CHEMIE

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Exploring biomarkers for the risk assessment of food constituents

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Food constituents of relevance for food safety

- **Organic Chemicals of natural origin**
  - polyphenols
  - alkaloids
  - saponins
  - steroidal/other compounds

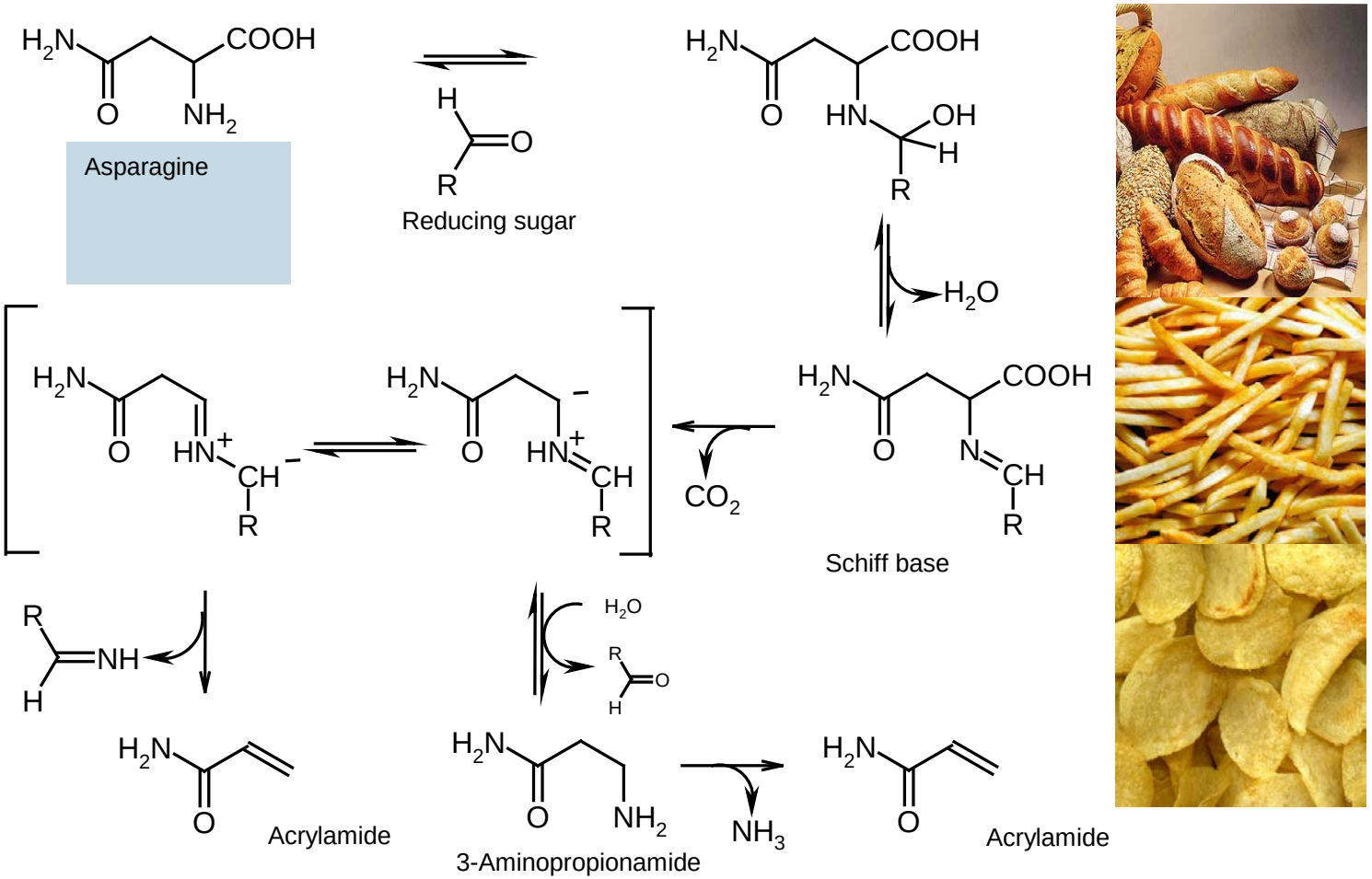
- **Inorganic chemicals**
  - minerals
  - nitrates, (thio)cyanates, halogenides
  - toxic metal/other compounds (eg arsenic, lead, cadmium…)

- **Contaminants, related to**
  - production, storage, transport
  - migration from contact materials
  - processing

Process related contaminants

- N-nitroso compounds
- Polycyclic aromatic hydrocarbons
- Heterocyclic amines
- Acrylamide (AA)
- Acrolein
- Furan
- Chloropropanols / esters
- Glycidol / esters
- ??

AA: Heat–induced formation in foods



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

AA: Toxicology

Mutagenicity

in-vitro: bacteria mostly negative; mammalian somatic/ germ cells

→ chrom. aberrations / micronuclei (at mM conc.)

in-vivo: effects observed in general at carcinogenic dose range

Non neoplastic: Neurotoxicity(peripheral neuropathy)

several species, incl humans; $BMDL_{10}$: 0.43 mg/kg/d

Neoplastic effects: 2 years studies in mice/ rats (0.5 - 2 mg/kg bw/d; $BMDL_{10}$: 0.17 mg/kg/d)

→ thyroid, mammary, adrenal, pituitary, Harderian gland, lung, peritesticular mesotheliomas

Classification: Category 2, DFG-Senate Commission on Health Hazards in the Work Area (MAK)

Group 2 A, Intern. Agency for Research on Cancer (IARC)

→ probably carcinogenic to humans

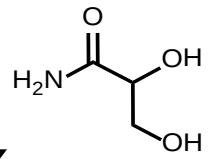
AA: risk characterisation (EFSA, 2015)

Dietary exposure sources :	potato fried products (contributing up to 50%); soft/crisp bread /cereal products; coffee/coffee substitutes
Exposure estimates (µg/kg b.w./day)	
infants, toddlers, children	0.5 - 1.9 (mean); 1.4 - 3.4 (95th%ile)
adolescents, adults, elderly	0.4 - 0.9 (mean); 0.6 - 2.0 (95th%ile)
Margins of exposure (MOE) → exposure / BMDL10	
Neurotoxicity	dietary exposure → no concern (MOE 1075 - 226, mean)
Neoplastic effects	dietary exposure → of concern (MOE 89 - 425, mean)

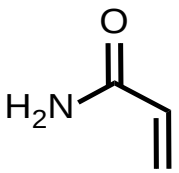
Acrylamide: toxification / detoxification

AA → GA: mouse > rat > human; GS-Adduct → mercapturic acid formation: humans > rodents

Reaction with proteins (Hemoglobin: Hb-adducts)
→ surrogate biomarker for DNA-adduct formation

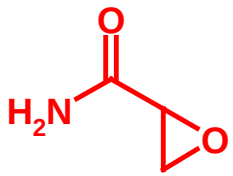


epoxide hydrolase (minor pathway, in humans)



acrylamide

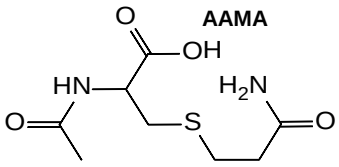
CYP450 2E1



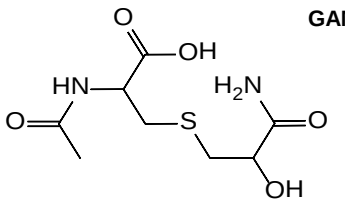
glycidamide

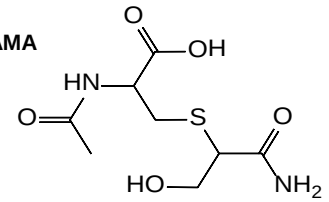
GSH-conjugation

mercapturic acids



AAMA





GAMA

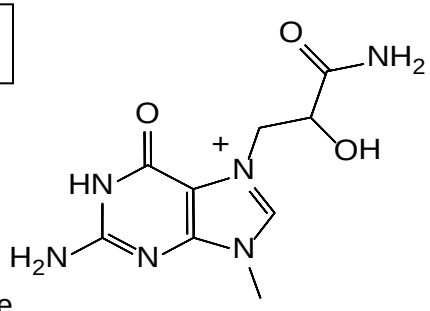
DNA damage

→ adducts

→ apurinic sites

→ ring opening formamidopyrimidine

DNA-N^7-guanine-adduct
(by far predominant)

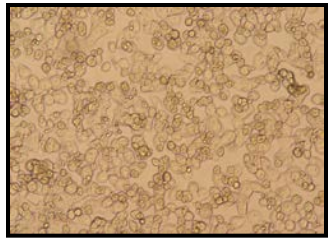


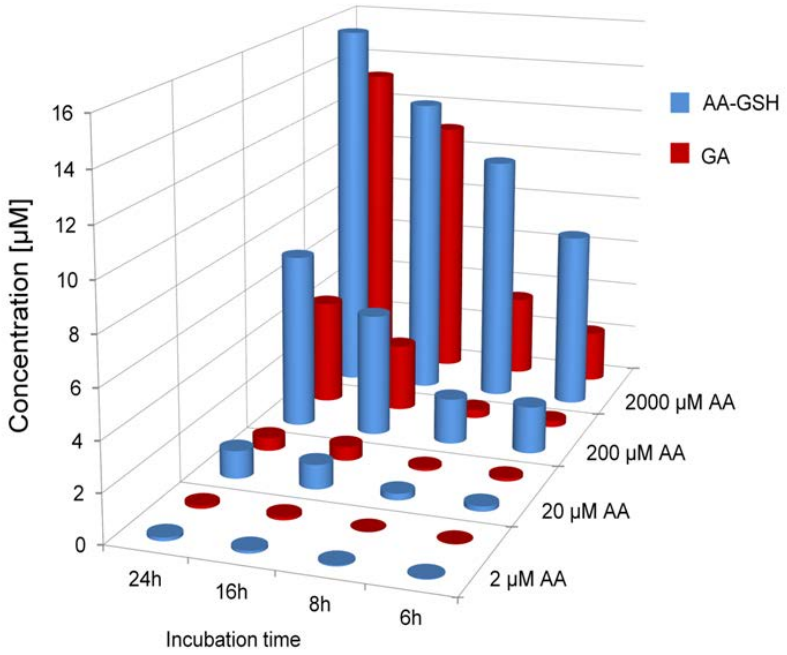
AA metabolism in primary rat hepatocytes

- Incubation : AA concentration: 0.2 - 2000 µm,
  37 °C, 15 mio. cells,

Metabolites : in culture medium, HPLC-ESI-MS/MS → AA-GSH (AAMA/GAMA)
Glycidamide

(stable isotope dilution)





→ AA-GSH-adduct formation 1.5-3 x faster than GA formation

[Watzek et al., Arch Toxicol 2013]

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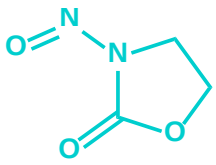
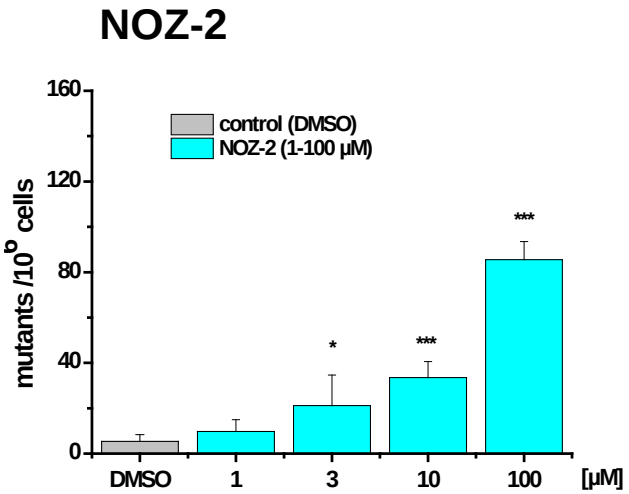
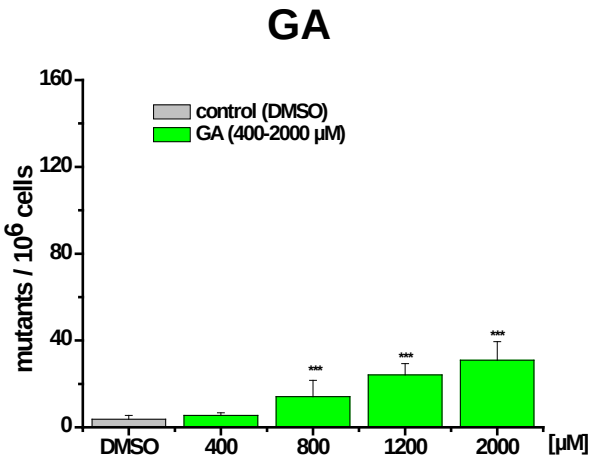
Induction of *hPRT*-mutations in V79 cells

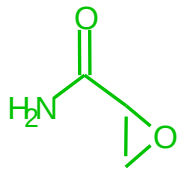
→ GA a weak mutagen, compared to activated forms of potent carcinogens



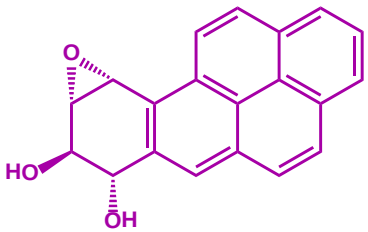
3-nitroso-oxazilodin-2-one (NOZ-2)



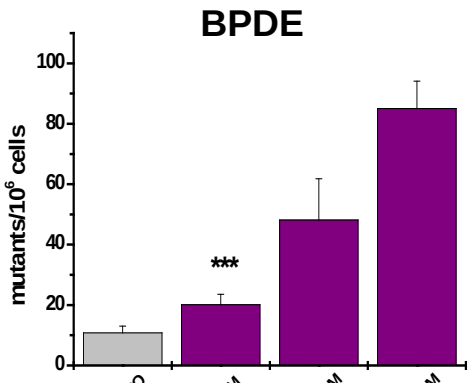




glycidamide



(+)-anti-B(a)P-7,8-D-9,1D-E



→ **NOZ-2 and BPDE**: potent mutagens (≥ 3 µM)

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

AA: genotoxicity in-vivo → biomarker monitoring

- Extended single dose/response study in rats
- Monitoring of biomarkers (LC-MS/MS)

→ DNA damage in liver, lung, kidney induced by the genotoxic metabolite, glycidamide
(GA → DNA *N*7-GA-Guanine)

→ mercapturic acids in urine (AAMA, GAMA)

Single oral dose-response study in rats
0.1 – 10 000 µg/kg bw

Study design

- Female SD rats 2 weeks on AA free diet before onset
  (< 0.5 µg/kg diet → dietary exposure ≤ 0.08 µg/kg bw/d)

- Low dose experiment (0.1 – 100 µg/kg bw):
  - 8 rats per group
  - doses of 0, 0.1, 1, 10, 100 µg AA/kg bw (gavage)

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

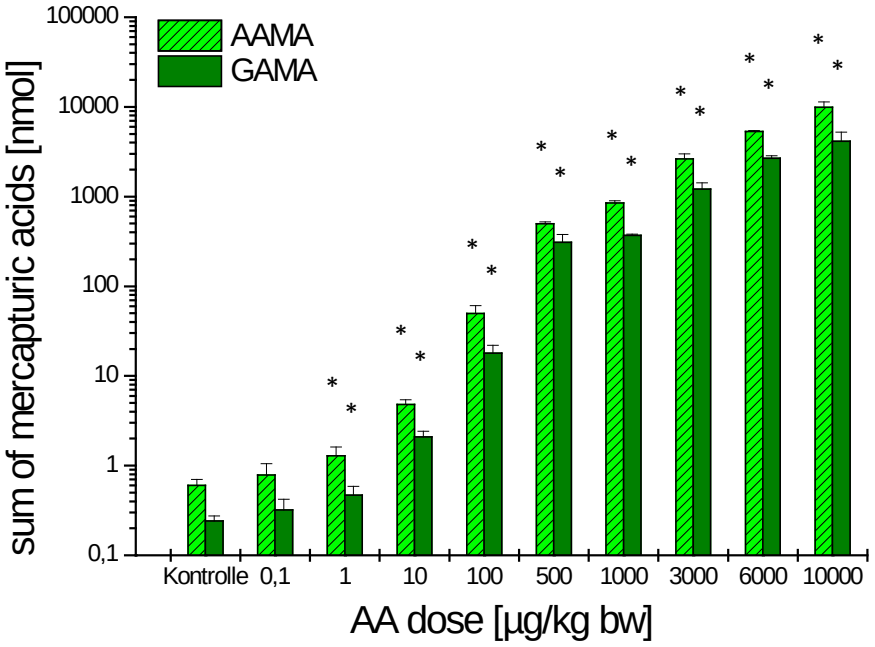
- 16 h after administration → urine collected; liver, lung, kidney samples taken;

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

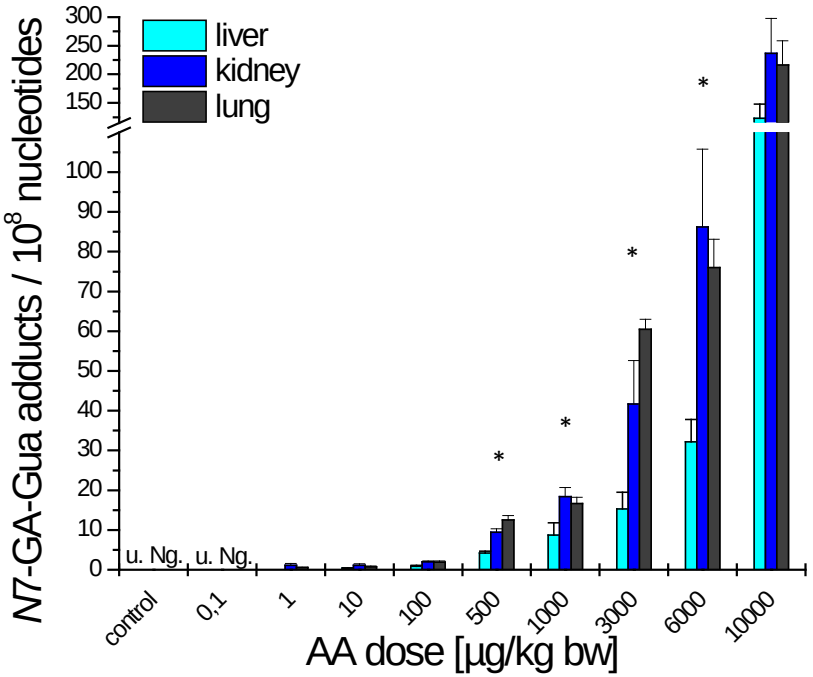
[Watzek et al., Chem. Res. Toxicol., 2012]

Mercapturic acids and N7-Ga-Gua adducts

Mercapturic acids



N7-Ga-Gua adducts



→ urinary MA

0.1 µg/kg bw: not different from control ; ≥1 µg/kg bw: dose dependent biomarker excretion

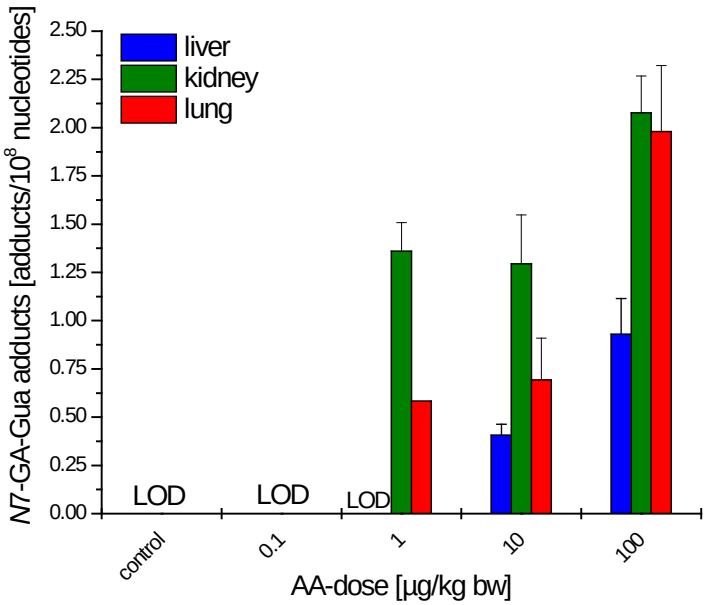
→ DNA-N7-GA-Gua

0.1 µg/kg bw: no difference from control; up to 100 µg/kg bw: no dose dependent increase

[Watzek et al., Chem. Res. Toxicol., 2012]

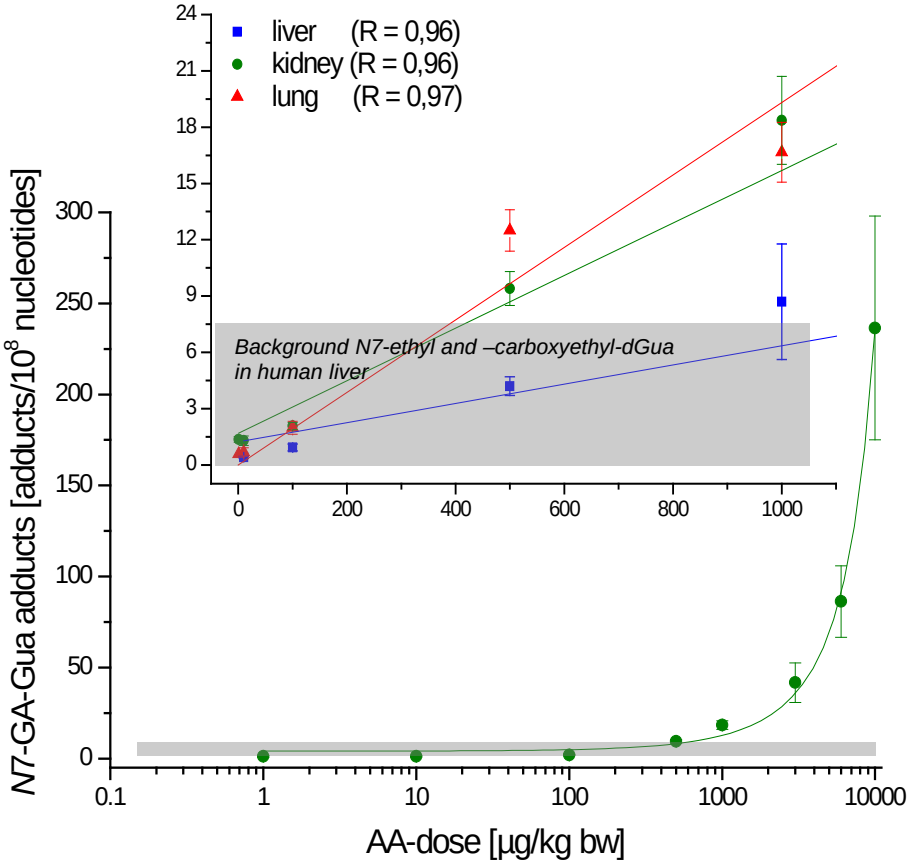
a adducts in tissues of rats

16 h after AA dosage (via gavage)



Dose range: 0 - 100 µg/kg bw

(mean values +/- SD; n=8)



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

→ ***LOD:*** *0.15 N7-GA-Gua /10^8 nclt (8 fmol / µmol Gua);* ***LOQ:*** *0.25 N7-GA-Gua /10^8 nclt (13 fmol / µmol Gua)*

[Watzek et al., Chem. Res. Toxicol., 2012]

DNA lesions in human tissues/body fluids

Lesion	Human Tissue / Body Fluid	presumed agent exposed to	Level (adducts/10^8 nucleotides	References
N7-methyl-dG	Human lymphoblastoid (cultured)	Endog.methylating agents	224	Sharma et al., 2014
7-(2-hydroxyethyl)-dG	Liver, lymphocytes	Endogenous Ethene/ ethylene oxide	58 48	Wu et al., 1999
7-(2´-Carboxyethyl)-dG	liver	acrylic acid/acrolein?	7.5	Cheng et al., 2010
N7-ethyl-dG	liver	?	0.8	Chen et al., 2007
N^2-Ethyl-dG	blood cells	ethanol/acetaldehyde (non drinkers) (drinkers)	269 527	Balbo et al., 2010
	granulocytes & lymphocytes	ethanol (0.05-0.07% blood ethanol)	150 up to about 5 fold	Balbo et al., 2012a,b
N^2-Ethylidene-dG	liver	acetaldehyde	10	Wang et al., 2006
Etheno-base adducts (1,N^6-etheno-A; 3,N^4-etheno-C; 1,N^2-ethenoxy-G)	leukocytes	lipid peroxidation products	~ 36 (averaged mean values)	Monien et al., 2014
3,N^4-etheno-C	lung	lipid peroxidation products	~ 80	Monien et al .,2014
1,N^6-etheno-A	lung		~ 48	Monien et al., 2014
N^2-(Methylisoeugenol-3‘-yl)-2‘-G	liver	methyleugenol	~ 11	Monien et al .,2014

Wrap up: experimental studies

- GA, the genotoxic metabolite of AA, is a mutagen of rather modest potency
- Primary rat hepatocytes: AA-GSH formation faster than GA formation
- In rats: dose range of 0.1 - 100 µg AA/kg bw

  → **no** dose dependence of N^7-GA-Gua adducts (≤ 2 adducts / 10^8 nucleotides)
- up to 100 µg AA/kg bw → N^7-GA-Gua DNA adduct levels within background range of similar DNA lesions in human and rat tissues

AAMA excretion after 2 weeks washout indicative for AA endogenous background in rats ?

(estimate:1.6 – 2 nmol = 0.6 – 0.7 µg/kg bw of AA)

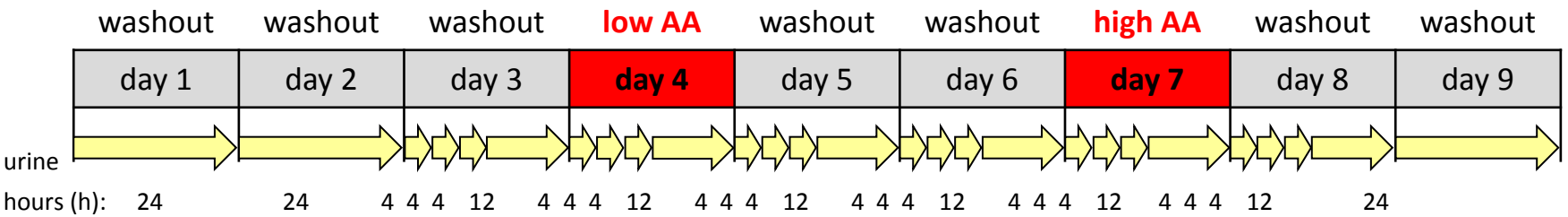
Human biomarker studies

- **Intervention : AA exposure through food intake using duplicate diet dosimetry**

- **→ relation to urinary mercapturic acids excretion** *(Ruenz et al., Arch Toxicol 2015)*

Monitoring mercapturic acids as biomarkers of human dietary exposure to AA in combination with acrylamide uptake assessment based on duplicate diets

Intervention study in male volunteers (9d; N=14; age 20-44; BMI 19-25 kg/m^2):

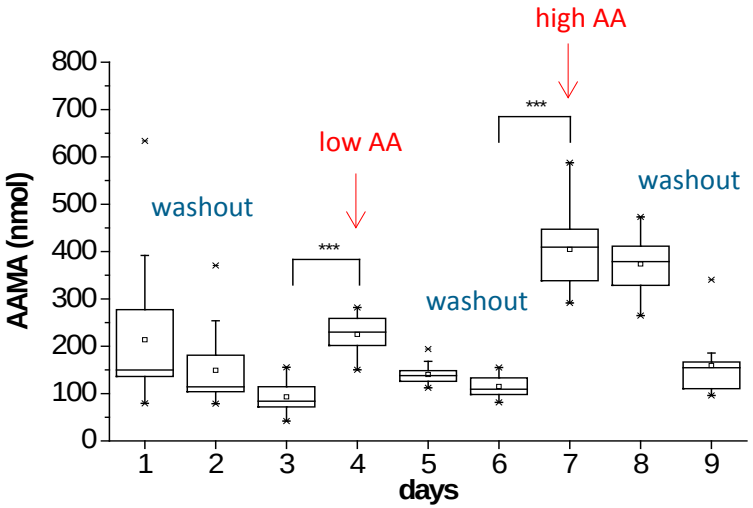


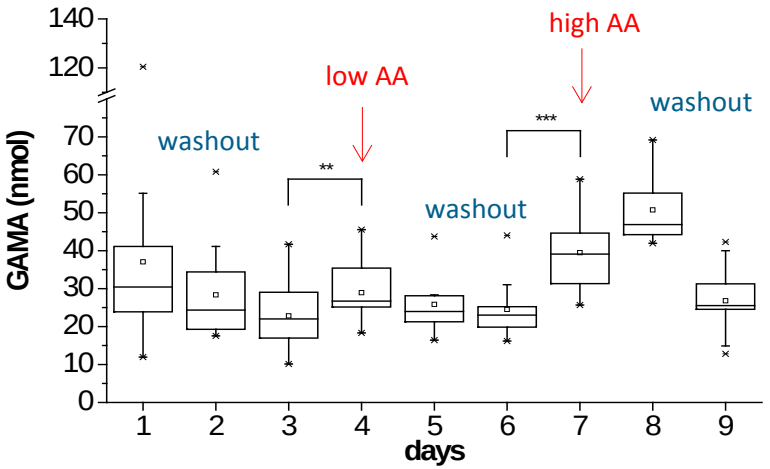
Collection of urine:

- 24h period (day 1, 2 & 9)
- 3 x 4 h periods + 12 h period (day 3, 4, 5, 6, 7 & 8)

[Ruenz et al., 2015, Arch Toxicol]

Human intervention study





„washout“: max. AA intake: 0.02 - 0.04 µg/kg bw/d (2.2 ± 0.01 µg/day→ < 5% of normal mean)

„low AA“ : 0.6 - 0.9 µg/kg bw → normal mean intake → urinary exposure biomarkers:

24h 30% AAMA + 4% GAMA;

72h 58% AAMA + 10% GAMA

„high AA“ :1.3 - 1.8 µg/kg bw →intake within 95th%ile → 24h 27% AAMA + 3% GAMA;

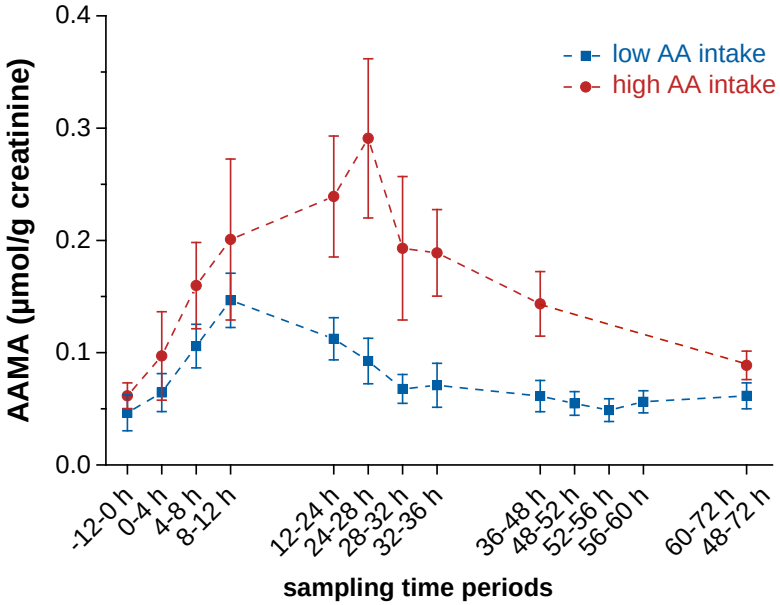
72h 58% AAMA + 7% GAMA

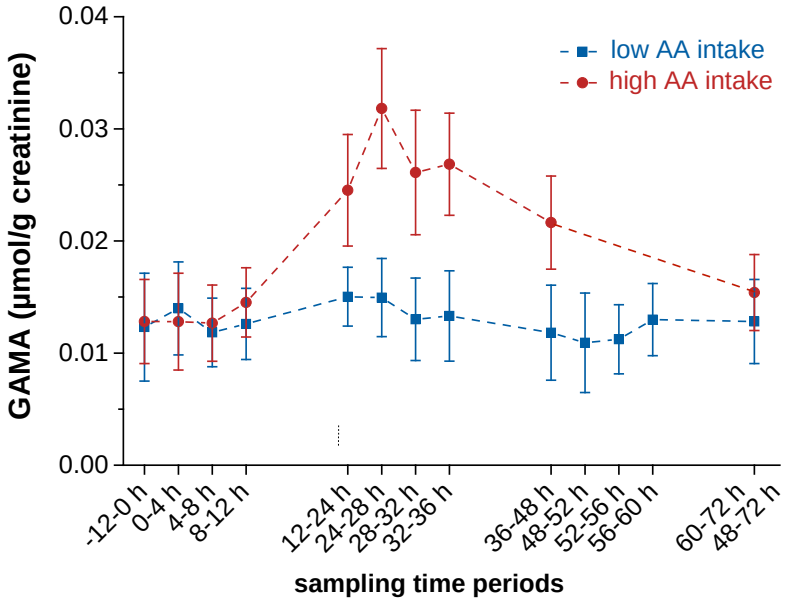
Baseline day 3 of initial washout → equivalent to 0.2 - 0.3 µg/kg/d putative net AA exposure on washout day 2 , assuming 30% of AA excreted within 24 h as AAMA

[Ruenz et al., 2015, Arch Toxicol]

Human intervention study

Urinary biomarker kinetics after controlled dietary AA intake in a duplicate diet study





For comparison:

Hartmann et al. (2008): N=91 (45 male,46 female), 6 to 80 years (median: 36 years)

AAMA <LOD - 0,59 µmol/g (median 0,13); GAMA <LOD-0,015 µmol/g Crt (median=0,04)

calculated median AA intakes: 0.43 (0.21-1.04) µg/kg bw/d based on Hb adducts; 0.51 (<LOD-2.32) µg/kg bw/d based on mercapturic acids

DFG-MAK (2015) *Biological occupational reference value* ***BAR: AAMA 0,43 µmol/g Crt*** *(100 µg/g Crt)*

(Biologischer Arbeitsstoff-Referenzwert: ***background exposure in reference population of occupationally unexposed individuals*** *)*

[Ruenz et al., 2015, Arch Toxicol]

Exploring biomarkers for the risk assessment of genotoxic carcinogens in food: the example of AA →conclusions

- **Urinary mercapturic acids**:

  short term exposure biomarkers, reflecting toxification and detoxification

  → of specific value for intervention studies
- easily validated by duplicate diet dosimetry
- Useful to address short term kinetics and question of background exposure
- **DNA lesions:**

  first key event in the chain of cellular processes

  leading (eventually, not necessarily) to malignant transformation
- Dosimetry at current levels of consumers exposure
- → providing the perspective to relate DNA damage induced

  in humans by exposure to a specific genotoxic agent

  to background DNA damage and

  to apply read across methodology at level of DNA lesions

Acknowledgement

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