U.S. FOOD & DRUG ADMINISTRATION

DIVISION OF BIOCHEMICAL TOXICOLOGY

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DIVISION OF BIOCHEMICAL TOXICOLOGY

STAFF

Government Positions (full-time employees)

- Research Scientists, Staff Fellows & Visiting Scientists: 30
- Support Scientists: 13
- Administrative: 2
- FDA Commissioner Fellows: 1

ORISE Post Docs, Graduate Students, etc: 17

Total = 63

DIVISION OF BIOCHEMICAL TOXICOLOGY

OUTREACH

Collaborations:

- NCTR Divisions of Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Microbiology, Neurotoxicology, and Systems Biology, and the Office of Scientific Coordination
- CBER, CDER, CDRH, CFSAN, CTP, and CVM
- NIEHS/NTP, NCI, EPA, CDC, and various universities

Global leadership/outreach:

- IARC
- WHO
- EFSA
- OECD

DIVISION OF BIOCHEMICAL TOXICOLOGY

MISSION

Mission: To conduct fundamental and applied research designed to define the biological mechanisms of action underlying the toxicity of products regulated by FDA.

Goals: To characterize the toxicities and carcinogenic risks associated with chemicals, specifically those of interest to FDA.

Strategies: Bioassays, mechanistic studies, computational modeling

DIVISION OF BIOCHEMICAL TOXICOLOGY

Recently completed or ongoing toxicological assessments:

- Retinyl palmitate (CFSAN)
- Aloe vera (CFSAN)
- Acrylamide/glycidamide (CFSAN)
- Melamine/cyanuric acid (CFSAN and CVM)
- Triclosan (CDER and CFSAN)
- Bisphenol A (CFSAN and CDRH)
- Furan (CFSAN)
- Arsenic (CFSAN)
- Brominated vegetable oils (CFSAN)
- Nattokinase/lumbrokinase (CFSAN)

ACCOMPLISHMENT #1: ALOE VERA

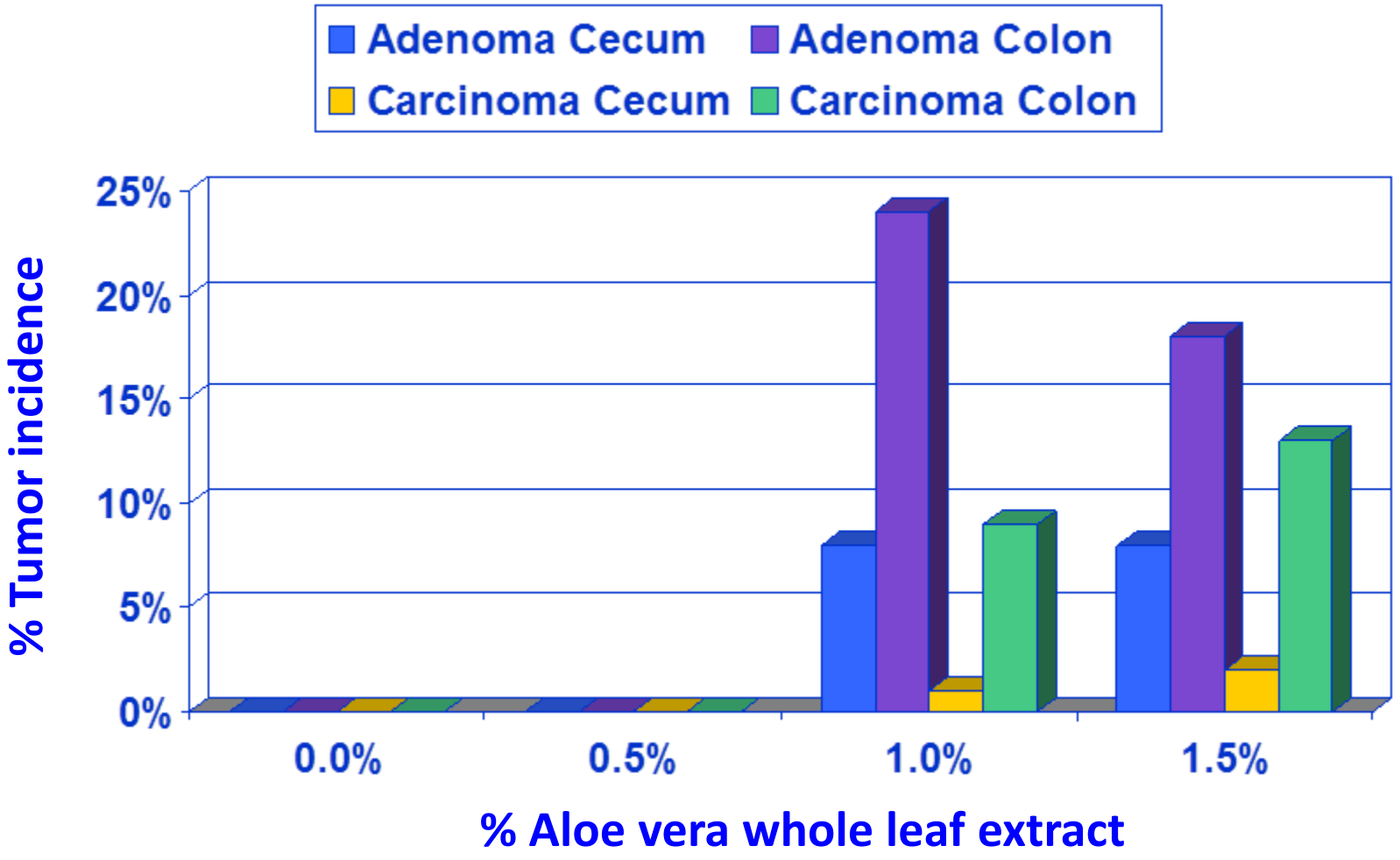
Aloe vera (*Aloe barbadensis* Miller) plant leaf extracts are used as dietary supplements to prevent disease or to relieve symptoms of disease.

Consumer products for oral consumption contain various Aloe vera leaf parts in their formulations and are offered at various concentrations in liquid, powder, and tablet forms.

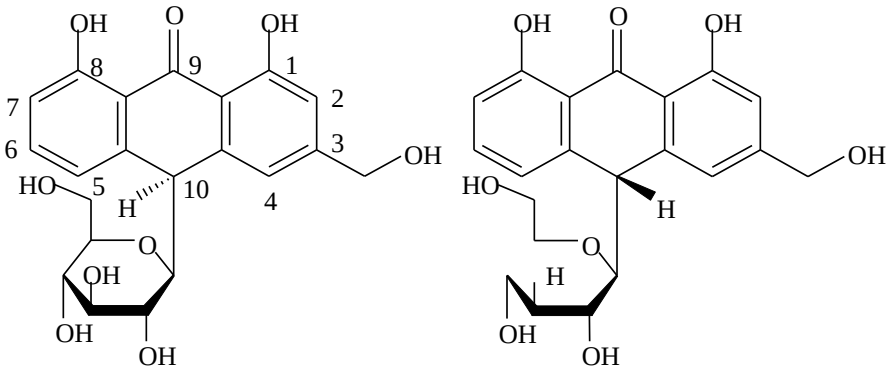
A 2-year chronic bioassay was conducted to evaluate the toxicity and carcinogenic potential of oral exposure to Aloe vera plant extracts in mice and rats.

Drinking water was selected as the route of administration because human exposure is likely to occur by the oral route.

ALOE VERA BIOASSAY



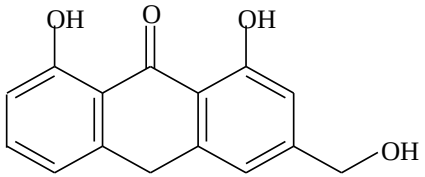
METABOLISM OF ALOIN



Aloin A (Barbaloin)

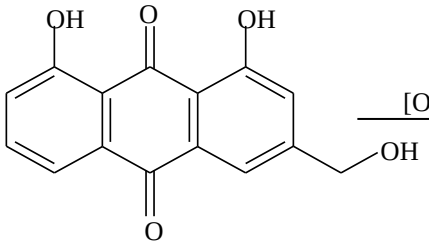
Aloin B (Isobarbaloin)

Hydrolysis of the β-glysosidic bond by intestinal bacteria



Aloe-emodin-9-anthrone

[O]



Aloe-emodin

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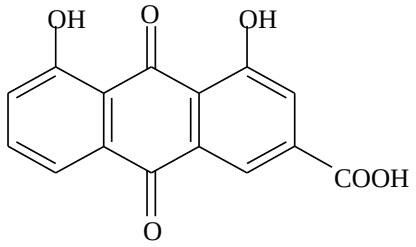
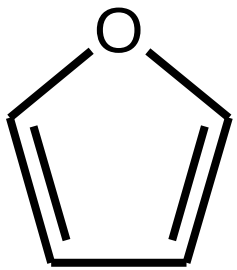


Rhein

ALOIN – GOBLET CELL/MUCOSAL HYPERPLASIA

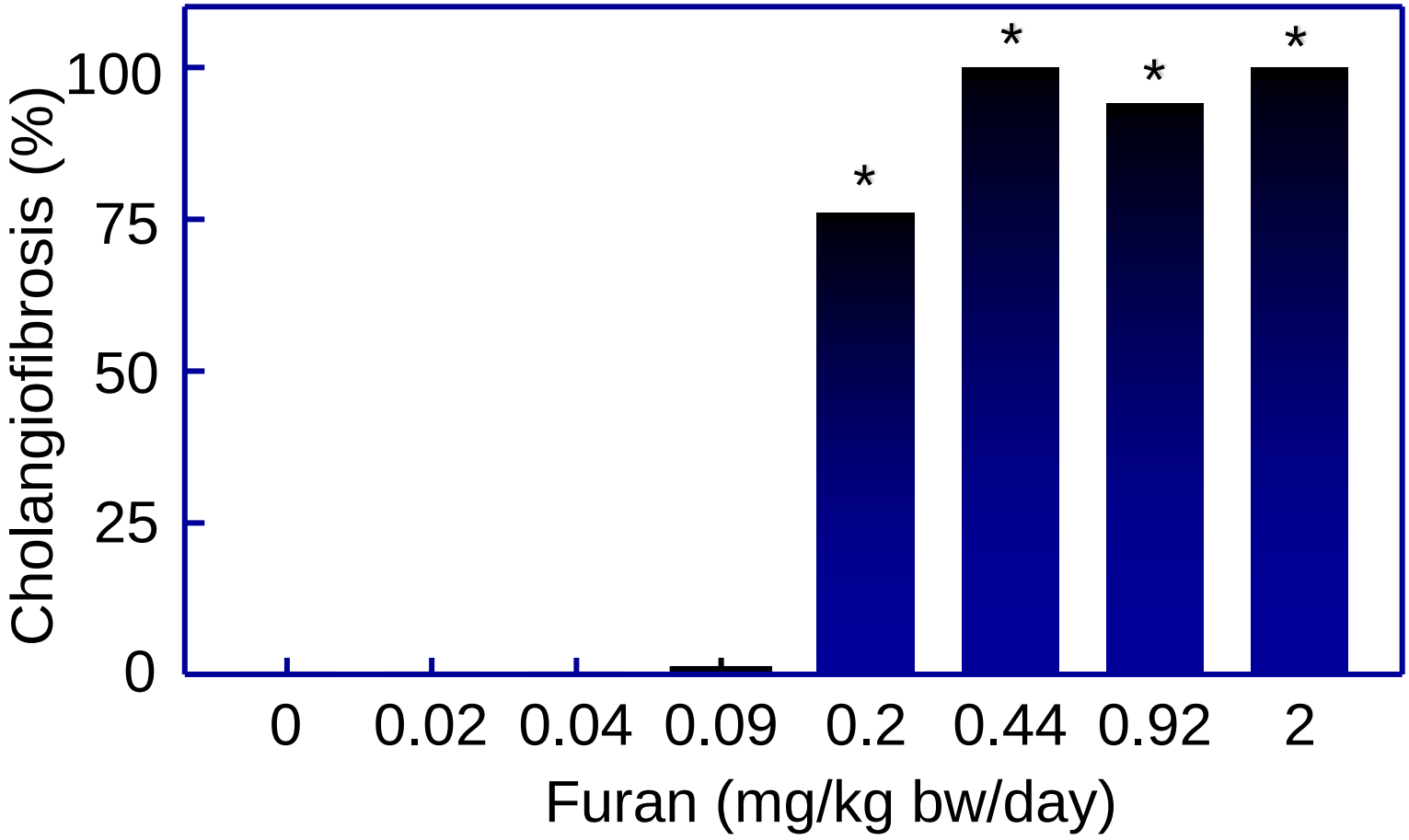
Aloin (ppm)	0	6.95	13.9	27.8	55.7	111	223	446
Dose equivalent of Aloe vera whole leaf extract	0%	0.03%	0.06%	0.13%	0.25%	0.50%	1.00%	2.00%
Cecum	0	0	0	0	0	20% (2.0)	70% (2.4)	100% (2.5)
Ileo-cecal-colic junction	0	0	0	0	10% (1.0)	90% (2.0)	90% (2.7)	100% (3.3)
Ascending Colon	0	0	0	10% (2.0)	60% (1.6)	100% (2.9)	100% (3.8)	100% (4.0)
Transverse Colon	0	0	0	10% (2.0)	60% (1.5)	100% (2.6)	100% (3.3)	100% (3.9)
Descending Colon	0	0	0	0	10% (2.0)	100% (2.3)	100% (2.6)	100% (3.6)
Rectum	0	0	0	0	0	60% (1.6)	100% (1.9)	100% (2.9)

ACCOMPLISHMENT #2: FURAN

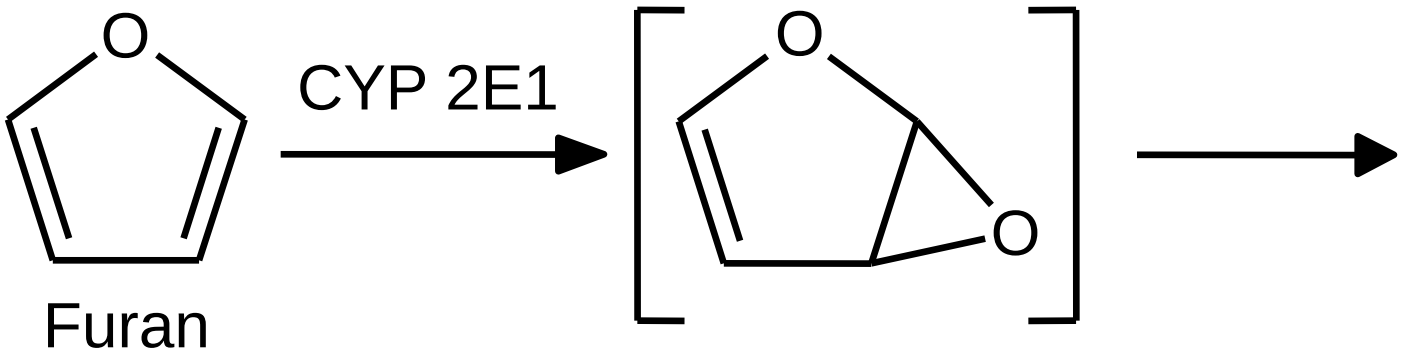


- Synthetic intermediate in the production of pesticides, stabilizers, and pharmaceuticals
- Constituent of tobacco smoke
- Contaminant of many common foods
  - Coffee, baked or fried cereal products, canned and jarred foods, baby food, and infant formula
  - 260 ng/kg bw/day

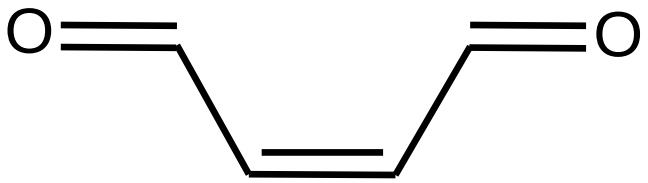
CHOLANGIOFIBROSIS IN RATS ADMINISTERED FURAN



METABOLISM OF FURAN



Furan

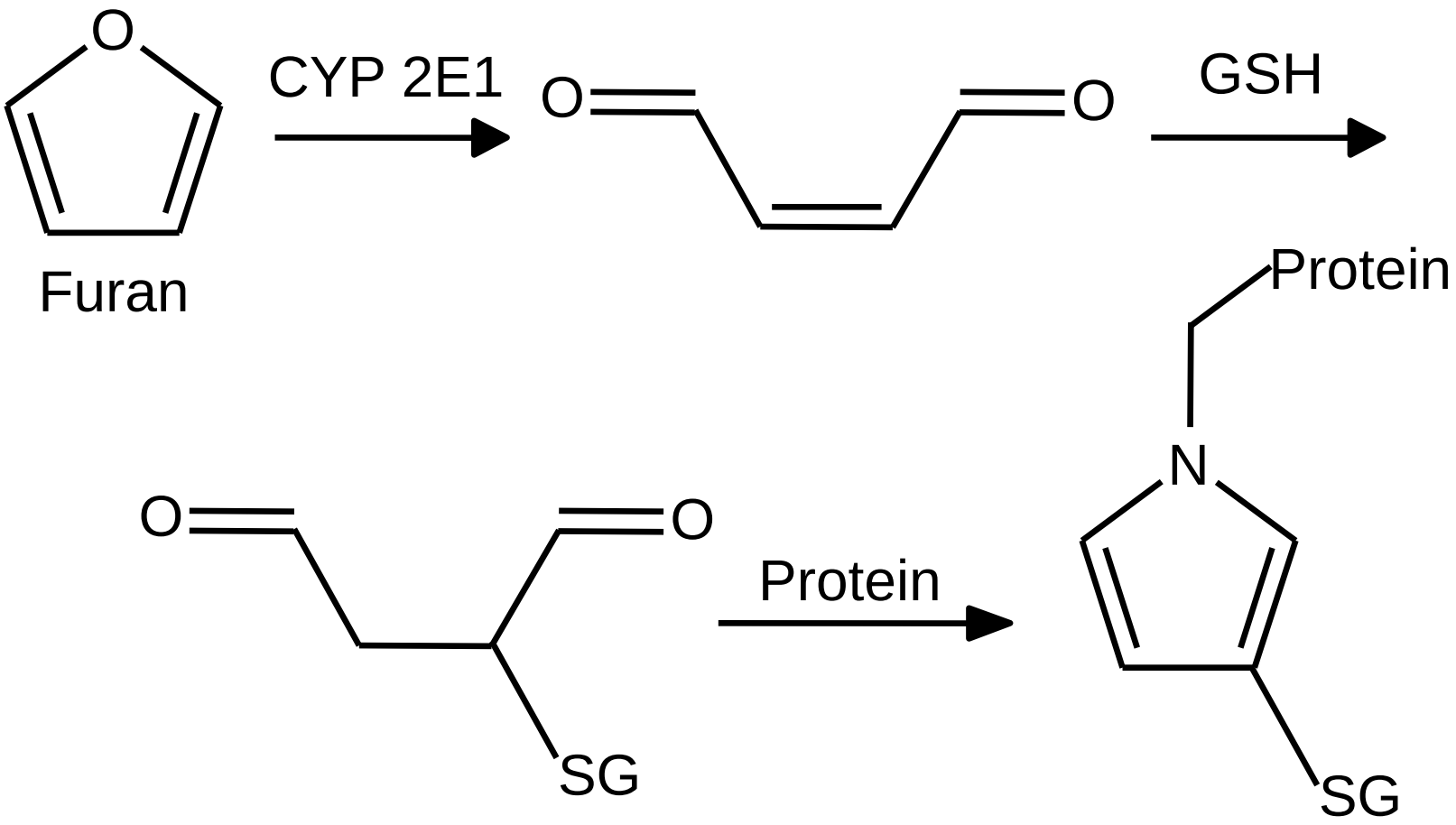


DNA

DNA adducts

cis-2-butene-1,4-dial

METABOLISM OF FURAN



NEW INITIATIVE #1: BVO

Brominated vegetable oil (BVO) is a food additive used in a number of popular soft drinks in the U.S., including *Mountain Dew*, *Fanta Orange*, *Fresca*, *Squirt*, *Sun Drop*, *Sunkist*, and many generic fruit-flavored drinks.

Due to its high density (*ca.* 1.33 g/cm^3), BVO can be mixed with lighter-than-water flavoring oils, affording an oily mixture with a density that matches that of the drink, enabling the formation of a stable emulsion.

BROMINATED VEGETABLE OIL

- BVO was generally recognized as safe (GRAS) in the U.S. until 1970 when it was removed from the GRAS list primarily due to cardiotoxicity observed in rat studies.
- FDA issued a regulation for the interim use of BVO at a maximum of 15 ppm in fruit-flavored beverages, pending the outcome of additional studies.
- Following receipt of additional studies (*e.g.* 2-year studies in dogs and miniature pigs), the cardiotoxicity issue was considered resolved.
- Since then, other animal studies of BVO have reported effects, including: reproductive impairment; enlargement of liver, heart, kidneys, and spleen; arrested testicular development; thyroid hyperplasia; myocarditis; accumulation of brominated fatty acids in tissues; and elevation of bromide ion (Br^-) in blood.

BROMINATED VEGETABLE OIL

90-Day Toxicity Study – Dose Response Arm Design

Dose group (%BVO in feed w/w)	Males	Females
0	10	10
0.002*	10	10
0.02	10	10
0.1	10	10
0.5	10	10

Sprague-Dawley rats will be fed BVO diet for 90 days.

*Dose corresponding to 10-fold the 90% percentile value for human exposure.

BROMINATED VEGETABLE OIL

90-Day Toxicity Study – Dose Response Arm Endpoints

- Feed consumption
- Body and organ weights
- Histopathological analysis – formalin-fixed/H&E, and frozen sections in heart and liver for lipid stains
- Clinical chemistry
- Terminal serum bromide ion levels
- Terminal serum TSH, T3, and T4
- Brominated triglycerides in liver, heart, and inguinal fat

BROMINATED VEGETABLE OIL

90-Day Toxicity Study – Bioaccumulation Arm Design

- Sprague-Dawley rats will be fed 0.002% and 0.5% BVO diet for 90 days.
- Brominated triglycerides in liver, heart, and inguinal fat will be assessed at 15, 30, 60, and 90 days, and at 15, 30, 60, 120, and 240 days after dosing ends.
- Other endpoints the same as in the 90-day dose-response arm.

NEW INITIATIVE #2: ARSENIC

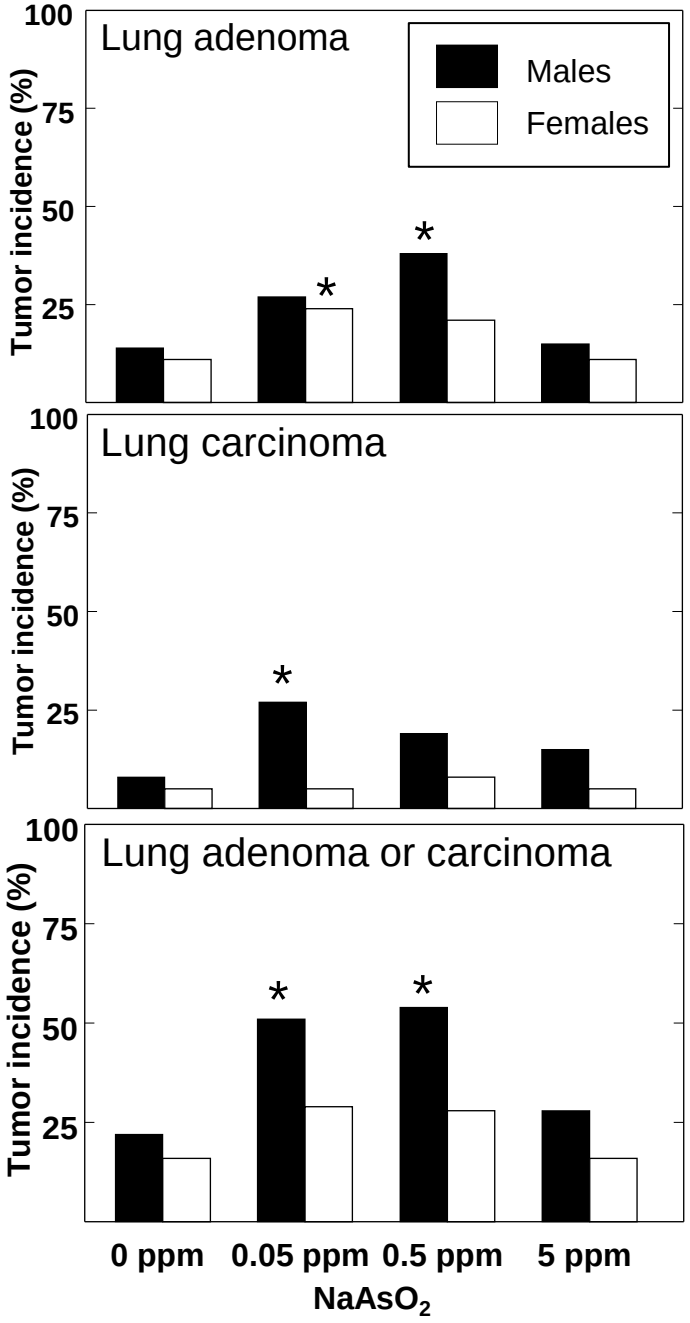
Background

- Average arsenic content in drinking water in the U.S. is 2 ppb, with some areas having concentrations >1000 ppb.
- EPA maximum contaminant level and WHO guideline value for inorganic arsenic in drinking water is 10 ppb.
- Estimated mean daily exposure to inorganic arsenic in U.S.
  - Adults, 0.08 - 0.20 µg/kg bw/day (95th percentile, 0.16 - 0.34 µg/kg bw/day)
  - Children, ages 1 - 6, 0.11 - 0.32 µg/kg bw/day
  - Children, < 1 year old, 0.24 - 1.19 µg/kg bw/day

TUMORIGENICITY OF $NaAsO_2$ IN MICE

"Whole life exposure": male and female F_0 CD1 mice exposed to $NaAsO_2$ in drinking water before breeding and during breeding; F_0 dams during pregnancy and lactation; F_1 mice from weaning until 2 years of age.

Waalkes *et al*., *Arch. Toxicol*. 88, 1619-1629, 2014.



PROPOSED $NaAsO_2$ BIOASSAY

- Repetition of Waalkes *et al*. (2014) “whole life” exposure bioassay

  Sires and dams before breeding and during breeding; dam during pregnancy; drinking water

  Pups PND 1 - 21; gavage

  Weaning - 2 years; drinking water

  0, 0.05, 0.16, 0.5, 1.6, 5, and 16 ppm $NaAsO_2$

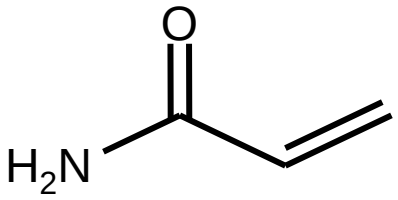
- “Perinatal only” exposure bioassay

  Sires and dams before breeding and during breeding; dams during pregnancy; drinking water

  Pups PND 1 - 21; gavage

  0, 1.6, 5, and 16 ppm $NaAsO_2$

NEW INITIATIVE #3: MUTATIONAL SIGNATURES

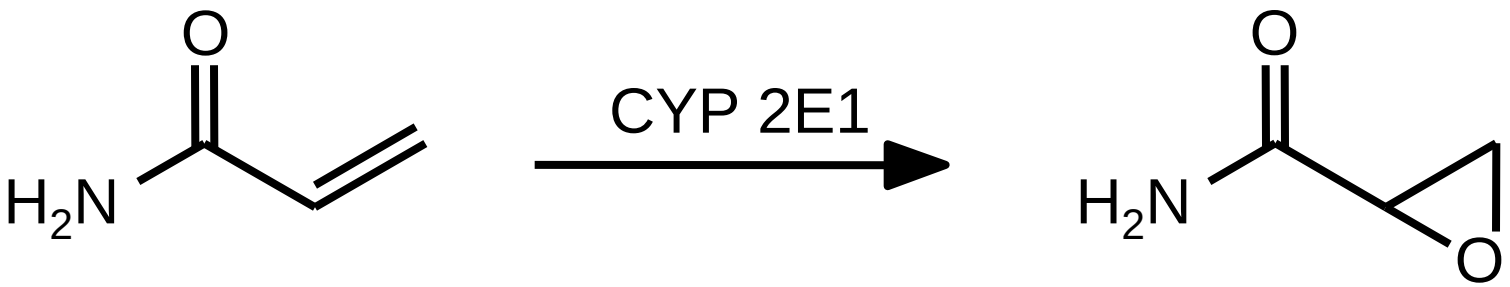


Acrylamide

Background:

- High-production chemical (>200 Gg/yr)
- Polymeric forms used in water treatment, crude oil and pulp-paper processing, concrete and grouts
- Monomeric acrylamide used for PAGE
- Cigarette smoke (3.1 µg/kg bw/day)
- Food (French fries, potato chips, bread, cereals; 0.44 µg/kg bw/day)

METABOLISM OF ACRYLAMIDE



Acrylamide

Glycidamide

DNA

DNA adducts

TUMORS IN B6C3F$_1$ MICE ADMINISTERED ACRYLAMIDE OR GLYCIDAMIDE

- Harderian gland adenoma (males and females)
- Lung alveolar/bronchiolar adenoma or carcinoma (males and females)
- Skin neoplasms (males and females)
- Forestomach squamous cell neoplasms (primarily papilloma) (males)
- Mammary gland adenoacanthoma or adenocarcinoma (females)

RISK ESTIMATE FOR DIETARY CARCINOGENS

Carcinogen	$BMDL_{10}$ in rodents (μg/kg bw/day)	Mean human dietary exposure (ng/kg bw/day)	Margin of exposure
PhIP	710	15	47,000
Benzo[*a*]pyrene	290	10	29,000
Aflatoxin B1	0.31	0.3	1,000
Acrylamide	159-173	440	360-390

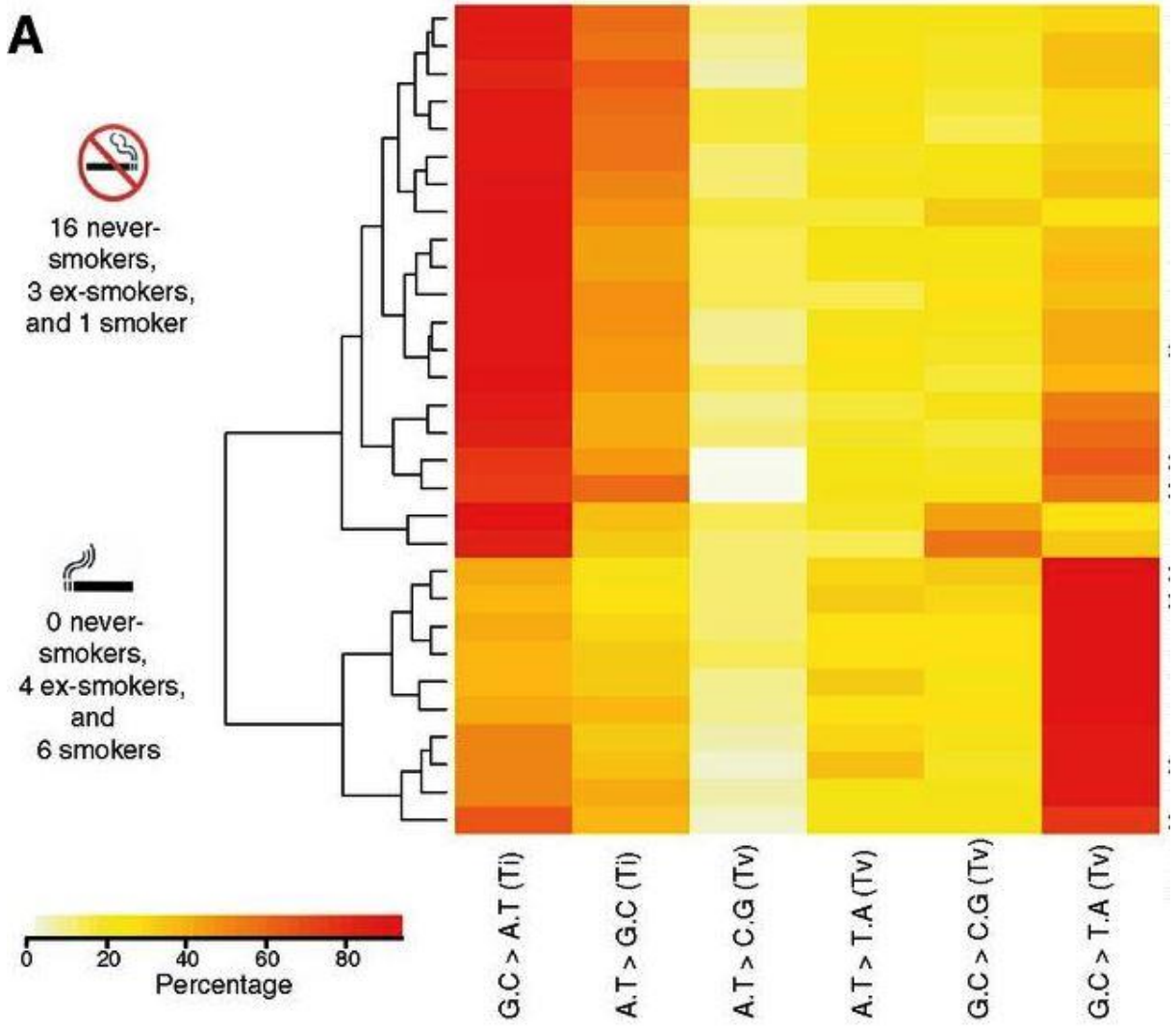
MUTATIONAL SIGNATURES PROTOCOL

Specific Aim 1: To determine the mutational signatures of tumors induced in experimental animals by acrylamide and glycidamide.

- Male B6C3F$_1$ mice: lung, forestomach, and Harderian gland tumors
- Female B6C3F$_1$ mice: lung, mammary gland, and Harderian gland tumors
- Male F344/N rats: thyroid and testes tumors
- Female F344/N rats: thyroid and mammary gland tumors
- Tumor DNA will be assessed by whole exome next-generation sequencing.

Specific Aim 2: To compare the mutational signatures obtained from acrylamide and glycidamide in experimental animals with mutational signatures of human tumors in published databases.

MUTATIONAL SIGNATURES



Krishnan *et al*., *Cancer Res*. 74, 6071-6081, 2014