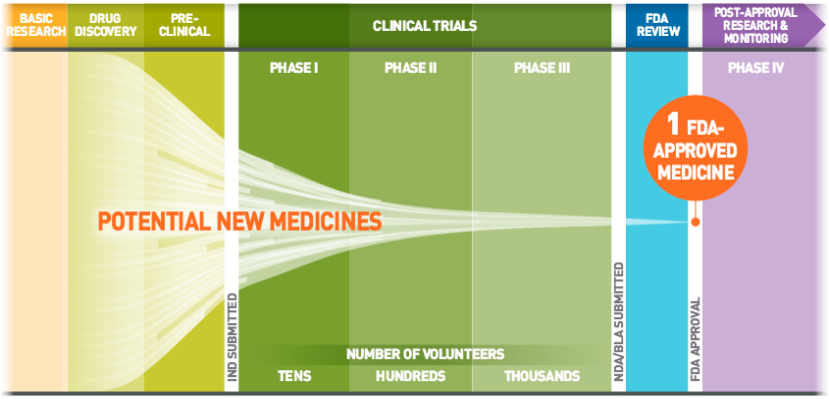
# Intermezzo 2: Algemene principes

- 1ste algemene principe, van *Paracelsus* (~ 1500):
  - 'alle stoffen zijn giftig, alleen de dosis bepaalt of een stof niet giftig is'.
  - Alcohol is een voedingsmiddel (< 0,1% cellulaire energie), een pijnstiller (een-malige hoge dosis) en toxisch (langdurig hoge dosis)
- 2de algemeen principe (~ PAKs en kanker)
  - *Percival Pott* ontdekte in 1775 dat Engelse schoorsteenvegers vaak scrotum kanker kregen. Pas in 1933 werden PAKs, waaronder Benzo(a)pyrene (B(a)P), geisoleerd uit roet en in 1960 werd ontdekt dat B(a)P wordt afgebroken tot metabolieten. In de jaren '80 werden adducten van reactieve B(a)P metabolieten aan DNA geidentificeerd en in verband gebracht met het ontstaan van kanker. In de jaren '90 werden DNA-adducten van B(a)P metabolieten uit tabaksrook direct gelinked aan long kanker.
  - Ondertussen staat vast dat B(a)P en andere PAKs uit tabaksrook oorzaak zijn van long kanker en ook verschillende andere kankersoorten in de mens kunnen veroorzaken
  - De toxicologie heeft zich langzaam ontwikkeld van een fenomenologische/waarnemende tot een verklarende wetenschap. De naam *Moleculaire Toxicologie* bestaat pas sinds 1979.
  - Toxiciteit van chemicalien (en ook van geneesmiddelen) is vaak niet het gevolg van de moederverbinding, maar van metabolieten die door het lichaam zelf worden gemaakt. Dit proces *heet 'bioactivatie' of 'toxificatie'*

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

# Drug Discovery & Development: Process & Attrition

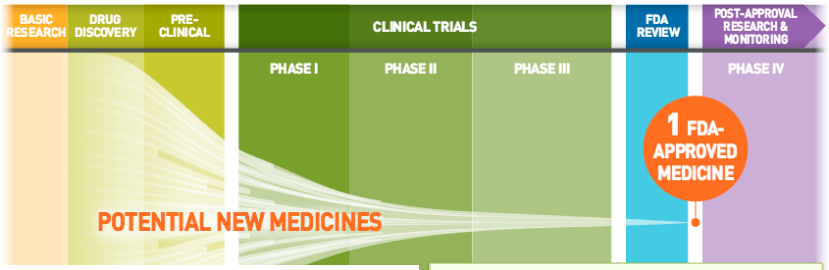


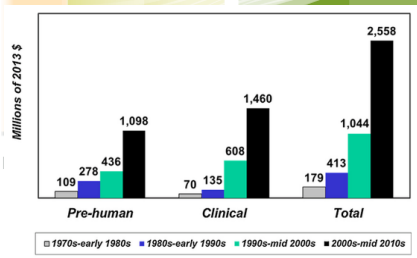

Key: IND: Investigational New Drug Application, NDA: New Drug Application, BLA: Biologics License Application

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

# Drug Discovery & Development: Process & Attrition

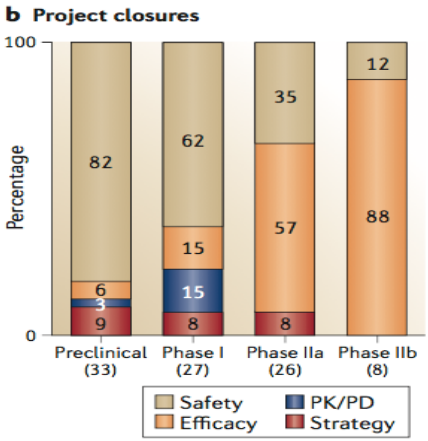



**Dimasi et al., J. Health Econ., 2016**

- 106 randomly selected drugs from 10 firms
- Pre-human, clinical and total R&D costs (~ 4 decennia)
- Costs of abandoned compounds linked to approved
- Total pre-approval cost estimate $ 2558 million
- With estimated post-approval costs $ 2870 million

# Reasons for Attrition during Drug Discovery & Development




**a) Project success rates 2005-2010**

- Preclinical, Phase I, II and III
- AstraZeneca against PBF benchmark for industry
- AZ: 120 projects

**b) Project closures & reasons**

- Safety (toxicology or clinical safety
- Efficacy (failing or insufficient)
- PK/PD properties
- Strategy (commercial or …)
- 33 closed before and 61 during clinical testing

Amsterdam Institute for Molecules Medicines and Systems

Cook et al., Nat. Revs. Drug Disc., 2014

VU UNIVERSITY AMSTERDAM

# Hepato- / Livertoxic drugs in man: Blackbox warning labels or even withdrawn from market

**Drugs with black warnings for hepatotoxicity***

| drug | dose (mg/day) | reactive products |
|---|---|---|
| acitretin | 25-50 | no |
| bosentan | 125-250 | no |
| dacarbazine | 140-315 | yes |
| dantrolene | 300-400 | yes |
| felbamate | 1200 | yes |
| flutamide | 750 | yes |
| gemtuzumab | 9 $mg.m^{-3}$ | yes (?) |
| isoniazid | 300 | yes |
| ketoconazole | 200 | yes |
| naltrexone | 50 | no |
| nevirapine | 200 | yes |
| tolcapone | 300 | yes |
| trovafloxacin | 100-500 | no |
| valproic acid | 1000-2400 | Yes (10/14 = 71%) |

*Definition: a black box warning is the strongest type of warning that the FDA can require for a drug and is generally reserved for warning prescribers about adverse drug reactions that can cause serious injury or death. An issue here is the benefit/risk ratio.

**Drugs withdrawn for hepatotoxicity**

| drug | date | dose (mg/day) | reactive products |
|---|---|---|---|
| cincopher | 1930 | 300 | no |
| iproniazid | 1959 | 25-150 | yes |
| pipamazinc | 1969 | 15 | no |
| fenclozic acid | 1970 | 300 | yes |
| oxyphenisatin | 1973 | 50 | no |
| nialamide | 1974 | 200 | yes |
| tienilic acid | 1980 | 250-500 | yes |
| benoxaprofen | 1982 | 300-600 | yes |
| nomifensine | 1986 | 125 | yes |
| chlomezanone | 1996 | 600 | no |
| bromfenac | 1998 | 25-50 | yes |
| troglitazone | 2000 | 400 | yes |
| nefazodone | 2004 | 200 | yes |
| pemoline | 2005 | 38-110 | no |

## Hepato- / Livertoxic drugs in man: Blackbox warning labels or even withdrawn from market

**Drugs with black warnings for hepatotoxicity***

| drug | dose (mg/day) | reactive products |
|---|---|---|
| acitretin | 25-50 | no |
| bosentan | 125-250 | no |
| dacarbazine | 140-315 | yes |
| dantrolene | 300-400 | yes |
| felbamate | 1200 | yes |
| flutamide | 750 | yes |
| gemtuzumab | 9 mg.m$^{-3}$ | yes (?) |
| isoniazid | 300 | yes |

**Drugs withdrawn for hepatotoxicity**

| drug | date | dose (mg/ day) | reactive products |
|---|---|---|---|
| cincopher | 1930 | 300 | no |
| iproniazid | 1959 | 25-150 | yes |
| pipamazinc | 1969 | 15 | no |
| fenclozic acid | 1970 | 300 | yes |
| oxyphenisatin | 1973 | 50 | no |
| nialamide | 1974 | 200 | yes |
| tienilic acid | 1980 | 250-500 | yes |

| Drug (Company) | Year | DILI finding | Impact | Time to onset |
|---|---|---|---|---|
| fasiolifam/ TAK-875 (Takeda) | 2013 | liver enzyme elevations | Ph-III termination | > 1 Month[1] |
| LY2888721 (Eli Lilly) | 2013 | liver enzyme elevations | Ph-II termination | > 1 Month[1] |
| sovaprevir; (Achillion) | 2013 | liver enzyme elevations | Ph-II, clinical hold | > 1 Month[1] |
| sitaxsentan, (Pfizer) | 2011 | liver enzyme elevations; hepatotoxicity | Market withdrawal | > 3 Months |
| lapatanib, (GSK) | 2009 | liver enzyme elevations; hepatotoxicity | Label -black box warning | <13 weeks |

**Drug-induced Liver injury (DILI)**

- Major cause of liver failure & transplantation
- Implications for patients & health care professionals
- 75% of idiosyncratic DILI results in transplantation or dead
- 29% of drugs withdrawn between 1998 - 2008

## Incidence of Adverse Drug Reactions (ADRs)

- **In USA 2001:**
  **- Over 2 MILLION serious ADRs yearly (= 6.7% of hospital admissions)**
- **100,000 DEATHS yearly:**
  - **ADRs 4th - 6th leading cause of death**
- **Causes of ADRs by class:**
  **669.559 drugs of abuse (incl. alcohol)**
  **220.289 psychotherapeutics 99.317 narcotics**
  **39.165 acetaminophen 22.663 OTC NSAIDs**
- **UK, Sweden (2008): 5-7% of all hospital admissions due to ADRs**
- **NL/VWS (2012): 5-7% of all hospital admissions due to ADRs**


1) Institute of Medicine, Nat. Acad. Press, 2000 Lazarou J et al.
2) *JAMA* 1998; 279 (15): 1200–1205;
3) Gurwitz JH et al. *Am J Med* 2000;109:87–94; 4) Einarson, Ann.Pharmacother. 27, 832, 1993

## Intermezzo 3: Ontwikkeling van geneesmiddelen

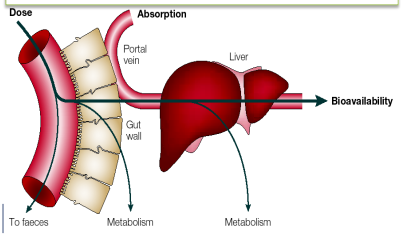
- *'Drug discovery & development' (DD&D)* van nieuwe geneesmiddelen is langdurig (10-12 jaar) en erg kostbaar (van 1 – 2,5 miljard $)
- Het DD&D proces is feitelijk nog steeds 'inefficient' omdat er tijdens het proces zeer veel geneesmiddel-kandidaat moleculen afvallen (v.b. niet het gewenste effect in de mens, toxiciteit in de mens, of om commerciele redenen)
- Bijwerkingen van geneesmiddelen (*'Adverse Drug Reactions*', ADRs) komen zeer vaak voor: 5-7 % van de ziekenhuis opnames is een gevolg van dergelijke bijwerkingen
- Het komt ook nog steeds voor dat ADRs pas na de introductie van een nieuw geneesmiddel op de markt wordt ontdekt. Deze *idiosyncratische* bijwerkingen *(iADRs)* zijn niet voorspelbaar op basis van het traditionele DD&D proces, meestal zeer ernstig en soms dodelijk
- Levertoxiciteit ('drug-induced liver injury', DILI) is een van de belangrijkste vormen van ADRs/iADRs, met overeenkomstige *'black-box warnings'* of *'drug-witdrawals'* als gevolg

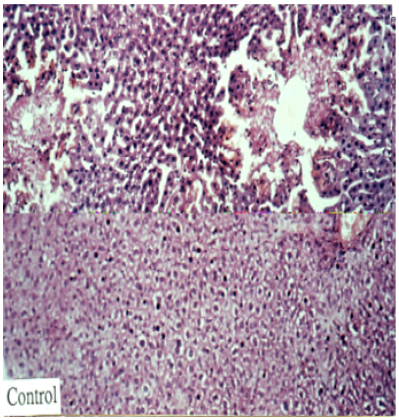
Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

## Paracetamol: Livertoxicity upon overdose

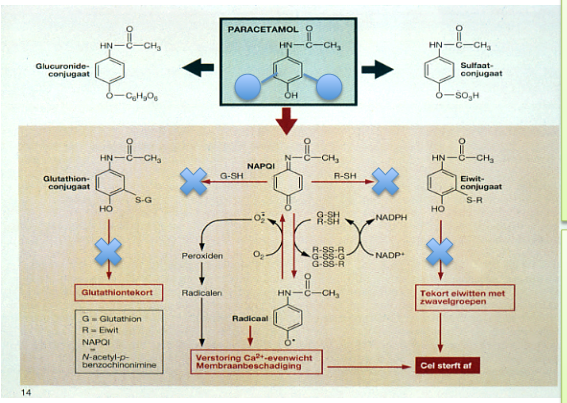
- **Introduced 1957; 1st intoxication 1966**
- **Recommended daily dose < 4 g**
- **Toxic dose > 4g and higher**
- **Centrilobular damage**
- **Most common form DILI in US & UK**
- **400-500 deaths/yr,**
- **70-90,000 hospital visits/yr**
- **2600 notifications at NVIC / Utrecht**
- **Excellent translational 'tool'**




VU UNIVERSITY AMSTERDAM

# Paracetamol: Mechanisms and mechanism-based protection




**MolTox end 1980's:**

- Parent compound, pharmacologically active
- Bioinactivation and bioactivation pathways are shown
- NAPQI, a chemically reactive metabolite is leading to liver toxicity, upon overdose

- Modulation of toxicity by molecular design
- 3,5-di-alkyl analogues
- Blocking covalent binding to GSH and proteins

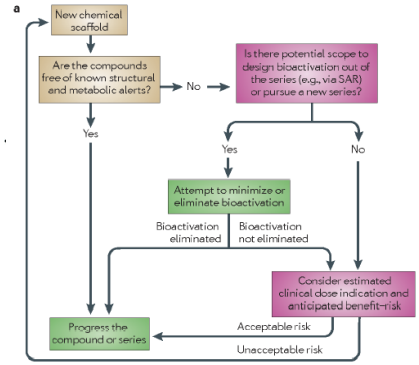
-> non-toxic, but still analgesically active!

Amsterdam Institute for Molecules Medicines and Systems

Van der Straat, Bessems, 1987 - 1992

VU UNIVERSITY AMSTERDAM

# Mechanisms-based mitigation of undesired properties and risks




**Stepan et al., CRT., 2011**

- 200 marketed drugs analysed
- Structural alerts identified
- Mitigation strategies for ADR risks by chemical or molecular design

**Park et al., Nat. Revs. Drug Disc., 2011**

- Managing the challenge of chemically reactive metabolites (CRMs) in drug development

-> Modulation of drug safety risks is widely now used in drug discovery & development

Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

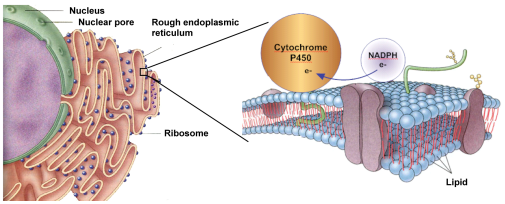
# Intermezzo 4: Moduleren van toxiciteit

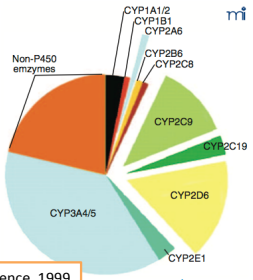
- Ons *oorspronkelijke doel van de Moleculaire Toxicologie* was onder andere: het ontwerpen van preventieve en therapeutische strategieën tegen toxiciteiten, en het ontwerpen van efficiëntere veiligere geneesmiddelen en (agro-) chemicaliën
- Op *inzicht in moleculaire mechanismen* gebaseerde modificatie van de Paracetamol structuur heeft eind jaren '80 met succes geleid tot niet-levertoxische analoga
- Ondertussen leiden dergelijke inzichten veelvuldig tot modulatie van ongewenste werkingen van geneesmiddel-kandidaat moleculen tijdens 'drug discovery & development'
- Dit geldt ook en met name voor het voorkomen van ongewenste bioactiveringsprocessen en voor het optimaliseren van ADME-PK (absorptie, distributie, metabolisme en eliminatie - farmacokinetiek) eigenschappen van geneesmiddel-kandidaat moleculen



# Cytochrome P450s: Short introduction

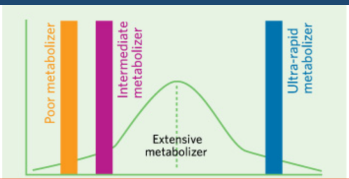
- **> 11.500 distinct proteins ; > 150 crystal structures; well conserved; in plants, bacteria, archaea, eukariota and mammalians (Nelson: http://drnelson.utmem.edu/CytochromeP450.html, 2009)**
- **In human 18 CYP families, 44 sub-families; ~10/60 responsible for metabolism of 75 % drugs on the market**
- **Nomenclature: e.g. CYP3A4: 3, family identifier, A: sub-family identifier, 4: individual number**




Evans et al., Science, 1999

# CYP2D6 genetic polymorphisms: consequenses for drug metabolism and drug therapy




**CYP2D6 and the European population**

20-30 million subjects have no CYP2D6 enzymes (PMs)

15-20 million subjects have *CYP2D6 gene* duplications (UMs)

*resulting in*

- Too slow drug metabolism
- Too high drug levels at ordinary dosage
- High risk for ADRs
- No response from certain prodrugs (e.g. codeine)

- Too rapid drug metabolism
- No drug response at ordinary dosage - Non-responders

Relevant for 15 % of all drugs used

**Decreased / increased clearance:**

- tricyclic anti-depressants, haloperidol
- anti-arrhytmic drugs, perphenazine
- perhexiline, SSRIs, S-mianserin, tolterodine

**Adverse effects:**

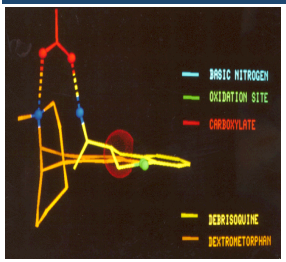
- cardiotoxicity by tricyclic anti-depressants
- parkinsonism by haloperidol?
- neuropathy by perhexiline
- arrhytmias by anti-arrhytmic drugs
- nausea by SSRIs

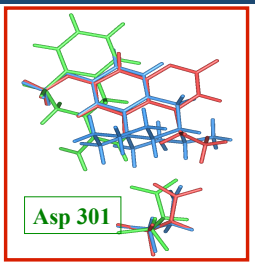
**Reduced prodrug activation:**

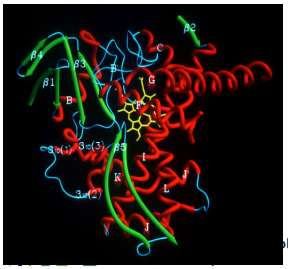
- tramadol, codeine, ethylmorphine

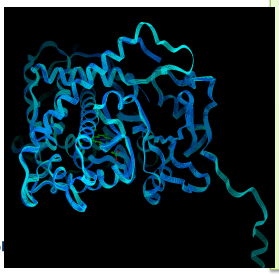
**-> drugs with CYP2D6 involvement constitute a potential risk for drug therapy** (M. Ingelman-Sundberg)
**-> WE: better exclude CYP2D6 drugs from discovery**

# Computational prediction of CYP 2D6 substrates and metabolites





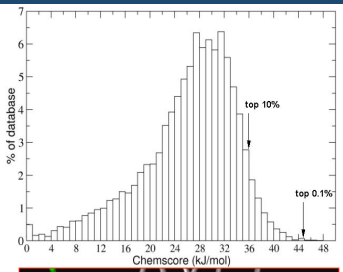
**History at MolTox**

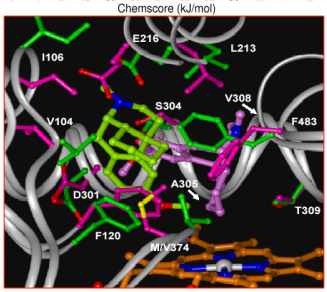
- 1992: pharmacophore model of Koymans
- 1996: Predicted protein model of CYP2D6 (de Groot)
- 2000: Improved CYP2D6 pharmacophore model (Venhorst)
- 2002: improved CYP2D6 protein model (Venhorst)

**Resulted in:**

- Relatively accurate prediction of sites of metabolism
- Elimination of CYP2D6 substrates during drug discovery
- New ideas for the active structure of CYP2D6 and mechanisms of catalysis
- New hypotheses for CYP2D6 mutation experiments

# Docking and virtual screening of substrates / inhibitors of CYP2D6




- **Library of ~ 200.000 compounds**
- **FlexX and Gold (scoring functions)**
- **Including active site water molecules**
- **Experimentally verified hits in top 0.1 % and top 10 %**

**(e.g. De Graaf et al., J. Med. Chem., 2005)**

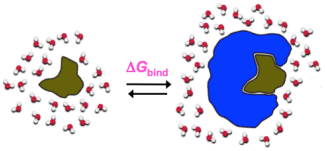
**2006: New CYP2D6 protein homology model (HM) and 1st crystal structure (CS)**

- **Good overlap between essential amino acids: CS: magenta; HM: green**
- **Dextromethorphan (Dex) orientation was different:**
  - **CS: 7-hydroxylation of Dex**
  - **HM: O-dealkylation of Dex**
- **Reason: CS crystallized substrate free!**

De Graaf, Oostenbrink, J Med Chem., 2006

UNIVERSITY AMSTERDAM

# LIE model for CYP2D6 substrate / ligand affinity prediction



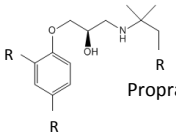

Åqvist *et al*.

LIE calculation using Molecular Dynamics (MD) and: $\Delta G_{bind} = \alpha\langle\Delta V_{vdw}\rangle + \beta\langle\Delta V_{ele}\rangle$

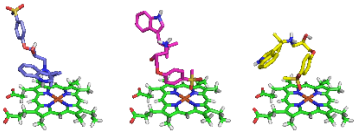
- Ligand in water and in protein and water
- Multiple protein & ligand conformations -> MD

*Recent years:* Good correlations between calculated and experimental values for affinities $\Delta G_{bind}$

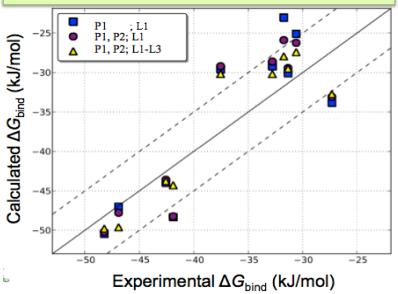
*Relevance:* prediction substrates, inhibitors and e.g. drug-drug interactions



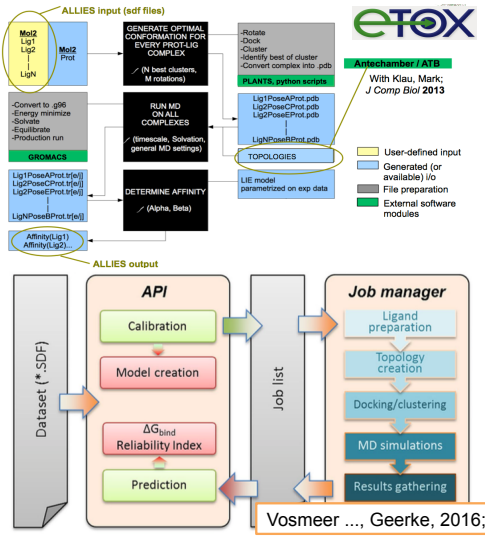

Propranolol-analogues



Vosmeer, Geerke *IJMS*, **2014**



# eTOX ALLIES: automated, unsupervised pipeline for LIE simulations



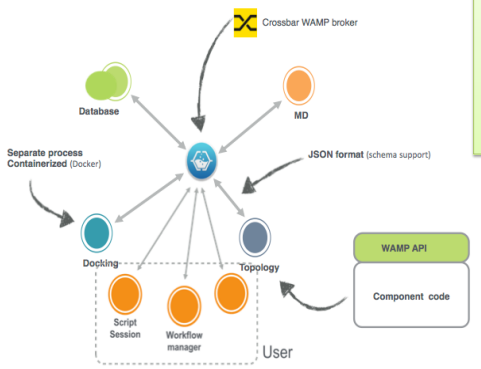

- In IMI-eTOX project *eTOX ALLIES* developed and implemented in eTOXsys (~ Molecular Networks/Erlangen)
- *User-friendly open source framework for automated prediction of* $\Delta G_{bind}$
- Based on the iterative LIE/MD approach
- Requires only ligand structures as input

- Initiation with a compound dataset (training or test)
- Model creation & calibration
- Job manager takes care of required steps to interaction energies
- Model evaluation & reliability indexation is included

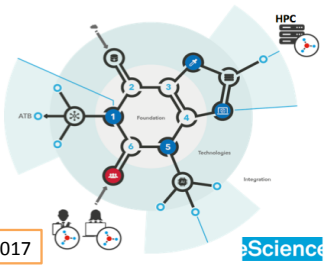
Vosmeer ..., Geerke, 2016; Capoferri ..., Geerke *J.Chem.Inf.*, 2017

# ***MDStudio:*** Microservice environment for MD workflows

A modular and scalable system that provides easy access to state-of-the-art Molecular Dynamics (MD) methods




1. Plug-and-Play extendible, e.g. ATB modules
2. Data provenance: track who, what, where and when
3. Easy access to advanced (MD) methods
4. Easy access to HPC infrastructures
5. Data analysis and machine learning methods
6. Multi-user, multi-platform support, browser-based interface




Amsterdam Institute for Molecules, Medicines and Systems

Van Dijk, Geerke 2017

eScience

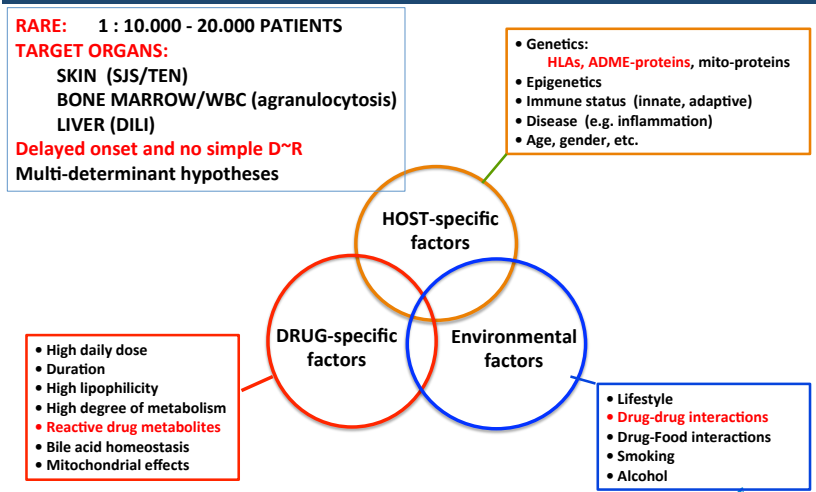
# Intermezzo 5: computer modeling

- Een van de *oorspronkelijke doelen van onze Moleculaire Toxicologie* was het ontwerpen van efficiëntere, veiligere geneesmiddelen en (agro-)chemicaliën.
- Cytochrome P450 is het belangrijkste metabole enzymsysteem voor de afbraak van geneesmiddelen. Voor CYP2D6 werd in de jaren '80 al een, genetisch bepaalde, variabiliteit ontdekt (~ 'poor' en 'extensive' metabolizers). Voor bestaande en nieuw te ontwikkelen geneesmiddelen was voorspelling van CYP2D6 betrokkenheid hoog nodig.
- Eind jaren '80 hebben wij hiervoor 'farmacofoor modellen' ontwikkeld (alleen kijkend naar de structuur en eigenschappen van moleculen) en in de jaren '90 en '00 hebben we, steeds verbeterde, CYP2D6 eiwitmodellen voorspeld en gebouwd.
- Met de eiwitmodellen kon de plaats (site) voor metabolisme in moleculen steeds beter worden voorspeld. Er werden ook methodes ontwikkeld om de affiniteit (bindingssterkte) van moleculen in de CYP2D6 active site te berekenen. De eiwitmodellen werden ook gebruikt voor 'virtueel' screenen van chemische bibliotheken op CYP2D6 binders.
- Tot slot gaven computersimulaties veel inzicht in werkingsmechanismen en de flexibiliteit van CYP2D6, en hebben ze geleid tot nieuwe hypotheses voor experimentele studies.
- Recentelijk zijn geheel nieuwe, geautomatiseerde applicaties (~ eTOX ALLIES) ontwikkeld die CYP2D6 betrokkenheid van nieuwe moleculen kunnen voorspellen. Mede door deze ontwikkelingen, worden er nu vrijwel geen 'CYP2D6-geneesmiddelen' meer ontwikkeld.



# *Idiosyncratic* Adverse Drug Reactions (iADRs): Susceptibility and variability

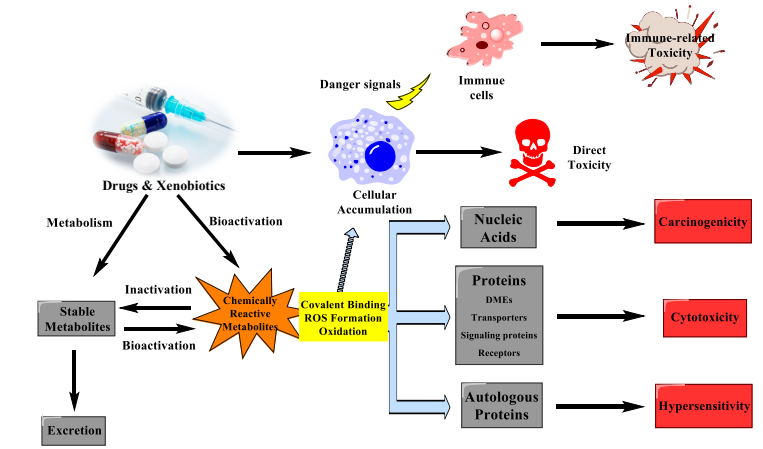
**RARE:** **1 : 10.000 - 20.000 PATIENTS**
**TARGET ORGANS:**
**SKIN (SJS/TEN)**
**BONE MARROW/WBC (agranulocytosis)**
**LIVER (DILI)**
**Delayed onset and no simple D~R**
**Multi-determinant hypotheses**





Commandeur, 2015, 2016

# Chemically Reactive Metabolites (CRMs) in Adverse Drug Reactions (ADRs)

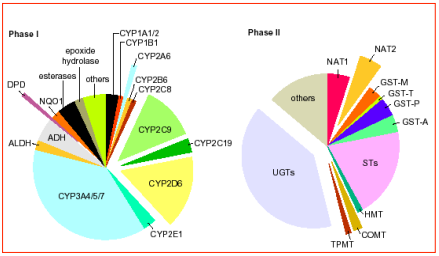



Amsterdam Institute for Molecules, Medicines and Systems

Yongjie Zhang, Thesis (2018)

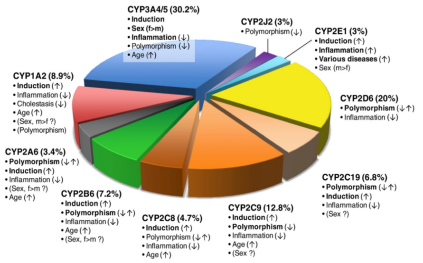
VU UNIVERSITY AMSTERDAM

# Drug metabolism enzymes in Human Liver




- Multiple human drug metabolizing enzymes (DMEs)
- Classification in Phase I and Phase II
- Some DMEs are located in ER-membranes (e.g. CYPs), others in cytosol (e.g. GSTs and STs)
- Size of cake-pieces: relative contributions to metabolism of marketed drugs

(Evans & Reading, Science, 1999)

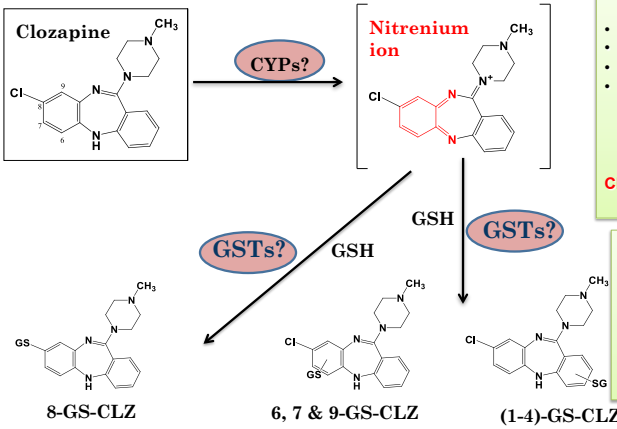



- Relative amounts of DMEs vary continuously, up and down
- Cause of variations: e.g. genetic polymorphisms, enzyme induction, diseases, smoking, inflammation, age, sex, etc

(Zanger and Schwab, Pharmacol. Ther., 2013)

VU UNIVERSITY AMSTERDAM

# CLOZAPINE: Agranulocytosis & hepatoxicity




**Antipsychotic drug**

- Only 2nd line use
- Severe adverse effects
- *agranulocytosis (1-2%)*
- *mild hepatotoxicity (37%) (0.06% progress to liver failure)*

**CRM: Nitrenium ion (via MPO and CYPs)**

**Research question:**

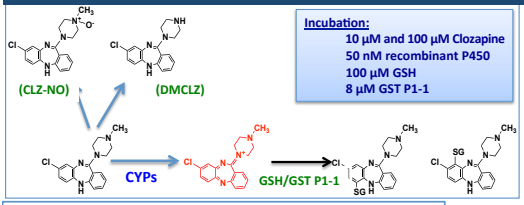
- Are genetically polymorphic enzymes involved?
- What is the variability in these enzymes?

Amsterdam Institute for Molecules, Medicines and Systems

Dragovic, thesis 2013 and others

VU UNIVERSITY AMSTERDAM

## METABOLISM OF CLOZAPINE BY HL-micros (n=100) and recombinant CYPs

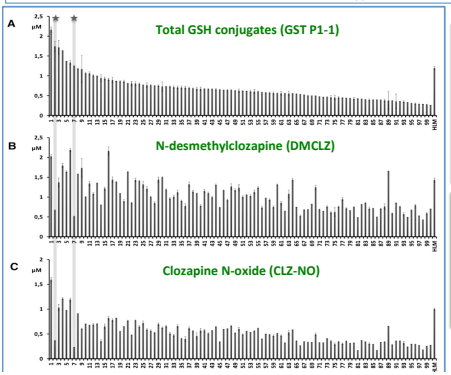



**Incubation:**
10 µM and 100 µM Clozapine
50 nM recombinant P450
100 µM GSH
8 µM GST P1-1

Dragovic e.a. DMD 41, 651 (2013)

Human liver microsomes (n=100); ~ Ingelman-Sundberg

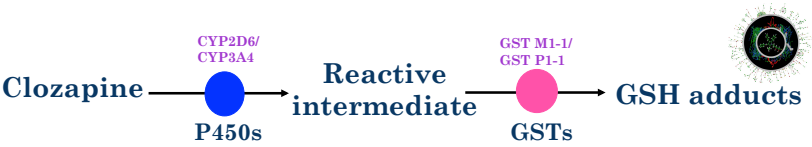
CYP dependent metabolic pathways to stable metabolites and reactive nitrenium ion, trapped as GSH-conjugates




- With recombinant/expressed hCYPS: Clint 2D6 > 3A4. However, 3A4 levels 20 x 2D6 in human liver!
- CYP-selectivity supported by strong inhibition 3A4 in pooled livers.

- Relatively high bioactivation measured (as GSH-conjugates *by GST P1-1*)
- Variability in bioactivation > 8,5 fold; 2 individuals (# 2 and 7) even more bioactivation

VU UNIVERSITY AMSTERDAM




**CYP3A4 and CYP2D6 play a major role in the bioactivation of Clozapine**

***Risk factors:***

- ✓ ***Multicopy genotype of CYP2D6 might be risk factor***
- ✓ ***Enzyme induction of CYP3A4***

**Two polymorphic GSTs play a significant role in the inactivation of reactive clozapine metabolite.**

- ✓ ***GST M1-1: deficient in 50% of population***
- ✓ ***GST P1-1: different alleles; phenotype to be determined***

**Combination of polymorphisms at level of bioactivation (P450s) and inactivation (GSTs) may explain variability in susceptibility of patients to ADRs of Clozapine**



# Intermezzo 6: Idiosyncratie en gevoeligheid

- *Idiosyncratische ADRs* zijn onvoorspelbaar op basis van pre-klinisch en klinisch onderzoek, komen weliswaar in-frequent voor, maar zijn doorgaans zeer ernstig, vaak zelfs dodelijk
- *Verschillen in gevoeligheid* voor ADRs/iDRS zijn een gevolg van het feit dat de mechanismen van deze bijwerkingen niet bekend, multi-factorieel en complex zijn: geneesmiddel-specifieke, patient-specifieke en omgevingsfactoren spelen een rol
- Hoewel er verschillende hypotheses voor onderliggende mechanismen bestaan, is de vorming van *chemisch reactieve metabolieten (CRMs)* meestal van belang
- De blootstelling van cellen en organen aan CRMs, en de variabiliteit daarin wordt bepaald door *balans van de snelheid van vorming* (bioactivering of toxificatie) *en de snelheid van de afbraak* of ontgifting (bio-inactivering of detoxificatie) door metabole enzymen, zoals de CYPs en de GSTs
- Inzicht in de balans van vorming en afbraak van CRMs, en de variatie daarin is daarom cruciaal. Dit wordt als voorbeeld geillustreerd met Clozapine

# Diclofenac: an old, but challenging model drug / model toxin

**Literature:**

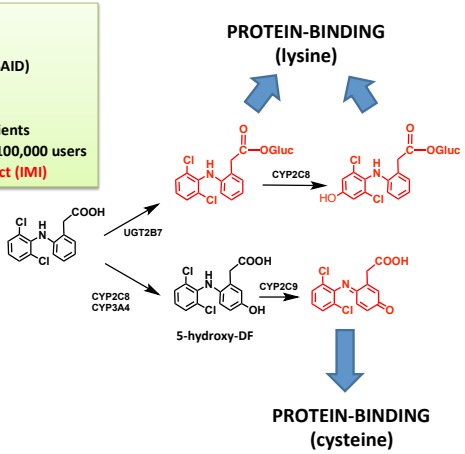
- Non-steroidal anti-inflammatory drug (NSAID)
- daily dose: 200 mg/kg
- Extensive metabolisme: > 50% first pass
- Elevated plasma transaminases: 15% patients
- *Severe idiosyncratic liver injury:* 4-6 per 100,000 users

(a Training compound in the MIP-DILI project (IMI)

**Association studies:**

| | |
|---|---|
| > 150 mg/day | OR 5.0 |
| UGT2B7*2 | OR 8.0 |
| ABCC2 C-24T | OR 5-6 |
| CYP2C8 | OR 3.7 |

Daly AK et al., *Gastroenterol.*, 2007

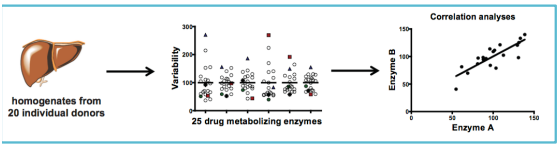


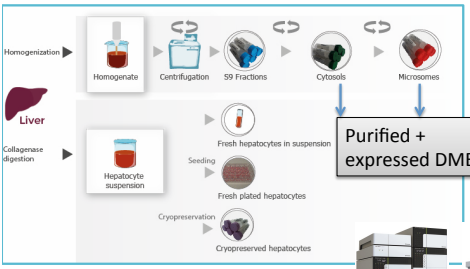

Amsterdam Institute for Molecules, Medicines and Systems

Mechanism based Integrated systems for the Prediction of Drug Induced Liver Injury
MIP-DILI

VU UNIVERSITY AMSTERDAM

# DRUG METABOLIZING ENZYMES (DMEs) IN HUMAN LIVER DONORS: Balance and variability




MIP-DILI project: M. Den Braver, S. Sewradj, S. Dekker

**GOALs:**

- Measure and model the balance in the liver between bioactivating and protective enzymes
- Measure the variability between individuals

**APPROACH:**

- Isolate liver fractions from 20 human liver donors
- Measure activities and expression levels of DMEs

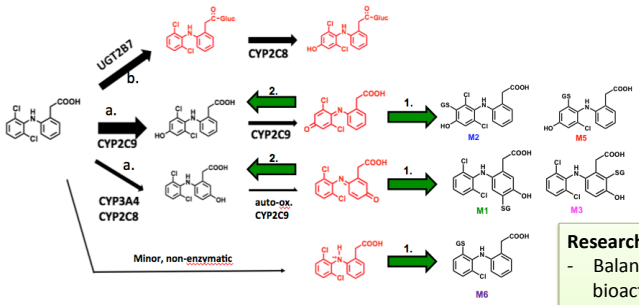
**25 DMEs**

- 9 CYPs (in microsomes)
- 6 UGTs (in microsomes)
- 6 GSTs (in cytosol)
- 2 STs
- 2 NQOs

# DICLOFENAC: Bioactivating and Protective enzymes

a. Cytochromes P450 ☑
b. Glucuronyl transferases ☑

1. Glutathione transferases ☑
2. NAD(P)H Quinonereductase 1 ☑

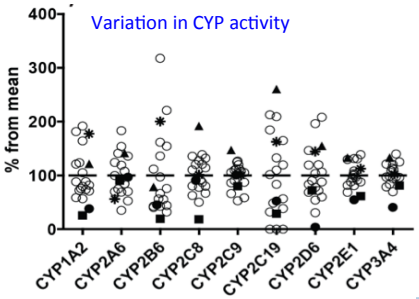



Our studies identified:
- individual CYPs and UGTs as responsible for bioactivation
- new chemically reactive metabolites (CRMs)
- GST- and NQO-enzymes as protecting enzymes

(Dragovic, Venkataraman, Vredenburg, Den Braver, Sewradj)

**Research questions:**
- Balance between bioactivation and protection?
- Variability in this balance in human livers?
- Can the variability be determined experimentally?

# CYP activities and contents in livers of 20 human donors




| Enzyme | Mean Activity ± SD (pmol/min/mg) | CV (%) | Range (pmol/min/mg) |
|---|---|---|---|
| CYP1A2 | 15.4 ± 7.4 | 47.9 | 3.9 – 29.5 |
| CYP2A6 | 32.6 ± 12.4 | 38.2 | 11.5 – 59.8 |
| CYP2B6 | 5.3 ± 4.1 | 77.4 | 1.0 – 16.9 |
| CYP2C19 | 10.7 ± 8.4 | 78.7 | 0.0 – 27.9 |
| CYP2C9 | 586 ± 137.1 | 23.4 | 310.9 – 863.4 |
| CYP2D6 | 74.0 ± 38.1 | 51.4 | 2.9 – 154.2 |
| CYP2E1 | 140 ± 34.1 | 24.4 | 76.5 – 194.1 |
| CYP3A4 | 409 ± 95.3 | 23.3 | 166.7 – 572.2 |

**Example:**

**CYPs**
- 9 CYPs
- Variation in activity: 4 donors high-ligted for comparision
- Mean activities + ranges
- CYP levels →

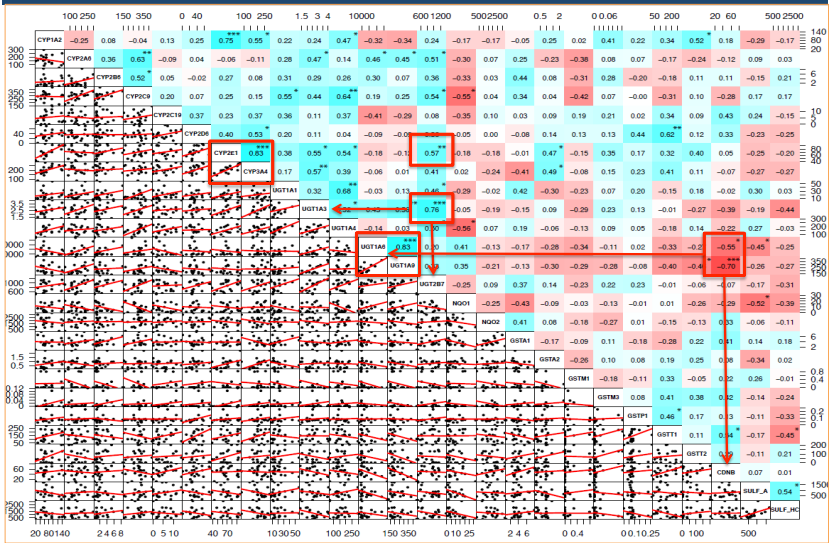
**Methods:**

*a) Activities:* Cocktail approach, (~ Spaggiari et al., 2014)

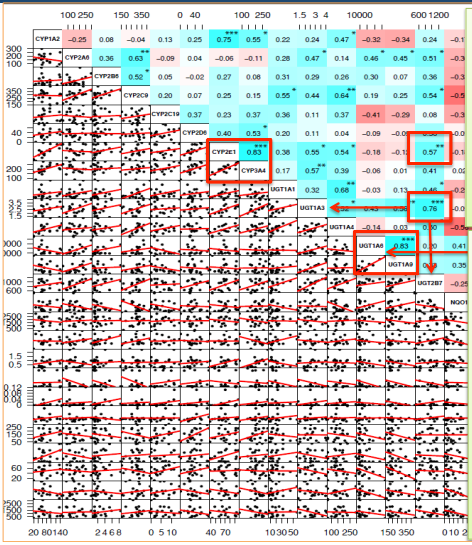
*b) Content:* CO Spectra in liver microsomes (Guengerich et al., 2009, Nature protocols)

| Donor ID | nmol P450/mg |
|---|---|
| S1399 ✸ | 0,21 |
| S1449 | 0,14 |
| S1352 | 0,17 |
| S1342 | 0,19 |
| S1327 | 0,21 |
| S1405 | 0,23 |
| S1336 ● | 0,19 |
| S1404 | 0,29 |
| S1332 | 0,40 |
| S1356 | 0,00 |
| S1446 | 0,52 |
| S1329 | 0,27 |
| S1339 | 0,49 |
| S1402 ■ | 0,06 |
| S1343 | 0,48 |
| S1442 | 0,18 |
| S1344 | 0,31 |
| S1441 ▲ | 0,61 |
| S1334 | 0,19 |
| R1341 | 0,34 |

# Correlation matrix of 25 DMEs in Livers of 20 donors



# Correlation matrix of 25 DMEs in Livers of 20 donors



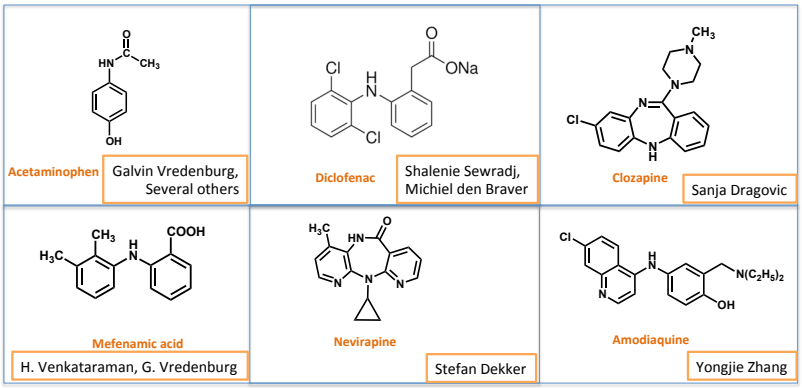
**Correlation matrix**

- 25 DMEs on diagonal
- *Left:* Scatter plot of DME pairs, fitted linearly
- *Right:* Pearson's product moments, with sifnificance *(p=0.05*, p=0.01** and p=0.001***)*
- *Correlations*: blue/positive, red/negative, intensity/strength
  - *positive: CYP2E1~CYP3A4; UGT1A6~UGT1A9*
  - *negative: UGT1A6/UGT1A9 ~ CDNB/GSTs*

**Summary:**

- Unique, comprehensive protocol to determine expression levels and activities of 25 DMEs in livers.
- Unique sets of data, per individual donor and per group of 20 donors.
- Allows the assessment of inter-donor variability and inter-correlations.
- Will allow to model the balance between bioactivation to and protection against CRMs, with PKPD modeling.

# Drugs showing ADRs and strong variability in liver CYPs, GSTs, UGTs, STs and NQOs *in vitro*



| Acetaminophen | Diclofenac | Clozapine |
|---|---|---|
| Galvin Vredenburg, Several others | Shalenie Sewradj, Michiel den Braver | Sanja Dragovic |

| Mefenamic acid | Nevirapine | Amodiaquine |
|---|---|---|
| H. Venkataraman, G. Vredenburg | Stefan Dekker | Yongjie Zhang |

Amsterdam Institute for Molecules, Medicines and Systems

Jan Commandeur & Chris Vos; MIP-DILI (IMI)

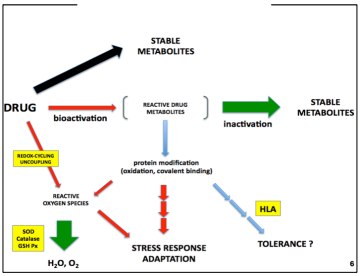
VU UNIVERSITY AMSTERDAM

# Intermezzo 7: Diclofenac & variabiliteit

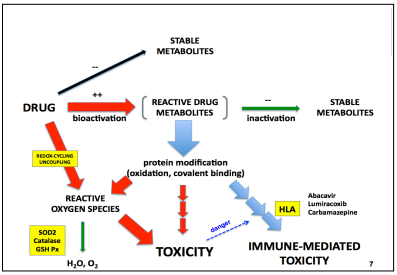
- *Diclofenac* is een veel gebruikt NSAID, dat in 15% van de gebruikers verhoogde transaminase veroorzaakt en in 4-6/100.000 gebruikers ernstige leverschade (*iDILI)*
- Diclofenac wordt snel gemetaboliseerd en daarbij ontstaan o.a. chemisch reactieve metabolieten (CRMs), die mogelijk een rol spelen in de iDILI reacties
- Om de balans tussen tussen de bioactivering tot CRMs en de bescherming daartegen, en vooral ook de variabiliteit daarin te bepalen hebben we in levers van 20 humane donoren 25, daarbij betrokken, biotransformatie enzymen (9 CYPs, 6 UGTs, 6 GSTs, 2 STs en 2 NQOs) gemeten
- Het onderzoek, dat onderdeel was van MIP-DILI, een groot Europees consortium, heeft een uniek protocol opgeleverd om expressie niveaus en activiteiten van 25 enzymen te meten, en een unieke set van data, per individue en per groep
- Inter-donor variaties werden vastgesteld en de data maken het mogelijk om de balans tussen de bioactivering tot CRMs en de bescherming daartegen te moduleren, met PKPD modeling. Het laatste moet nog gebeuren
- Ook moet de relatie met toxiciteit en variatie daarin nog worden vastgesteld

# SUSCEPTIBILITY and EXPOSURE to CRMs (or ROS)
# Balance between bioactivation and protection

*Non - susceptible individual*




*Susceptible individual: same dose of drug, higher exposure to CRM ?*




"The reactive dose makes the poison" ?

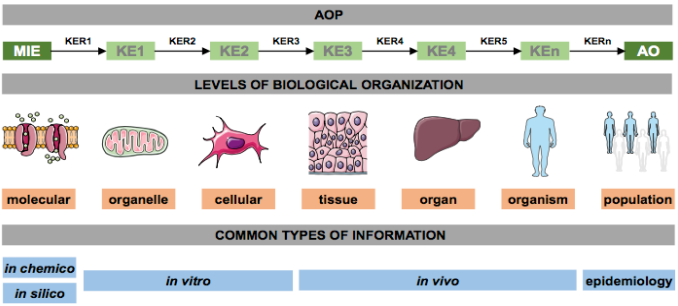
Also for:
Non-sensitive vs sensitive animal species
Non-responsive vs responsive in vitro model

Amsterdam Institute for Molecules, Medicines and Systems

Commandeur, 2015, 2016

VU UNIVERSITY AMSTERDAM

# Adverse Outcome Pathways (AOPs)



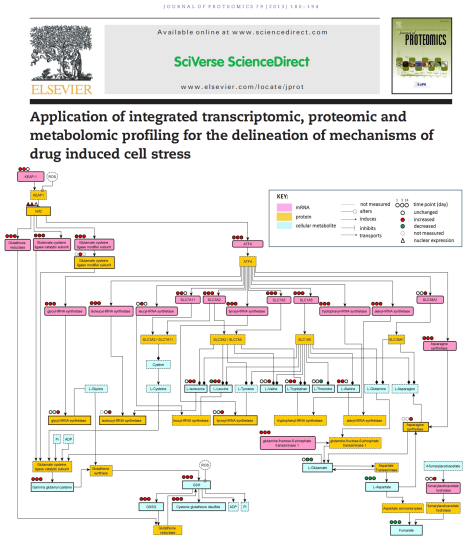

- AOPs provide a mechanistic representation of toxicological effects propagating over different layers of biological organisation, from chemical to target to adverse outcome (AO) at individual or population level
- Important components: MIEs (molecular initiating events) and AOs (adverse outcomes)
- KEs (key events) and KERs (key event relationships) link steps between the MIEs and the AOs
- AOPs constitute a relatively new mechanism-based toxicological strategy and can play an interesting role in translational toxicology and risk assessment

Amsterdam Institute for Molecules, Medicines and Systems

Vinken et al., Arch. Toxicol., 2017

VU UNIVERSITY AMSTERDAM

# Integrative Omics Strategies in Toxicology




**An example from the Jennings Lab:**

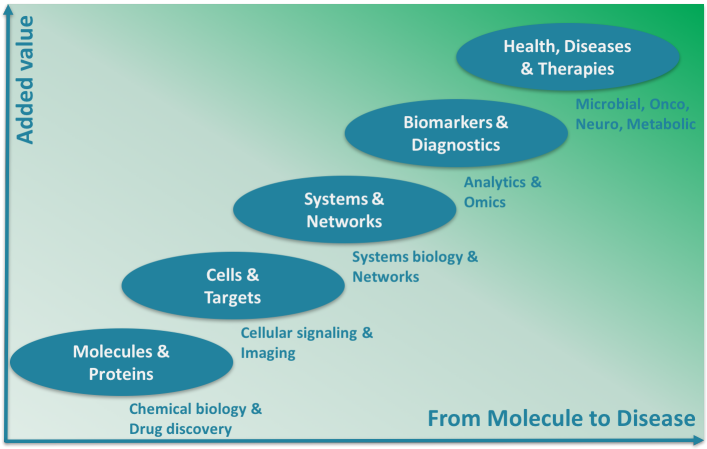
- Cyclosporine (CsA) widely used immunosuppressant)
- Metabolised via CYP3A4/5 and extruded by MDR1 transporter
- CSA is cytotoxic

Outcomes:

- Mechanistically: mitochondrial, oxidative and ER-stress high CsA concentrations
- In vitro cell cultures, combined with PK-data and HC-Omics approaches provide valuable insights in pharmacological and toxicological drug effects.

Anja Wilmes, J. Proteomics, 2013)

# AIMMS: Translational Value Chain




Amsterdam Institute for Molecules, Medicines and Systems

VU UNIVERSITY AMSTERDAM

Amsterdam Institute for Molecules, Medicines and Systems

VU university amsterdam

# Division of Molecular & Computational Toxicology

- dr. Jan N.M. Commandeur
- dr. Daan G. Geerke
- dr. J. Chris Vos
- prof. Nico P.E. Vermeulen

- 10 - 12 PhD-students
- 3-5 post-docs / PAs
- 1 technician
- 1-2 visiting scientists

**July 1st, 2017: prof. Paul Jennings**
**- 5 PhD-students / postdocs**

eScience center

TI PHARMA ACTS iBOS MIP-DILI SafeNanoSciMET

ASAT Organon MERCK Be well Bayer HealthCare Roche imi eTOX

SOLVAY BASF The Chemical Company VERTEX BD Synthon

China Scholarship Council www.csc.edu.cn

MARIE CURIE ACTIONS NWO Netherlands Organisation for Scientific Research