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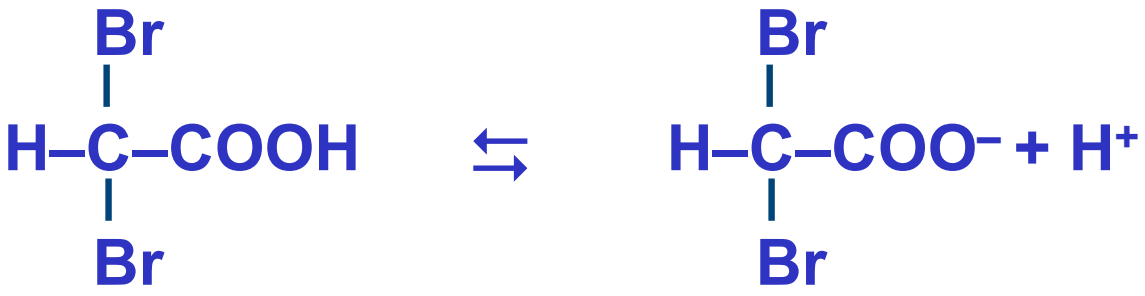
Toxicology and Carcinogenesis Studies of Dibromoacetic acid (CAS NO. 631-64-1) in F344/N Rats and B6C3F$_1$ Mice (Drinking Water Studies)



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DBA: Human Exposure

- Drinking water disinfection by-product: formed primarily by the reaction of chlorine and naturally occurring organic matter in the presence of bromide
- Concentrations in finished water: up to 18 μg/L
- Concentrations in water at Southern Res. Inst.
  - Total DHAs = 45 ± 23 μg/L
  - DBA = 3.8 ± 2.9 μg/L
- EPA's maximum contaminant level for haloacetic acids in drinking water is 60 μg/L (MCA, DCA, TCA, MBA, and DBA)

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DBA: Study Rationale

- Nominated to the NTP by EPA for toxicity and carcinogenicity studies because:
  - Widespread human exposure to DBPs
  - DCA is carcinogenic to the liver of rats and mice
- Drinking water is primary route of human exposure

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2-Week Study in Rats

- Water concentrations of DBA: 0, 125, 250, 500, 1,000, and 2,000 mg/L
- No effects on survival, clinical signs, water consumption, or final mean body weight
- Hepatocyte cytoplasmic alteration in males at 500 mg/L or higher and in females at 2,000 mg/L
- Delayed spermiation, retained spermatids, and large residual bodies in testes of males exposed to 500 mg/L or higher concentrations

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3-Month Study in Rats

- Water concentrations of DBA: 0, 125, 250, 500, 1,000, and 2,000 mg/L
- No effects on survival or clinical signs
- Water consumption and body weights were reduced in 2,000 mg/L males and females
- Liver: incidence of hepatocellular cytoplasmic vacuolization was increased in males at 500 mg/L and higher and in females at 2,000 mg/L
- Testes: decreased weight and atrophy of germinal epithelium at 2,000 mg/L (also epididymal hypospermia and reduced sperm motility); delayed spermiation and retained spermatids at 500 and 1,000 mg/L

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2-Year Study in Rats

Conc, mg/L	Survival %	Av. Terminal Wt. (% of control)	Av. daily dose mg/kg
Males			
0	68	509	-
50	48	504 (99)	2
500	60	457 (90)	20
1000	57	435 (86)	40
Females			
0	70	351	-
50	78	349 (97)	2
500	70	336 (96)	25
1000	64	306 (87)	45

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2-Year Study in Rats: Incidence of Neoplasms

Concentration (mg/L)	0	50	500	1,000
Male	N = 50	50	50	50
Mesothelioma[a]	3 (7)**[d]	1 (2)	0 (0)	10 (23)*
Mononuclear cell leukemia[b]	17 (37)	31 (66)**	24 (56)	13 (30)
Female	N = 50	50	50	50
Mononuclear cell leukemia[c]	11 (24)**	13 (27)	16 (35)	22 (47)*

[a] Historical incidence in 2-year drinking water controls: 6.0 ± 4.2%, range 0-12%
[b] Historical incidence in 2-year drinking water controls: 31.6 ± 3.3%, range 26-34%
[c] Historical incidence in 2-year drinking water controls: 23.5 ± 4.4%, range 20-30%
[d] Poly-3 adjusted incidence
* P≤0.05, ** P≤0.01

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2-Year Study in Rats: Nonneoplastic Lesions

Concentration (mg/L)	0	50	500	1,000
Male	N = 50	50	50	50
Liver, cystic degeneration	3 (1.0)[a]	9* (1.4)	11* (1.5)	15** (1.3)
Female	N = 50	50	50	50
Lung, alveolar epithelial hyperplasia	3 (1.3)	7 (1.9)	13** (1.7)	14** (1.9)
Kidney, nephropathy	18 (1.1)	32** (1.3)	37** (1.4)	40** (1.3)

[a] average severity: 1=minimal, 2=mild, 3= moderate
* P≤0.05, ** P≤0.01

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2-Week Study in Mice

- Water concentrations of DBA: 0, 125, 250, 500, 1,000, and 2,000 mg/L
- No effects on survival, clinical signs, water consumption, or final mean body weight
- Thymic atrophy in males at 1,000 or 2,000 mg/L and in females at 2,000 mg/L
- Spermatid retention and atypical residual bodies in the testes of males exposed to 1,000 or 2,000 mg/L

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3-Month Study in Mice

- Water concentrations of DBA: 0, 125, 250, 500, 1,000, and 2,000 mg/L
- No effects on survival or clinical signs
- Body weight gains were reduced in 2,000 mg/L males and females
- Liver: severity of hepatocellular cytoplasmic vacuolization was increased in males and females at 1,000 and 2,000 mg/L
  - Males: 1.6, 1.6, 1.5, 1.5, 2.1, 2.9
    [1=minimal, 2=mild, 3=moderate, 4=marked]
  - Females: 1.5, 1.5, 1.5, 1.7, 2.3, 2.7
- Testes: spermatid retention and atypical residual bodies at 1,000 and 2,000 mg/L; no effect on epididymal sperm concentration or sperm motility

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2-Year Study in Mice

Conc, mg/L	Survival %	Av. Terminal Wt. (% of control)	Av. daily dose mg/kg
Males			
0	63	46.7	-
50	76	50.0 (107)	4
500	68	51.1 (109)	45
1000	62	46.6 (100)	87
Females			
0	76	59.0	-
50	70	59.5 (101)	4
500	64	57.9 (98)	35
1000	64	56.6 (96)	65

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2-Year Study in Mice: Liver Neoplasms

Concentration (mg/L)	0	50	500	1,000
Male	N = 49	50	50	50
Hepatocellular adenoma or carcinoma[a]	28 (61)**[d]	41 (86)**	42 (88)**	47 (96)**
Hepatoblastoma[b]	0 (0)**	4 (9)	6 (13)*	18 (39)**
Female	N = 49	50	50	49
Hepatocellular adenoma or carcinoma[c]	22 (48)**	28 (61)	37 (80)**	37 (80)**

[a] Historical incidence in 2-year drinking water controls: 49.7 ± 31.1%, range 48-85%
[b] Historical incidence in 2-year drinking water controls: 4.5 ± 6.2%, range 0-13%
[c] Historical incidence in 2-year drinking water controls: 44.4 ± 18.1%, range 20-63%
[d] Poly-3 adjusted incidence
* P≤0.05, ** P≤0.01

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2-Year Study in Mice: Lung Lesions

Concentration (mg/L)	0	50	500	1,000
Male	N = 49	50	50	50
Alveolar epithelial hyperplasia	2 (1.5)[a]	6 (1.7)	6 (2.3)	7 (1.9)
Alveolar/bronchiolar adenoma or carcinoma[b]	12 (28)[c]	12 (26)	22 (49)*	17 (37)
Female	N = 50	50	50	50
Alveolar/bronchiolar adenoma or carcinoma[d]	2 (4)	5 (11)	5 (11)	7 (15)

[a] average severity: 1=minimal, 2=mild, 3= moderate
[b] Historical incidence in 2-year drinking water controls: 16.5 ± 10.7%, range 12-26%
[c] Poly-3 adjusted incidence
[d] Historical incidence in 2-year drinking water controls: 6.4 ± 3.9%, range 2-12%
* P≤0.05, ** P≤0.01

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Conclusions: 2-Year Study of DBA in Rats

- **Level of evidence of carcinogenic activity**
  - Male rats: malignant mesothelioma = some evidence
  - Female rats: mononuclear cell leukemia = some evidence
- **Nonneoplastic effects**
  - Male rats - cystic degeneration of the liver
  - Female rats - alveolar epithelial hyperplasia, nephropathy

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Conclusions: 2-Year Study of DBA in Mice

Level of evidence of carcinogenic activity

- **Male mice:**

  Hepatocellular neoplasms, hepatoblastomas = clear evidence

  Lung neoplasms = exposure related

- **Female mice:**

  Hepatocellular neoplasms = clear evidence

  Lung neoplasms = may have been related

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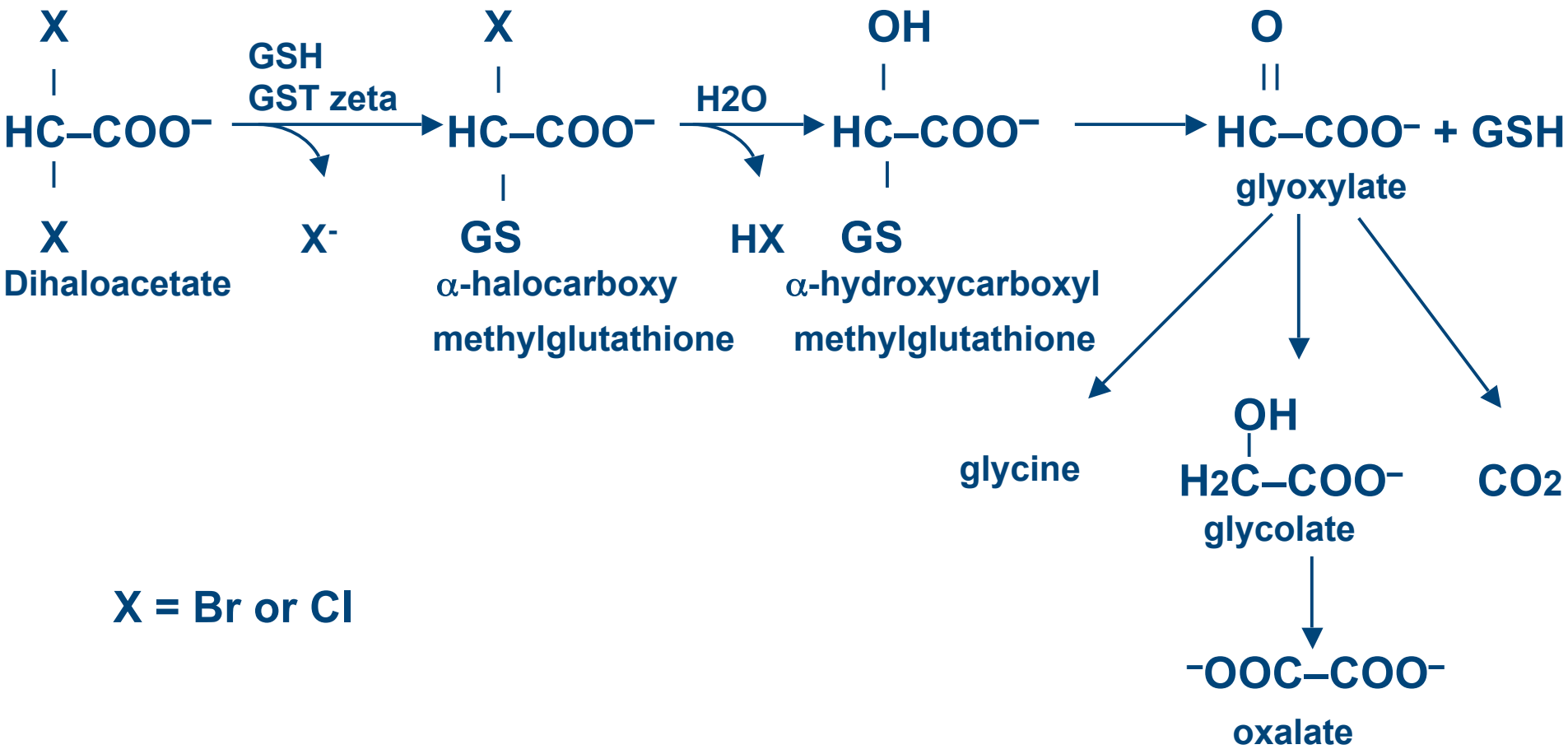
Metabolism of Dihaloacetates



X = Br or Cl

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Toxicokinetic Studies on Dihaloacetates

- **Chemical, sex and species**
  - DCA - male rats and female mice
  - DBA - female rats and male mice
  - BCA - male and female rats and mice
- **Measurements**
  - Plasma time courses of parent DHA and metabolites (glyoxylate and oxalate)
  - Urine analyses of parent DHAs and metabolites after gavage treatments
- **Treatments**
  - Single iv of each DHA
  - Single gavage of each DHA
  - Single iv of glyoxylate
  - 2 week drinking water exposures with each DHA followed by gavage
  - 2 week drinking water exposures with each DHA

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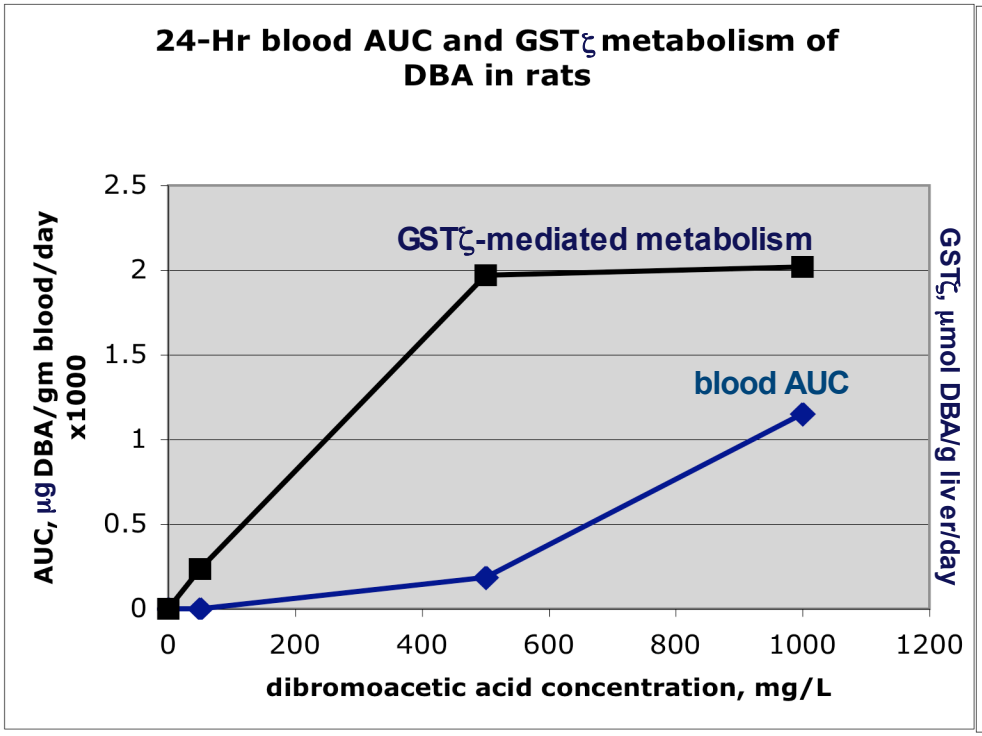
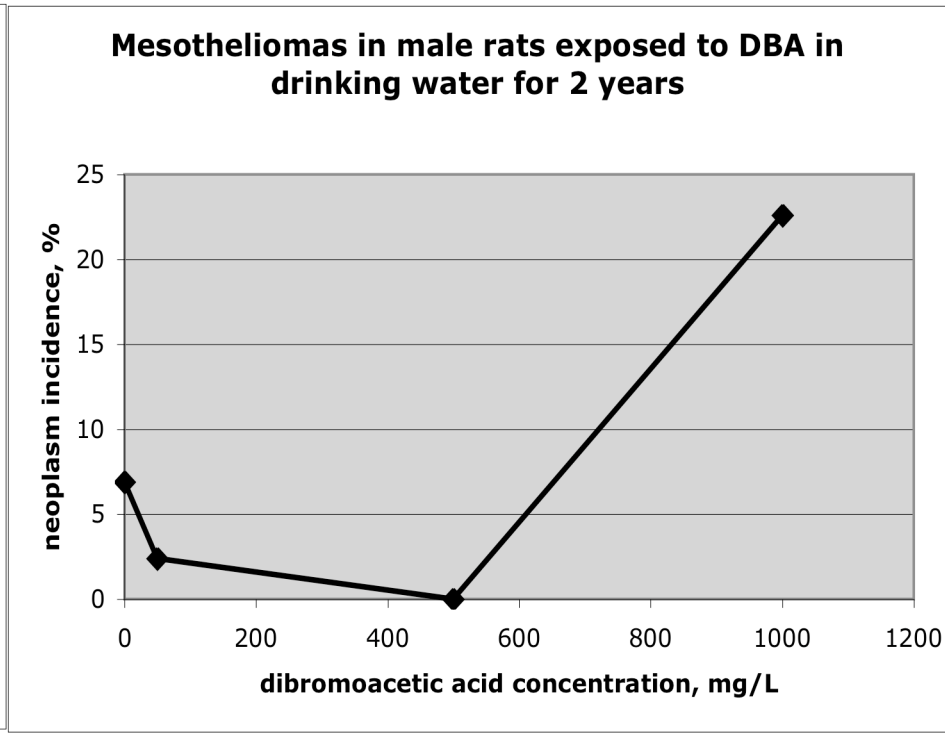
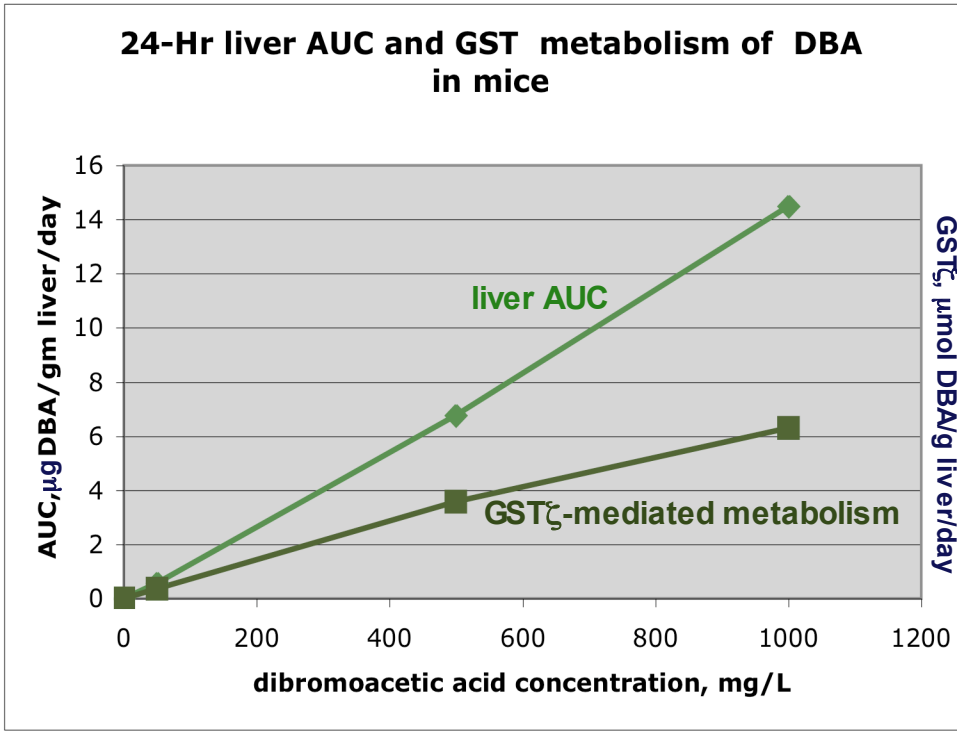
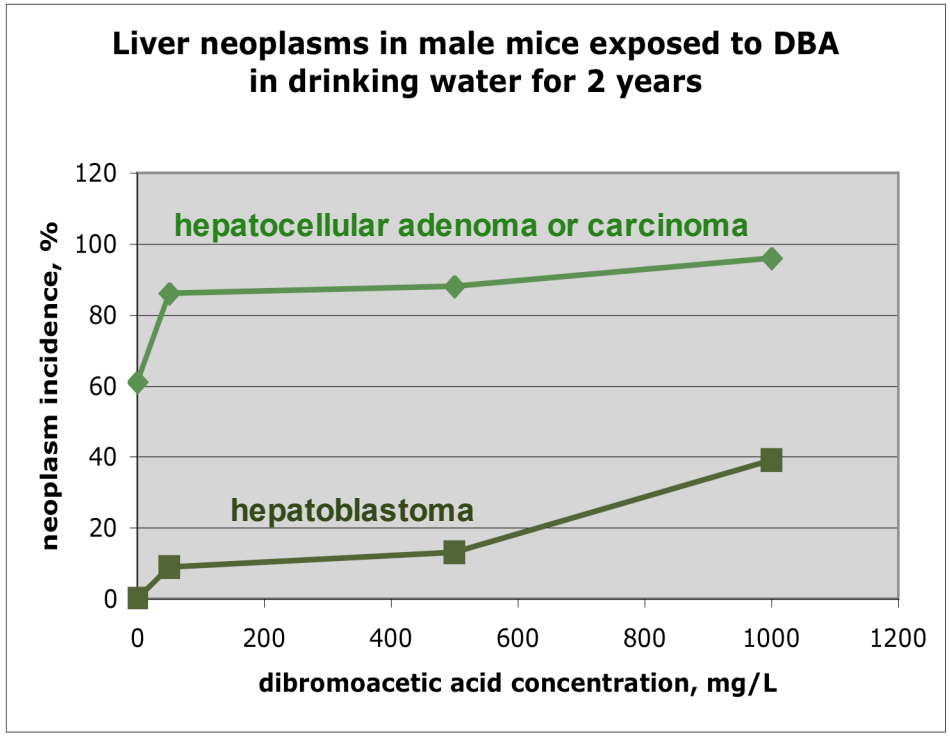
Features of the Preliminary PBPK Model of DBA

- Oral absorption
- Multi compartment distribution (flow-limited)
  - stomach, liver, kidney, slowly perfused tissues, rapidly perfused tissues
- Metabolism
  - GST-zeta kinetics with suicide inhibition, degradation and resynthesis
  - Non GST-zeta kinetics
- Urinary elimination
  - Glomerular filtration
  - Saturable reabsorption
- Published parameters
  - Cardiac output, urine flow, glomerular filtration, organ volumes (as fraction of body weight), organ blood flow (as fraction of cardiac output), tissue partition coefficients


24-Hr blood AUC and GSTζ metabolism of DBA in rats
AUC, µg DBA/gm blood/day x1000
GSTζ, µmol DBA/g liver/day
GSTζ-mediated metabolism
blood AUC
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
1200
dibromoacetic acid concentration, mg/L


Mesotheliomas in male rats exposed to DBA in drinking water for 2 years
neoplasm incidence, %
25
20
15
10
5
0
0
200
400
600
800
1000
1200
dibromoacetic acid concentration, mg/L


24-Hr liver AUC and GST metabolism of DBA in mice
AUC,μg DBA/gm liver/day
GSTζ, μmol DBA/g liver/day
liver AUC
GSTζ-mediated metabolism
dibromoacetic acid concentration, mg/L
0
2
4
6
8
10
12
14
16
0
200
400
600
800
1000
1200


Liver neoplasms in male mice exposed to DBA in drinking water for 2 years
neoplasm incidence, %
hepatocellular adenoma or carcinoma
hepatoblastoma
dibromoacetic acid concentration, mg/L
0
20
40
60
80
100
120
0
200
400
600
800
1000
1200

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NTP Technical Reports Review Subcommittee Meeting

Dibromoacetic acid