Integration of Internal Dosimetrics into Risk Assessment of Dietary Contaminants through use of PBPK Modeling

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Combining Dietary Intake Assessment with PBPK Modeling and Benchmark Dose Analysis of Rodent Carcinogenicity for Risk Assessment of Acrylamide in Food

OUTLINE

- History
- Dietary exposure
- Mechanistic studies linking glycidamide (GA) as the proximate genotoxic metabolite of acrylamide (AA)
- PK studies linking metabolism and disposition of AA and GA to biomarkers of internal exposure
- Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling for extrapolation from rodents to humans
- Rodent carcinogenicity bioassays for AA and GA
- Benchmark dose (BMD) modeling of tumorigenicity
- Cancer risk assessment
- Legacy from studies of “cooking carcinogens”

History of Acrylamide Research and Risk Assessment

- 1986; 1995 Two 2-y carcinogenicity bioassays for AA in F344 rats
- 1994 – IARC: AA probably carcinogenic to humans
- 1995-7 Occupational exposures – peripheral neuropathy
- 2002 Discovery of ppm levels of AA in many baked and fried starchy foods (M. Törnqvist et al./Swedish Food Authority)
- 2003 FDA priority nomination to NTP – mechanistic studies and chronic bioassays (AA and GA in rodents)
- 2005 JECFA – genotoxic and carcinogenic; low MOEs
- 2009 U.S. EPA IRIS – genotoxic carcinogen
- 2010 JECFA – genotoxic and carcinogenic; low MOEs
- 2013-2015 NCTR/NTP 2-yr chronic carcinogenicity studies in F344 rats and B6C3F1 mice published
- 2015 EFSA risk assessment for acrylamide in food
- 2016 FSCJ risk assessment for acrylamide in food

FDA's Regulatory Concerns - 2003

- Wide distribution in food supply
- Neurotoxicity - low dose, long duration?
- Mutagenicity – somatic and germ cells
- Carcinogenicity in chronic rodent bioassays
- Genotoxic vs. non-genotoxic mechanisms?
- Interspecies & dose extrapolations - toxicokinetics, exposure biomarkers (PBPK model)
- Risk assessment/management
- Totality of "cooking carcinogens"

Dietary Intake of Acrylamide

- FDA: 2004/2006
  population mean (2+) 0.44 µg/kg bw/d;
  90th percentile 0.95 µg/kg bw/d
- JECFA: 2005/2010: mean 1 µg/kg bw/d;
  95th percentile 4 µg/kg bw/d
- EFSA: 2015 mean 0.4-1.9 µg/kg bw/d;
  95th percentile 0.6-3.4 µg/kg bw/d
- Food Safety Commission Japan: 2016
  point estimate 0.24 µg/kg bw/d

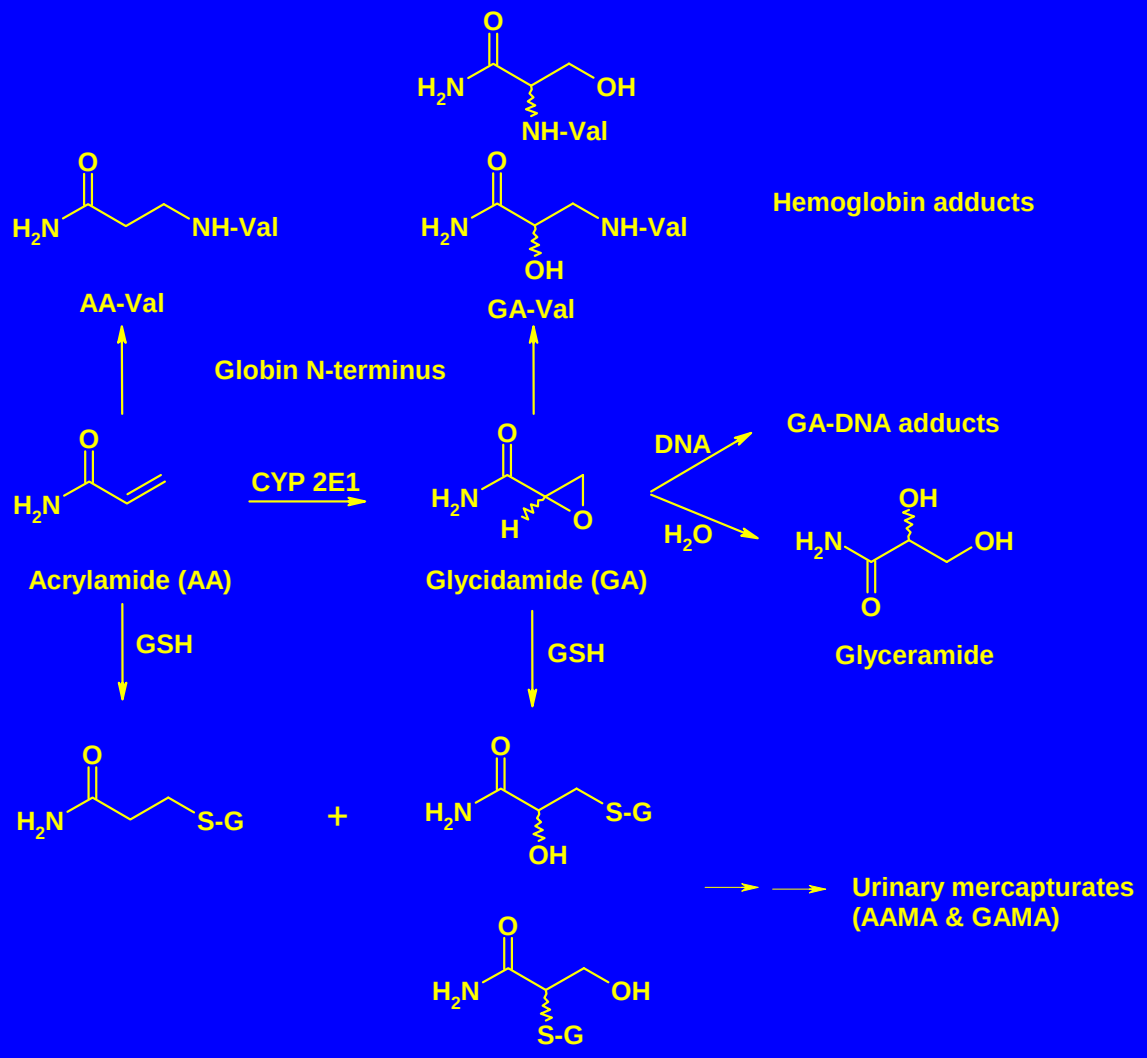
Di Novi What-If Scenarios
CSFII, 1994-96, 98; 2+ Population

- Overall Mean = 0.44 µg/kg bw/d
- Remove AA from all French Fries
  - Mean – 0.32 µg/kg bw/d
- Remove AA from all Snack Foods
  - Mean – 0.37 µg/kg bw/d
- Remove AA from Breakfast Cereal
  - Mean – 0.40 µg/kg bw/d
- Remove AA from Coffee
  - Mean – 0.41 µg/kg bw/d

Mechanistic Studies Indicate that GA is the Genotoxic Metabolite of AA

- No evidence for increased cell proliferation by AA in target tissues secondary to endocrine disruption in male rats (thyroid & testes), even at high doses
- Similar structure and rodent tumor sites to other low molecular weight epoxide carcinogens (urethane, ethylene oxide, glycidol)
- GA reactivity with DNA bases >> AA
- GA-DNA adducts (N7-Gua & N3-Ade) in all tissues tested that accumulate with repeated dosing
- GA more mutagenic than AA *in vitro* (*Salmonella*, Big Blue mouse embryonic fibroblasts)
- GA causes DNA adducts, micronuclei & DNA damage (comet assay), germ cell mutations & dominant lethality in *wt*, but not CYP2E1 ko mice
- AA & GA = genotoxic mutagens in Big Blue mice (lymphocytes, liver, lung, testes) and rats (lymphocytes, bone marrow, thyroid); neonatal *Tk*$^{+/-}$ mice (lymphocytes)
- GA is a genotoxic carcinogen in neonatal B6C3F$_1$ mice
- Tumor site concordance in rats and mice treated chronically with equimolar concs of AA or GA

Metabolism of AA and Potential Biomarkers of Exposure



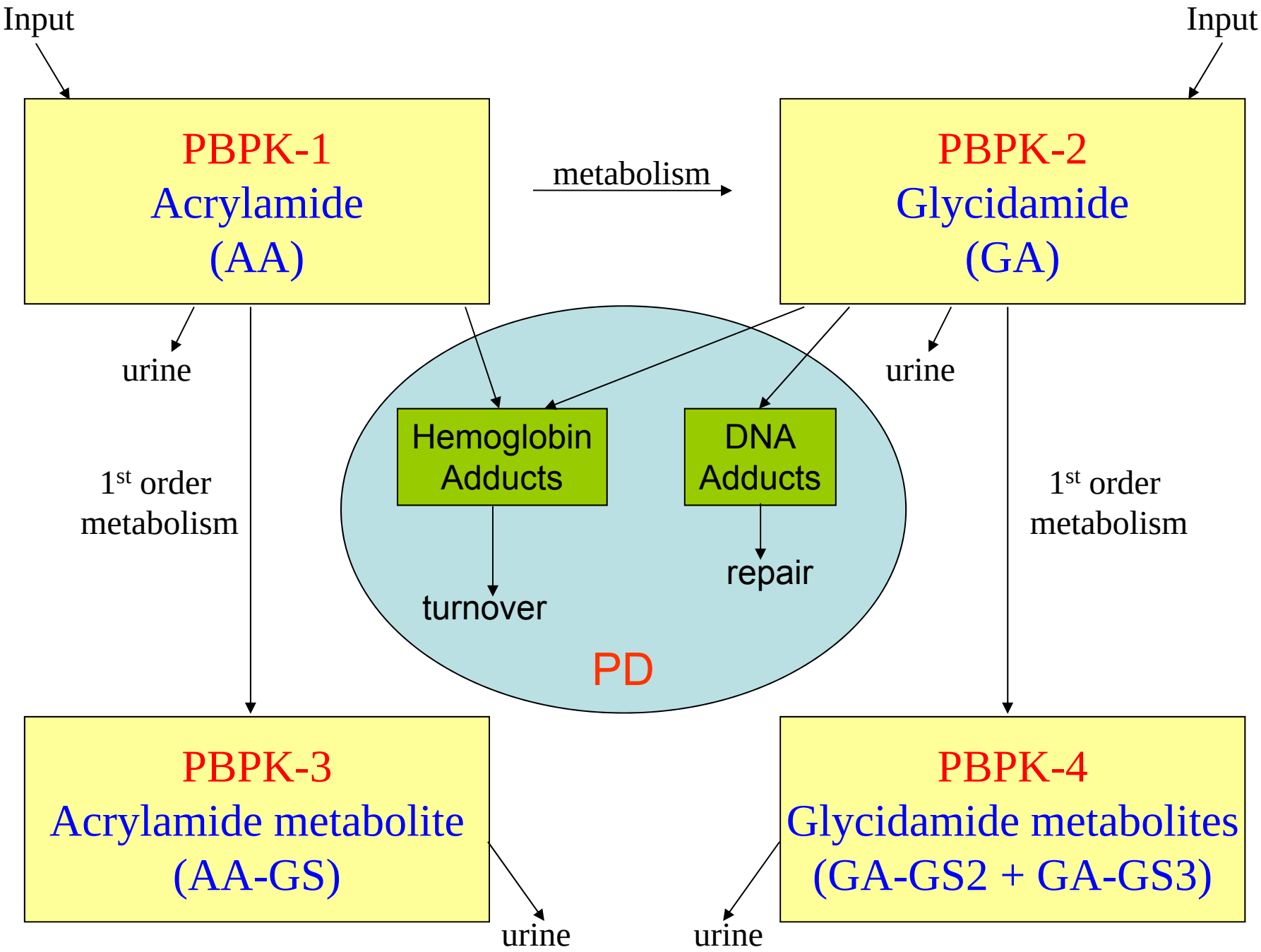
PK Studies Validate Biomarkers of Internal Exposure for AA and GA

- Hb adducts proportional to AUC for either AA or GA (single dose)
- Hb adduct removal in rats and mice are dependent on erythrocyte lifespan
- Hb adducts accumulate to apparent steady state with repeat dosing
- GA-Hb adducts, but not AA-Hb, are proportional to DNA adducts at steady state
- DNA adducts proportional to GA AUC in rodents
- DNA adduct removal in rats and mice dependent on spontaneous depurination
- DNA adducts accumulate to apparent steady state with repeat dosing

PBPK/PD Model for AA and Its Metabolites In Mice, Rats, and Humans

Chem. Res. Toxicol. (2007)

J.F. Young, R.H. Luecke, D.R. Doerge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Input
PBPK-1
Acrylamide
(AA)
metabolism
PBPK-2
Glycidamide
(GA)
urine
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metabolism
Hemoglobin
Adducts
DNA
Adducts
1st order
metabolism
repair
turnover
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Acrylamide metabolite
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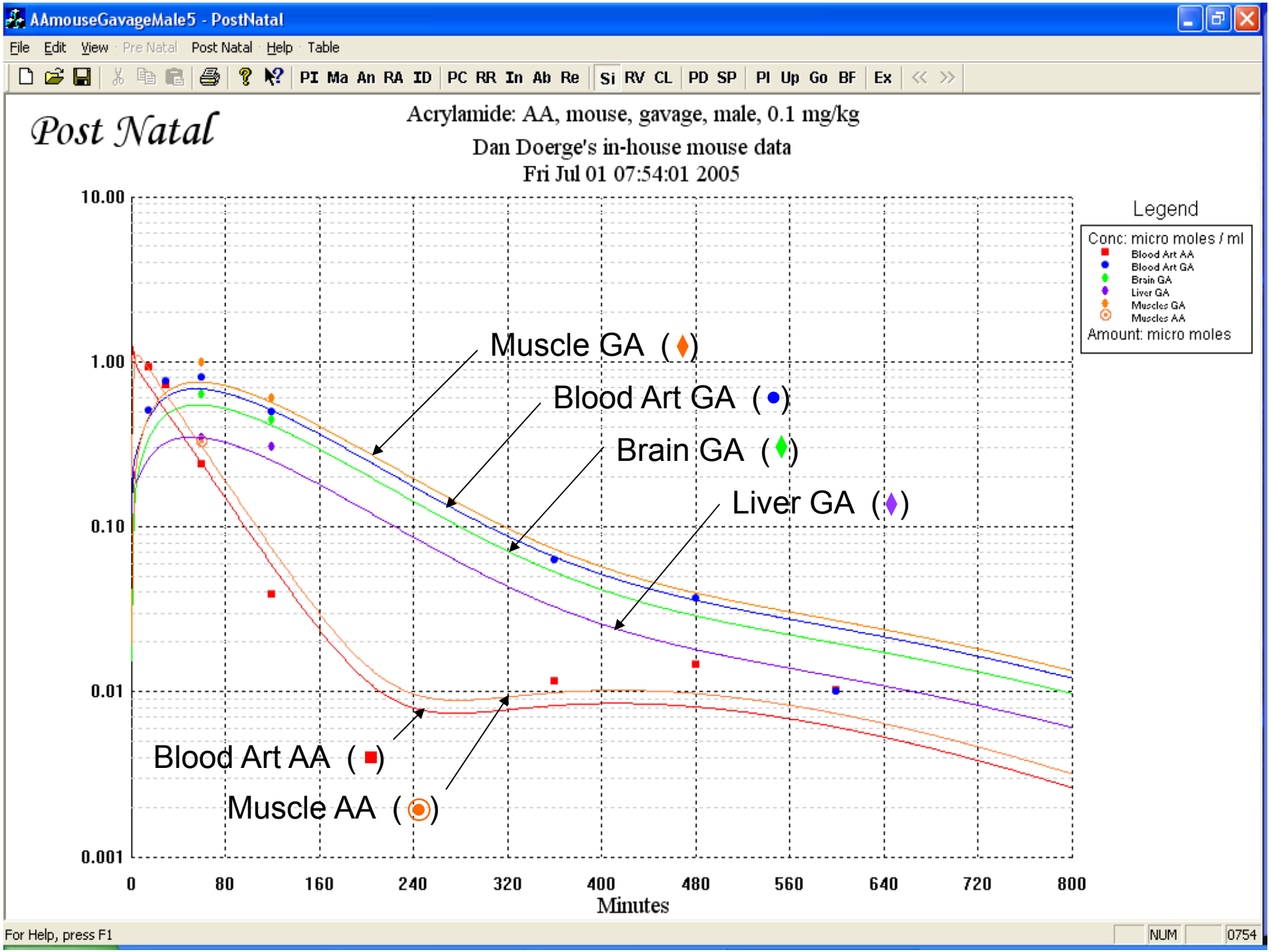
Glycidamide metabolites
(GA-GS2 + GA-GS3)
urine
urine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Post Natal
Acrylamide: AA, mouse, gavage, male, 0.1 mg/kg
Dan Doerge's in-house mouse data
Fri Jul 01 07:54:01 2005
Legend
Conc: micro moles / ml
Blood Art AA
Blood Art GA
Brain GA
Liver GA
Muscles GA
Muscles AA
Amount: micro moles
Muscle GA ( )
Blood Art GA ( )
Brain GA ( )
Liver GA ( )
Blood Art AA ( )
Muscle AA ( )
10.00
1.00
0.10
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80
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240
320
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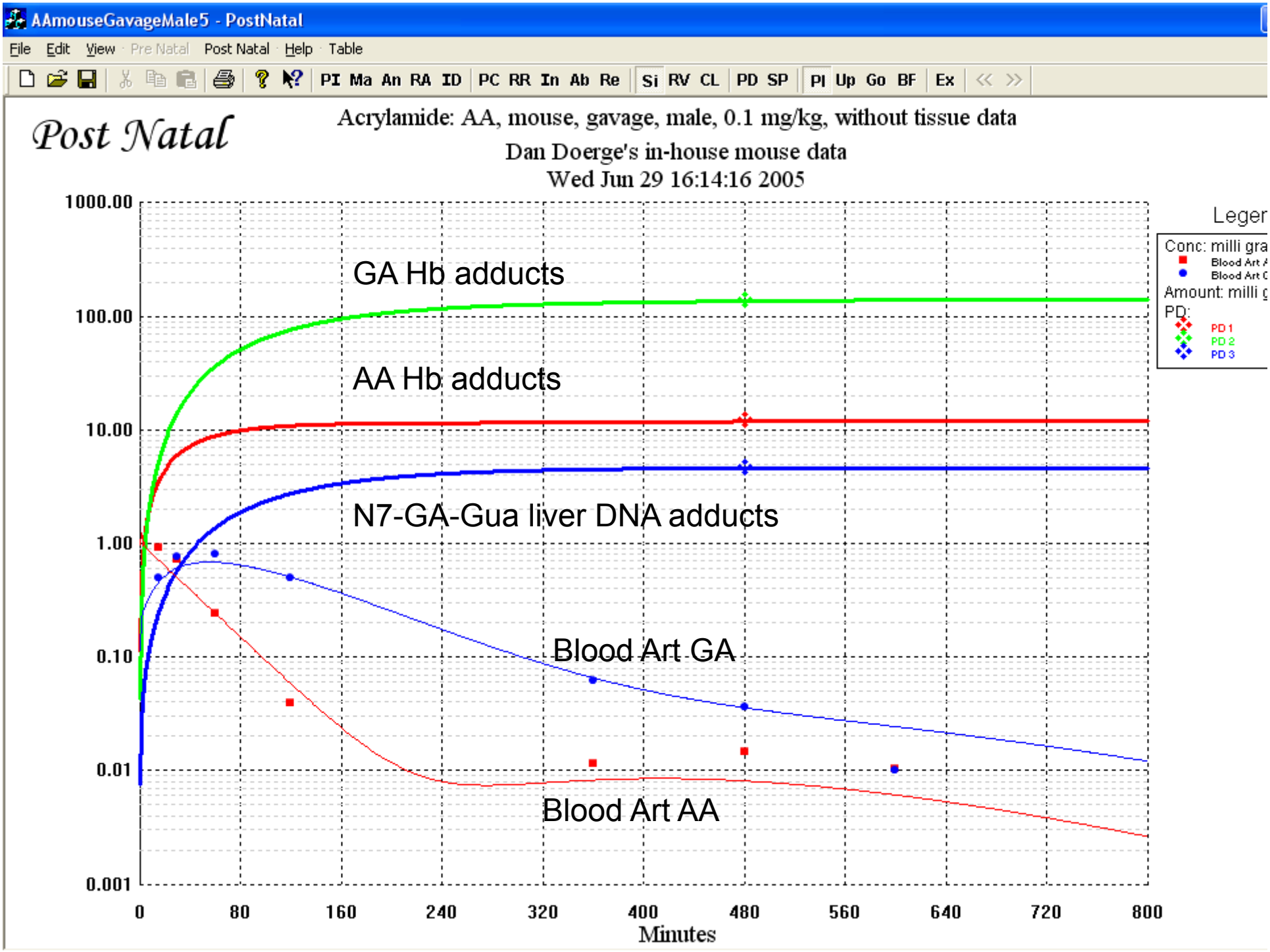
AAmouseGavageMale5 - PostNatal
File Edit View Pre Natal Post Natal Help Table
PI Ma An RA ID PC RR In Ab Re Si RV CL PD SP PI Up Go BF Ex
Post Natal
Acrylamide: AA, mouse, gavage, male, 0.1 mg/kg, without tissue data
Dan Doerge's in-house mouse data
Wed Jun 29 16:14:16 2005
1000.00
100.00
10.00
1.00
0.10
0.01
0.001
GA Hb adducts
AA Hb adducts
N7-GA-Gua liver DNA adducts
Blood Art GA
Blood Art AA
0
80
160
240
320
400
480
560
640
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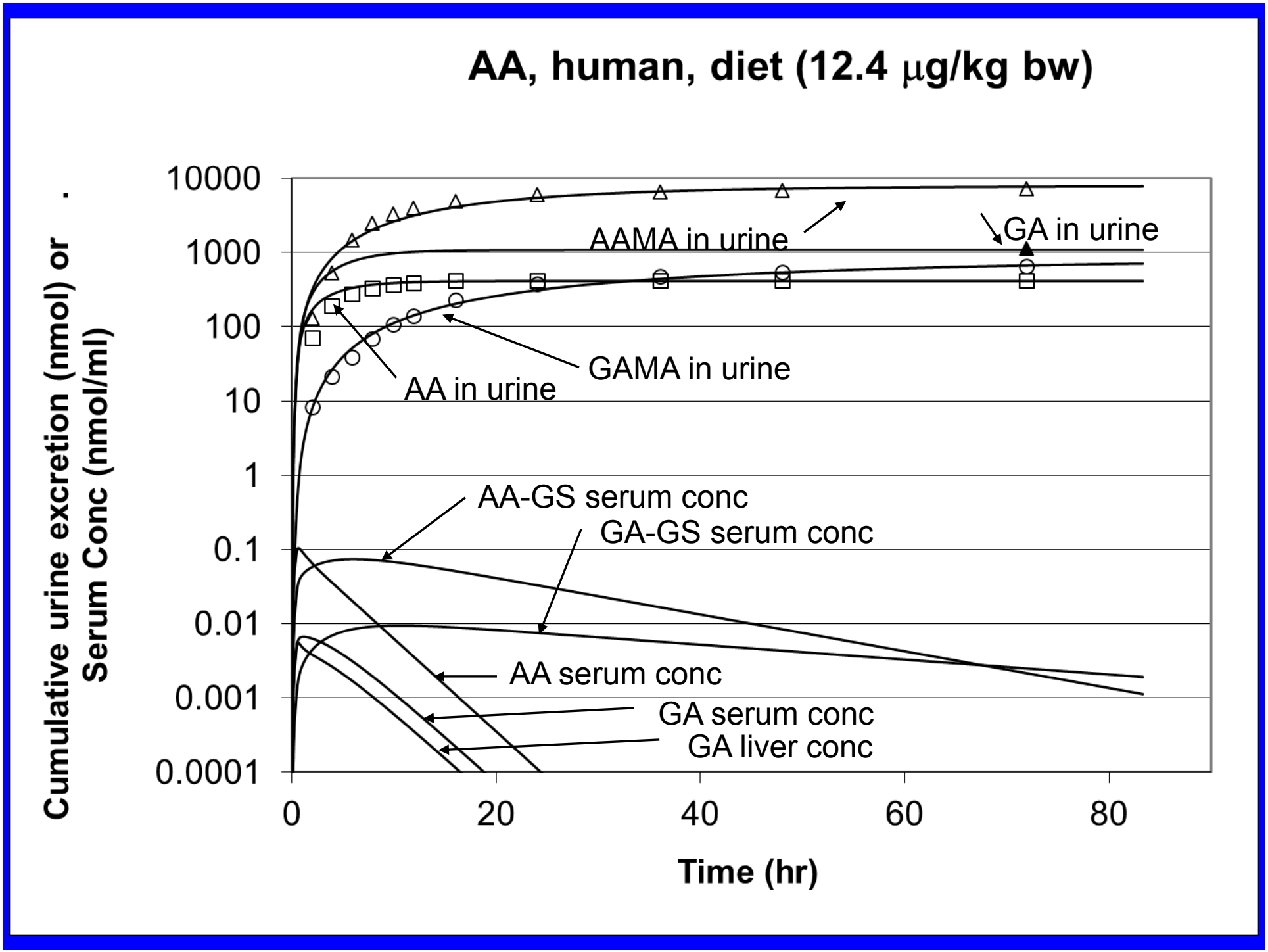
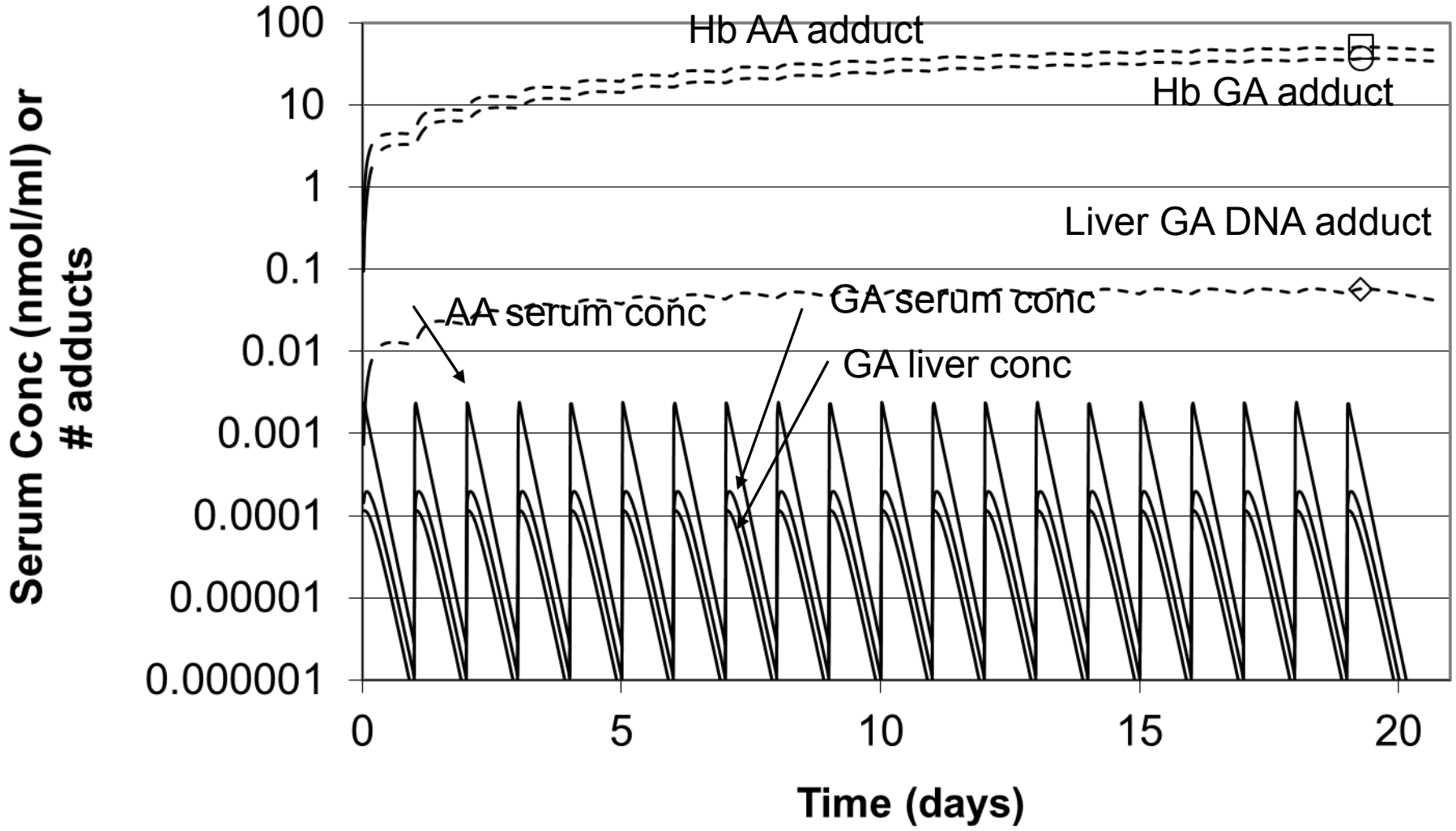
800
Minutes
Leger
Conc: milli gra
Blood Art A
Blood Art G
Amount: milli g
PD:
PD 1
PD 2
PD 3


AA, human, diet (12.4 μg/kg bw)
Cumulative urine excretion (nmol) or
Serum Conc (nmol/ml)
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0
20
40
60
80
Time (hr)
AAMA in urine
GA in urine
GAMA in urine
AA in urine
AA-GS serum conc
GA-GS serum conc
AA serum conc
GA serum conc
GA liver conc

AA, human dietary exposure (0.23 µg/kg bw/d)



PBPK/PD Prediction of Steady State Human DNA Adducts from Dietary Exposures

- PBPK/PD using urinary metabolites & Hb adducts from non-smokers – 0.4 µg/kg bw/d 0.46 N7-GA-Gua/10^8 nucleotides (extra-hepatic)
- Empirical relationship between rodent GA-Hb & DNA adducts with human GA-Hb adducts - 0.2-0.5 N7-GA-Gua/10^8 nucleotides

Chronic Carcinogenicity Study Doses
$B6C3F_1$ Mice – Drinking Water

Test agent	Sex	Dosed water (mM)	Daily AA intake (mg/kg bw)
AA	Male & female	0.70	8.9-10
		0.35	4.1-4.7
		0.175	2.2
		0.0875	1.0-1.1
		0	0

Chronic Carcinogenicity Study Doses
F344 Rats – Drinking Water

Test agent	Sex	Dosed water (mM)	Daily intake (mg/kg bw)
AA	Male & female	0.70	2.7-4.0
		0.35	1.3-1.8
		0.175	0.7-0.9
		0.0875	0.3-0.4
		0	0

Chronic Carcinogenicity Study Doses
B6C3F$_1$ Mice – Drinking Water

Test agent	Sex	Dosed water (mM)	Daily AA intake (mg/kg bw)
GA	Male & female	0.70	9.6-13
		0.35	5.1-5.6
		0.175	2.7-2.9
		0.0875	1.2-1.4
		0	0

Chronic Carcinogenicity Study Doses
F344 Rats – Drinking Water

Test agent	Sex	Dosed water (mM)	Daily intake (mg/kg bw)
GA	Male & female	0.70	3.3-4.7
		0.35	1.6-2.2
		0.175	0.8-1.1
		0.0875	0.4-0.5
		0	0

Benchmark Dose Modeling of AA Carcinogenicity in B6C3F$_1$ Mice (Harderian gland adenoma)

Table 3: Benchmark dose modeling of neoplastic incidences in male and female B6C3F$_1$ mice administered 0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide in the drinking water for two years[a].

Species	Endpoint	Sex	Model	AIC[b]	Fitted model[c]	GOF[d]	BMD[e]	BMDL[f]
B6C3F$_1$ mice	Harderian gland adenoma	Male	Log-Logistic	237.2	0.404	0.399	0.365	0.173
			Log-Probit	237.5	0.345	0.342	0.383	0.159
		Female	Log-Logistic	223.5	0.428	0.427	0.473	0.282
			Log-Probit	223.6	0.417	0.418	0.519	0.230

Benchmark Dose Modeling of GA Carcinogenicity in B6C3F$_1$ Mice (Harderian gland adenoma)

Benchmark dose modeling of neoplastic incidences in B6C3F$_1$ mice administered 0, 0.0875, 0.175, 0.35, or 0.70 mM GA in the drinking water for two years.

Species	Endpoint	Sex	Model	AIC	Fitted model[c]	GOF	BMD	BMDL
B6C3F$_1$ mice	Harderian gland adenoma	Male	Log-Logistic	245.1	0.418	0.425	0.48	0.22
			Log-Probit	244.9	0.459	0.464	0.51	0.22
		Female	Log-Logistic	253.5	0.099	0.100	0.40	0.29

Benchmark Dose Modeling of AA Carcinogencity in F344 Rats (Female mammary fibroadenoma)

Table 4: Benchmark dose modeling of neoplastic incidences in female F344/N rats administered 0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide in the drinking water for two years[a].

Species	Endpoint	Sex	Model	AIC[b]	Fitted model[c]	GOF[d]	BMD[e]	BMDL[f]
F344/N rats	Mammary gland fibroadenoma	Female	Gamma	321.6	0.587	0.584	0.706	0.441
			Logistic	321.8	0.546	0.543	0.914	0.649
			Log-Logistic	321.5	0.609	0.607	0.550	0.296
			Log-Probit	323.4	0.411	0.410	0.431	0.008
			Multistage	321.6	0.587	0.584	0.706	0.441
			Probit	321.8	0.547	0.544	0.911	0.650
			Weibull	321.6	0.587	0.584	0.706	0.441

Benchmark Dose Modeling of GA Carcinogencity in F344 Rats (Female mammary fibroadenoma)

Benchmark dose modeling of neoplastic incidences in female F344/N rats administered 0, 0.0875, 0.175, 0.35, or 0.70 mM GA in the drinking water for two years[a].								
Species	Endpoint	Sex	Model	AIC[b]	Fitted model[c]	GOF[d]	BMD[e]	BMDL[f]
F344/N rats	Mammary gland fibroadenoma	Female	Log-Logistic	306.5	0.140	0.141	0.21	0.12

PBPK/PD-Simulated DNA Adduct Levels in Rodents at the BMDLs and in Humans Exposed to AA in the Diet (0.4 μg/kg bw/d)

Tissue-Sex	BMDL for increased tumor incidence (mg/kg BW/d)	N7-GA-Gua (per 10^8 nucleotides at BMDL)	N7-GA-Gua (from avg human dietary exposure)
Harderian Gland-Male Mouse	**0.16**	**32**	**0.46**
Harderian Gland-Female Mouse	**0.23**	**45**	**0.46**
Mammary Gland-Female Rat	**0.30**	**140**	**0.46**
Thyroid Gland-Male Rat	**0.34**	**134**	**0.46**

Using BMD & PBPK/PD Modeling of DNA Adduct Levels to Derive Margins of Exposure for Rodent Tumor Incidences in Humans Exposed to Dietary AA

Tumor Site	Margin of Exposure (MOE) (BMDL/0.4 µg/kg bw/d)	Margin of Internal Exposure (rodent DNA adducts at BMDL/human DNA adducts at 0.4 µg/kg bw/d)
Harderian Gland Adenoma (male mouse)	**400**	**70**
Harderian Gland Adenoma (female mouse)	575	98
Mammary Fibroadenoma (female rat)	**750**	**304**
Thyroid Adenoma+Carcinoma (male rat)	850	291

CONCLUSIONS

- AA = multi-site, multi-species genotoxic carcinogen in both sexes of rodents via metabolism to GA
- GA produces nearly identical tumor profiles (sites and BMDLs)
- Humans produce GA at measureable levels
- Tumor incidences and MOEs from rodent bioassays (“external” dose)
- GA-Hb adduct measurements in humans exposed through diet only (“internal” dose)
- PBPK predictions of steady state GA-DNA adduct levels in human tissues (“effective” dose)
- Cancer risk assessment
- Dietary AA is likely a significant contributor to population-based carcinogenic risks

REMAINING QUESTIONS - AA

- What to do about a genotoxic carcinogen that is pervasive throughout the diet?
- Significant proportion of total caloric content of global agriculture in cereals and tubers
- Diet-Cancer linkage robust
- Optimal use of rodent carcinogenicity bioassays
- Sufficiently powered epidemiological studies to relate AA in diet with cancers at specific sites are unlikely
- Despite strong linkages between mechanism, dose-response, and risk assessment – large unresolved societal issues remain

REMAINING QUESTIONS – COOKING CARCINOGENS

- What about exposure to other known cooking carcinogens?
  furan (300 ng/kg bw/d), MCPD (>30), HAAs (15), B[a]P (4)
- Unknown compounds?
- Holistic risk assessment for all “cooking carcinogens”
- Risk-Benefit continuum – cooking:
  ↓ microbial pathogens; ↑ flavor, ↑ color,
  ↑ chemopreventative compounds

ACKNOWLEDGEMENTS

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- NTP

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LIMITATIONS OF EPIDEMIOLOGY TO ESTABLISH A LINK BETWEEN DIETARY AA AND HUMAN CANCERS

- Wide distribution of AA in common foods (substitution)
- Variability of AA content in individual foods from commercial and home-made sources; agronomic and seasonal factors; cooking methods and preferences
- Variability in GAmetabolism (intra- and inter-individual) from changes in age, lifestyle, disease, etc.
- Poor correlation between FFQs with validated biomarkers of internal exposure to AA & GA (concurrent)
- Age affects food choices and cancer susceptibility
- Rodent tumor sites poorly predict human cancers

Using PBPK/PD-Simulated DNA Adduct Levels in Rodents and Humans Exposed to Dietary AA (0.4 μg/kg bw/d) to Estimate Excess Tumor Incidences

Tissue-Sex	N7-GA-Gua (per 10^8 nucleotides at BMDL)	N7-GA-Gua (avg human from dietary exposure)	Tumor Incidence (avg human from dietary exposure)
Harderian Gland-M mouse	**32**	**0.46**	**14×10^{-4}**
Harderian Gland-F mouse	45	0.46	10×10^{-4}
Thyroid-M rat	134	0.46	3.4×10^{-4}
Mammary-F rat	**140**	**0.46**	**3.3×10^{-4}**

Carcinogenicity Bioassay Results AA in $B6C3F_1$ Mice

Table 1: Incidence of neoplasms in male and female $B6C3F_1$ mice administered 0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide in the drinking water for two years[a].

Neoplasm	Sex	Acrylamide (mM)				
		0	0.0875	0.175	0.35	0.70
Harderian gland adenoma	Male	2/46 (4%)***	13/46 (28%)**	27/47 (57%)***	36/47 (77%)***	39/47 (83%)***
	Female	0/45 (0%)***	8/44 (18%)**	20/48 (42%)***	32/47 (68%)***	31/43 (72%)***
Lung alveolar/bronchiolar adenoma	Male	5/47 (11%)***	6/46 (13%)	13/47 (28%)*	10/45 (22%)	19/48 (40%)***
	Female	1/47 (2%)***	4/47 (9%)	6/48 (13%)	11/45 (24%)***	19/45 (42%)***
Stomach (forestomach) squamous cell papilloma	Male	0/46 (0%)**	2/45 (4%)	2/46 (4%)	6/47 (13%)*	6/44 (14%)**
	Female	4/46 (9%)***	0/46 (0%)	2/48 (4%)	5/45 (11%)	8/42 (19%)
Mammary gland adenoacanthoma or adenocarcinoma	Female	0/47 (0%)***	4/46 (9%)	7/48 (15%)**	4/45 (9%)*	17/42 (41%)***
Skin fibrosarcoma, hemangiosarcoma, liposarcoma, myxosarcoma, neurofibrosarcoma, or sarcoma	Female	0/48 (0%)***	0/46 (0%)	3/48 (6%)	10/45 (22%)***	6/43 (14%)**

Carcinogenicity Bioassay Results
AA in Fischer 344 Rats

Table 2: Incidence of neoplasms in male and female F344/N rats administered 0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide in the drinking water for two years[a].

Neoplasm	Sex	Acrylamide (mM)				
		0	0.0875	0.175	0.35	0.70
Thyroid gland follicular cell adenoma or carcinoma	Male	1/47 (2%)**	3/48 (6%)	4/47 (9%)	6/48 (13%)	9/48 (19%)**
	Female	0/48 (0%)**	0/48 (0%)	2/48 (4%)	3/48 (6%)	4/47 (9%)*
Heart malignant Schwannoma	Male	1/48 (2%)*	2/48 (4%)	3/48 (6%)	4/48 (8%)	6/48 (13%)*
	Female	2/48 (4%)*	1/48 (2%)	0/48 (0%)	2/48 (4%)	4/48 (8%)
Epididymis or testes malignant mesothelioma	Male	2/48 (4%)**	2/48 (4%)	1/48 (2%)	5/48 (10%)	8/48 (17%)*
Pancreatic islets adenoma	Male	1/46 (2%)*	2/48 (4%)	4/48 (8%)	1/48 (2%)	6/48 (13%)*
Mammary gland fibroadenoma	Female	16/48 (33%)***	18/48 (38%)	24/46 (52%)*	22/47 (47%)*	31/48 (65%)***
Clitoral gland carcinoma	Female	1/48 (2%)*	6/48 (13%)*	12/47 (26%)***	3/48 (6%)	8/47 (17%)**
Clitoral gland squamous cell papilloma	Female	0/48 (0%)**	0/48 (0%)	1/47 (2%)	0/48 (0%)	3/47 (6%)
Oral mucosa or tongue squamous cell papilloma or carcinoma	Female	0/48 (0%)**	2/48 (4%)	1/48 (2%)	3/48 (6%)	5/48 (10%)*
Skin (subcutaneous tissue) fibroma, fibrosarcoma, or sarcoma	Female	1/48 (2%)***	0/48 (0%)	0/48 (0%)	1/48 (2%)	5/48 (10%)*
Liver hepatocellular adenoma	Female	0/48 (0%)**	0/48 (0%)	1/48 (2%)	1/48 (2%)	3/48 (6%)