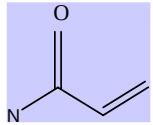


EFSA Scientific Colloquium n°11
Acrylamide carcinogenicity – new evidence in relation to dietary exposure

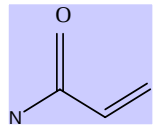
Tabiano, Italy, 22-23 May 2008

Objectives of the Colloquium

Josef Schlatter

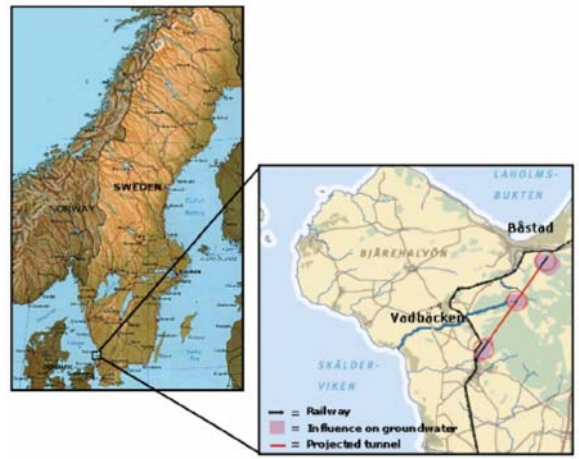
- ✓Short History
- ✓Neurotoxicity / Reproductive Toxicity
- ✓Mutagenicity / Carcinogenicity
- ✓Assessment by the 64 JECFA: MOE Calculation ⇒ BMDL, Intake

Acrylamide: History - 1997



Leakage of acrylamides from a tunnel construction work:
http://ean.cepn.asso.fr/pdf/program4/An-TORNQVIST.pdf

5. August–30. Sept 1997 Hallandsås Tunnel construction.
Application of Rhoca Gil due to water inrush



⇒ **Fish kill**

⇒ **3 cows with paralysis of the hindlimbs**

⇒ **High conc. of Rhoca Gil monomer in water**

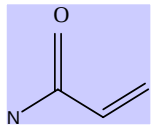
⇒ **Stopp of Rhoca Gil use on 30.9.1997**

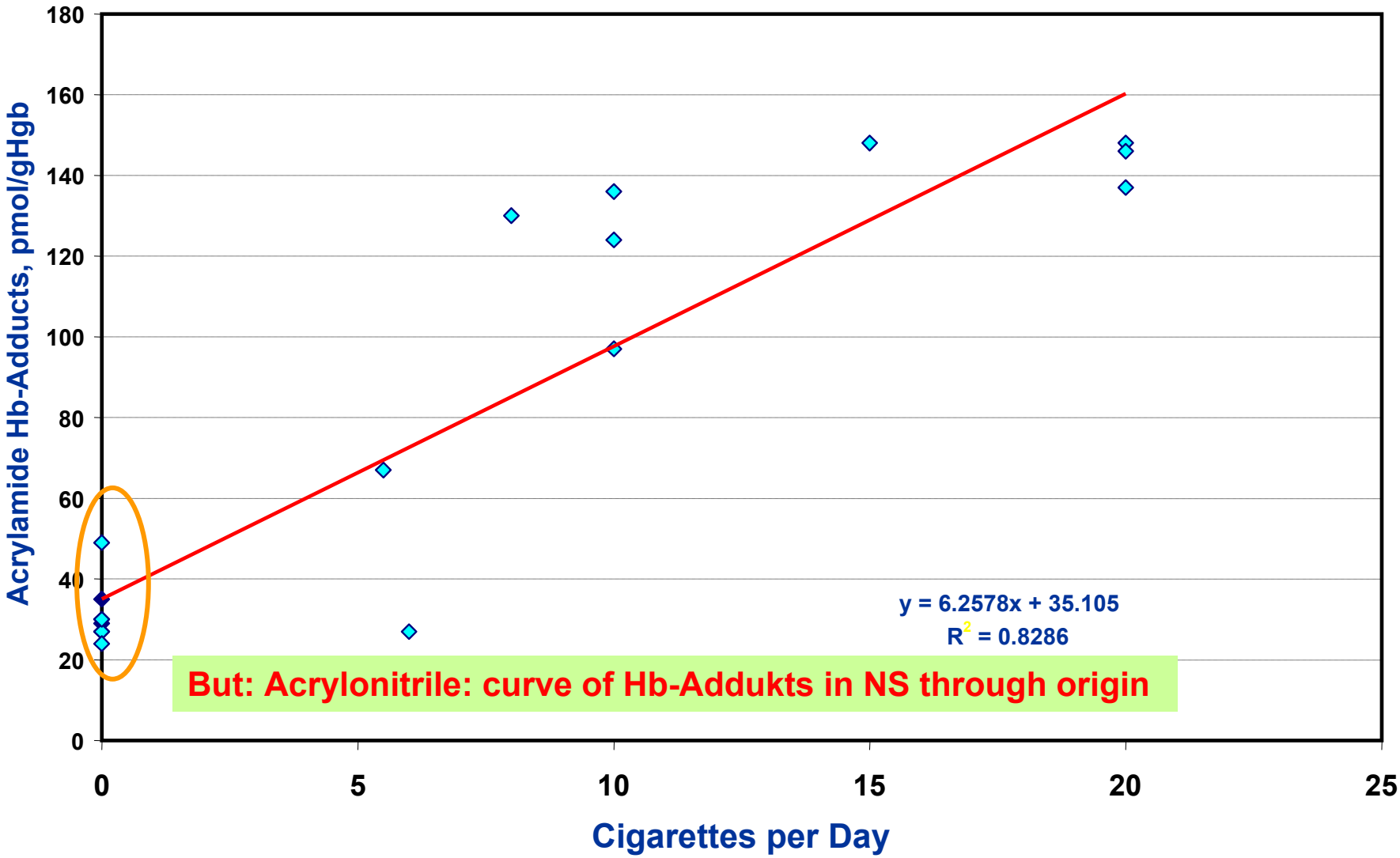
- **7. October: Tunnel construction stopped**
- **10. October: identification of Acrylamide-Hb-Adducts in cows**

➢ **Investigation of the tunnel-workers for Hb-Adducts [Median 250, maximum 3000 pMol/g Hb]**

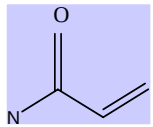
➢ **Adducts also found in non-smoking control grup [40 pMol/g Hb]**

Acrylamide: Hb Adducts in Smokers (Bergmark, 1997)





Acrylamide: History



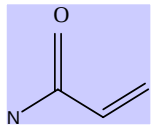
- 2000: Acrylamide Hb-Adducts in rats after feeding on fried feed
- Investigation of foodstuffs
- 24. April 2002:
  Press conference of the Swedish Authority

LIVSMEDELS VERKET
NATIONAL FOOD ADMINISTRATION

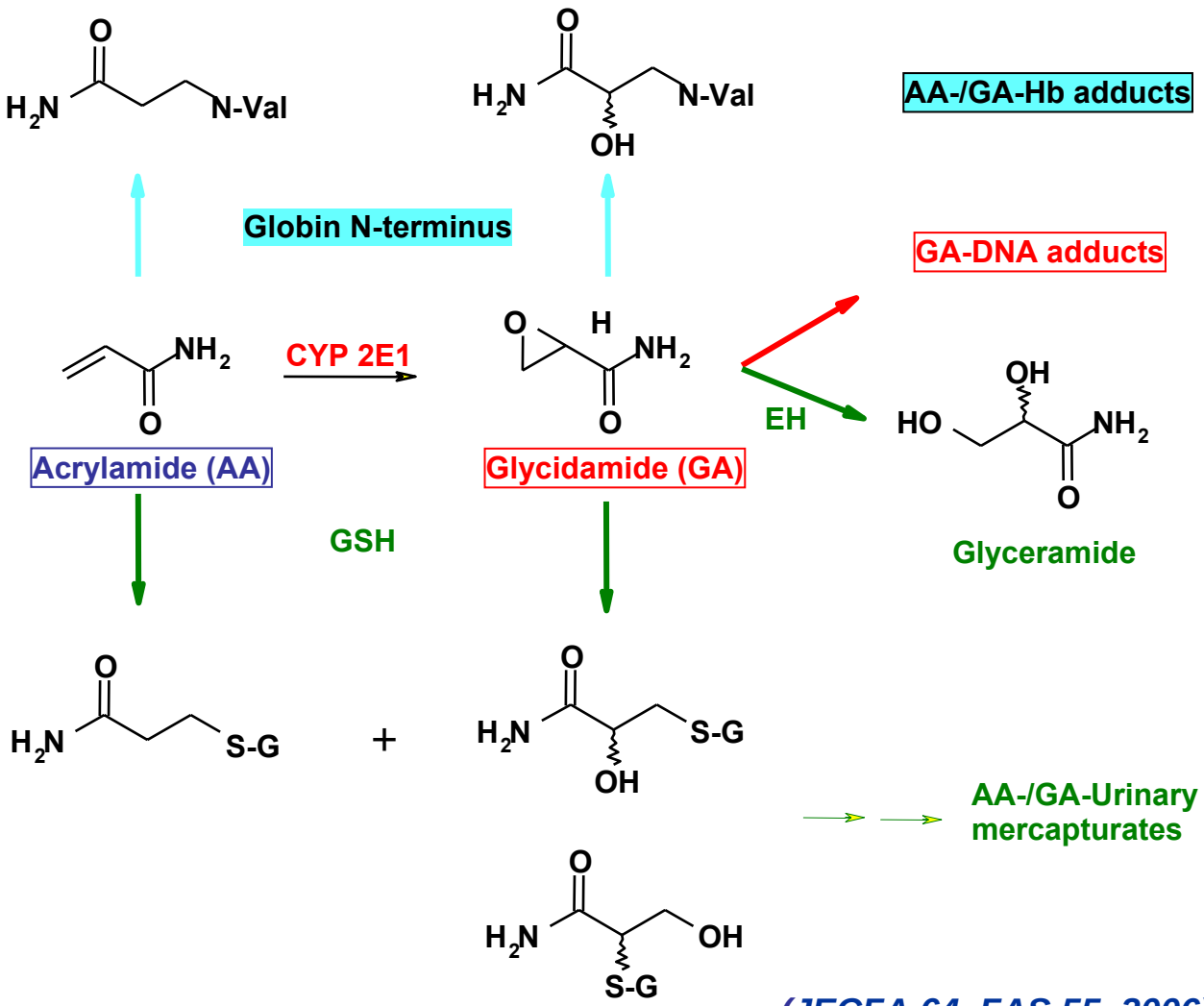
Elevated levels of acrylamide found in starch-containing and heated foodstuffs

- 25.-27. June 2002 Joint FAO/WHO Consultation, Geneva
- JIFSAN Workshop Chicago Okctober 2002, April 2004

Acrylamide: CAS Number: 79-06-1

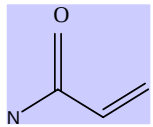


- Readily soluble in water
- Rapid and uniform distribution in the body including
  - breast milk
  - fetus
- Metabolism to the epoxide Glycidamide
  - is saturable
  - dose dependent
  - species differences (mice > rat)
- rel. fast elimination (hrs)



(JECFA 64, FAS 55, 2006)

Acrylamide: Neurotoxicity



- is neurotoxic upon repeated ”high" doses:
  Peripheral neuropathies (NOEL 0.5 mg/kg bw)
- Morphological nerve changes (EM): NOEL 0.2 mg/kg bw
- Mode of action: likely due to direct covalent binding to proteins

  [“Motor-proteins” important for membrane fusions
  → functionning of synapses, growth of neurons]

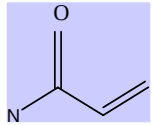
- Single neurotox. Dose:

  ≥100 mg/kg bw (convulsions)

- Reduced fertility
  & effects on reproductive organs:

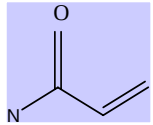
  repeated 10-15 mg/kg bw
  NOEL 2 mg/kg KG

Acrylamide: Genotoxicity / Carcinogenicity



- AA mainly **negative** in prokaryotic *in vitro* test systems but predominantly **positive** in mammalian and in *in vivo* tests
- Most of the genotoxicity of AA mediated by GA
- causes gene mutations *in vivo* & *in vitro* (somatic & germ cells)
- causes chromosomal aberrations (breaks) *in vivo* & *in vitro* (induction of micronuclei)
- is genotoxic
- increases tumour incidence in rats at doses of 1-2 mg/kg bw
- IARC: Group 2A: *Probably carcinogenic to humans*

DNA Adduct Summary-Rodents



- Wide tissue distribution of DNA adducts
- Reactivity with DNA bases GA > AA
- GA produces higher levels of DNA adducts in rodents than AA
- DNA adducts proportional to GA AUC for rats and mice
- DNA adducts accumulate - repeated dosing
- DNA adduct removal in rats and mice - spontaneous depurination (N7 & N3)
- Species differences in DNA adducts apparent at high dose of AA – not at low dose (rats less sensitive than mice)
  ⇒ Link to metabolism

Acrylamide: carcinogenicity *(JECFA 64, FAS 55, 2006)*

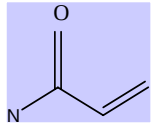


Table 3. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years

Type of tumour	Sex	Dose[a] (mg/kg bw per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	M	1/60	0/58	2/59	1/59	7/59*
Peritesticular mesotheliomas	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland,[b] pheochromocytomas	M	3/60	7/59	7/60	5/60	10/60*
Mammary tumours	F	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumours	F	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	F	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	F	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	F	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas[c]	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas[b]	F	25/59	30/60	32/60	27/60	32/60*

Data from Johnson et al. (1986), as compiled by Rice (2005)

F, female; M, male

[a] Asterisk (*) indicates $P = 0.05$; pair-wise Mantel-Haenszel comparison with the control group adjusted for mortality.

[b] The historical incidence of adrenal gland pheochromocytomas in males was 8.7% (range, 1.2–14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2–46.9%).

[c] Only clitoral glands with gross lesions were examined histologically.

Acrylamide: carcinogenicity *(JECFA 64, FAS 55, 2006)*

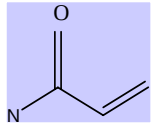


Table 4. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years[a]

Type of tumour	Sex	Dose[b] (mg/kg bw per day)						
		0	0	0.1	0.5	1.0	2.0	3.0
Peritesticular mesotheliomas	M	4/102	4/102	9/204	8/102	–	13/75*	–
Brain and spinal cord, glial neoplasms[c]	M	1/102[d]	1/102[d]	2/204[e]	1.102[f]	–	3/75[d]	–
	F	0/50[g]	0/50[g]	–	–	2/100[g]	–	2/100[g]
Thyroid gland, follicular adenomas	M	2/100	1/102	9/203	5/101	–	15/75*[h]	–
	F	0/50	0/50	–	–	7/100	–	16/100*[h]
Thyroid gland, follicular cell carcinomas	M	1/100	2/102	3/203	0/101	–	3/75	–
	F	1/50	1/50	–	–	3/100	–	7/100
All follicular cell neoplasms	M	3/100	3/100	12/203	5/101	–	17/75	–
	F	1/50	1/50	–	–	10/100	–	23/100*
Mammary gland, fibroadenomas and adenocarcinomas	F	7/46	4/50	–	–	21/94*	–	30/95*

Data from Friedman et al. (1995), as compiled by Rice (2005)

[a] Certain tumours that occurred at increased incidence in treated rats in the previous study (Johnson et al., 1986) were not reported as occurring at increased incidences in this study. These included papillomas of the oral cavity in females, adenomas of the clitoral gland and uterine adenocarcinomas. Numbers of these neoplasms were not given.

[b] Asterisk (*) indicates statistical significance, $P < 0.001$.

[c] Does not include seven rats with "malignant reticulosis" of the brain, including five dosed females, one dosed male and one control male.

[d] All brains of high-dose rats and all control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51–75 high-dose spinal cords were examined.

[e] Only 98/204 brains and 68/204 spinal cords were examined.

[f] Only 50/102 brains and 37/102 spinal cords were examined.

[g] All brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords in control, control, low- and high-dose females, respectively, were examined. The study used two groups of control animals in an effort to increase the statistical power of the study and to obtain a better description of the dose–response curve.

[h] Includes three male rats and one female rat with multiple tumours in the highest dose groups.

64 JECFA BMDL-calculations mammary tumours

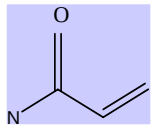
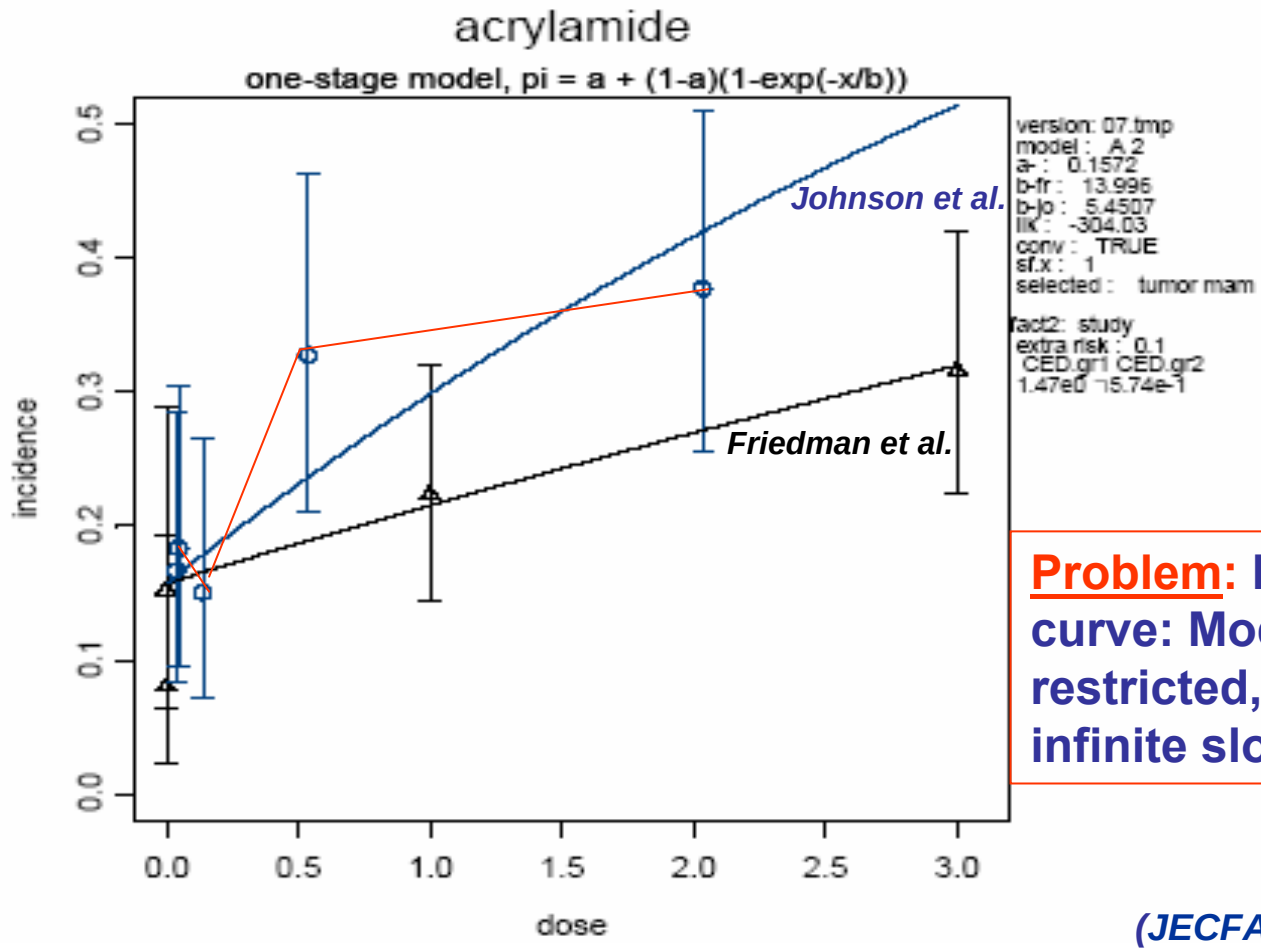


Figure 8. Incidences of total mammary tumours, with fitted one-stage model. Circles: Johnson et al. (1986); triangles: Friedman et al. (1995). Dose is expressed in mg/kg bw per day.



Problem: Dose-response-curve: Model needs to be restricted, otherwise infinite slope at 0 !!

(JECFA 64, FAS 55, 2006)

Acrylamide: 64 JECFA BMDL-calculations

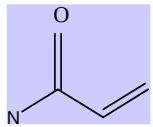


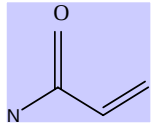
Table 17. Summary of the results of dose–response modelling for induction of selected tumours in rats given drinking-water containing acrylamide

Tumour	Study			
	Johnson et al. (1986)		Friedman et al. (1985)	
	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)
Total mammary tumours	0.48–0.57	0.30–0.46	1.4–1.5	0.89–1.1
Peritesticular mesothelioma	0.97	0.63–0.97	NA	NA
Thyroid follicular adenoma	NA	NA	0.88–1.2	0.63–0.93
Central nervous system tumours of glial origin	1.9–2.0	1.3–1.6	NA	NA

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls; NA, not applicable

(JECFA 64, FAS 55, 2006)

Acrylamide: 64 JECFA intake estimates: Summary

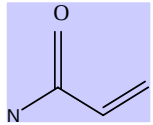


Major contributing foods to total human exposure:

(most countries)

- Potato chips (french fries) 16-30%
- Potato crisps (chips) 6-46%
- Coffee 13-39%
- Pastry and sweets biscuits (cookies) 10-20%
- Bread, rolls, toast 10-30%
- Others <10%

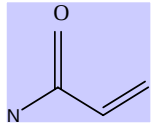
Acrylamide: 64 JECFA intake estimates: Summary



- Average **national intake** 0.3 - 2.0 µg/kg bw per day
- 90. - 97.5 percentile: 0.6 - 3.5 µg/kg bw per day
- 99. Percentile: up to 5.1 µg/kg bw per day
- children: about 2–3x higher than adults on bw basis
- **international average intake** 3.0–4.3 µg/kg bw per day (5 GEMS/Food regional diets, bw 60 kg).

JECFA: concluded that based on national estimates, an intake of acrylamide of **1 µg/kg bw per day** could be taken to represent the average for the general population and that an intake of **4 µg/kg bw per day** could be taken to represent consumers with a high intake. Children are also included in these estimates for average to high intake.

Acrylamide: resulting MOEs



- **MOE 200 and 50** for morphologic nerve changes **(NOEL 0.2 mg/kg bw)**
- **MOE 2000 and 500** for reproductive, developmental and other non-neoplastic effects **(NOEL 2 mg/kg bw)**

JECFA concluded that adverse effects were unlikely at the estimated average intakes, but that morphological changes in nerves could not be excluded for some individuals with a very high intake

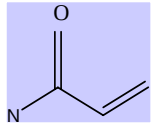
- **MOE 300 and 75** for carcinogenic effects (breast tumours) **(BMDL 0.3 mg/kg bw)**

MOE 750 and 200 at intakes of 0.4 and 1.5 µg/kg bw

JECFA considered these MOEs to be low for a compound that is genotoxic and carcinogenic and that this may indicate a human health concern.

Therefore, appropriate efforts to reduce concentrations of acrylamide in food and beverage should be continued.

EFSA- CONTAM Statement April 2005

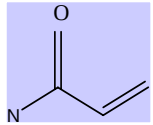


JECFA cautioned that there are uncertainties in its conclusion as the toxicological database is incomplete and recommended that:

- **acrylamide be re-evaluated when results of ongoing carcinogenicity and long-term neurotoxicity studies become available.**
- **work should be continued on using PBPK-modelling to better link human biomarker data with exposure assessments and toxicological effects in experimental animals.**
- **appropriate efforts to reduce acrylamide concentrations in food should continue.**

The CONTAM Panel noted the use of the MOE approach that incorporated data from European countries, including information gathered under collaborative initiatives between the Commission and EFSA. The Panel agrees with the principal conclusions and recommendations of the JECFA and concludes that at present an additional evaluation by EFSA is not necessary.

What-If Scenarios (M. Di Novi, US FDA)

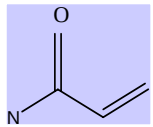


Based on food consumption survey CSFII, 1994-96, 98, 2+ Population

		mean	90th Percentile
Estimated exposure		0.43	0.92 µg/kgbw-d
▪ Remove AA from French Fries	→	0.37	0.78 µg/kgbw-d
▪ Remove AA from Snack Foods	→	0.38	0.85 µg/kgbw-d
▪ Remove AA from Breakfast Cereal	→	0.38	0.84 µg/kgbw-d
▪ Remove AA from Coffee	→	0.40	0.88 µg/kgbw-d

http://www.jifsan.umd.edu/presentations/acry2004/acry_2004_dinovihoward.pdf

Risk-Benefit Considerations of Mitigation Measures on Acrylamide Content of Foods – A Case Study on Potatoes, Cereals and Coffee



Brit. J. Nutrition 2008 Vol 99 Iss S2, S1-S46- ILSI Europe Process Related Compounds Task Force

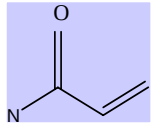
C. J. Seal[1], A. de Mul[2], G. Eisenbrand[3], A. J. Haverkort[4], K. Franke[5], S. P. D. Lalljie[6], H. Mykkänen[7], E. Reimerdes[5], G. Scholz[8], V. Somoza[9], S. Tuijtelaars[10], M. van Boekel[11], J. van Klaveren[2], S. J. Wilcockson[1], L. Wilms[12]

Table 11. Exposure to acrylamide for different mitigation scenarios, based on labscale experiments

	Mitigation measure	Acrylamide reduction	Acrylamide exposure (μg/kg bw per day) P50	P95	P99
Original scenario			0·44	1·15	1·58
Mitigation scenarios					
Wheat bread	2 h yeast fermentation	80 %	0·40	1·07	1·52
Crisp bread	Asparaginase	80 %	0·43	1·12	1·55
Biscuits	Different measures	69 %	0·38	1·03	1·47
Ginger bread	Sugar → sucrose	90 %	0·41	1·09	1·51
Potato crisps	Combination of measures	50 %	0·41	1·03	1·40
Coffee	Storage	20 %*	0·42	1·12	1·57
Total		All scenarios	0·27	0·74	1·11

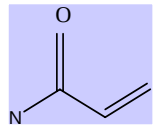
*It has to be stressed that reaction mechanisms leading to storage loss are not an option to date to reduce acrylamide concentration in coffee, since is it directly linked to quality and organoleptic properties and, consequently, consumer acceptability. However, for the purpose of this paper, an estimated degree of potentially achievable decrease is used in the modelling approach in order to assess the impact of acrylamide mitigation in coffee on human exposure and MOE.

The objectives of the Colloquium are:



1. To discuss the epidemiological evidence relating acrylamide exposure to cancer risk in humans including discussions on uncertainties.
2. To discuss the applications of biomarkers for acrylamide and PBPK models in relation to exposure, metabolism and elimination (toxicokinetics) and the mode of action (toxicodynamics) of acrylamide in experimental animals and humans.
3. To discuss the state of the art on genotoxic and non-genotoxic mechanisms for the carcinogenicity of acrylamide including new *in vitro/in vivo* evidence in experimental animals and humans.
4. To discuss the current knowledge on dietary exposure to acrylamide across Europe and to explore if there are possibly new potential food sources contributing to the dietary intake.

⇒ To explore whether the additional information that has become available since the 64 JECFA in 2005 in epidemiology, carcinogenicity and exposure would call for a revision of the previous risk assessment of acrylamide in food.



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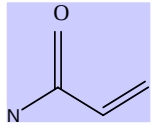
Thank you for your attention !!!

SESSION 1: INTRODUCTORY PLENARY SESSION



09.00-09.10	Welcome and introduction to EFSA	*Riitta Maijala*
09.10-09.30	Objectives of the Colloquium	*Josef Schlatter*
09.30-10.00	Dietary exposure to acrylamide and cancer risk: a summary of recent epidemiological evidence	*Jenny Barrett*
10.00-10.30	Application of biomarkers for dietary exposure to acrylamide	*Jan Alexander*
10.30-11.00	COFFEE/TEA BREAK	
11.00-11.30	Genotoxic and non-genotoxic mechanisms for acrylamide carcinogenicity	*Daniel Doerge*
11.30-12.00	Dietary exposure across Europe - current situation	*Thomas Wenzl*
12.00-12.20	General discussion	
12.20-12.30	Introduction to discussion groups	*Stef Bronzwaer*
12.30-14	LUNCH	