Low Dose Effects of the Dietary Carcinogen Acrylamide (AA)

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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“

- colorants
- taste
- antioxidants
- modified proteins
- flavour

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

Asparagine

Reducing sugar

Schiff base

Acrylamide

3-Aminopropionamide
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);
Critical endpoints for toxicity (animal studies):

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

Benchmark dose $\rightarrow \text{BMDL}_{10} : 0,43 \text{ mg/kg b.w.};$

**Carcinogenicity:** Harderian gland tumors in mice

Benchmark dose $\rightarrow \text{BMDL}_{10} : 0,17 \text{ mg/kg b.w.};$

AA is considered a [genotoxic carcinogen](https://example.com), 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

**Benchmark dose (BMD):** dose related to a defined (benchmark) response (e.g., 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“

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*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\(_{10}\)*

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

Acrylamide: toxification / detoxification

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

Reaction with proteins (Hemoglobin: Hb-adducts)

→ surrogate – biomarker for DNA-adduct formation

Acrylamide → CYP450 2E1 → Glycidamide → epoxide hydrolase

GSH-conjugation → mercapturic acids

DNA-N'-guanine-adduct (by far predominant)

→ apurinic sites
→ ring opening formamidopyrimidine
Genotoxicity/ Mutagenicity – a comparison at the level of activated carcinogens

- Glycidamide (GA) the genotoxic AA metabolite,
  - preferentially alkylation $N^7$ of guanine

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

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

[Thielen et al. 2006, Baum et al. 2008]
GA a potent mutagen?
Comparison of induction of \textit{hPRT}-mutations in V79 cells

\begin{itemize}
\item \textbf{NOZ-2: potent mutagen (\geq 3 \, \mu M)}
\item \textbf{GA: much less potent (\geq 800 \, \mu M)}
\item \textbf{BPDE: potent mutagen (\geq 3 \, \mu M)}
\end{itemize}

[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)

Biomarkers
  → Hemoglobin-(Hb-)adducts in blood (AA-Val/GA-Val)
  → Mercapturic acids (MA) in 24 h urine

SD rats: 3 animals/group (male, about 200 g)
Acrylamide biomarkers of exposure
(Hb-adducts, mercapturic acids)

AA uptake: Drinking water (DW), French Fries (FFS, FFR)

GI-tract

AA/GA-Hb-Adducts

systemic distribution via blood

liver

GA + GSH-Add.

kidney

urine

AA/GA-Mercapturic acids

tissue

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

- **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)

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

[Berger et al., Mol Nutri Food Res 2010]
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
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 1-14C-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

[Watzek et al., Chem. Res. Toxicol., 2012]
Mercapturic acids and N7-Ga-Gua adducts

**Mercapturic acids (MA)**

- **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 adducts**

- **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 to 100 µg/kg bw : no dose related response

[Watzek et al., Chem. Res. Toxicol., 2012]
N7-GA-Gua adducts in tissues of rats 16 h after AA dosage (via gavage)

**Dose range: 0 - 100 µg/kg bw**

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]
Human „background“ DNA damage:

<table>
<thead>
<tr>
<th>Adduct</th>
<th>Adducts/10^8 nucleotides</th>
<th>fmol/µmol guanine</th>
<th>Tissue [human]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Oxo-dGuo Epe, 2002</td>
<td>100</td>
<td>5330</td>
<td>lymphocytes</td>
</tr>
<tr>
<td>7-(2'-Carboxyethyl)guanine Cheng et al., 2010</td>
<td>7</td>
<td>373</td>
<td>liver</td>
</tr>
<tr>
<td>N²-ethylidene-dGuo Wang et al., 2006</td>
<td>10</td>
<td>534</td>
<td>liver</td>
</tr>
<tr>
<td>7-ethyl-Gua Chen et al., 2007</td>
<td>0.8</td>
<td>42</td>
<td>liver</td>
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<tr>
<td>N²-ethyl-dGuo Wang et al., 2006</td>
<td>0.2</td>
<td>12</td>
<td>liver</td>
</tr>
<tr>
<td>1,N²-propano-dGuo Zhang et al., 2006</td>
<td>0.3</td>
<td>15</td>
<td>liver</td>
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<tr>
<td>N²-hydroxymethyl-dA Wang et al., 2010</td>
<td>7</td>
<td></td>
<td>leucocytes</td>
</tr>
</tbody>
</table>
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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References:

Mutagenicity/ Genotoxicity

Bioavailability from food: Hemoglobin / Mercapturic acid biomarker response in rats

Dose/Response study: DNA damage and mercapturic acid excretion

Toxicokinetics in rat hepatocytes

Biomarker based human exposure comparison: Acrylamide/ Acrolein

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