Current Status on Acrylamide Research in Food
- Analytics, Formation, Toxicity -

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How it started

Swedish National Food Agency (Tareke et al., JAFC, April 2002):
• High concentrations of acrylamide in highly heated, carbohydrate-rich foods, particularly in fried and baked foods like French fries, potato crisps and crisp bread.
• No acrylamide was found in raw and boiled foods.

Molecule: C₃H₅NO, MW = 71.08
Melting point: 84.5 °C
Boiling point: 125 °C (25 mm Hg)
Solubility (water): 2.215 g/L (30 °C)
Stability: Polymerization above m.p.
Reactivity: Reacts with acids, bases, oxidizing agents
Use: Polyacrylamide production (clarifying water, gels for laboratories, soil stabilization, etc.)
Exposure: Dermal adsorption (solution), inhalation (dry monomer, aerosol), ingestion (food)
Scope

- Progress in Safety Assessment
  - Toxicity
  - Dietary intake
- Progress in Analytics:
  - Analytical methods
  - Occurrence in food
- Progress in Formation and Mitigation
  - Mechanisms and precursors
  - Food chemistry and physics
  - Reduction of acrylamide
- Conclusions and Outlook
Reactivity and metabolism

- Epoxidation to glycidamide (plasma half life of 2 h, oral administration in rat).

- Reaction with glutathione (Michael-type nucleophilic addition), excreted via the urine.

- AA and glycidamide form covalent adducts with DNA and proteins (e.g. haemoglobin → biomarkers).

- Only glycidamide-DNA adducts identified \textit{in vivo}.

\textbf{Friedman, J. Agric. Food Chem., 2003}
**Neurotoxicity**

- Acrylamide is a human neurotoxin, described for occupational exposure.
- Mechanism: Disruption of axonal transport and interference with synaptic transmission.
- NOAEL = 0.2 mg/kg bw/day, LOAEL = 1.0 mg/kg bw/day (rat subchronic drinking water study).

(Hagmar et al., 2001)
Toxic effects

*Mutagenicity*
- AA induces gene mutations and chromosomal aberrations in germ cells, somatic cells, cultured cells *in vitro* and induces cell transformation in mouse cell lines. *(Dearfield et al., Mut. Res., 1995)*
- Only glycidamide is mutagenic in bacterial test systems.
- Dietary intake too low to cause damage (epidemiological study needed).

*Cancerogenicity*
- AA produces cancer in various organs (mammary gland, testis, thyroid) at high concentrations (1-2 mg/kg bw/day) in long-term exp. animal studies.

*Teratogenicity*
- AA is not teratogenic.
Exposure

• Low level human exposure to AA via food from food packaging and polyacrylamide treated drinking water.

• Higher exposures documented in occupational settings in the chemical industry.

• Low dietary exposure
  0.3-0.8 μg/kg bw/day (FAO/WHO)
  0.43 μg/kg bw/day (FDA/NTP)
  0.5 μg/kg bw/day (Svensson et al.)
  No ADI fixed

• However, exposure to young individuals (13-18 years) by a factor of 3 higher.

(Svensson et al., Food Chem. Toxicol., 2003)
Safety assessment: Current status

Classification

Group 2A carcinogen = probably carcinogenic to humans (International Agency for Research on Cancer)
Category 2 carcinogen and category 2 mutagen (EU)
→ No link between dietary acrylamide and some cancers.

Neurotoxic with LOAEL = 1.0 mg/kg bw/day
→ No neurotoxic effects to be expected from dietary AA

WHO

No recommendation for change in dietary habits
Food should not be cooked excessively
Balanced and varied diet recommended
Quantification by GC-MS and LC-MS

Review:

Approach:
Use of internal standards labeled with stable isotopes (e.g. $^{13}$C$_3$-AA, $^2$H$_3$-AA) → Isotope Dilution Assay

Methods:
GC-MS, usually after derivatization, e.g. bromination (Castle et al., 1991)
e.g. silylation (Lagalante & Felter, 2004)

LC-MS, usually w/o derivatisation (Becalski et al., 2003; Riediker & Stadler, 2003; Jezussek & Schieberle, 2003)
### Typical amounts of acrylamide in food

<table>
<thead>
<tr>
<th>Food</th>
<th>AA (μg/kg)</th>
<th>Median</th>
<th>Food</th>
<th>AA (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato crisps</td>
<td>330 - 2300</td>
<td>980</td>
<td>Potato crisps</td>
<td>170 - 3700</td>
</tr>
<tr>
<td>French fries</td>
<td>300 - 1100</td>
<td>410</td>
<td>French fries</td>
<td>200 - 12000</td>
</tr>
<tr>
<td>Fried potato</td>
<td>30 - 700</td>
<td>300</td>
<td>Fried potato</td>
<td>1270</td>
</tr>
<tr>
<td>Biscuits, wafers</td>
<td>&lt;30 - 640</td>
<td>230</td>
<td>Biscuits, wafers</td>
<td>30 - 3200</td>
</tr>
<tr>
<td>Crisp bread</td>
<td>&lt;30 - 1900</td>
<td>135</td>
<td>Crisp bread</td>
<td>800 - 1200</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>&lt;30 - 1400</td>
<td>100</td>
<td>Breakfast cereals</td>
<td>30 - 1350</td>
</tr>
<tr>
<td>Popcorn</td>
<td>360 - 720</td>
<td>390</td>
<td>Gingerbread</td>
<td>90 - 1660</td>
</tr>
<tr>
<td>Bread</td>
<td>&lt;30 - 160</td>
<td>40</td>
<td>Bread</td>
<td>70 - 430</td>
</tr>
<tr>
<td>Boiled potato</td>
<td>&lt;30</td>
<td></td>
<td>Snacks</td>
<td>30 - 1920</td>
</tr>
<tr>
<td>Boiled rice</td>
<td>&lt;30</td>
<td></td>
<td>Beer, malt</td>
<td>30 - 70</td>
</tr>
<tr>
<td>Meat balls</td>
<td>&lt;30</td>
<td></td>
<td>Fish products</td>
<td>30 - 40</td>
</tr>
</tbody>
</table>

Extraction and clean-up are critical steps

Improved NRC method - Flow diagramme - Key Steps

2 g (60°C, 10 mL H₂O) + 2 mL H₂O

Ultra Turrax

Carrez I & II (1 mL each)

Swirling

CH₂Cl₂ (5 mL)

Stirring, Evaporation, N₂

EtAc extract

Aqueous layer (6 mL) (NaCl, 1.8 g, EtOAc, 13 mL)

Centrifugation

Salting-out

Stirring, Centrifugation

Organic layer

SPE clean-up

2 x Extraction

H₂O

Multimode (SPE, 0.5 g)

Eluting H₂O

Conc. 40°C

LC-ESI-MS/MS

Eluate, Aliquote 180 uL + 90 uL MeOH

Protein precipitation

Improved NRC method (LC-MS/MS)


Current method (Delatour et al., 2004)
Cocoa powder (130 µg/kg)

Transition $m/z$ 72 → 27 taken for quantitation

LoD : 4.6 µg/kg
LoQ : 7.9 µg/kg

Repeatability (CV %):
15.8  (12.7 µg/kg)
6.1   (304 µg/kg)
5.4   (2504 µg/kg)

Recovery (%):
43    (12.7 µg/kg)
54    (304 µg/kg)
51    (2504 µg/kg)
Analytical capacities available today suffice

Majority of methods based on LC-MS/MS

Proficiency tests have highlighted problems with certain matrices (e.g. cocoa, coffee) and methods (e.g. GC/MS without derivatization)

Urgent need for fully validated reference method(s) as well as rapid screening methods.
Formation: Possible pathways to acrylamide

a) Hypothesis 1


\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{C} \quad \text{O} \\
\text{H} & \quad \text{C} \quad \text{O} \\
\text{H} & \quad \text{C} \quad \text{O} \\
\text{H} & \\
\text{Triglycerides}
\end{align*}
\]

→ \text{H}_2\text{C}=\text{CH}-\text{C}=\text{H} \quad \rightarrow \quad \text{H}_2\text{C}=\text{CH}-\text{C}=\text{OH}

\text{Acrolein} \quad \text{Acrylic Acid}

→ only ~5% of the yield compared to Asn

b) Hypothesis 2

\[
\begin{align*}
\text{COOH} & \quad \text{O} \\
\text{HC}-\text{CH}_2-\text{C}-\text{NH}_2 & + \quad \text{Glucose} \\
\text{NH}_2 & \\
\text{Asparagine}
\end{align*}
\]

Procter & Gamble (Sanders et al., Sept. 2002)
Univ. Reading / Leeds (Mottram et al., Oct. 2002)
Nestlé Research (Stadler et al., Oct. 2002)
Food control laboratory (Weisshaar et al., Nov. 2002)
Cantonal laboratory (Biedermann et al., Dec. 2002)
Strecker aldehyde versus $N$-glycoside

(Mottram, 2002)  (Stadler, 2002)

![Chemical structures](image)

Strecker aldehyde versus $N$-glycoside


![Graphs showing acrylamide formation](image)
Decarboxylated Amadori comp.

(Yaylayan, 2003)

(Zyzak, 2003)

Vinylogous compound (Styrene)

Strecker aldehyde

Strecker alcohol

m/z 251

(Blank et al., ACS Symposium, 2004)
Nestlé Research Center

2004-08-30 NRC/FCI - IBk

[Diagram showing chemical reactions]

AA (mmol/mol)

\[
\begin{align*}
\text{Glc} & \quad 0.6 \\
\text{Asn} & \quad 2.4 \\
\text{Asn} & \quad 0.1 \\
\text{Asn} & \quad \sim 0.3 \\
\text{Asn} & \quad 3-4 \\
\text{Asn} & \quad 0.2
\end{align*}
\]

(Blank et al., ACS Meeting, 2004)

[Chemical structures and reactions shown in the diagram]
3-Aminopropionamide

\[
\text{3-APA} \rightleftharpoons \text{NH}_2
\]

(Zyzak, 2003)

\[
\text{O} \quad \text{NH}_2
\]

\[
\text{NH}_2 \quad \text{NH}_2
\]

\[
\text{H}_2\text{N} \quad \text{OH}
\]

\[
\text{O} \quad \text{NH}_2
\]

\[
\text{NH}_2 \quad \text{NH}_2
\]

L-alaninamide

\[
\text{3-APA} \quad 292 \quad (\text{mmol/mol})
\]

\[
\text{+ Glc} \quad 109
\]

## Formation: Current status

<table>
<thead>
<tr>
<th>Route/Intermediate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycoconjugates of Asparagine (Asn)</strong></td>
<td></td>
</tr>
<tr>
<td>N-Glycosides of Asn ⇒ AA</td>
<td>Stadler <em>et al.</em>, 2002</td>
</tr>
<tr>
<td>Schiff base of Asn ⇒ AA</td>
<td>Zyzak <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Decarboxylation of the Schiff base + β-elimination of the Amadori product</td>
<td>Yaylayan <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><strong>3-Aminopropionamide from Asn</strong></td>
<td></td>
</tr>
<tr>
<td>3-APA ⇒ AA</td>
<td>Granvogl <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Acrylic acid and ammonia</strong></td>
<td></td>
</tr>
<tr>
<td>Acrylic acid + NH₃ ⇒ AA (~5% of yield from Asn)</td>
<td>Stadler <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Acrolein + NH₃ ⇒ AA</td>
<td>Yasuhara <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>2-Propenal ⇒ Acrylic acid</td>
<td>Vattem &amp; Shetty, 2003</td>
</tr>
<tr>
<td>Polyacrylamide is not a precursor (175°C)</td>
<td>Ahn <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Other amino acids</td>
<td>Yaylayan <em>et al.</em>, 2004</td>
</tr>
</tbody>
</table>
Mitigation: Effect of the type of sugar

Acrylamide amount in the sample (measured by HPLC-DAD)

- Fructose is more efficient in generating acrylamide under low moisture conditions!
- Chemical reactivity of sugar does not explain this phenomenon.

Hypothesis: Molecular mobility plays a key role in AA formation by solid-state Maillard reactions.

Acrylamide formation depends on melting point of the sugars.

Effect of moisture and pH in model systems

- Fructose is more reactive than glucose in generating acrylamide from asparagine.
- Moisture does influence acrylamide formation.

- Fructose is more reactive than glucose.
- Optimum for acrylamide formation at pH 8.
- Low pKₐ value of Asn (8.9), i.e. α-NH₂ less protonated

(Blank et al., *unpublished*, 2004)
Effect of pH in potatoes

Homogenized potatoes
T = 180 °C, t = 25 min

Homogenized potatoes
t = 25 min (pH ~5.8)

French fries
190 °C, 6.5 min


(Jung et al., J. Food Sci., 2003)
Effect of reaction time and temperature

Reaction time and temperature are covariant parameters. High amounts of acrylamide formed at 120-160 °C, depending on reaction time. (Robert et al., in press)

Acrylamide amounts in potatoes depend on surface-to-volume ratio (SVR) and processing time and temperature. (Taubert et al., 2004)
# Mitigation: Current status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relative effect</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>More asparagine</td>
<td>+++</td>
<td>Direct precursor (e.g. potato)</td>
</tr>
<tr>
<td>More red. sugars</td>
<td>+++</td>
<td>Initiating Maillard reaction</td>
</tr>
<tr>
<td>Add amino acids (Gly)</td>
<td>--</td>
<td>Consumption of red. sugars</td>
</tr>
<tr>
<td>Higher T</td>
<td>++/-</td>
<td>Pyrolysis / decomposition</td>
</tr>
<tr>
<td>Longer t</td>
<td>++/-</td>
<td>Pyrolysis / decomposition</td>
</tr>
<tr>
<td>pH</td>
<td>+/-</td>
<td>pH 8 optimum, low pH: less AA</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>?</td>
<td>No clear effect</td>
</tr>
<tr>
<td>Radical initiators</td>
<td>?</td>
<td>No clear effect</td>
</tr>
<tr>
<td>Water binding</td>
<td>--</td>
<td>Reduced pyrolysis</td>
</tr>
<tr>
<td>Asparaginase</td>
<td>---</td>
<td>Removal of asparaginate (patent)</td>
</tr>
<tr>
<td>Fermentation</td>
<td>--</td>
<td>Lowering the pH (patent)</td>
</tr>
</tbody>
</table>

Conclusion and outlook

- Importance to understand the unexpected presence of acrylamide in food
- Major pathways have been elucidated
- Investigate ways to reduce overall intake via food
- Further research on safety assessment of acrylamide in food needed (epidemiological studies)
- Insufficient data to justify changes in dietary advice
- Need to work in partnership and co-ordinate the research
Acknowledgments

Organization committee
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Analytics
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