Biomarkers of acrylamide

Jan Alexander
Department of Food Safety and Nutrition

Norwegian Institute of Public Health

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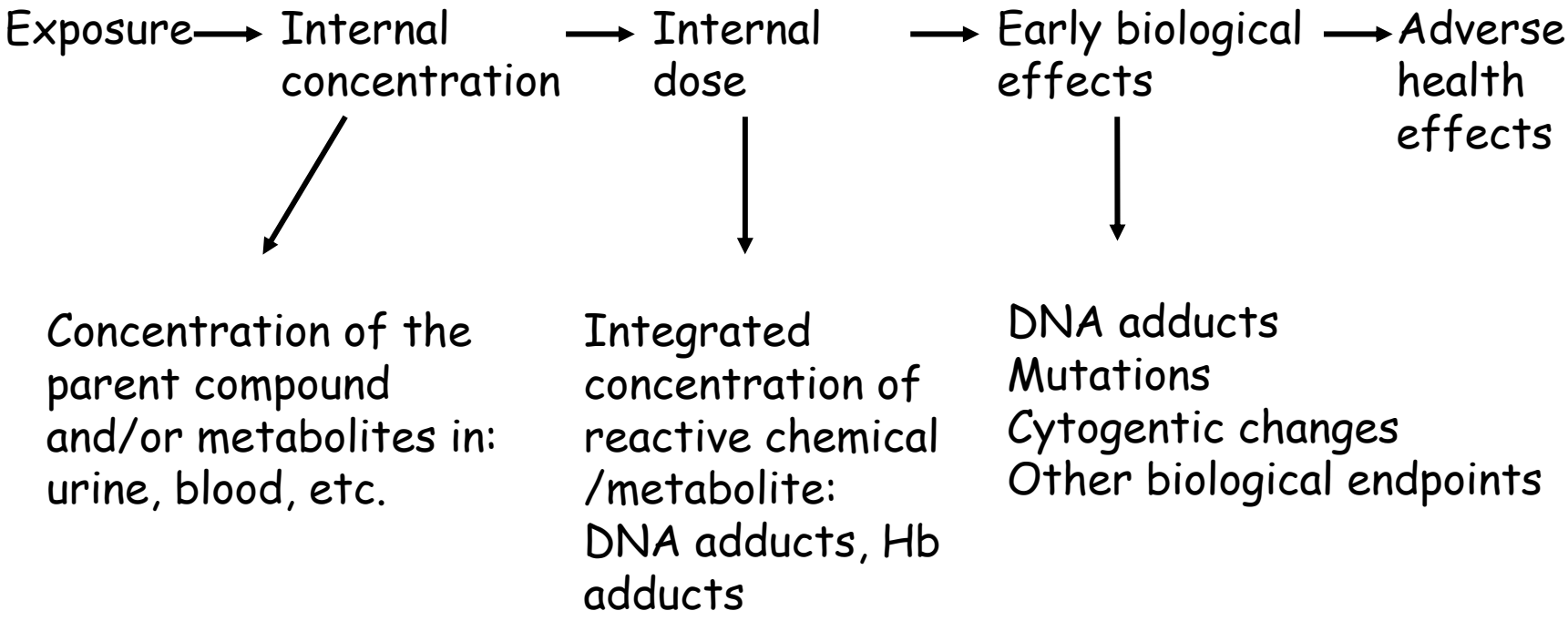
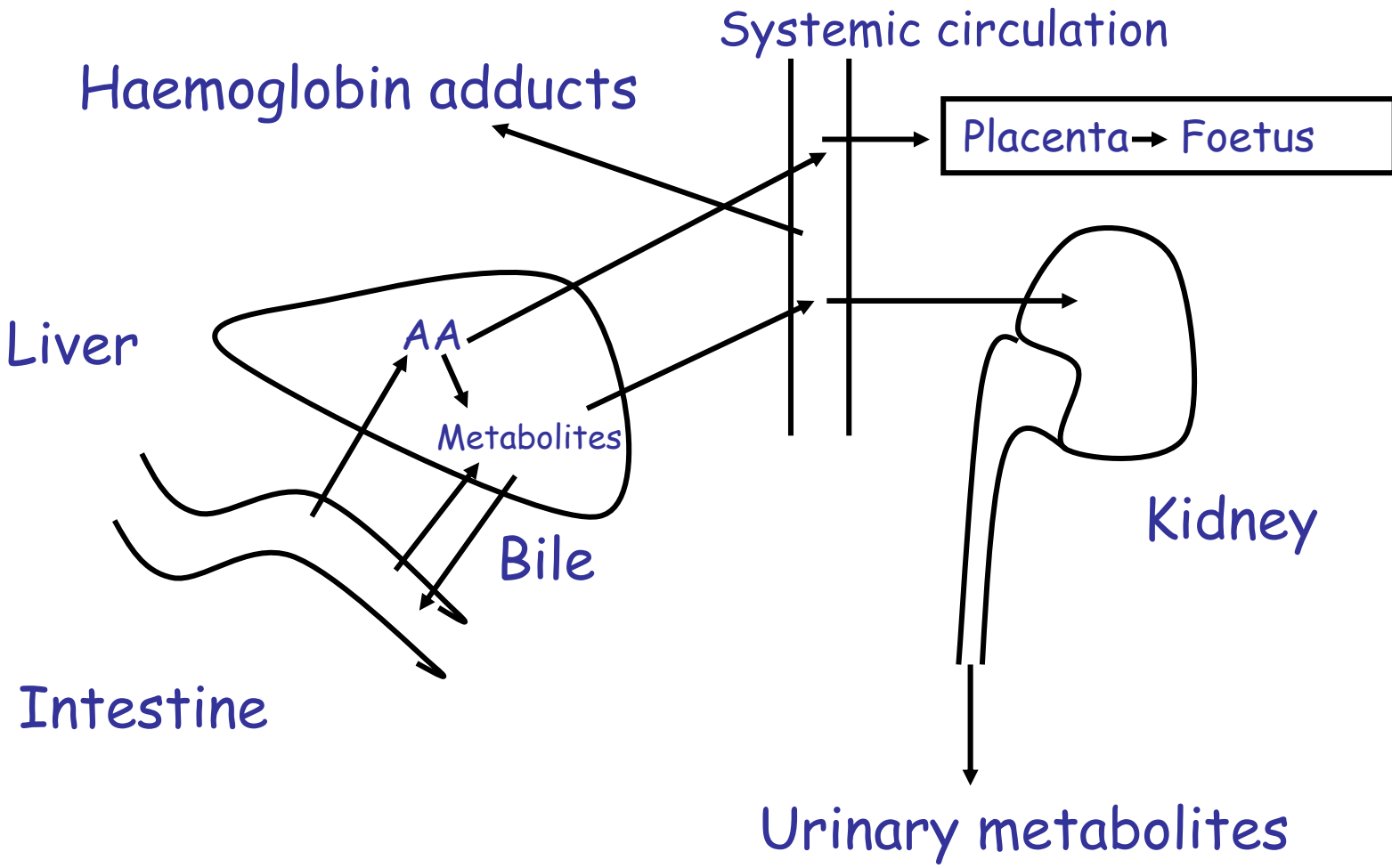
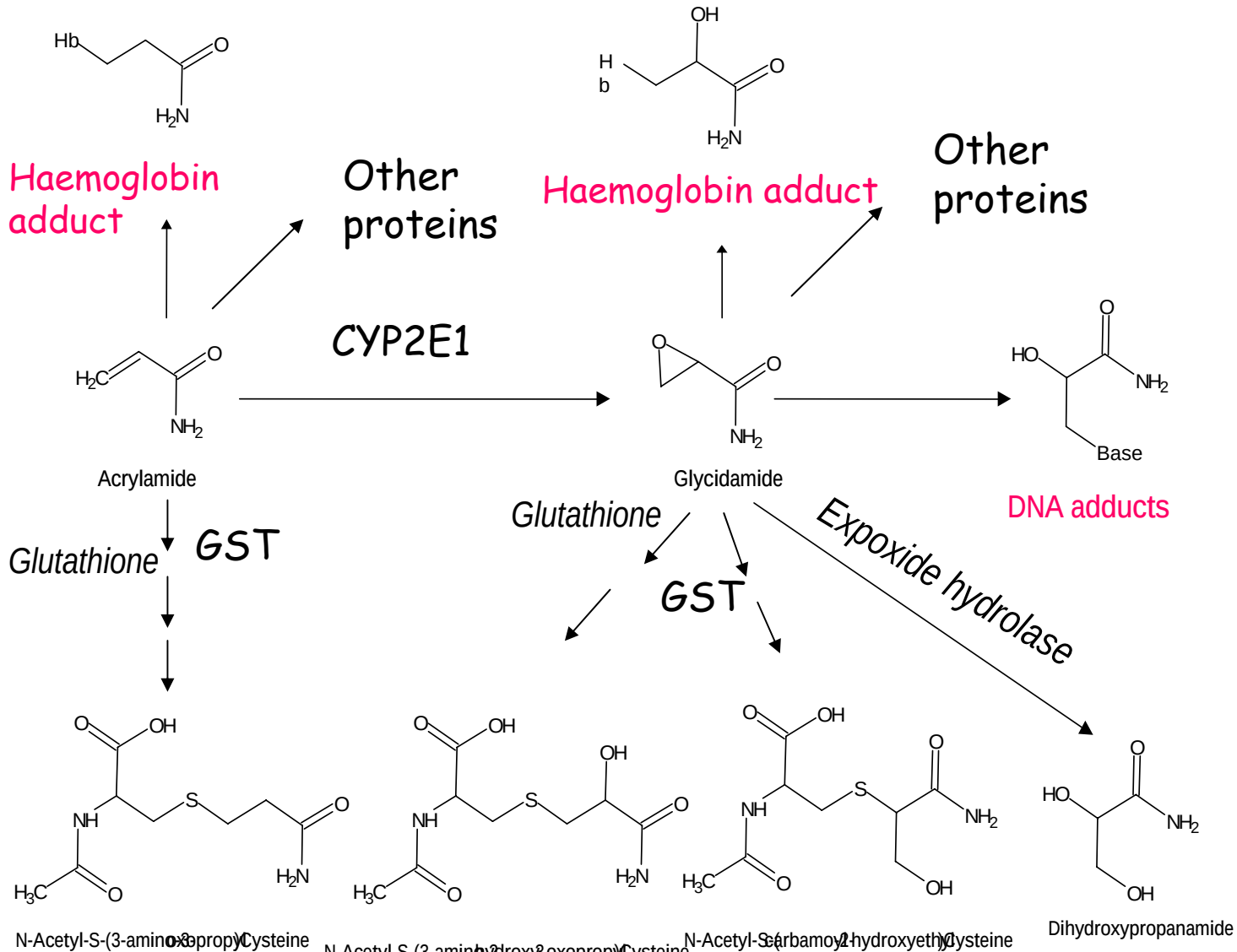
Scheme of biomarkers



Absorption, distribution, metabolism and excretion



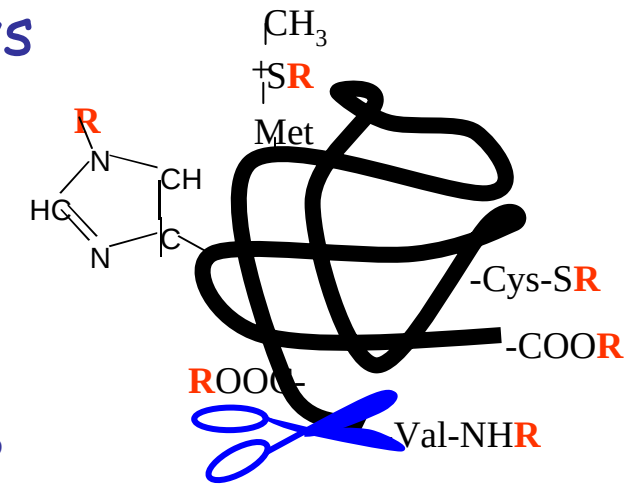
Biotransformation



Acrylamide – chemical biomarkers

- *AA* and *GA* are both reactive compounds, T1/2: 4.6 and 1.9 hrs in humans
- Urinary mercapturic acid, T1/2 ~ 3.5 hrs
- DNA adduct, major T1/2 ~ 4 days
- Hb-adducts – stable, life time that of red blood cells,
  - ~ 120 days humans,
  - rat 60 days, mouse 40 days

N-terminal valine adduct determined



Transplacental transfer

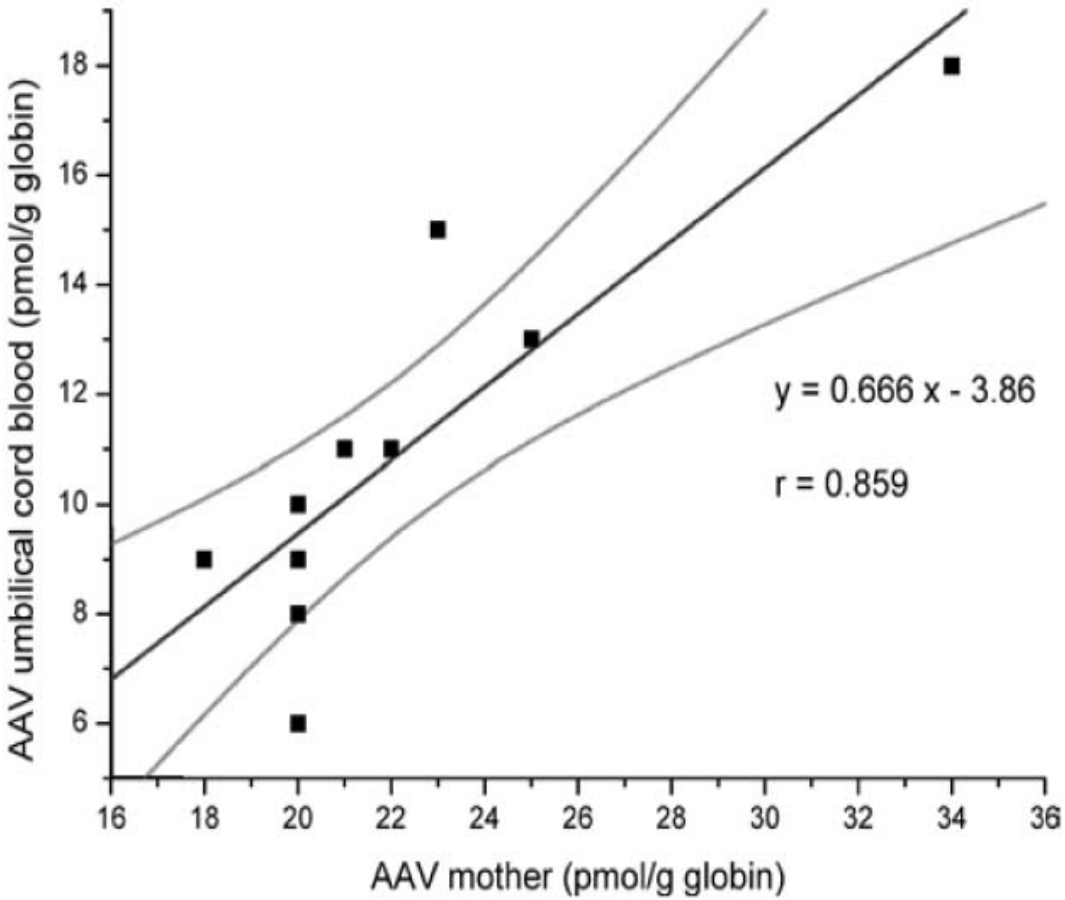


Fig. 1 Correlation between haemoglobin adducts of acrylamide (AAV) in mothers' blood and umbilical cord blood. Note that the results for the smoking woman (person no. 1) have been excluded in this correlation

Schettgen et al 2004

Determinants of biomarkers

- External exposure
- Modifiers:
  - Other sources of exposure than food, e.g. smoking
  - Metabolic capacity of enzymes
    - Developmental stage
      - newborn vs. children vs. adults
    - Genetic – polymorphisms
      - GST enzymes, Cyp 2E1, EPxH
    - Inducers, ketones, ethanol
    - Inhibitors, ethanol

Purpose of using biomarker

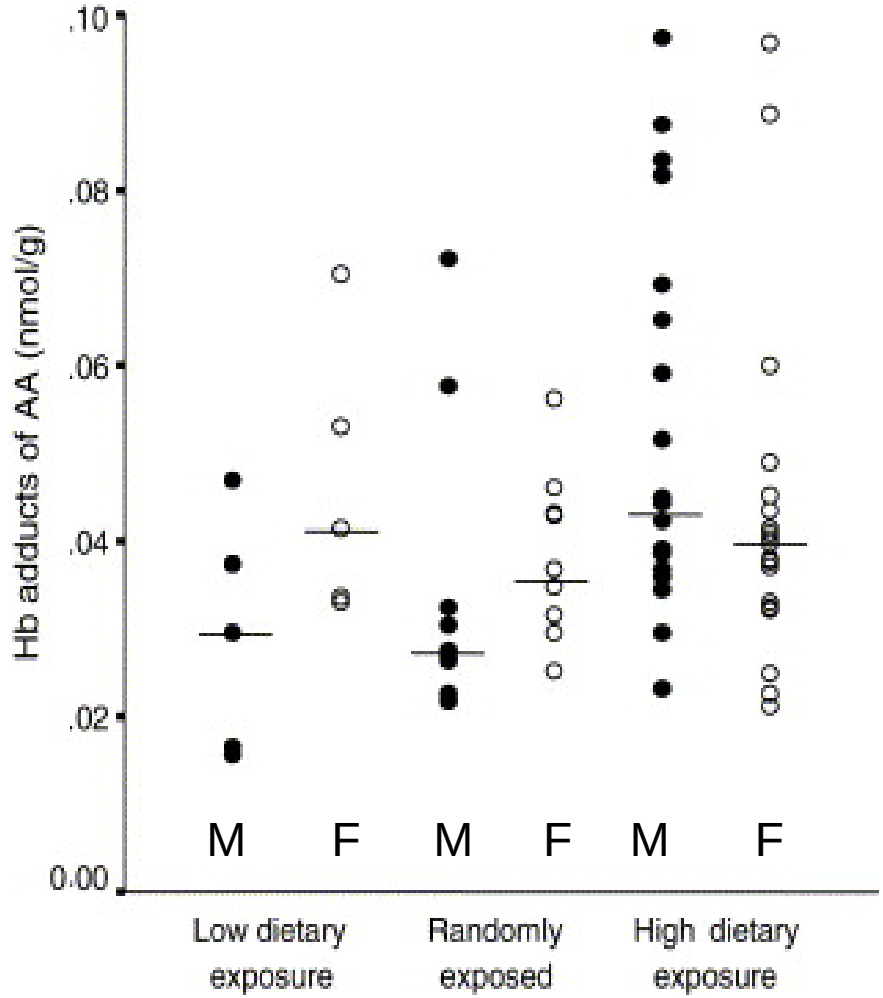
- Validate external dose estimate
- Determine food source
- Determine bioavailability
- Assess conversion rate from *AA* to *GA*
- Compare dose parameters with early biomarkers of effect
- Assess internal dose for use in epidemiological studies
- Extrapolation between species in risk assessment

Biomarkers of acrylamide

- Haemoglobin adducts of *AA* and *GA*
- Urinary metabolites
  - Mercapturic acids
- DNA adducts
  - Only used in animal studies
- Chromosomal abberations
  - Studies on workers exposed to acrylamide
- Micronucleus assay,
  - Studies following dietary exposure
- Sister chromatide exchange and mutations
  - Only used in *in vitro* or animal studies

External exposure vs. biomarkers

Intake estimates by food frequency questionnaire vs haemoglobin adducts



Hagmar et al. 2005

External exposure vs. biomarkers

- Bjellaas et al 2007: Hb adducts did not correlate with estimated dietary intake, (n=50)

- Wirfält et al 2008: Hb adducts: Strong correlation with smoking, weaker correlation with estimated dietary acrylamide in non-smokers (n=40)

- Kütting et 2008: Hb adducts: Strong correlation with smoking, weak but significant correlation with estimated dietary exposure in non-smokers, r_{sp} = 0.178 and 0.168 in females and males, respectively (n=828)

External exposure vs. biomarkers

- Non- or weak correlations between dietary intake estimates and AA-Hb adducts. Correlation dependent on accuracy of intake estimate.
- Strong influence of smoking

External exposure at population level fits well with biomarkers:

Background Acrylamide Hb adducts:

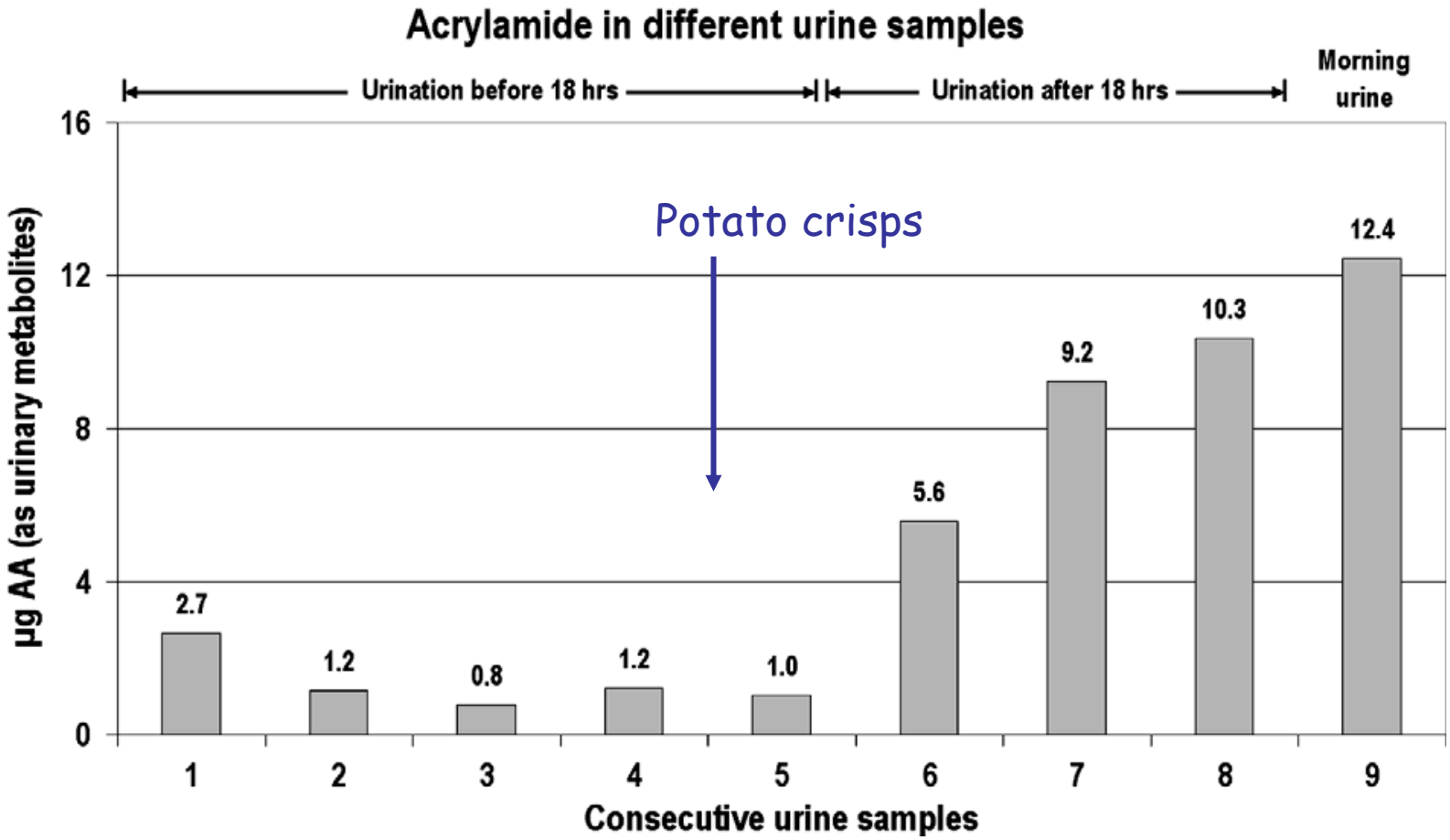
0.01 – 0.05 nmole/g, average: 0.03 nmole/g

Corresponds to an intake of about:

80 µg/ day or 1.1 µg/kg bw/day

Taken from Törnqvist

External exposure and urinary biomarkers



Bjellaas et al 2007

External exposure and urinary biomarkers

- In a study of 47 non-smoking persons estimated acylamide intake did not correlate with urinary biomarkers.
  - 24 hrs AA derived urinary metabolites: 16 (7-47) µg
  - dietary intake 24 hr recall: 21 (13-178) µg

Bjellaas et al 2007

- In a study of 119 pregnant non-smoking women the dietary median and (95 perc.) intake of acrylamide was:
  - 0.48 (0.92) µg/kg bw per day as estimated by FFQ
  - 0.41 (0.82) µg/kg bw per day as estimated by Food diary
  - 0.42 (0.70) µg/kg bw per day as estimated by probabilistic approach

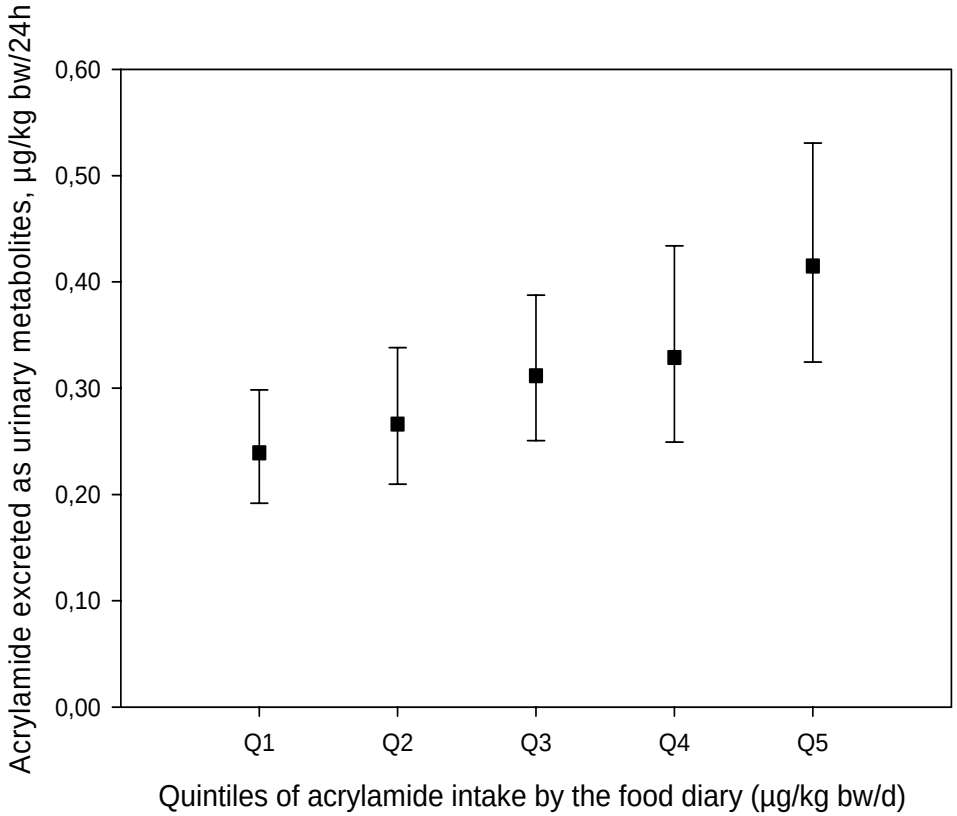
- total 24 hrs AA-derived urinary metabolites was:
  - 0.16 (0.50) µg/kg bw per day
  - Assuming 55 % recovery: 0.30 (0.91) µg/kg bw per day

  Significant correlation between biomarker and estimated dietary intake

Brantsæter et al. in press

- Reason for discrepancy: New data on acrylamide in Norwegian food items in last study? In Bjellaas et al 2007, For some food items EU acrylamide date for food used. Better basis for more accurate intake estimates improves the correlation.

Ranking of dietary intake vs. urine biomarkers

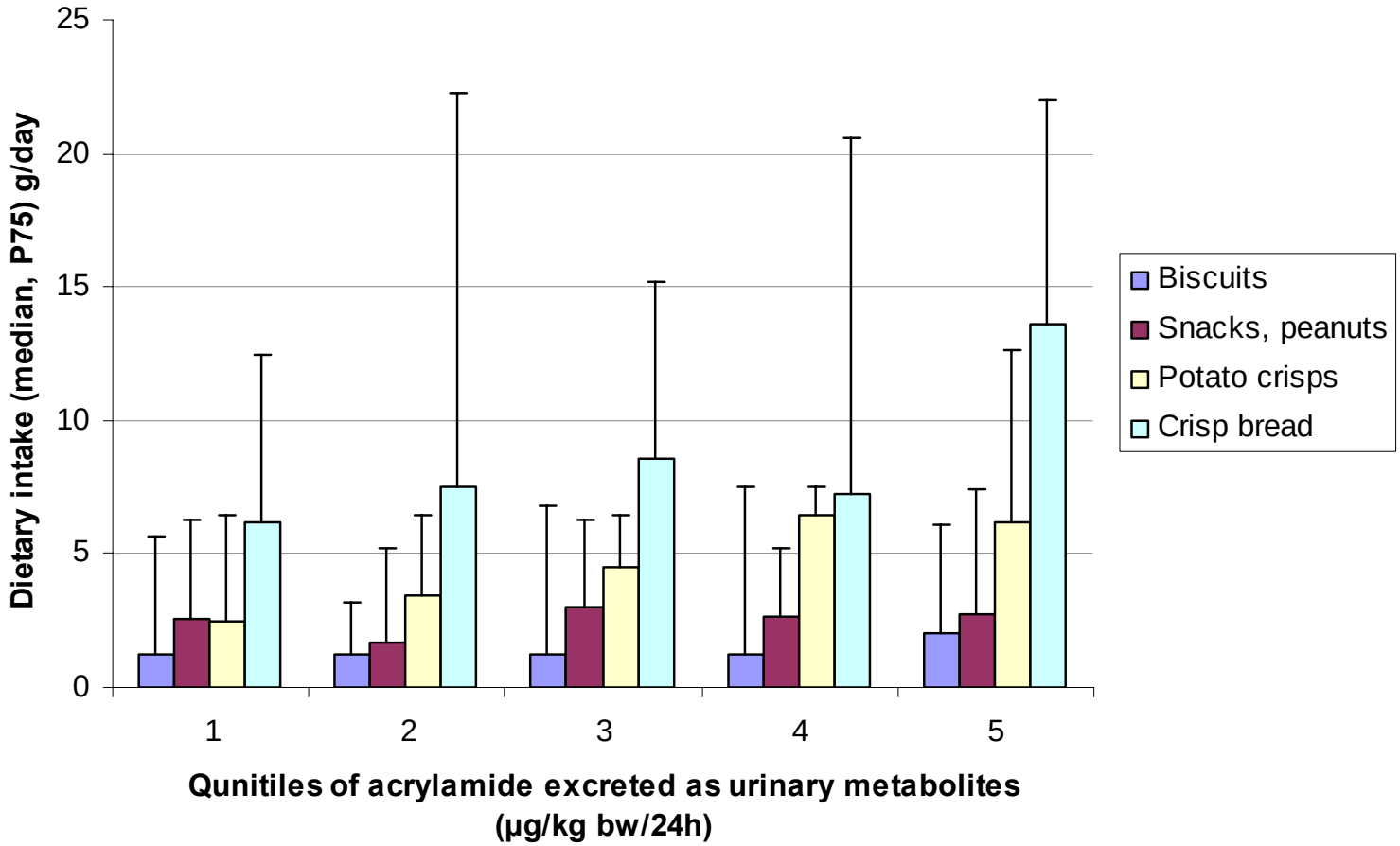


Classification into quintiles:

- 65 % classified in the same or adjacent quintile
- less than 10 % classified into opposing quintile

Brantsæter et al. in press

Contributing foods in various quintiles of urinary biomarker levels



Brantsæter et al. in press

Urinary biomarkers

Urinary biomarkers can be used for validation of dietary intake estimate (FFQ, FD Probabalistic) and ranking of of persons with regard to exposure

Brantsæter et al in press

Disadvantage with urinary biomarkers:

- Short term exposure only
- Not all major metabolites (e.g. glyceramide) determined

Foods contributing to Hb adducts or urinary biomarkers in Norway

- *AA*-Hb adducts: crisp bread, potato chips/snacks
- No correlations with *GA*-Hb-adducts (n=50)

Bjellaas et al 2007

- Total *AA*-derived urinary metabolites correlated with intake of aspartic acid, protein, starch and coffee (n=53)

Bjellaas et al 2007

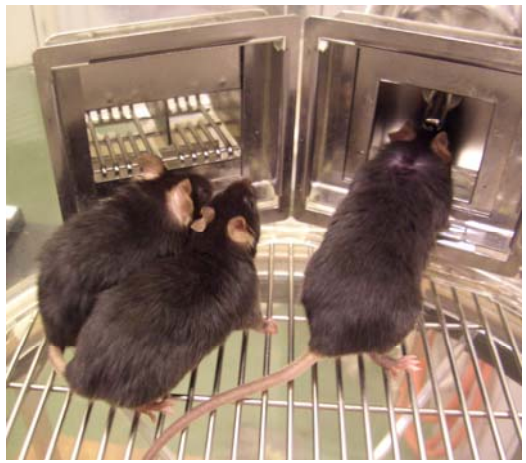
- Total *AA*-derived urinary metabolites correlated with intake of crisp bread, potato crisps, cooking oil and garlic. (n= 119 pregnant women)

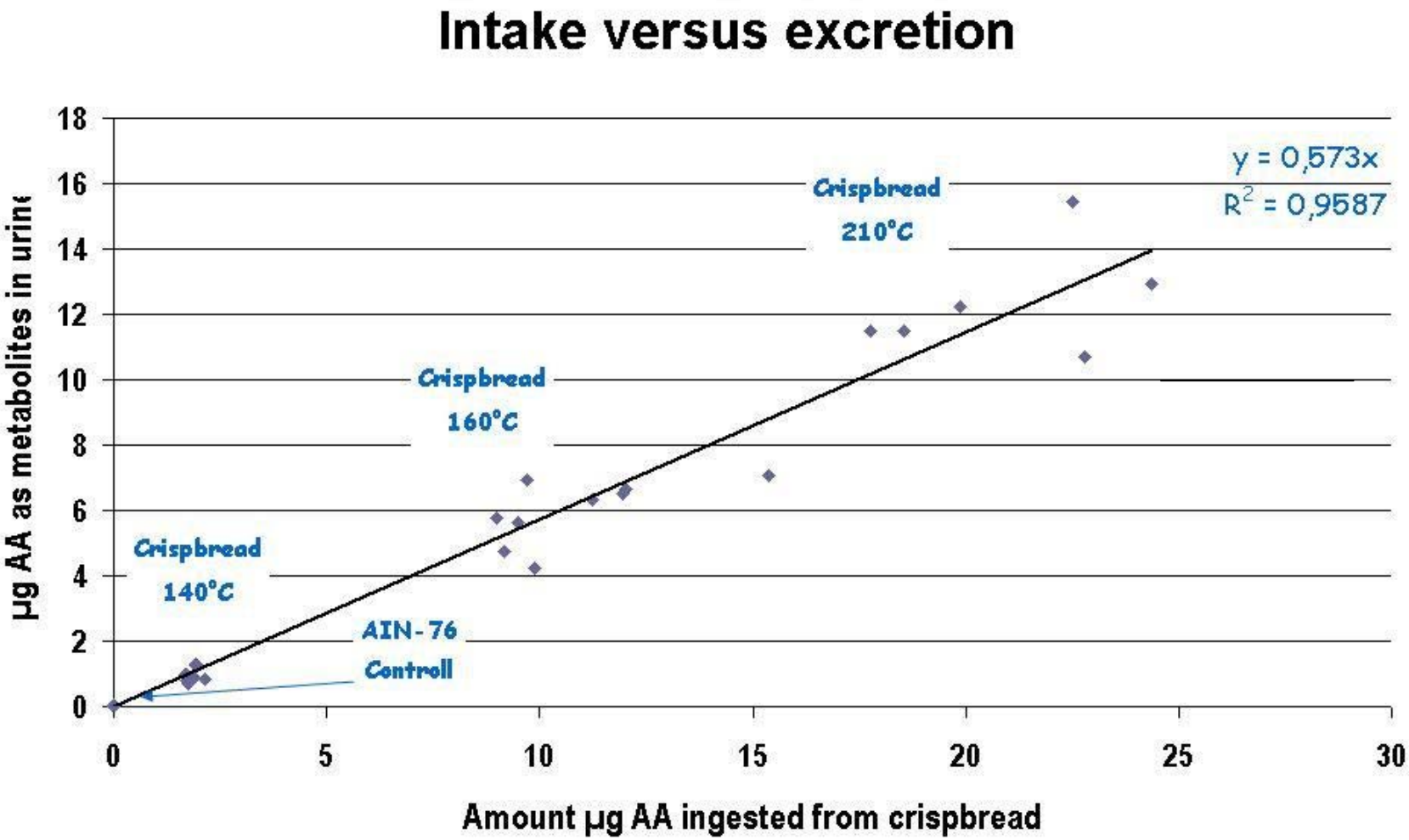
Bratsæter et al, in press

Biomarkers and bioavailability

Bioavailability of crisp bread in mice

– Crisp bread with different content of AA
  - 191 µg/kg,1020 µg/kg, 2650 µg/kg
– Steady-state feeding: 24 h intake crisp bread (4x)
– Steady-state excretion: 24 h urinary metabolite

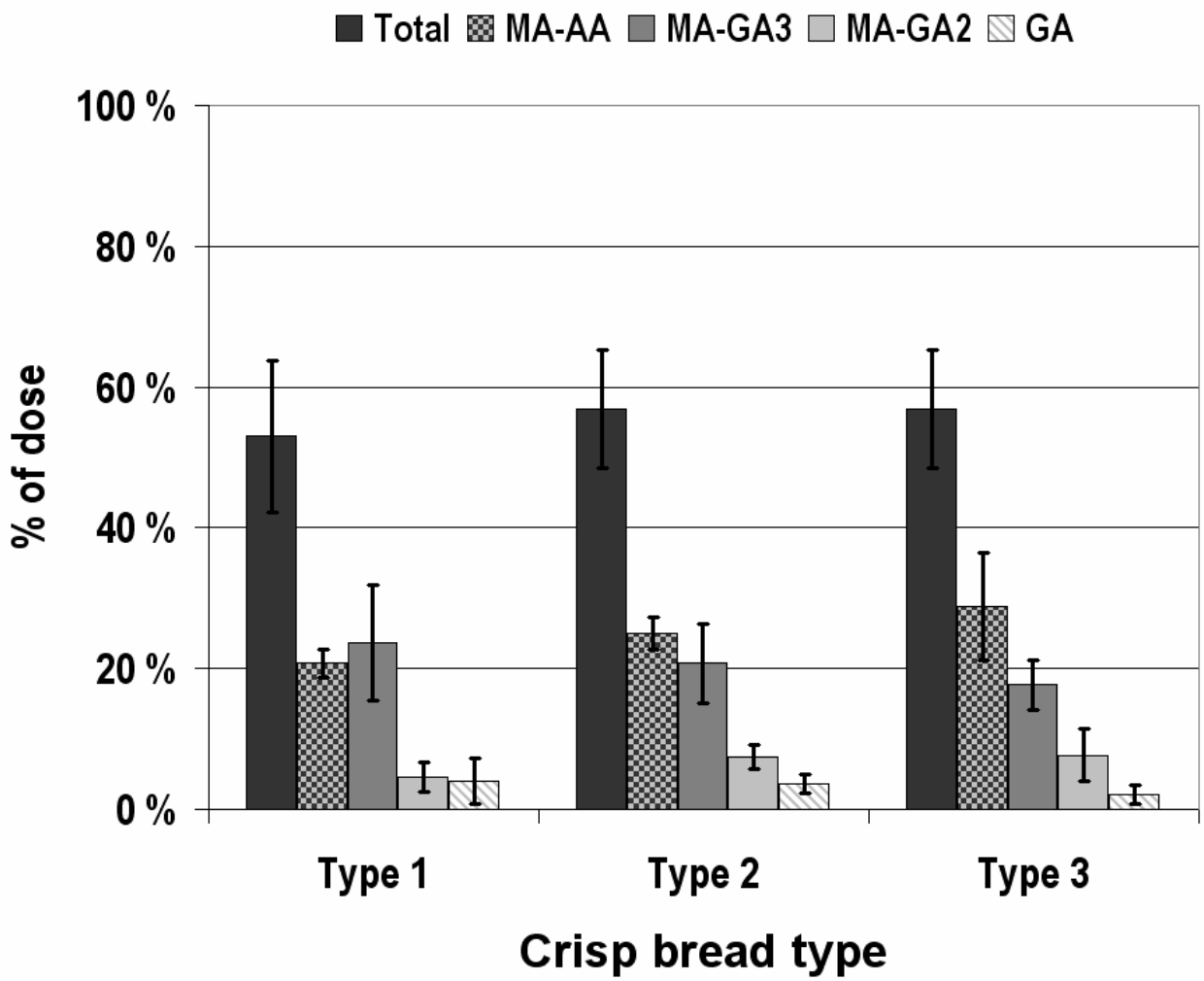




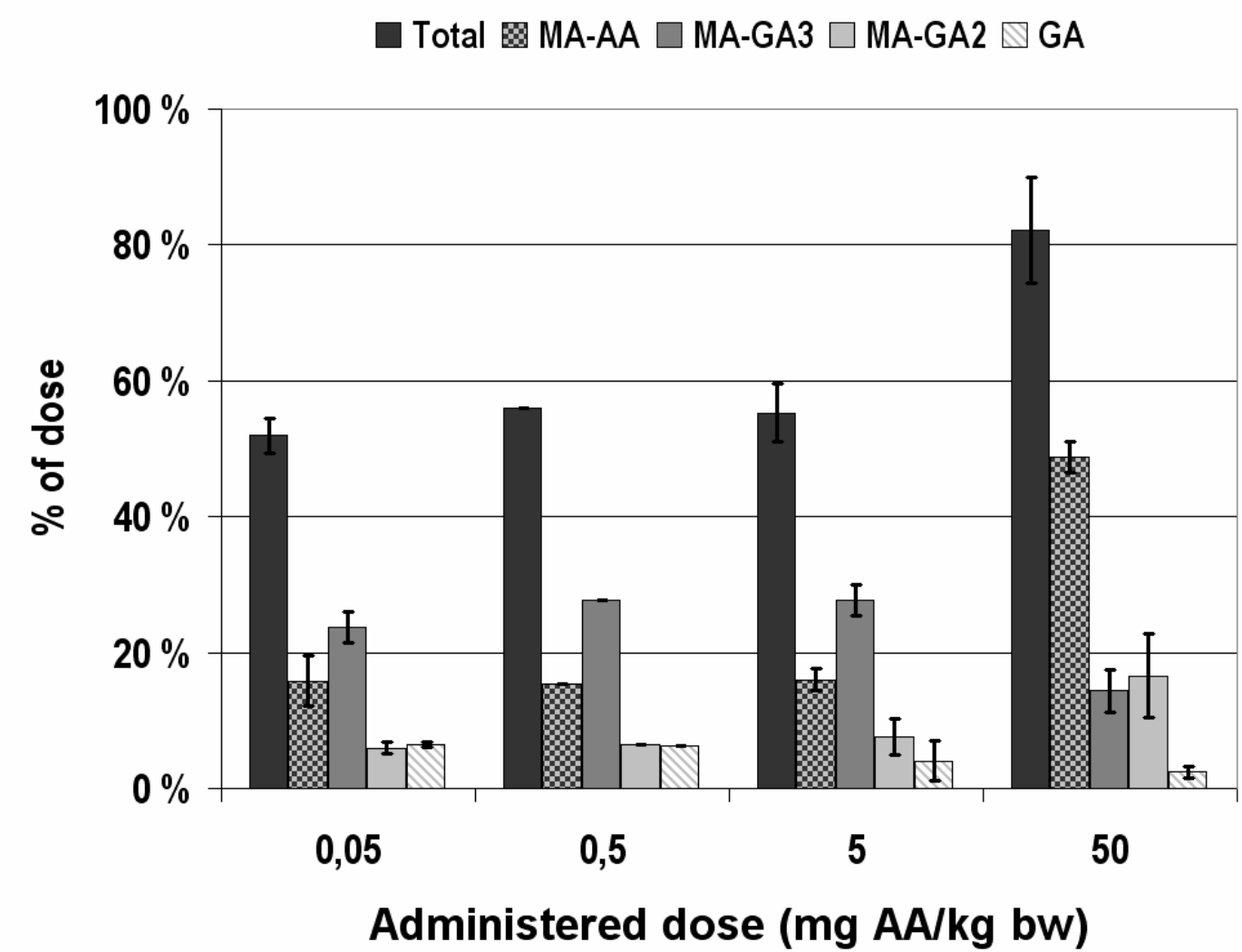
Recovery of urinary AA metabolites from crisp bread feeding = 55%
Recovery of urinary AA metabolites from sc AA injection (0.05 – 5 mg/kg bw) = 54%
Bioavailability ~ 100%

Bjellaas et al 2007

Recovery of AA as urine metabolites



Recovery of AA as urine metabolites



Enzymic polymorphism

- Hb adduct levels were studied *in vitro* with fresh blood from *GSTT1-/+* and *GSTM1-/+* donors followed by incubation with acrylamide.

- No effect of genotype or GST/EPXH inhibitor (effect with pos. control: ethylene oxide)

Paulsson et al 2005

- Tendency of influence on Hb adduct level of *GSTT1-/+* and *GSTM1-/+* status of tunnel workers exposed to acrylamide

Kjuus et al 2005

Internal dose and epidemiology

- Significant positive association between AA-Hb adduct level and oestrogen receptor positive breast cancer after adjustment for possible confounders
  - RR: 2.7 (CI 1.1-6.6) per 10 fold increase in adduct level.

Danish Diet, Cancer and Health Study: case control study, cases 374, controls 374

Olesen PT et al 2008)

Intake of food containing high level of acrylamide

- In a study from Sweden individuals were assigned to two groups
  - group 1: eating food, which were low in acrylamide
  - group 2: eating the same food as group 1, but fried, in addition potato crisps

  The eating were done for 4 days
  The acrylamide intake was about 15 times higher in group 2.
  Micronuclei (MN) were determined before and after.
  - The change was significantly higher in group 2
  - Incrased acrylamide in group 2 was confirmed by AA-Hb adducts

  But, according to author, compared with data on acrylamide doses inducing MN in mice, it is unlikely that the particular amount of acrylamide could have caused this increase

Zetterberg Abramsson et al, in press

Conclusion

- A lot of progress has taken place in the use of biomarkers for various purposes in studies on acrylamide
- Biomarkers can probably be very useful in various aspects of epidemiological studies
  - particularly as internal dose parameter
  - Validation of dietary intake calculations
  - For dietary acrylamide and cancer studies smoking is a confounder – influences biomarker level and influences outcomes because of high content of other carcinogens
- The use of biomarkers for extrapolation of risk from animals to humans is still limited as:
  - studies in animals of adverse outcomes have not used biomarkers
  - studies with early endpoints are still limited
  - PBPK modelling could facilitate the inter species comparison
- Analytical quality should be assured

Thank you!