TECHNISCHE UNIVERSITÄT KAISERSLAUTERN

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Low Dose Effects of the Dietary Carcinogen Acrylamide (AA)

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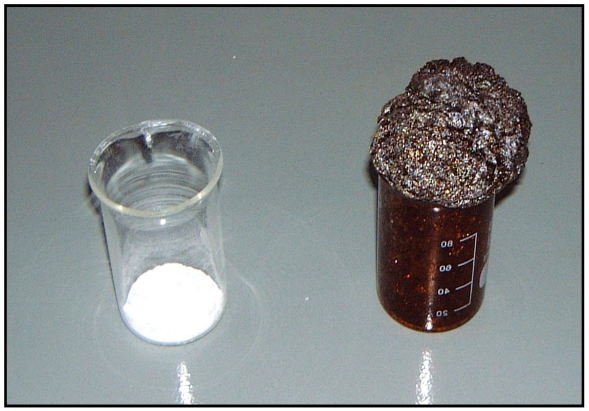
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Heat-treatment of food

*Non enzymatic browning → " **Maillard-reaction chemistry**"*
reaction of reducing sugars with amino acids

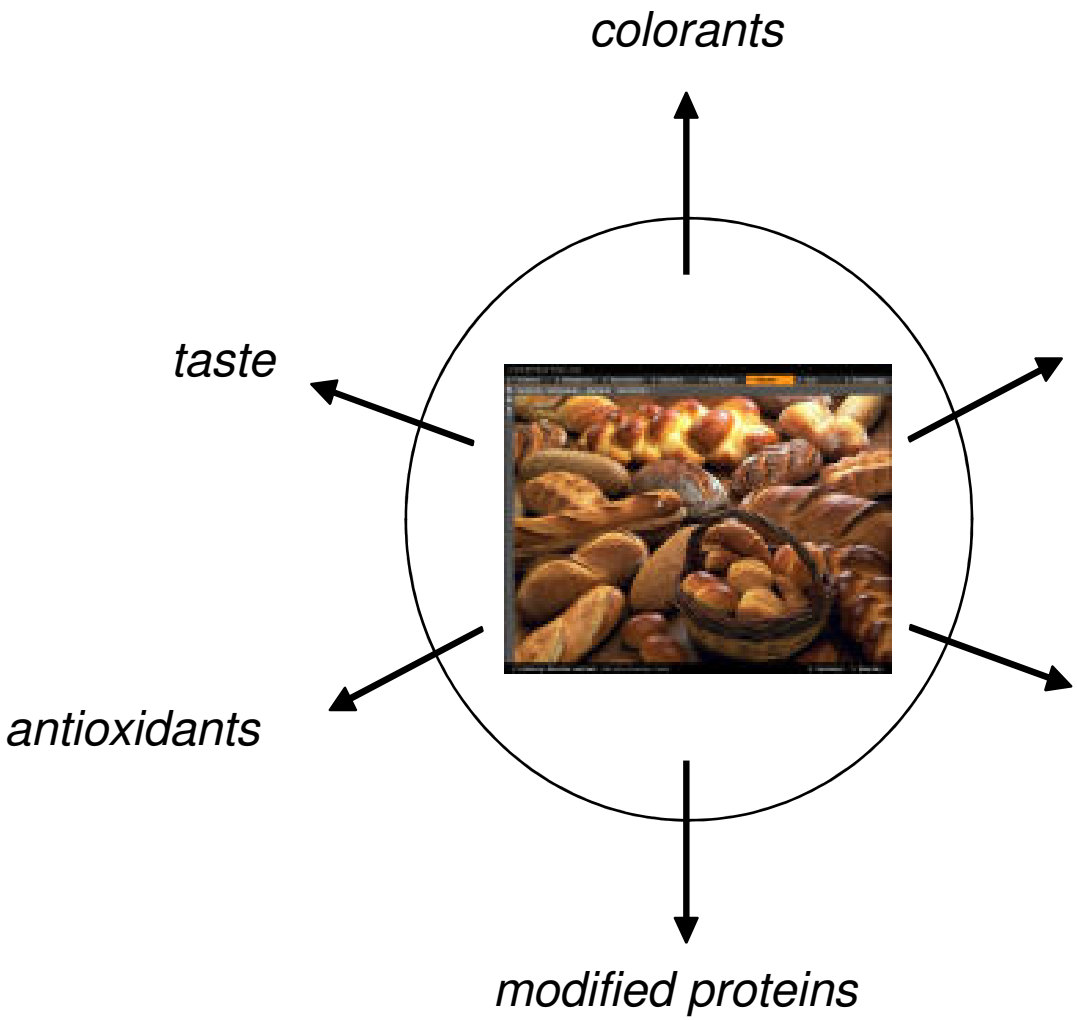


First described 1912 by the French chemist
Louis Camille Maillard



heating alanine and glucose → carbon dioxide+water and brown colour

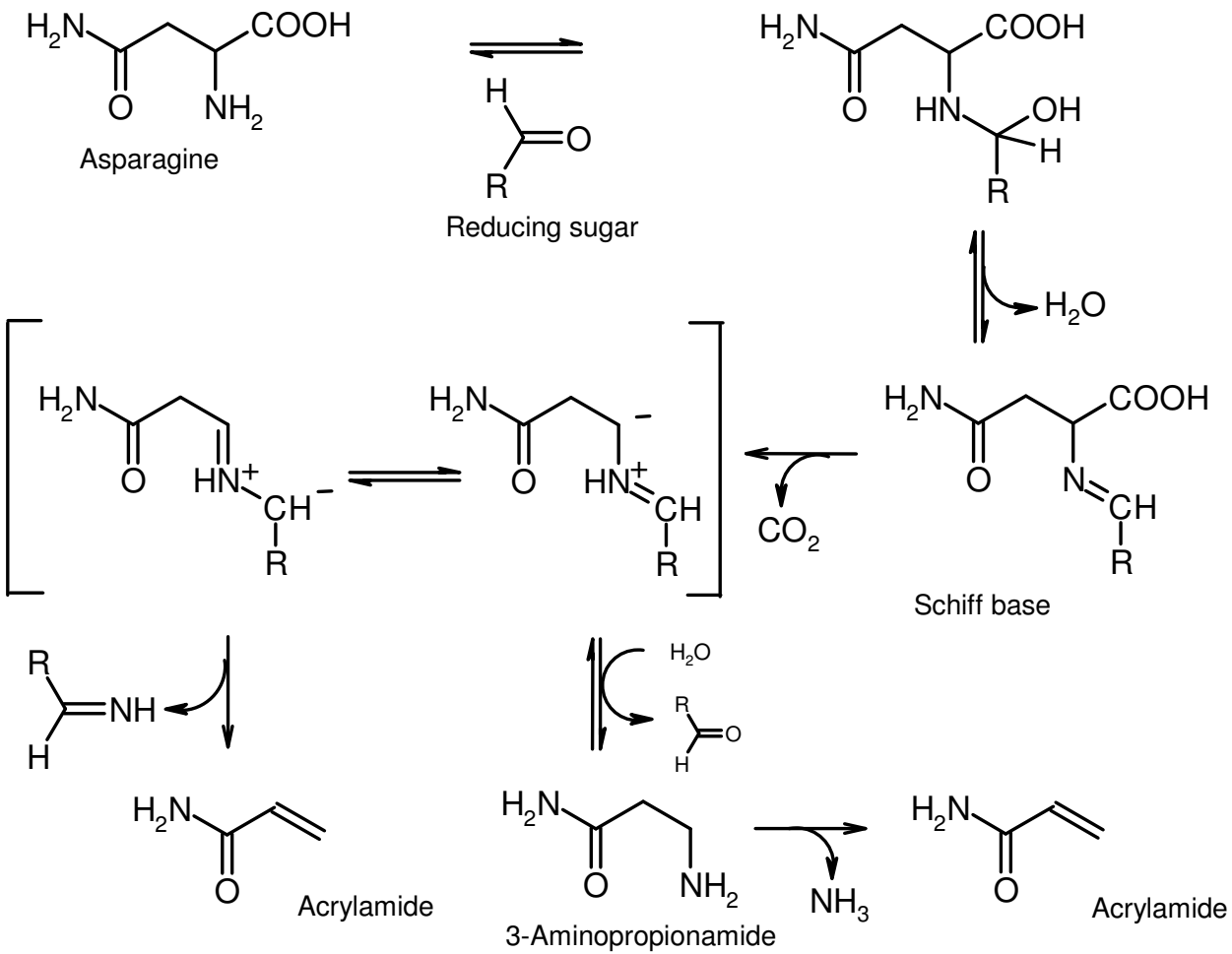
Heat Processing: formation of bioactive compounds in food by „Maillard-chemistry“



food-borne toxicants:
Acrylamide,
Furan,
Nitrosamines
Heterocyclic aromatic amines
Acrolein

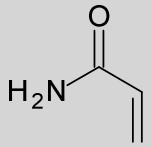
AA: Formation in foods by thermal treatment

Tareke et al 2002; Rosen and Hellenas, 2002; Zyzak et al., 2003





Exposure to Acrylamide (AA)



- ***Highly variable → Individual consumption habits / contents of individual food groups***
- ***Dietary sources: potato fried products (up to 50%), soft / crisp bread, biscuits, crackers other products based on cereals / potatoes,coffee / coffee substitutes***
- ***No noteworthy exposure to AA from environmental sources (except tobacco smoke)***
- ***Endogenous metabolic formation of AA ? scarcely studied, suggestive evidence in rats and humans***

→ Estimated daily uptake (µg/kg b.w./day ; EFSA, 2014)

Infants, toddlers,children:	***0.5-1.9 (average);1.4-3.4 (95th%ile);***
Adolescents, adults, elderly:	***0.3-0.9 (average);0.6-2.0 (95th%ile);***

AA: Hazard Characterisation

(EFSA Contam Panel, draft opinion on AA in foods, 2014)

Critical endpoints for toxicity (animal studies):

Neurotoxicity: **peripheral/central axono-/neuropathy in several species, including humans**

Benchmark dose →$BMDL_{10}$: 0,43 mg/kg b.w.;

Carcinogenicity: Harderian gland tumors in mice

Benchmark dose →$BMDL_{10}$: 0,17 mg/kg b.w.;

AA is considered a genotoxic carcinogen, acting through metabolic conversion to epoxypropaneamide (Glycidamide,GA), a DNA damaging (genotoxic) mutagen

Benchmark dose and Margin of Exposure (MoE) for Genotoxic Carcinogens in Food

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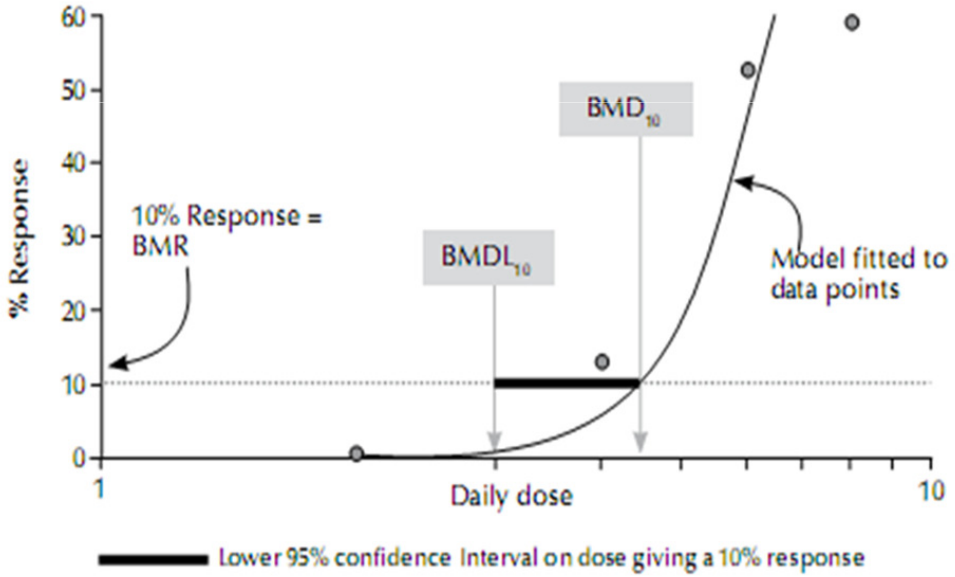
Bench**m**ark **d**ose (**BMD**) : **dose related to a defined (benchmark) response (eg 10% = BMD_{10})**

→ **Lower Confidence Limit ($BMDL_{10}$)**

MOE : **benchmark dose level divided by human exposure**

MOE ≥ 10.000 : ***„of low concern from a public health point of view…***

…"low priority for risk management"



Dose-response curve showing how the BMDL is derived. BMR, benchmark response; BMD_{10}, dose calculated to cause a 10 % increase in the background incidence of tumors; $BMDL_{10}$, lower confidence limit of the BMD_{10}

AA: risk characterisation

(EfSA Contam Panel, Draft opinion on AA in foods (2014)

Human dietary exposure	**: potato fried products (up to 50%), soft/ crisp bread, biscuits, crackers, other products based on cereals / potatoes, coffee/ coffee substitutes**
Infants,toddlers,children (µg/kg b.w./day)	**: 0.5-1.9 (average); 1.4-3.4 (95th%ile);**
Adolescents, adults,elderly	**: 0.3-0.9 (average); 0.6-2.0 (95th%ile);**
Margins of exposure (MOEs)	
Neurotoxicity	**: dietary exposure → no concern (thresholded → NOEL >100)**
Neoplastic effects	**: dietary exposure → of concern**
MOE mean exposure	**: 567 (min. lower bound) to 89 (max.upper bound)**
MOE 95th%ile exposure	**: 283 (min. lower bound) to 50 (max.upper bound)**

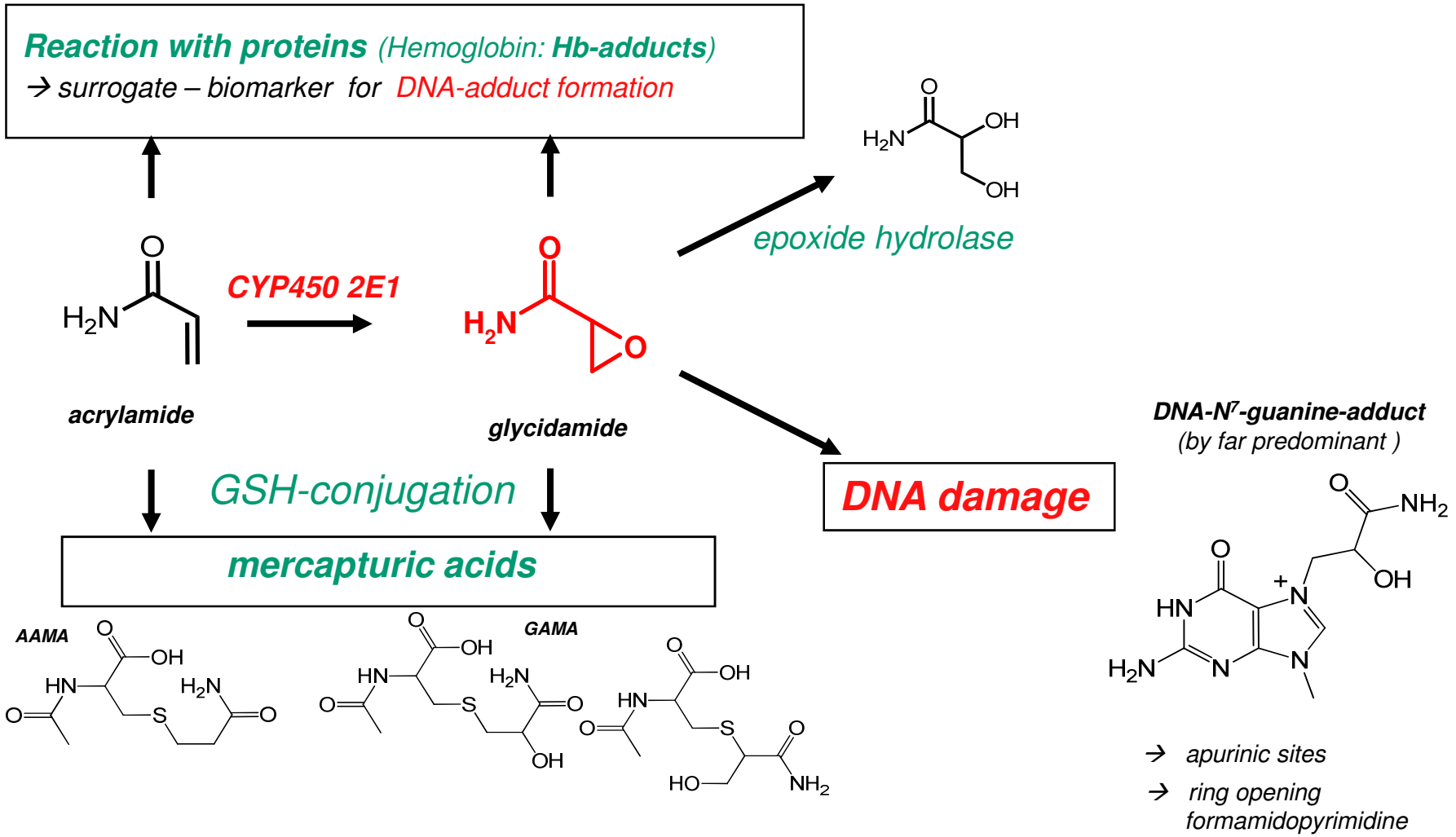
Epidemiological evidence for increased cancer risk associated with AA exposure?

Cancer is considered the critical lesion potentially associated with exposure to AA (EFSA Contam Panel, draft opinion on AA in foods, 2014):

„ In the epidemiological studies available to date AA intake was not associated with an increased risk of common cancers, including those of the gastrointestinal or respiratory tract, breast, prostate and bladder… A few studies suggested an increased risk for renal cell, endometrial and ovarian cancer (for the two latter particularly in never-smokers), but the evidence is limited and inconsistent… Occupational studies, with temporarily higher AA exposures, have not shown consistent increased risk for cancer.“

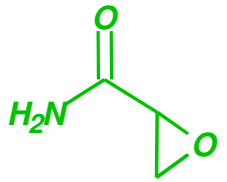
Acrylamide: *toxification* / *detoxification*

AA → GA : mouse > rat > human / mercapturic acid formation: humans > rodents



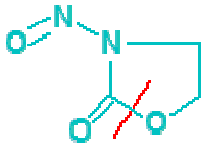
Genotoxicity/ Mutagenicity – a comparison at the level of activated carcinogens

➔ ***Glycidamide*** *(**GA**) the genotoxic AA metabolite,*
→ preferentially alkylating N^7 of guanine



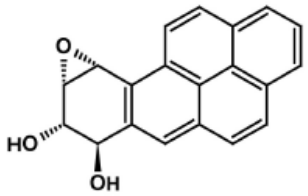
glycidamide

➔ ***activated nitrosamine NOZ-2***
→ N^7, O^6 of DNA guanine/ pyrimidine/ phosphodiester groups



3-nitroso-oxazolidin-2-one
(NOZ-2)

➔ ***Benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE)***

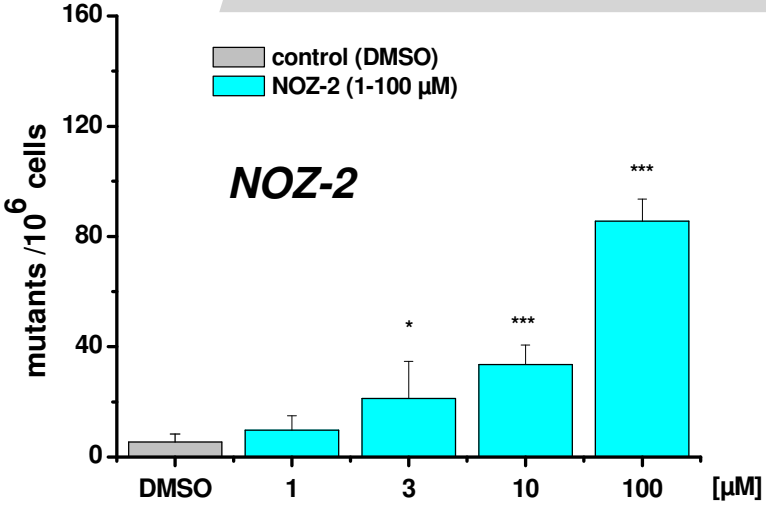


(+)-anti-B(a)P-7,8-D-9,10-E

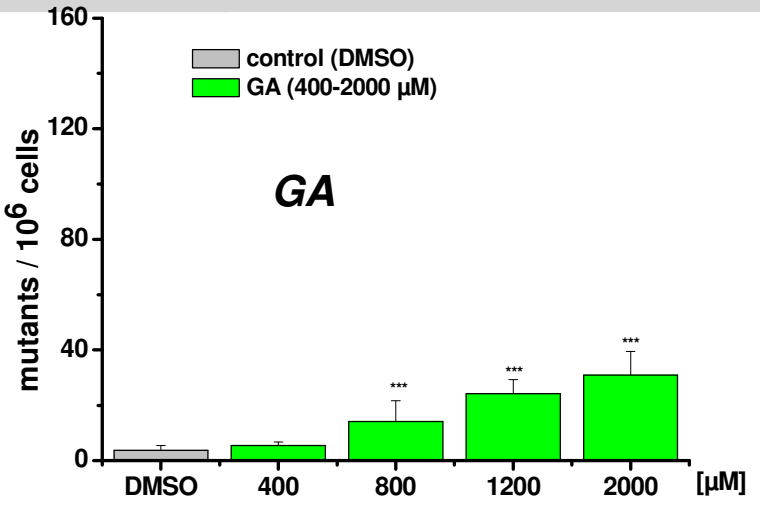
[Thielen et al. 2006, Baum et al. 2008]

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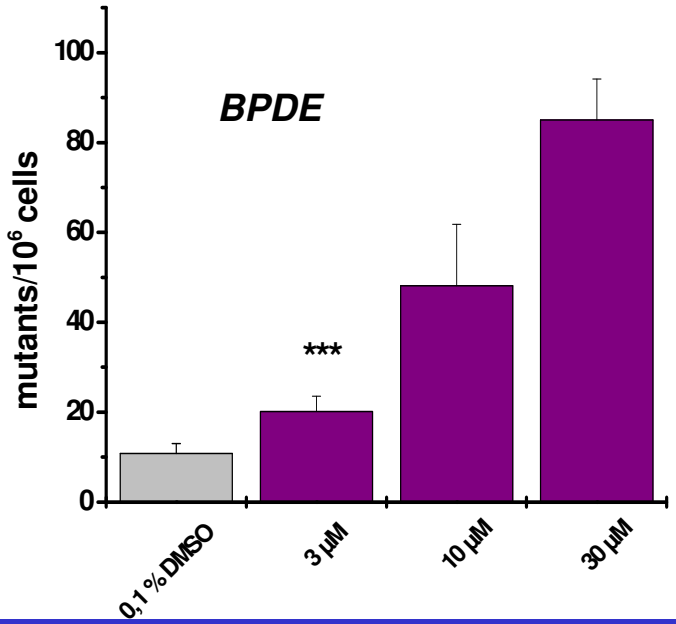
GA a potent mutagen?
Comparison of induction of *hPRT*-mutations in V79 cells



➔ ***NOZ-2:*** *potent mutagen (≥ 3 µM)*



➔ ***GA: much less potent (≥800 µM)***



➔ ***BPDE:*** *potent mutagen (≥ 3 µM)*

[Thielen et al. 2006, Baum et al. 2008]

In vivo studies

(rats)

Biomarker response in rats ingesting AA for 9 d in food/water

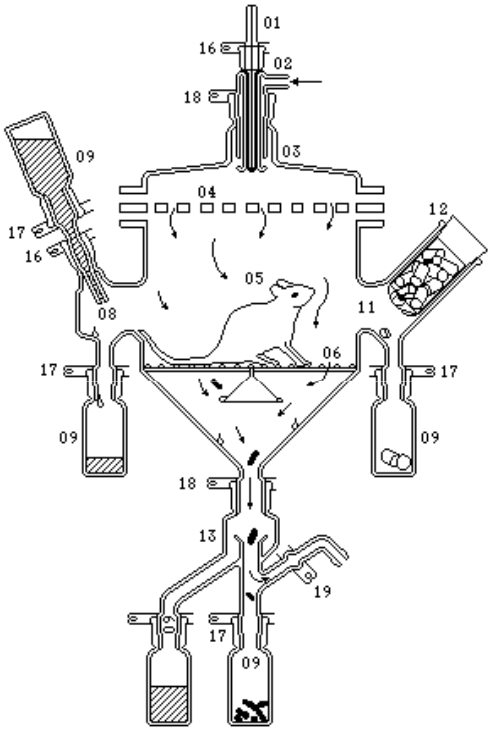
AA exposure

daily for 9 days in food: 100 µg/kg AA bw/d in food or water

single dose : 1 x 900 µg/kg in water

Food : French fries (sliced:FFS;reconstituted:FFR)

control : drinking water (DW)



SD rats: 3 animals/group (male,about 200 g)

Biomarkers

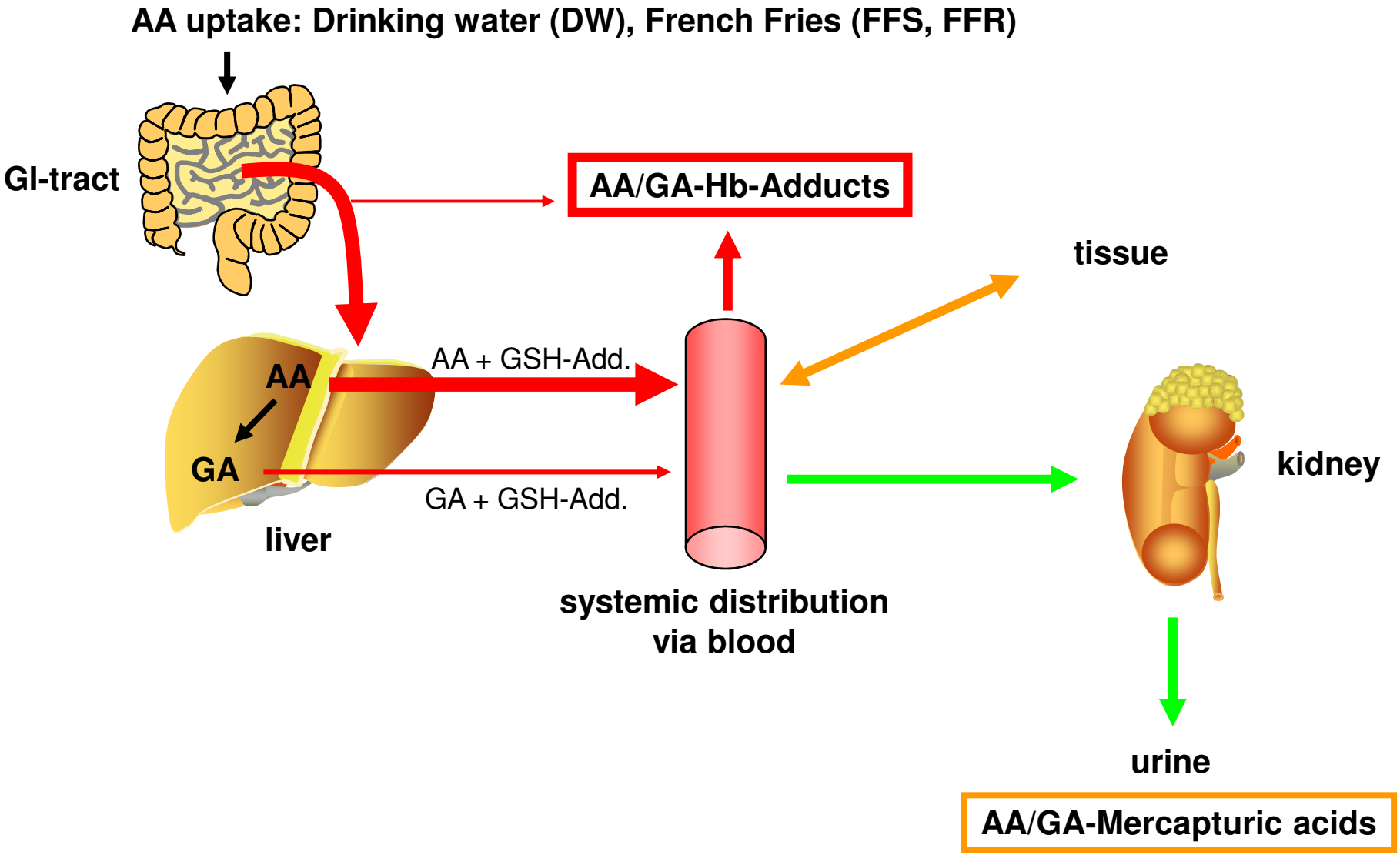
→ **Hemoglobin-(Hb-)adducts in blood (AA-Val/GA-Val)**

→ **Mercapturic acids (MA) in 24 h urine**

[Berger et al., 2010]

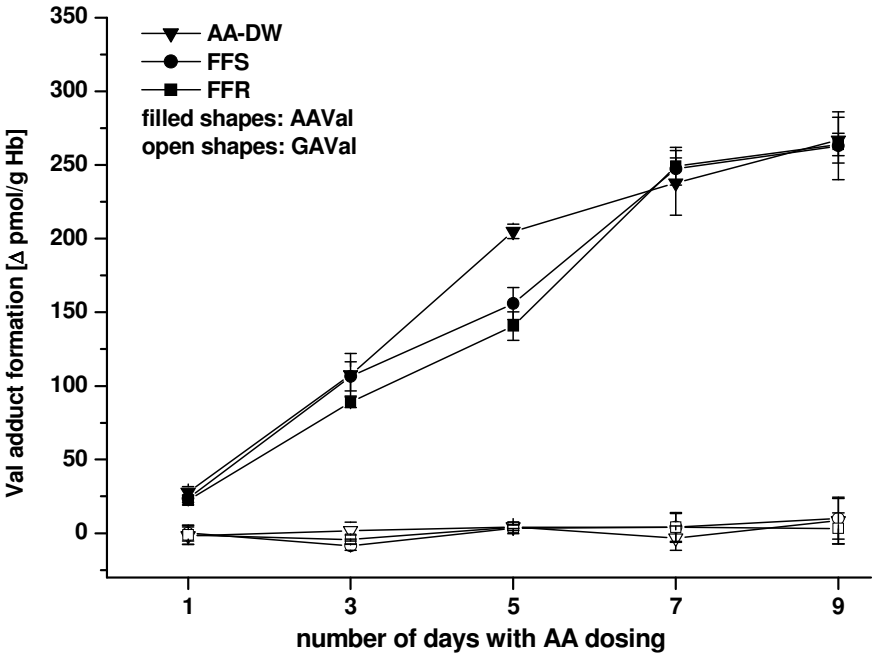
Acrylamide biomarkers of exposure

(Hb-adducts, mercapturic acids)

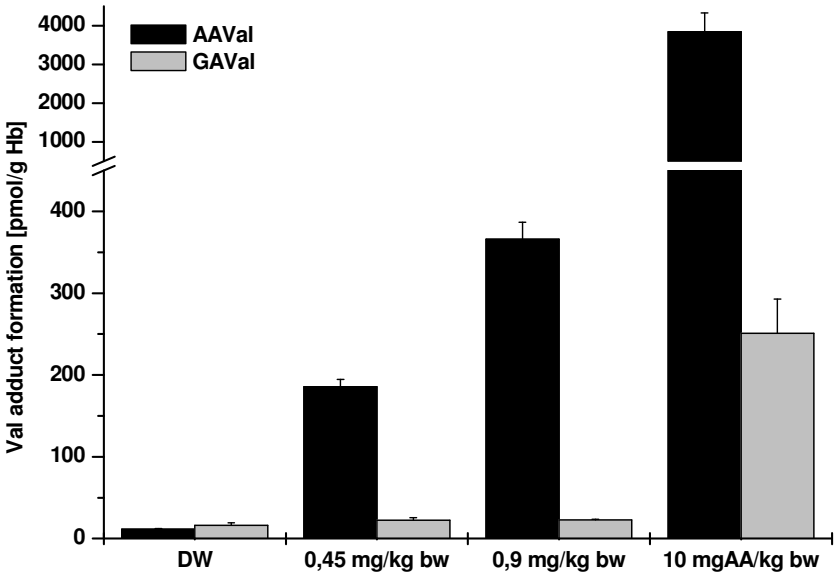


Biomarker - Hb-adducts
Repeated oral uptake of AA via water / food (9d)

AA for 9 d →100µg/kg bw p.o., in FFS/FFR (French fries) or drinking water



single high AA-dose (gavage)



- **Hb-Adducts :** **Monitored 24h after respective last AA intake**

  → AA-Val increases with cumulative AA uptake (linear with time);

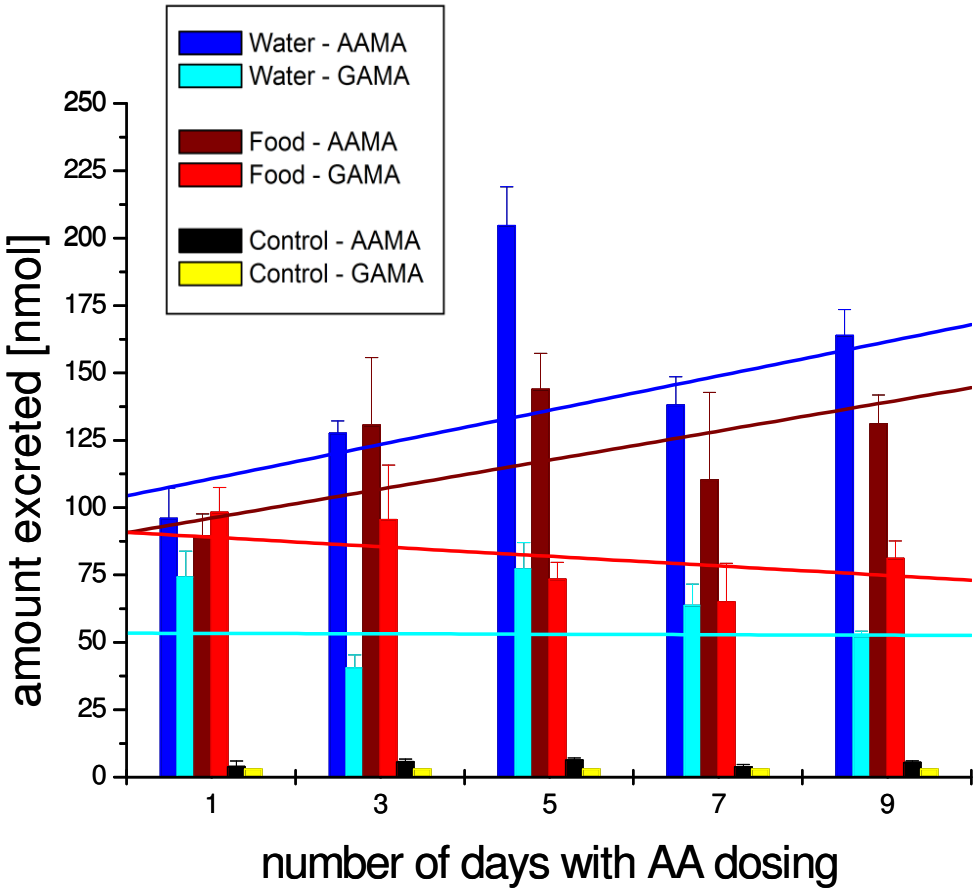
  → GA-Val : no difference to untreated controls at any dosage

  (9 x100µg/kg / 1x 900 µg/kg)

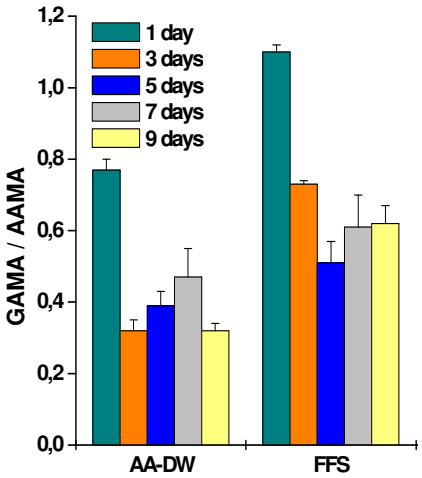
Biomarker– mercapturic acids in urine

Oral uptake via water / food

AA/GA-mercapturic acids, in 24h urine



→ *~50% of applied dose excreted as mercapturic acids (AAMA+GAMA)*



ratio:

GAMA / AAMA

→*Bioavailability of AA in foods comparable to drinking water (d5:accidental overdose)*

Biomarker response in rats: repeated oral uptake of AA via water / food

- no marked differences in bioavailability between water and food
- urinary mercapturic acids (AAMA and GAMA): clear indication for

  metabolic GA formation in the liver
- no significant increase in GA-Hb adducts up to total dose of 900µg/kg bw (repeated or single dosage)

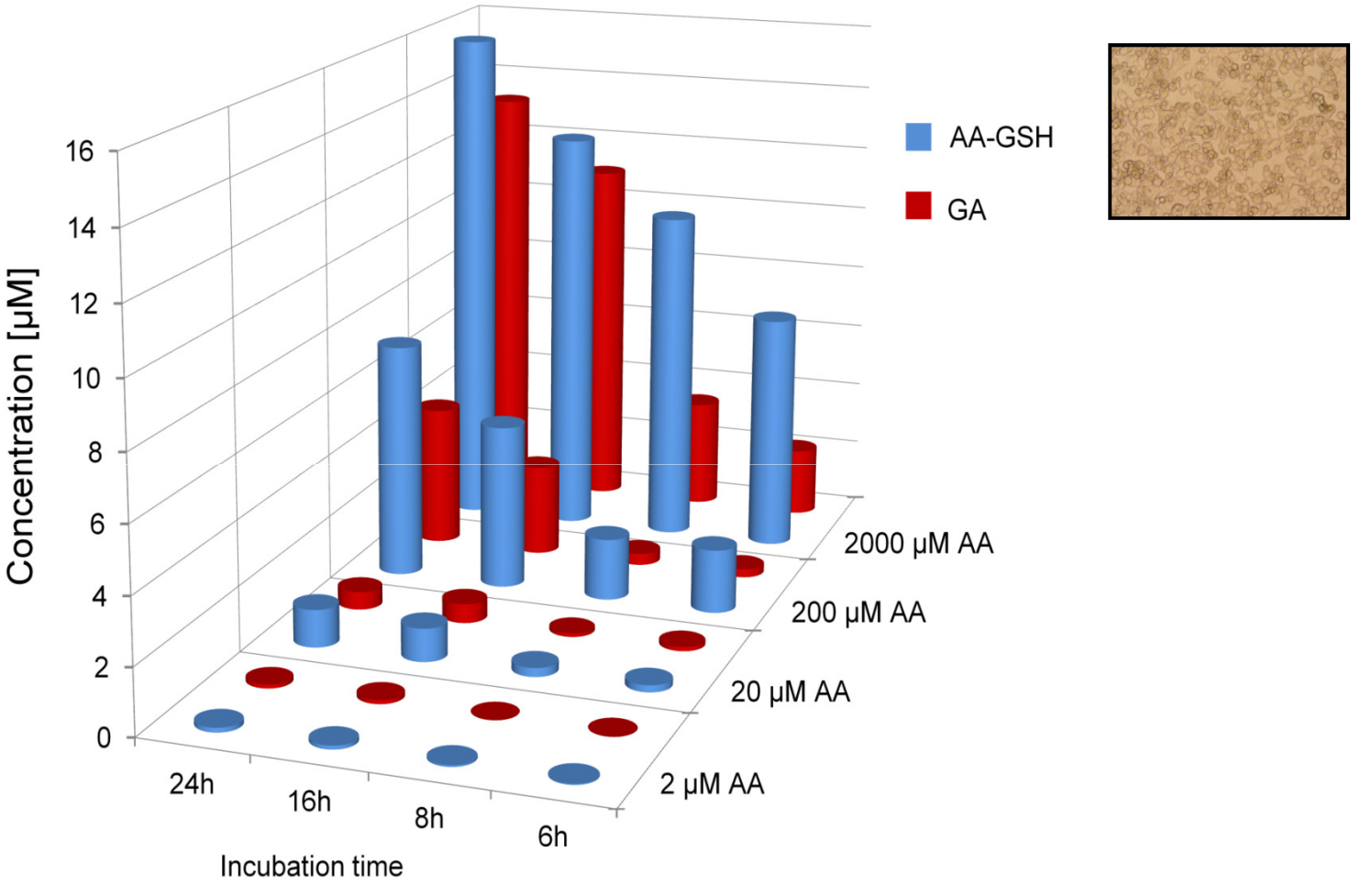
Conclusion

At AA-dosage of 100µg/kg b.w./ day:

any GA formed from AA is effectively coupled to GSH in rat liver

[Berger et al., Mol Nutr Food Res 2010]

Formation of phase I / II metabolites in primary rat hepatocytes: GA versus AA-GSH



- **AA-GSH formation at all AA concentrations faster than GA formation (1.5-3x in medium)**
- **at 2000 µM AA: steep GA increase at 8h to 16h → GSH depletion?**
- **only at this AA concentration → DNA N7-GA-Guanine detected (Cmax=16 h)**

[Watzek et al., Arch Toxicol, 2013]

AA: single oral dose-response study in rats 0.1 – 10 000 µg/kg bw



Design

- Female SD rats (n = 54; age 50 days, weight about 150-170g), kept on AA-minimized experimental diet (AA <0,5 µg/kg → uptake ≤ 0,08 µg/kg bw/d) for two weeks prior to start and during experiments with free access to (exp.) diet and water

- Low dose range (0 – 100 µg/kg bw, gavage):
  - 8 rats per group : 0, 0.1, 1, 10, 100 µg 1-^{14}C-AA/kg bw

- High dose range (500 – 10,000 µg/kg bw, gavage):
  - 3 rats per group: 500, 1000, 3000, 6000, 10000 µg AA/kg bw

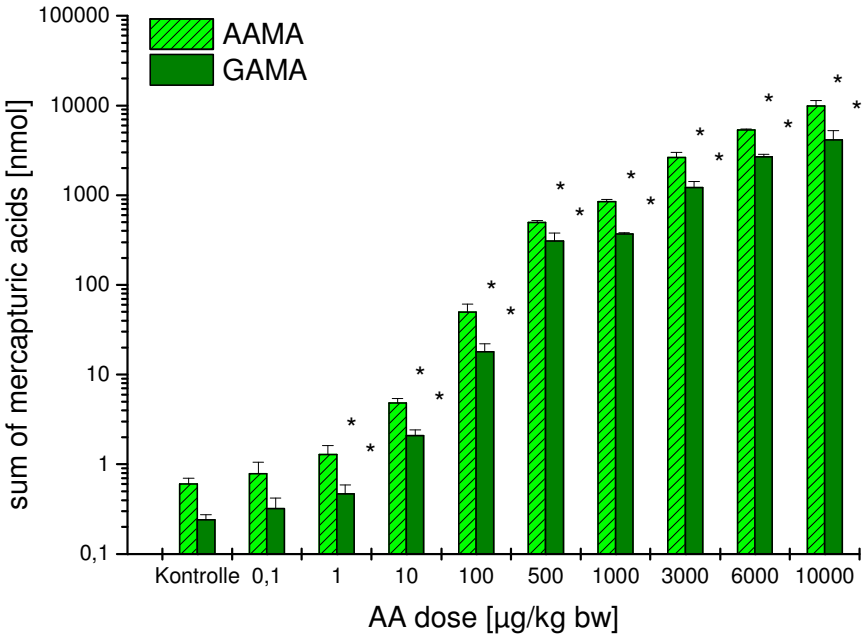
- Sacrifice 16 h after administration; urine collected; liver, lung, kidney samples taken;

- → biomarkers: mercapturic acids in urine; N7-GA-Gua DNA adducts in tissues

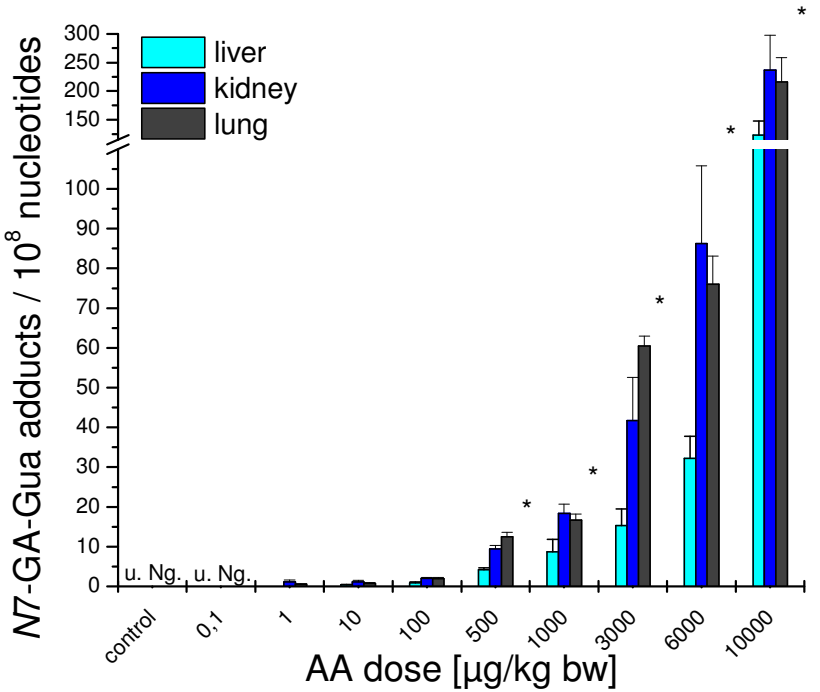
Mercapturic acids and N7-Ga-Gua adducts



Mercapturic acids (MA)



DNA- N7-Ga-Gua adducts



MA (AAMA/GAMA):

control: → background signal

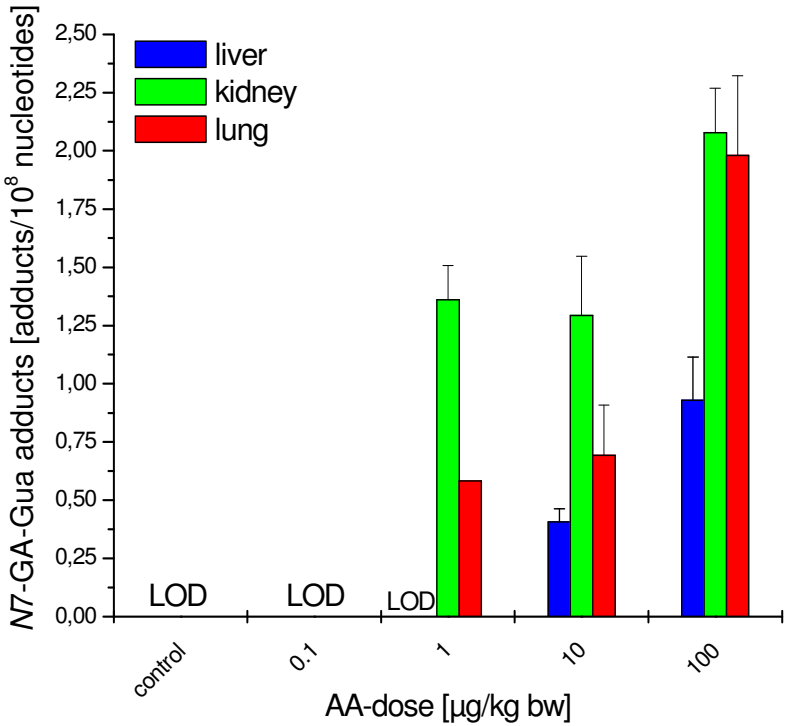
0.1 µg/kg b.w.: no difference to control

≥ 1 µg/kg b.w.: clear dose dependence

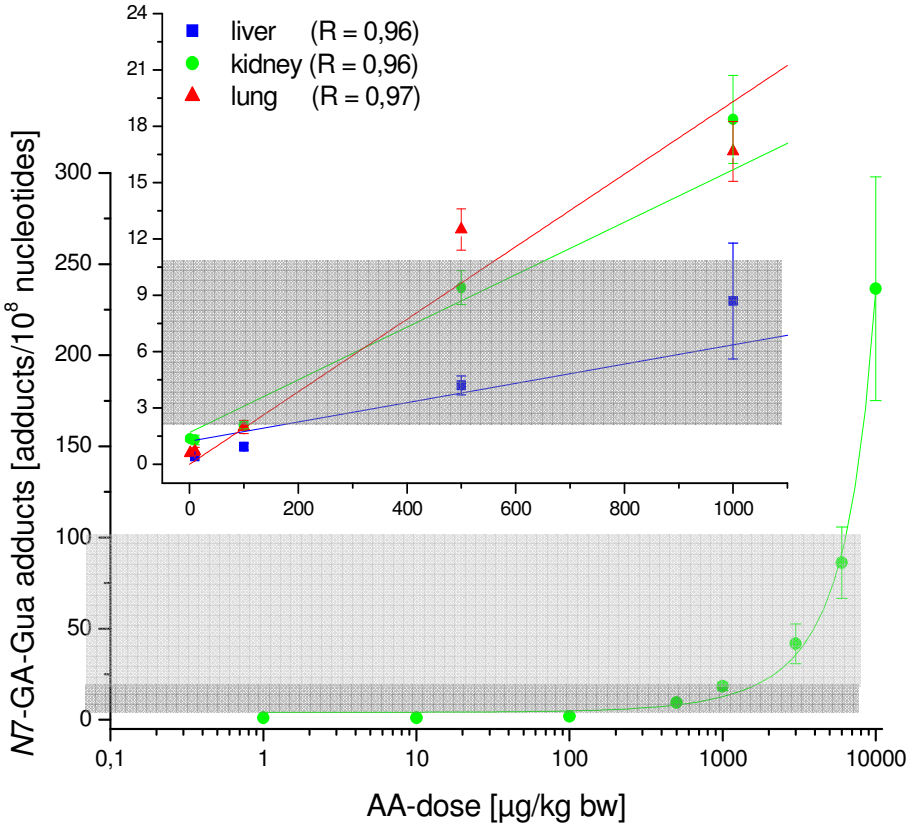
DNA-N7-GA-Gua: LOD = 0,15 add/10^8 ncts; LOQ = 0.25;
0.1 µg/kg bw : < LOD ; 1-10 µg/kg bw : <2 add/10^8 ncts

Up to100 µg/kg bw : no dose related response

N7-GA-Gua adducts in tissues of rats 16 h after AA dosage (via gavage)



Dose range: 0 - 100 µg/kg bw



Dose range: 0 - 10000 µg/kg bw (kidney)

LOD: 0.15 N7-GA-Gua /10⁸ nclt (8 fmol / µmol Gua);
LOQ: 0.25 N7-GA-Gua /10⁸ nclt (13 fmol / µmol Gua)
Mean+/-SD;n=8

[Watzek et al., 2012]

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„Background“ human DNA damage

Human „background“ DNA damage:

	adducts/10^8 nucleotides	fmol/µmol guanine	tissue [human]
8-Oxo-dGuo Epe, 2002	100	5330	lymphocytes
7-(2´-Carboxyethyl)guanine Cheng et al., 2010	7	373	liver
N^2-ethylidene-dGuo Wang et al., 2006	10	534	liver
7-ethyl-Gua Chen et al., 2007	0,8	42	liver
N^2-ethyl-dGuo Wang et al., 2006	0,2	12	liver
1,N^2-propano-dGuo Zhang et al., 2006	0,3	15	liver
N^2-hydroxymethyl-dA Wang et al., 2010	7		leucocytes

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Summary: experimental studies

- GA, the genotoxic metabolite of AA a mutagen of rather modest potency
- Primary rat hepatocytes: rate of AA-GSH formation exceeds rate of GA-formation (F ~ 1.5 -3)
- In vivo study : within dose range 0,1-100 µg AA/kg bw→ no dose related increase of N7-GA-Gua adducts (< 2 adducts/10^8 nucleotides); levels found close to lower bound background level reported for similar DNA lesions in human and rat tissues
- Human background DNA lesions → point of reference in future risk assessment ?

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Decl.of Interest:

Gerhard Eisenbrand serves as scientific consultant to
Institute of Scientific Information on Coffee, Switzerland (ISIC)
And Tchibo GmbH Germany;