BUNDESINSTITUT FÜR RISIKOBEWERTUNG

Risiken erkennen – Gesundheit schützen

The potential impact of toxicogenomics on modern chemical risk assessment – 3-MCPD and 3-MCPD fatty acid esters as examples

Prof. Dr. Dr. Alfonso Lampen

Department of Food Safety
German Federal Institute for Risk Assessment (BfR)
Berlin, Germany

Starting point: list of > 800 heat-induced food contaminants

→ no toxicity and exposure data available for most of them

→ (Q)SAR prediction for prioritization

→ Toxicokinetic studies

→ **Toxicogenomic studies** → MoA → AOP

→ Risk characterization of heat-induced food contaminants

Risk characterization of heat-induced food contaminants

Starting point: list of > 800 heat-induced food contaminants

→ experimental studies to gain data for <u>all</u> compounds not feasible (time, money)

→ <u>prioritization</u> for risk characterization required

→ use of computational tools (QSAR, read across) to <u>predict toxicity</u>

→ relevant endpoints: mutagenicity and carcinogenicity

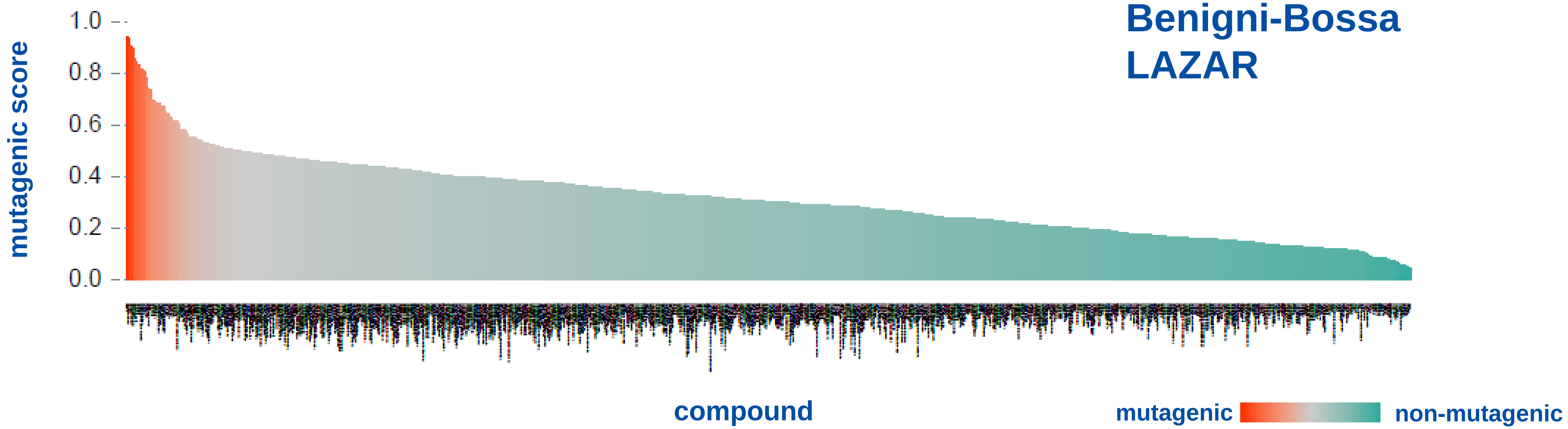
(Q)SAR predictions of mutagenicity of heat-induced compounds

→ **strategy to combine different *in silico* tools to increase <u>predictive power</u>**

→ freely available non-commercial (Q)SAR tools

→ <u>Higher confidence compared to any of the single models</u>

T.E.S.T.
SARy
CAESAR
Benigni-Bossa
LAZAR



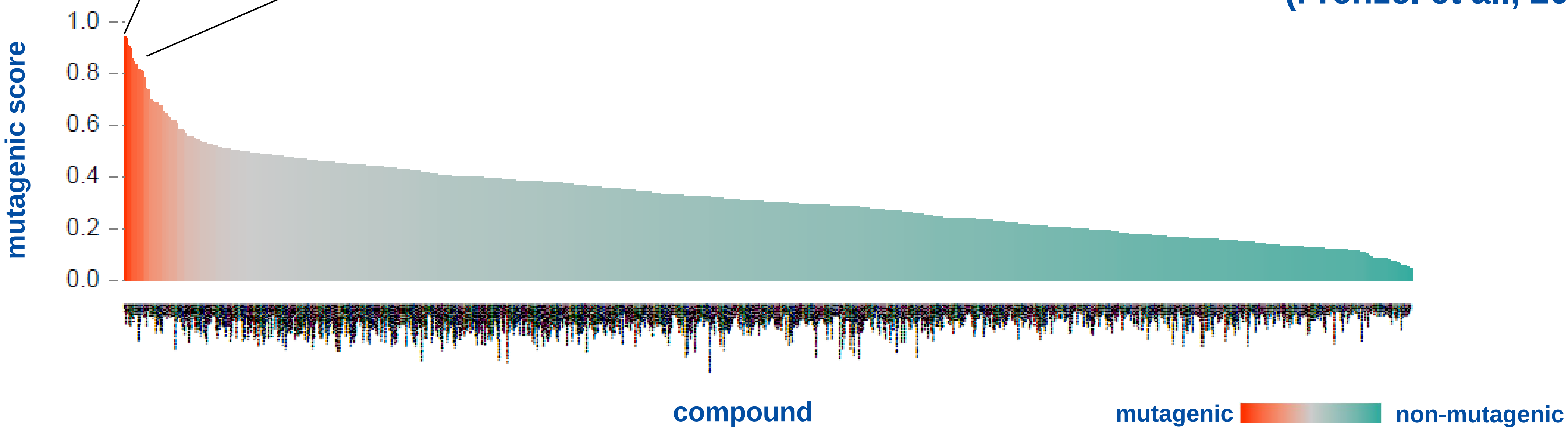
Mutagenicity of chloropropanoles

Rank	Compound	Mutagenic Score
1	1,3-dichloro-2-propanol (1,3-DCP)	0.94
2	glycidol	0.94
3	2,3-dichloro-1-propanol (1,2-DCP)	0.90
4	2-propenal	0.90
5	3-chloro-1,2-propandiol (3-MCPD)	0.90
6	[*E*,*E*]-2,4-hexadienal	0.84
7	[*E*]-2-butenal	0.84
8	[*Z*]-2-butenal	0.84
9	2-chloro-1,3-propandiol (2-MCPD)	0.74
10	2,4-pentadienal	0.74
11	5-hydroxymethyl-2-furfural (5-HMF)	0.82
12	2-furfural	0.81

→ **positive in Ames test**

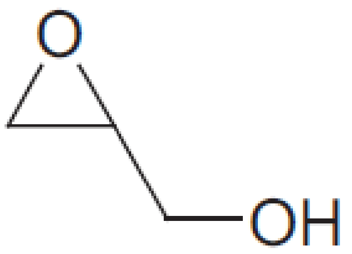
(bacterial reverse mutation assay)

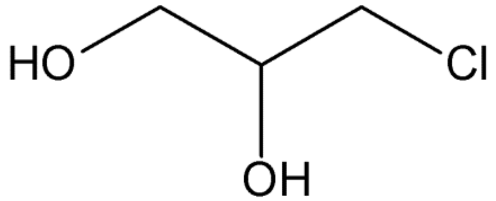
(Frenzel et al., 2017)

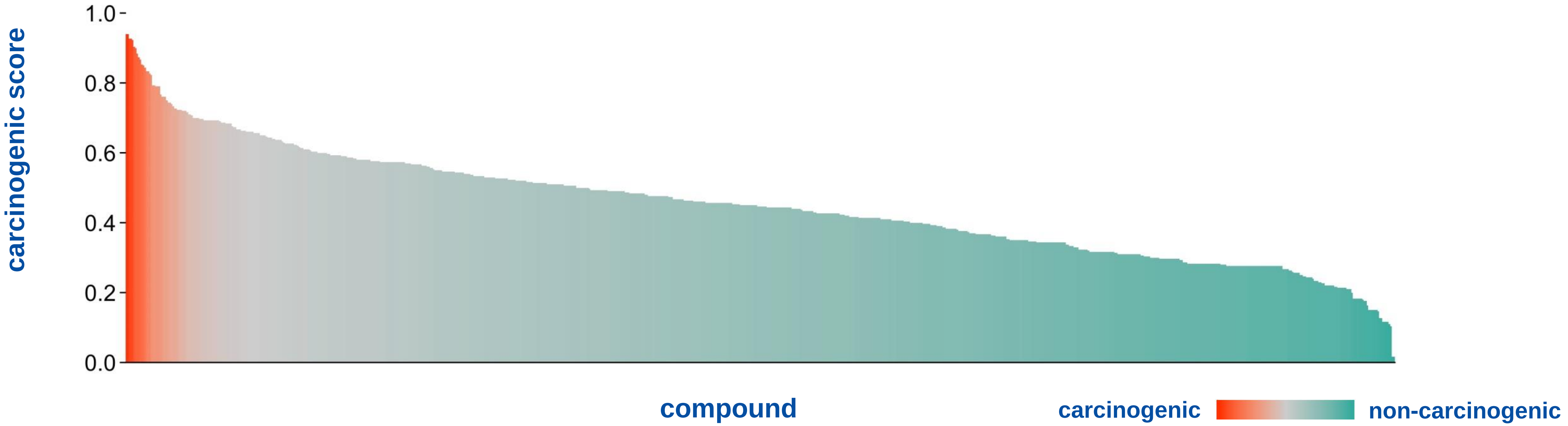


Carcinogenicity of chloropropanoles

Rank	Compound	Carcinogenic Score
1	Benzene	0.94
…	…	…
6	Glycidol	0.90
…	…	…
48	1,3-dichloro-2-propanol (1,3-DCP)	0.69
49	2,3-dichloro-1-propanol (1,2-DCP)	0.69
50	2-chloro-1,3-propandiol (2-MCPD)	0.69
…	…	…
554	3-chloro-1,2-propandiol (3-MCPD)	0.37

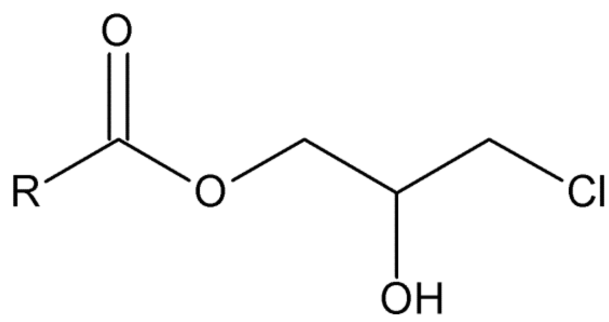




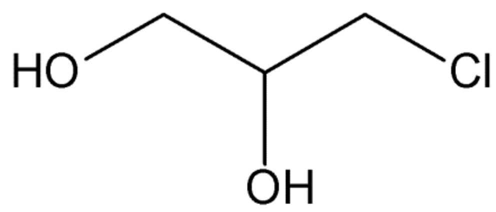


Formation and occurrence of MCPD/MCPD-FE

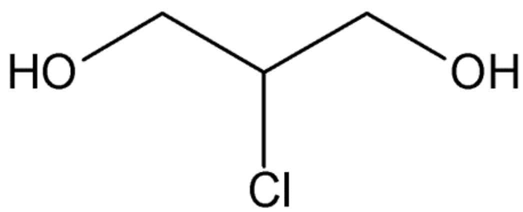
- **Monochloropranediols (MCPD) are process contaminants in various food**



3-MCPD fatty acid esters



3-MCPD



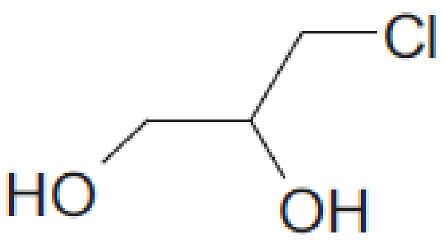
2-MCPD

- **MCPD-FE are mainly generated during the refinement of oils and fats**

Background: hazard potential of 3-MCPD

- Target organs for toxicity in animal studies:

  kidney and testis



- Tumor formation in rats/mice (hyperplasia in renal tubules)
- Non-genotoxic mechanism of tumor induction

Toxicological classification:

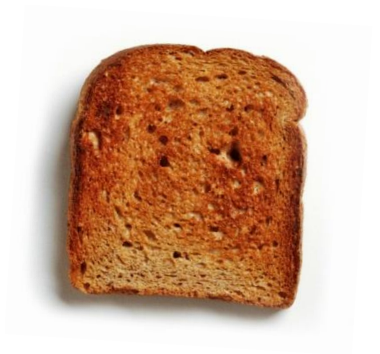
IARC (2012): „possibly carcinogenic to humans“(Group 2B)

Risk Assessment: definition of the TDI value

BfR (2007): 2 µg/kg body weight & day

SCF (2001): 2 µg/kg body weight & day

EFSA (2017): 2 µg/kg body weight & day

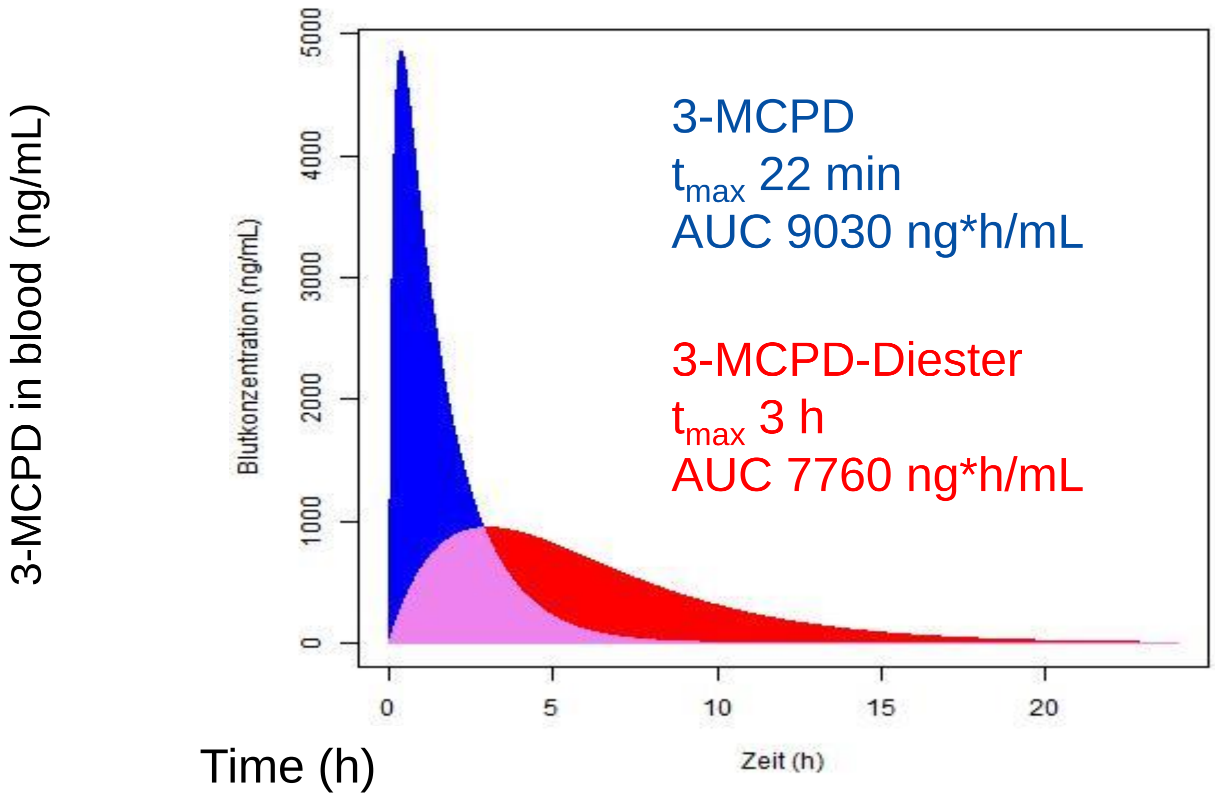


Toxicokinetics: hydrolysis of esterified 3-MCPD following oral ingestion

In vitro* studies (BfR): (Buhrke et al., 2011)*

***In vivo* studies** (BfR)

- Oral administration of 3-MCPD ester to rats
- Quantification of 3-MCPD biomarker in urine, feces, blood & various organs



Abraham et al. 2013

3-MCPD fatty acid esters (diester): almost complete (86%) hydrolysis

Molecular mechanism(s) of MCPD toxicity using Toxicogenomics

28-days feeding study with rats with 3-MCPD and 2-MCPD

=> kidney, testes, liver, heart

Proteomics

(2D-gels, protein spot identification with MS)

Transcriptomics

(gene array analysis; Affymetrix)

Bioinformatics

Systems Toxicology approach

Results:

=> Same effects for 3-MCPD and 3-MCPD dipalmitate

=> effects 3-MCPD > 2-MCPD

=> effects kidney > testes > heart > liver

=> inhibition of glycolysis

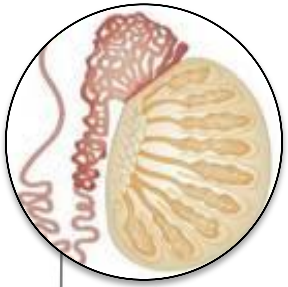
=> oxidative stress

- induction of ROS

- impact on glutathione metabolism

Frenzel et al. (2018) Food Chem Toxicol 116:354-359
Buhrke et al. (2018) Arch Toxicol 92:289-299
Schultrich et al. (2017) Arch Toxicol 91:3145-3155
Oberemm et al. (2017) Arch Toxiciol 91:3247-3260
Buhrke et al. (2017) Food Chem Toxicol 106:36-46
Sawada et al. (2016) Arch Toxicol 90:1437-1448
Sawada et al. (2015) Food Chem Toxicol 83:84-92
Braeuning et al. (2015) Food Chem Toxicol 86:374-484

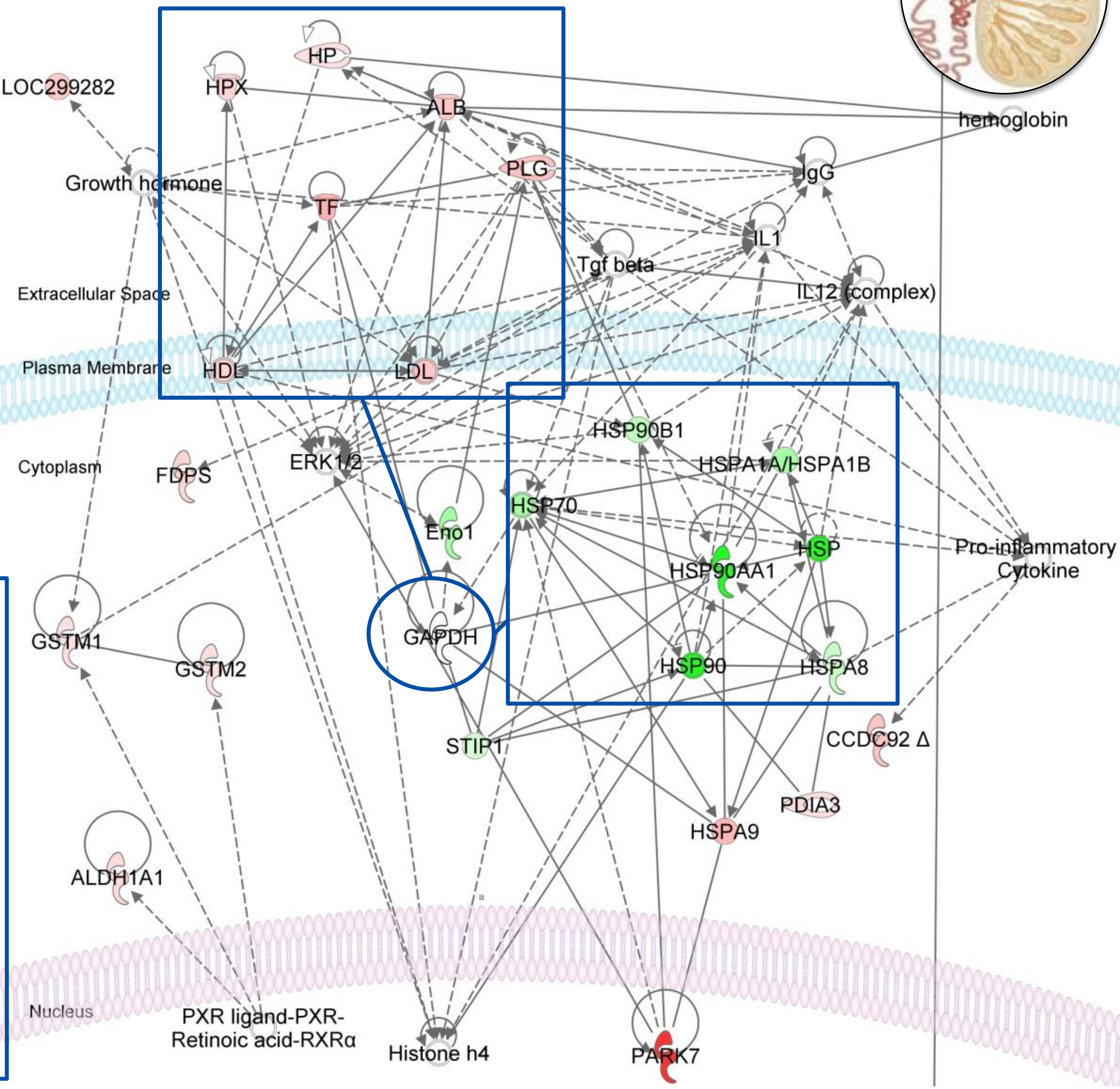
Results - testis



testis

3-MCPD group

overall network responses indicate reproductive systems disease and changes of lipid transfer



glycolytic protein GAPDH:

= cross-linking and multi-functional protein

= potential regulator of molecular mechanisms induced in testis

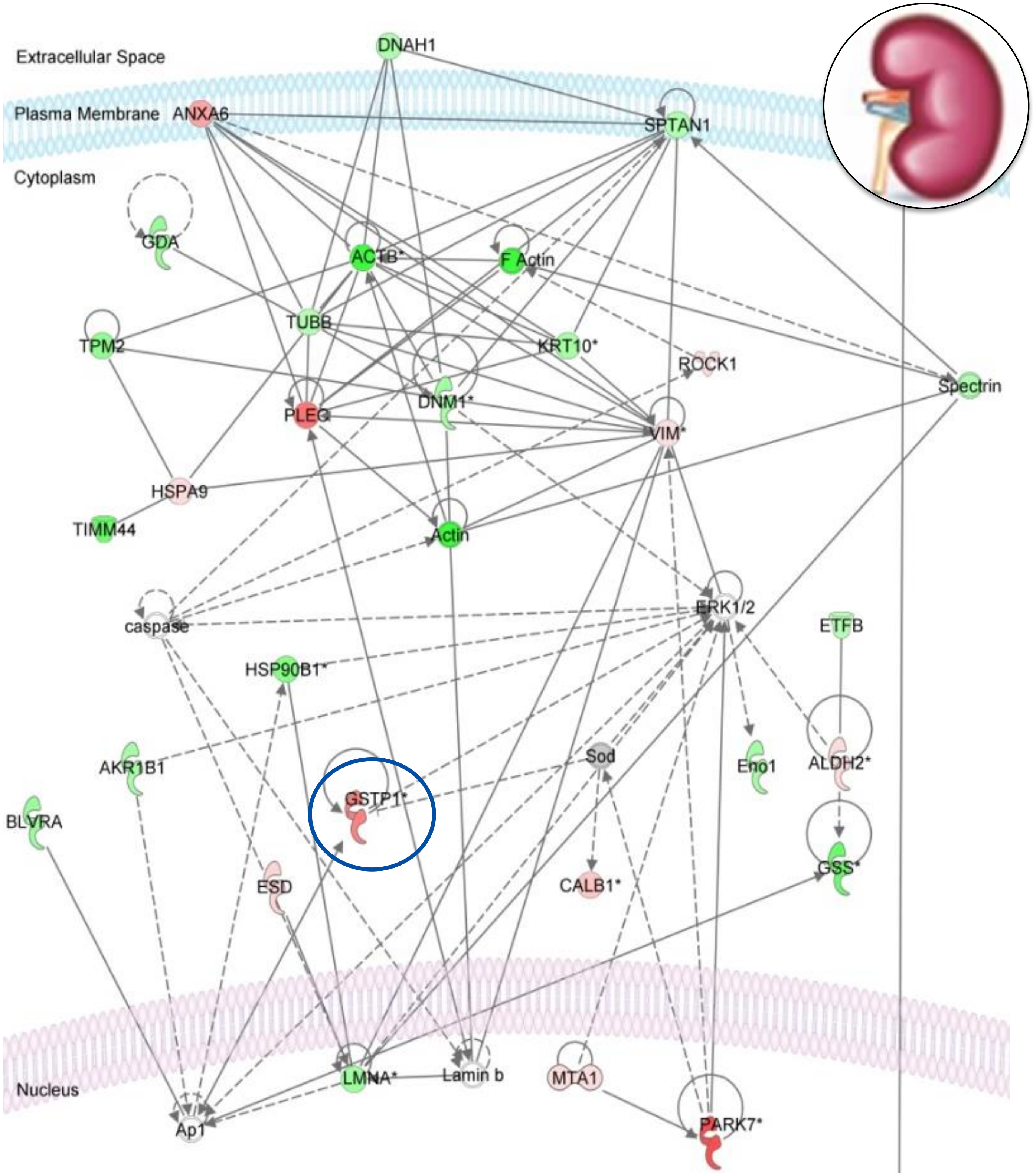
Results - kidney

IPA - bioinformatic analysis

kidney

3-MCPD group

general molecular changes indicate damage of kidney



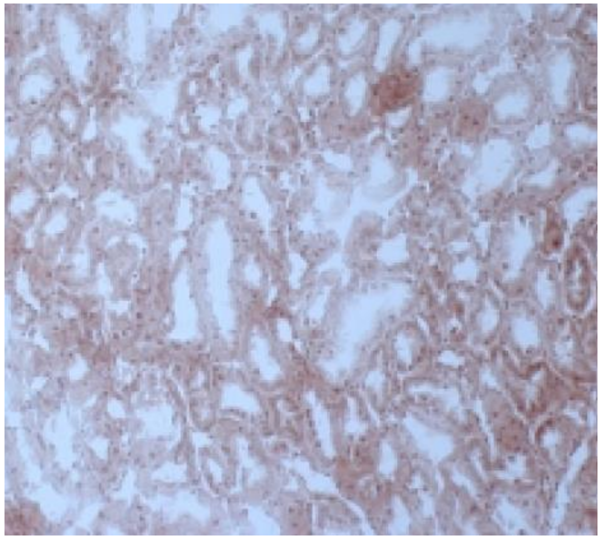
→upregulation of *glutathione-S-transferase P1 (GSTP1)*

= well-known marker for neoplasia/preneoplasia

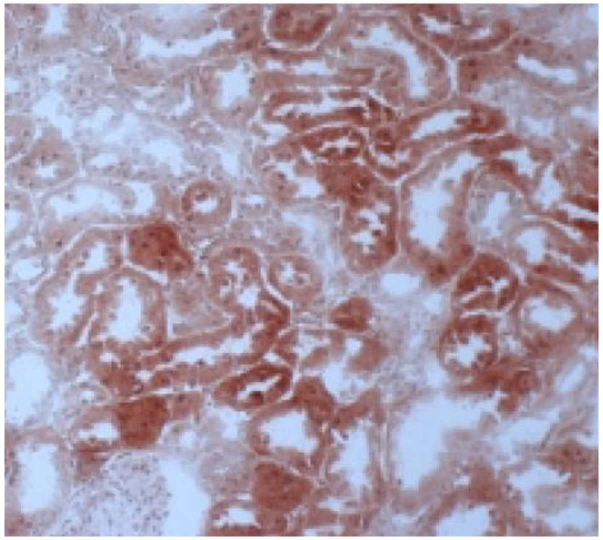
potential for binding of ERK

Cancer: 3-MCPD and 3-MCPD dipalmitate stimulate expression of the tumor marker GSTP1 in rat kidney

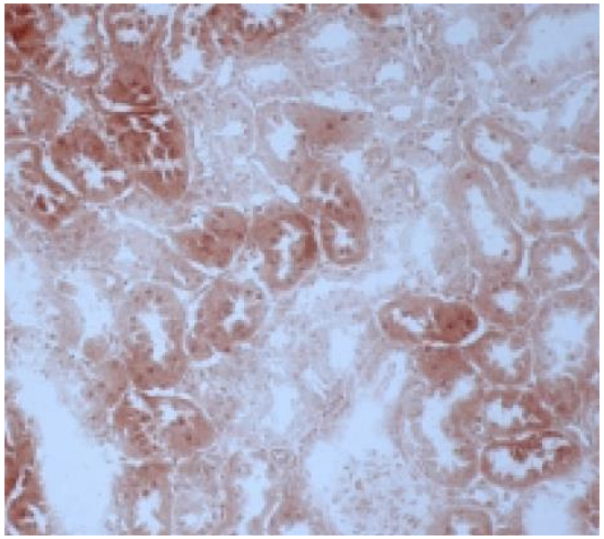
control



3-MCPD



3-MCPD dipalmitate



GSTP1 staining of rat kidney tissue

Results - kidney

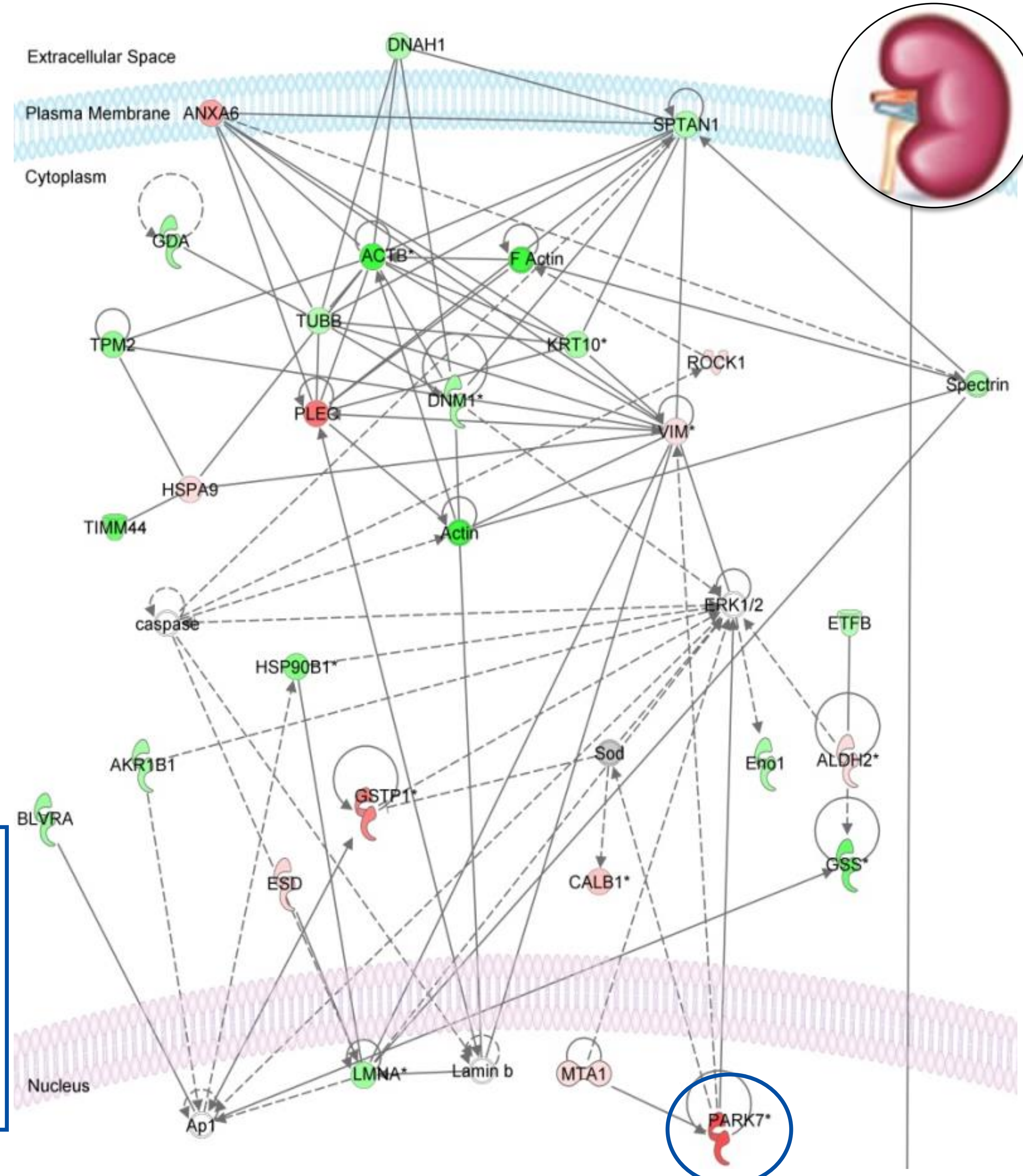
IPA - bioinformatic analysis

kidney

3-MCPD group

general molecular changes indicate damage of kidney

↑ PARK7 (DJ-1): multiple functions, oncogene oxidative stress response?



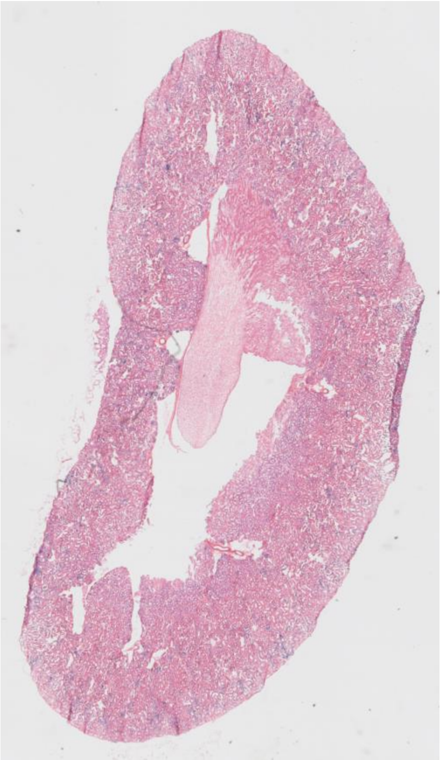
Induction of oxidative stress

→ animal study with HOTT-reporter mice: 28-days oral application of 3-MCPD

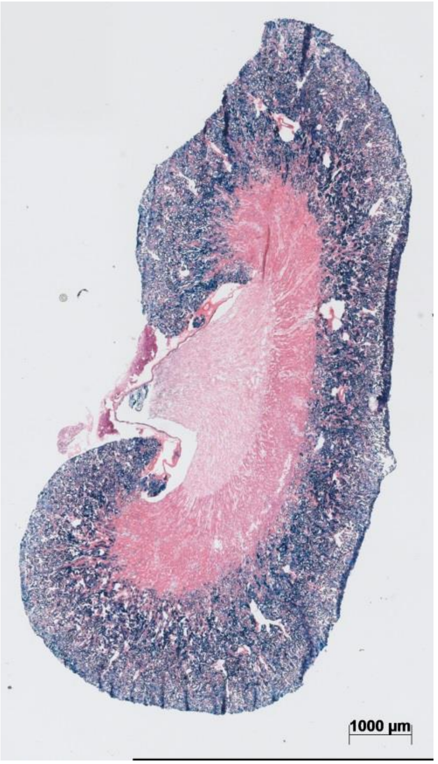
lacZ under the control of the ROS-sensitive HO-1 promoter, stably integrated into the genome

→ blue stain indicates oxidative stress (Henderson et al. (2015) Toxicol Sci 145:138-148)

Kidney

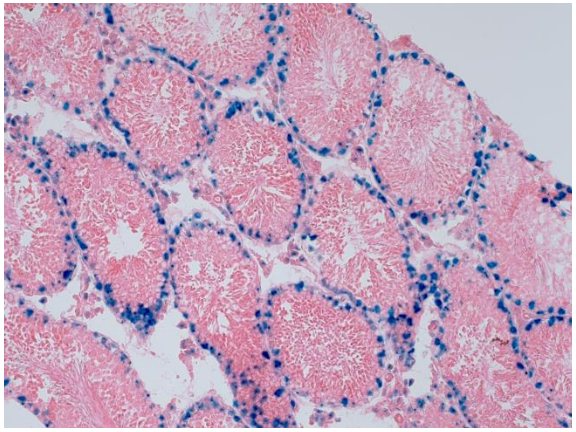


Control

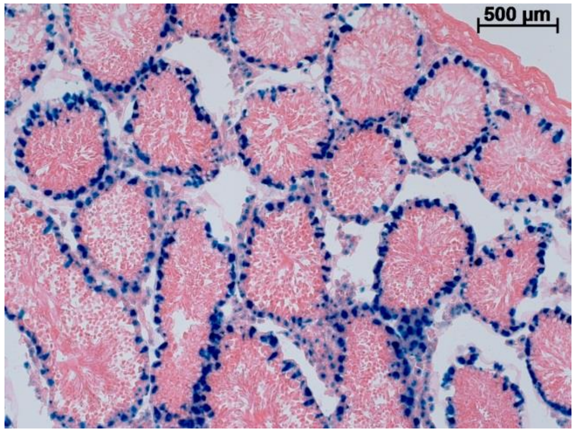


3-MCPD
100 mg/kg b.w. & day

Testes

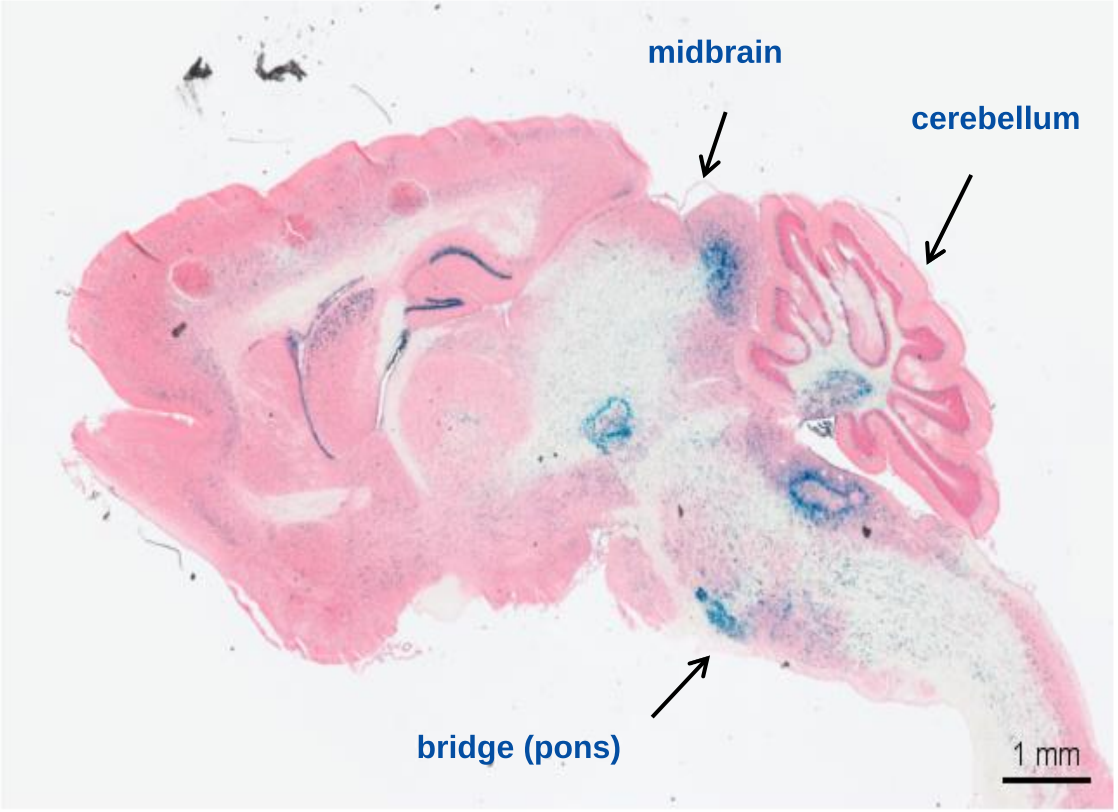


Control

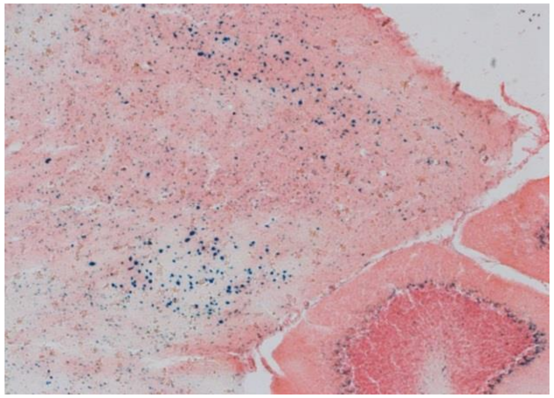


3-MCPD
100 mg/kg b.w. & day

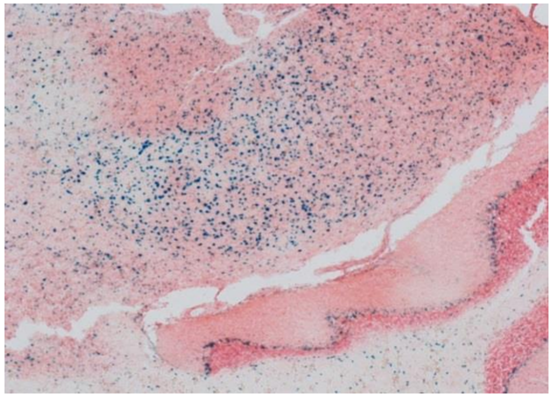
Oxidative stress response in HOTT mouse brain after 3-MCPD treatment



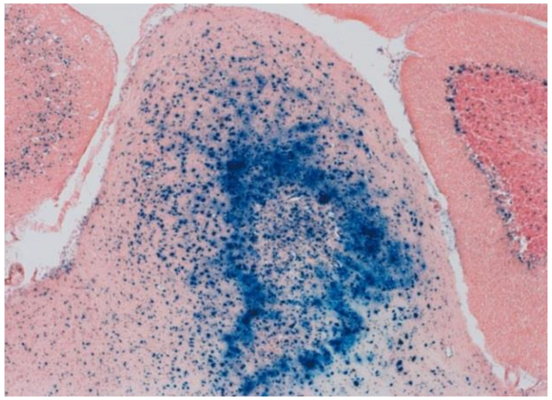
midbrain



control

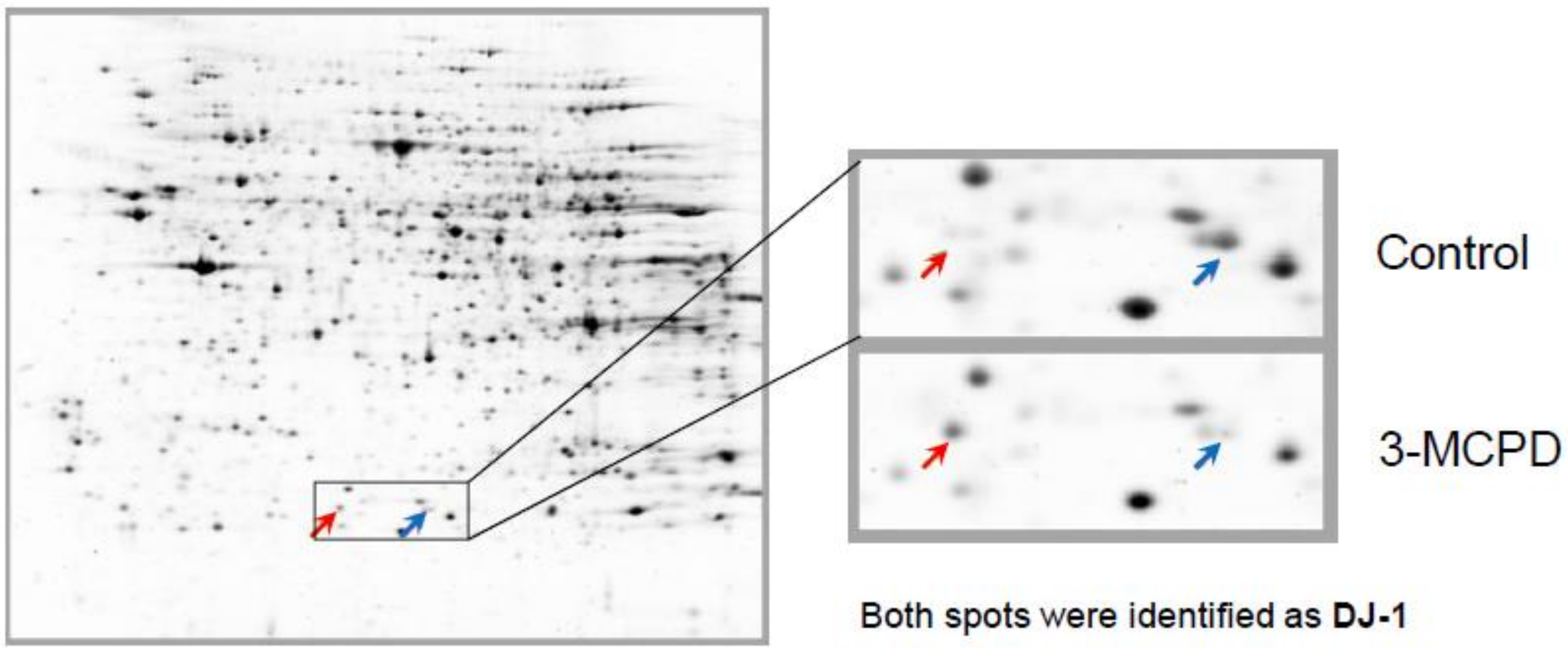


10 mg/kg b.w. & day 3-MCPD



100 mg/kg b.w.& day 3-MCPD

Oxidative irreversible inactivation of DJ-1 by 3-MCPD



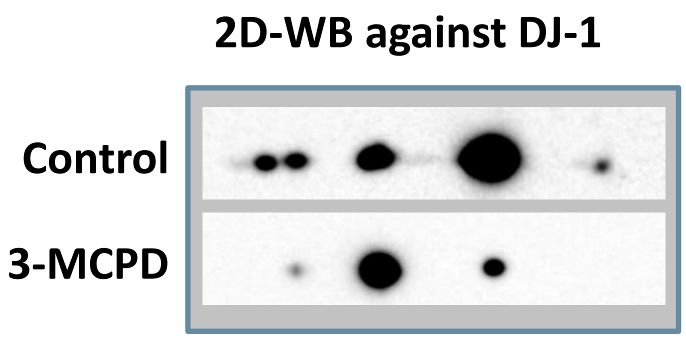
Both spots were identified as **DJ-1**

DJ-1/PARK7 is a multifunctional protein with a redox-active cysteine residue (Cys_{106})

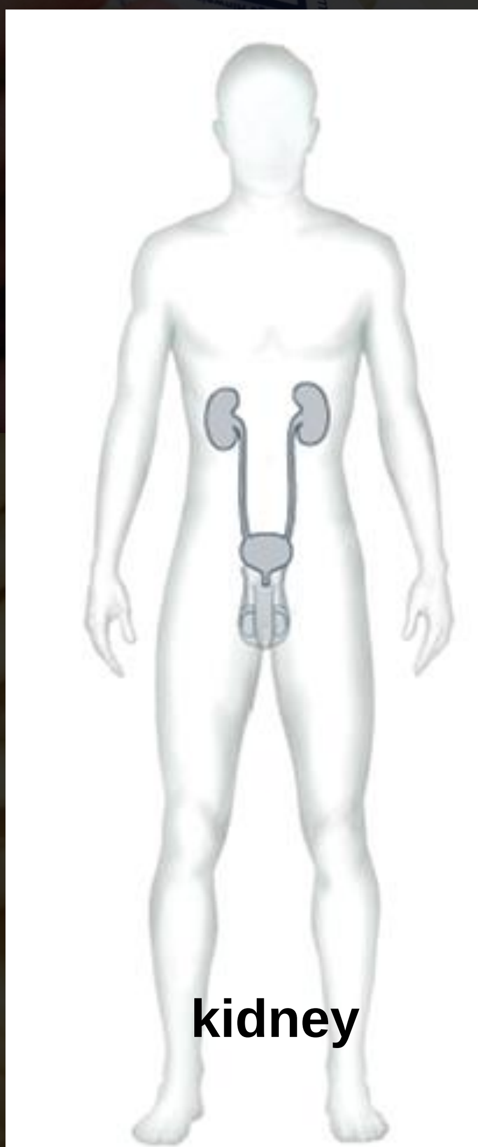
→ **Cys_{106}-SH is redox-active**

→ **Cys_{106}-SO_3^- is redox-inactive, loss of function, correlation with Parkinson‘s disease**

samples from rat kidney,
comparable results for testis, liver and heart,
and for 2-MCPD



Summary of „omics“ investigations (1)



kidney and testis

= target organs of 3-MCPD

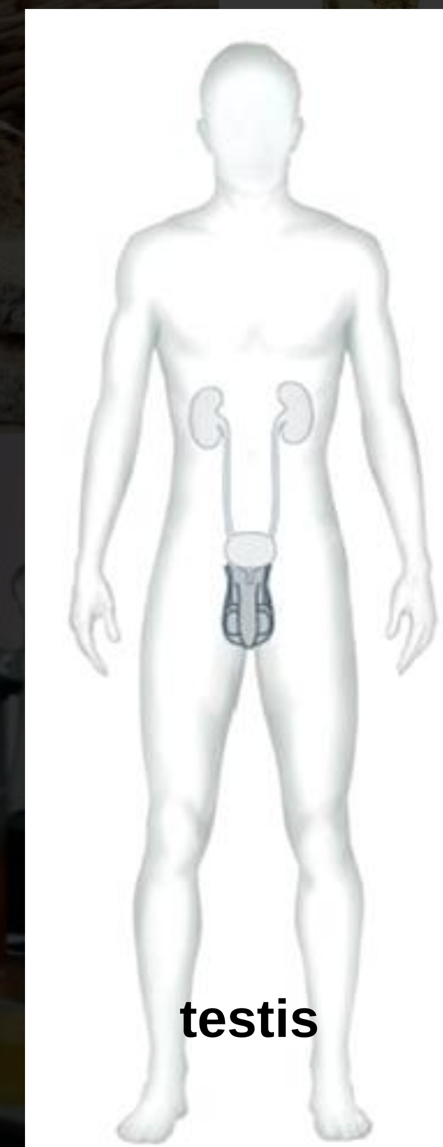
and 3-MCPD dipalmitate

→ 3-MCPD and 3-MCPD-dipalmitate induce similar effects, less pronounced with 3-MCPD dipalmitate

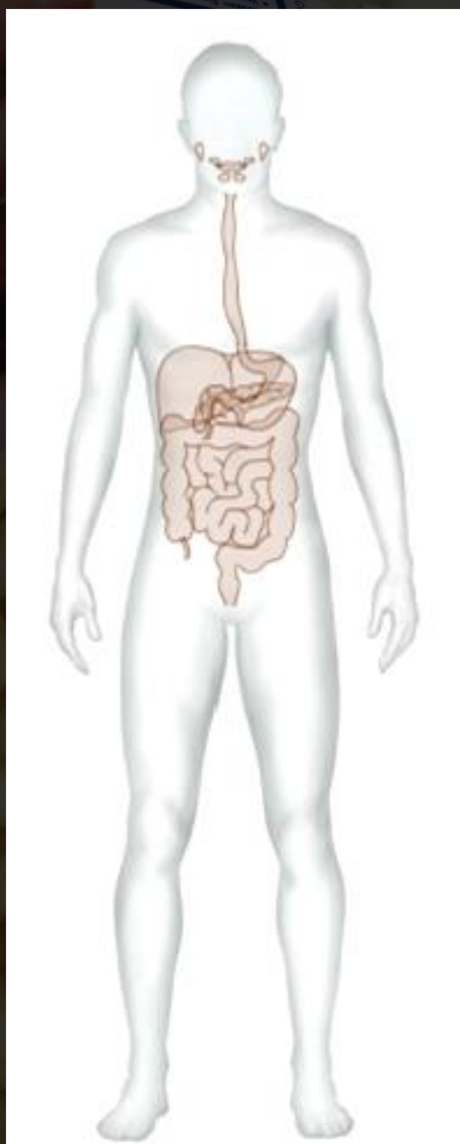
kidney → organ damage,

Nrf2 pathway is main target pathway of oxidative stress response

testis → infertility



Summary of „omics“ investigations (2)



liver

→similar key enzymes affected compared to kidney and testis

→ carbohydrate metabolism affected

→ despite global changes of protein expression, relatively minor impact on metabolic shifts

= no target organ for 3-MCPD and 3-MCPD-dipalmitate induced toxicity

---

heart

→ target tissue of effects by 3-MCPD and 2-MCPD with different MoA (mode of action)

→3-MCPD disturbs Park7/DJ-1 strongly

→but 2-MCPD only slighty

Conclusions

Starting point: list of > 800 heat-induced food contaminants

=> no toxicity and no exposure data available for most of them

QSAR/ Kinetics

• QSAR resulted that **chloropropanols** are important to prioritize
• Bioavailability data for 3-MCPD was available, for the fatty acid esters not
• biokinetic study resulted high bioavailability of 3-MCPD from uptake of the fatty acid esters

Toxicogenomics & MOA

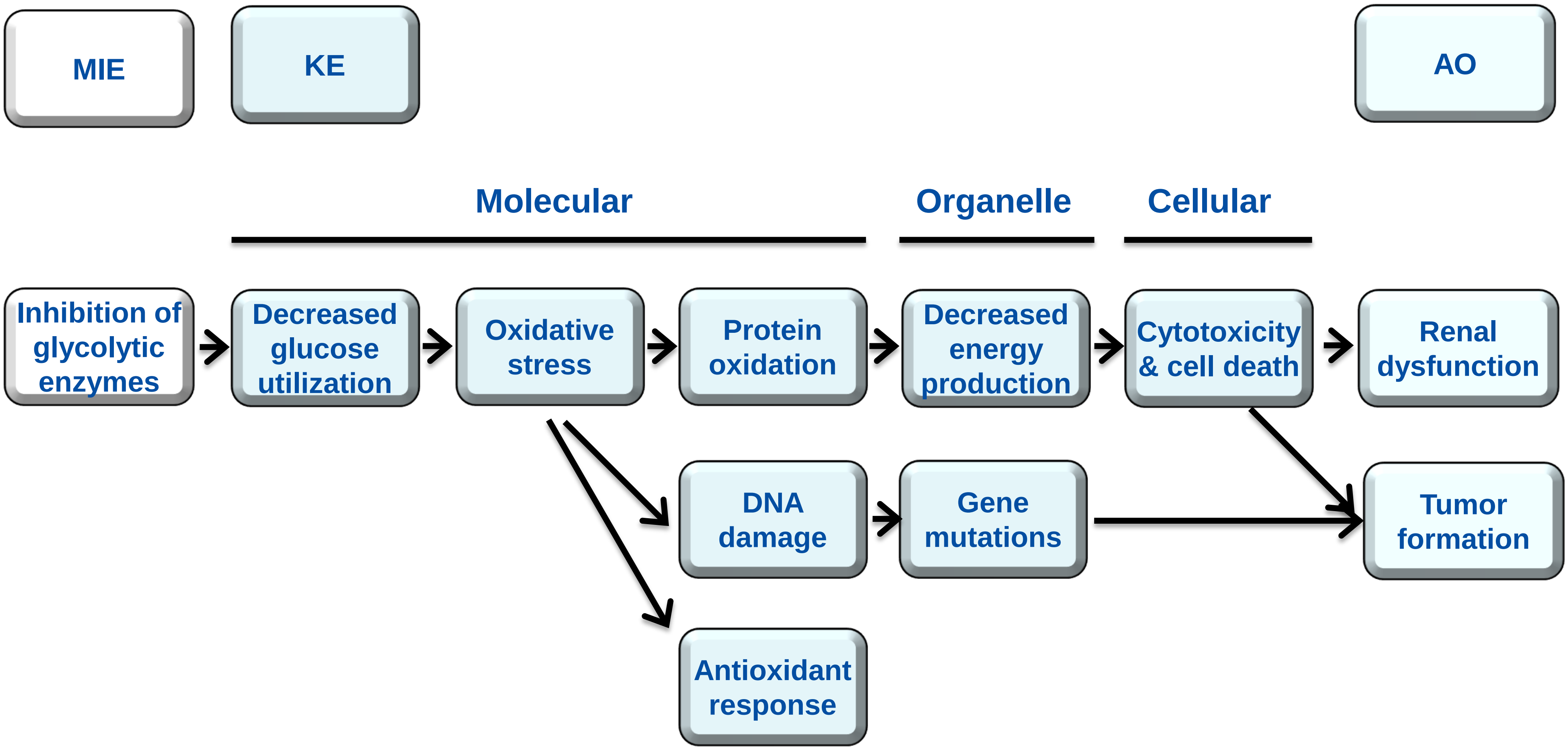
• proteomics and transcriptomics studies identified known and new molecular targets of 3-MCPD and 2-MCPD
• => effects 3-MCPD > 2-MCPD
• => effects kidney > testes > heart > liver
• Differences between 3-MCPD and 2-MCPD (e.g. in heart)
• 3-MCPD results an oxidative irreversible inactivation of DJ1

AOP

• MOA results may be a starting basis for development of kidney damage AOP
• Quantifative data…..

=> Risk characterization of heat-induced food contaminants

Provisional AOP draft for 3-MCPD kidney toxicity



Thank you for your attention

Acknowledgement:

Dr. Nadiya Bakhiya
Katharina Schultrich
Dr. Falko Frenzel
Dr. Torsten Buhrke
PD Dr. Albert Braeuning

Prof. Dr. Dr. Alfonso Lampen

Bundesinstitut für Risikobewertung

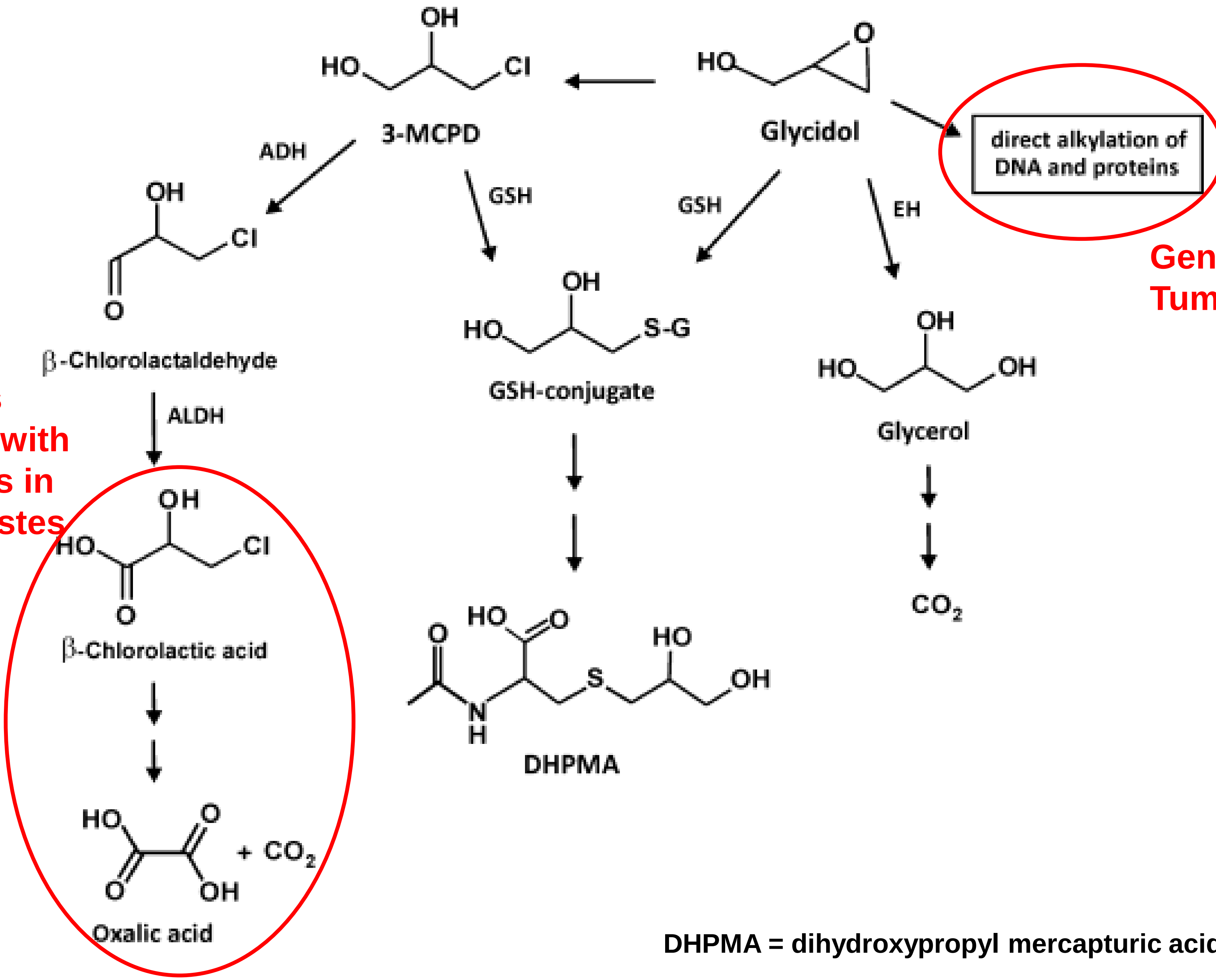
Abteilung Lebensmittelsicherheit

Max-Dohrn-Straße 8-10 • D-10589 Berlin

Tel. 0 30 - 84 12 - 0 • Fax 0 30 -184 12 - 30 03

Alfonso.Lampen@bfr.bund.de • www.bfr.bund.de

3-MCPD and Glycidol:
Metabolic pathways and Toxicity



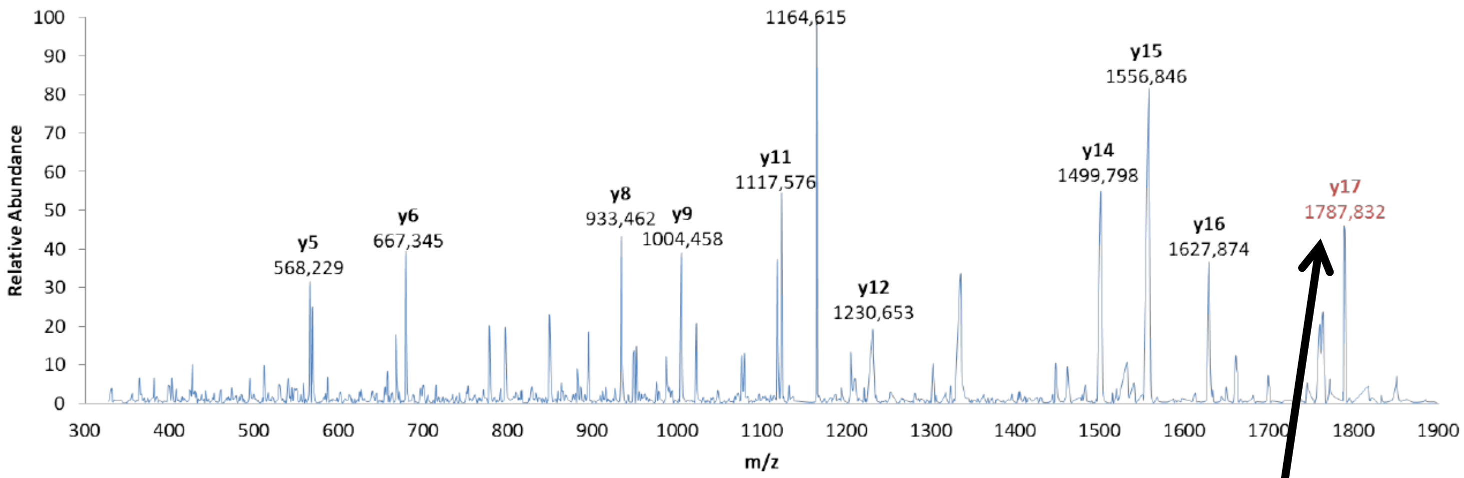
Genotoxicity
Tumor induction

Metabolites associated with toxic effects in kidney & testes

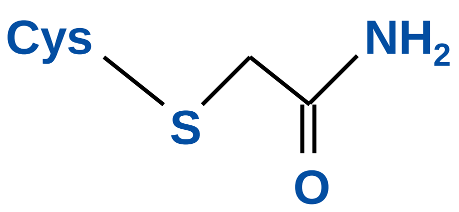
DHPMA = dihydroxypropyl mercapturic acid

3-MCPD induces the irreversible inactivation of DJ-1

GLIAAIC(CAM)AGPTALLAHEVGFGC(CAM)K



Carbamidomethylated (CAM)

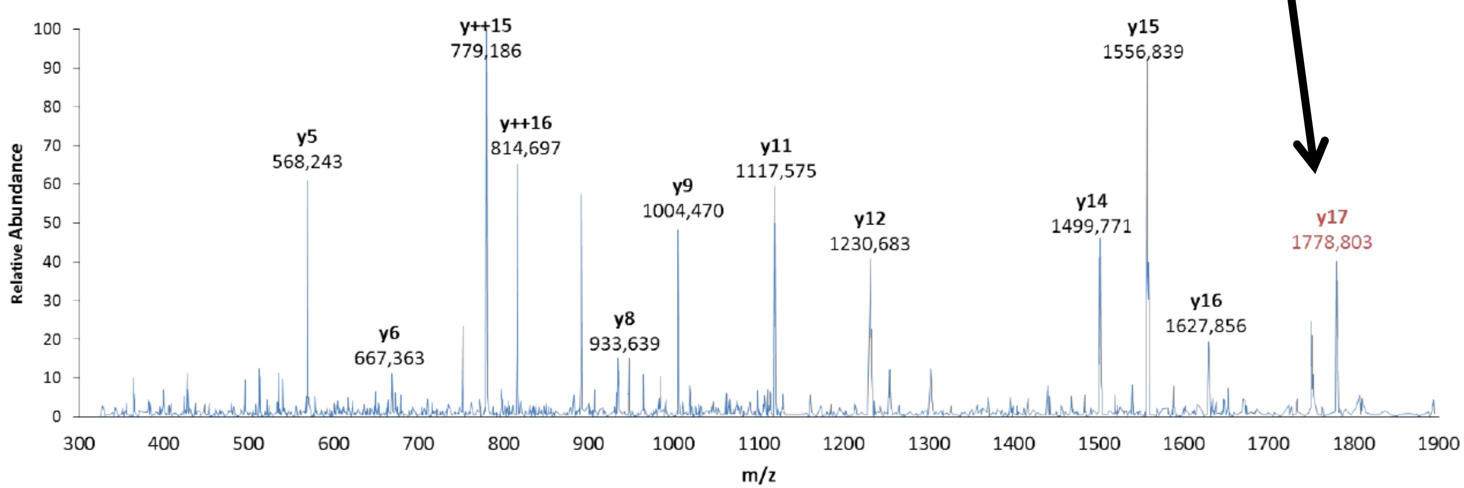


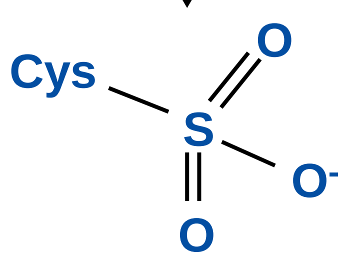
+ 57.021

Experimental Mass Difference: 9.029

Theoretical Mass Difference: 9.036

GLIAAIC(SO_3)AGPTALLAHEVGFGC(CAM)K





+ 47.985

Sulfonic Acid

BfR