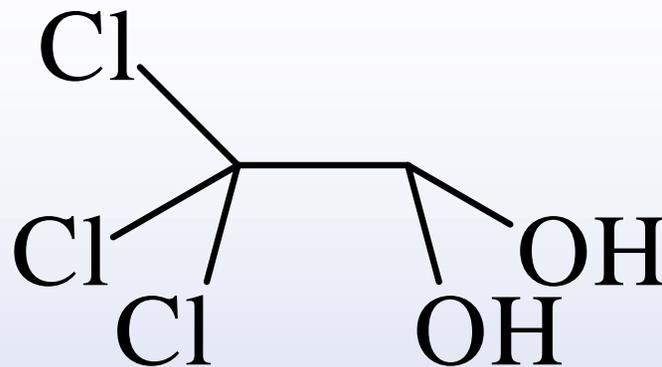


Chloral Hydrate



Molecular Weight: 165.4 CAS Reg. No.: 302-17-0



Use and Occurrence of Chloral Hydrate

- Sedative / hypnotic drug
 - Common pediatric use
 - Capsules, suppositories
 - 25 mg/kg +
- Chlorination by-product in water



Carcinogenicity of Chloral Hydrate

- **Carcinogenicity in humans**
 - No human data identified
- **Carcinogenicity in experimental animals**
 - Long-term drinking water studies in male and female rats, male mice
 - Long-term oral gavage studies in male and female mice
 - Single exposure oral studies in male and female mice
 - Neonatal mouse model studies in male and female mice



Tumors in B6C3F₁ Male Mice Receiving a Single Oral Dose of Chloral Hydrate at 15 Days of Age (Rijhsinghani *et al.*, 1986)

Tumor Site and Type		Dose, mg/kg		
		0	5	10
Liver	Adenomas	0/19	1/9	3/8
	Carcinomas	2/19	1/9	3/8
	Adenomas or Carcinomas	2/19 p=0.0007 ^a	2/9 (p=0.38) ^b	6/8 p=0.002 ^b

^a n value for trend (Mantel-Haenszel trend test).

^b p values for Fisher Exact Test relative to control group. Value is given in parentheses when not significant (p > 0.01).



Tumors in B6C3F1 Male Mice Receiving Chloral Hydrate (1 g/l in Drinking Water) for 104 Weeks (Daniel *et al.*, 1992)

Tumor Site and Type		Dose, mg/kg-day		
		0	166	
Liver	Hyperplastic Nodules	0/20	1/24	N.S.
	Adenomas	1/20	7/24	p = 0.04 ^a
	Carcinomas	2/20	11/24	p = 0.01
	Adenomas or Carcinomas	3/20	17/24	p = 0.0002

^a p-Values for Fisher's exact test relative to control group (N.S. = not significant).



Liver Tumors among Male B6C3F₁ Mice Treated with Chloral Hydrate in Drinking Water for Two Years and Surviving Beyond 78 Weeks (George *et al.*, 2000)

Tumor Type	Dose (mg/kg-day)				Trend ^a
	0	13.5	65.0	146.6	
Hepatocellular Adenoma	9/42	20/46 ^b	20/39 ^b	16/32 ^b	p = 0.019
Hepatocellular Carcinoma	23/42	25/46	23/39	27/32 ^b	p = 0.0018
Combined Hepatocellular Adenoma and Carcinoma	27/42	36/46	31/39	29/32 ^b	p = 0.0072

^a Exact test for linear trend.

^b Significantly increased above controls by pairwise comparison (Fisher's exact test, p < 0.05).



2002 NTP Studies of Chloral Hydrate

- **Female mice**
 - Long-term gavage study
 - Stop-exposure study
 - Single exposure studies with long-term follow-up
- **Male mice**
 - Long-term gavage studies of feed-restricted and *ad libitum*-fed mice
 - Single exposure study with long-term follow-up



Tumors among Female B6C3F₁ Mice Treated with Chloral Hydrate by Oral Gavage for Two Years (NTP, 2002a)

Tumor Type	Dose (mg/kg-day)				Trend ^a
	0	17.9	35.7	71.4	
Pituitary Gland Pars Distalis Adenoma	0/45	2/44	0/47	5/41 ^b	p = 0.0073
Malignant Lymphoma	9/48	7/48	8/48	15/48	p = 0.0455
Alveolar/Bronchiolar Adenoma	1/48	1/48	2/48	4/48	p = 0.0711
Hepatocellular Adenoma	1/48	2/48	3/48	2/48	N.S.
Hepatocellular Carcinoma	1/48	0/48	0/48	1/48	N.S.
Hepatocellular Adenoma or Carcinoma	2/48	2/48	3/48	3/48	N.S.

^a P-value of test for dose-related trend (NTP, 2002). N.S. = not significant (p > 0.05).

^b Significantly increased above controls by pairwise comparison (Poly-3 test, p < 0.05).



Tumors among Female B6C3F₁ Mice in Stop-Exposure Experiments Treated with Chloral Hydrate by Gavage (NTP, 2002a)

Tumor Type	Vehicle Control ^a	Dosing Period (Receiving 71.4 mg/kg-day)			
		3 Mo.	6 Mo.	12 Mo.	24 Mo. ^a
Pituitary Gland Pars Distalis Adenoma	0/45	3/36	1/36	1/33	5/41 ^b
Malignant Lymphoma	9/48	8/40	13/40	14/40	15/48
Alveolar/Bronchiolar Adenoma	1/48	2/40	4/40	7/40	4/48
Hepatocellular Adenoma	1/48	1/40	1/40	2/40	2/48
Hepatocellular Carcinoma	1/48	0/40	1/40	2/40	1/48
Hepatocellular Adenoma or Carcinoma	2/48	1/40	2/40	4/40	3/48

^a Control and 24-month incidences taken from previous slide.

^b Significantly increased above controls by pairwise comparison (Fisher's exact test, $p < 0.05$).



Tumors among Female B6C3F₁ Mice Treated with a Single Dose of Chloral Hydrate by Oral Gavage at 28 Days of Age, and Then Held Until Sacrifice at Two Years (“Regimen C”; NTP, 2002a)

Tumor Type	Dose (mg/kg)				Trend ^a
	0	10	25	50	
Malignant Lymphoma	8/48	16/48 ^b	6/48	16/48 ^b	N.S.
Hepatocellular Adenoma	3/48	3/48	2/48	2/48	N.S.
Hepatocellular Carcinoma	3/48	0/48	0/48	0/48	[p = 0.015]
Hepatocellular Adenoma or Carcinoma	6/48	3/48	2/48	2/48	N.S.

^a P value associated with trend test (NTP, 2002). Negative trends are indicated in brackets. N.S. = not significant.

^b Significantly increased above controls by pairwise comparison (Poly-3 test, p < 0.05).



Tumors among Female B6C3F₁ Mice Treated by Oral Gavage with a Single Dose of Chloral Hydrate at 15 Days of Age, and Then Held Until Sacrifice at Two Years ("Regimen D"; NTP, 2002a)

Tumor Type	Dose (mg/kg)				Trend ^a
	0	10	25	50	
Malignant Lymphoma	14/48	10/48	5/48 ^b	7/48	[p = 0.035]
Hepatocellular Adenoma	1/48	1/48	2/48	1/48	N.S.
Hepatocellular Carcinoma	0/48	2/48	1/48	1/48	N.S.
Hepatocellular Adenoma or Carcinoma	1/48	3/48	2/48	2/48	N.S.

^a P-value associated with trend test (NTP, 2002). N.S. = not significant. Brackets indicate negative trend.

^b Significant decrease in incidence relative to controls (p < 0.05, by Poly-3 test).



Liver Tumor Incidence among Both *Ad Libitum*-Fed and Dietary-Controlled Male B6C3F₁ Mice Treated by Oral Gavage with Chloral Hydrate in Water for Two Years (NTP, 2002b)

Diet	Tumor Type	Dose (mg/kg-day)				Trend ^a
		0	17.9	35.7	71.4	
Ad Libitum- Fed	Hepatocellular Adenoma	12/48	19/48 ^b	17/47	17/48	N.S.
	Hepatocellular Carcinoma	4/48	10/48 ^b	10/47 ^b	7/48	N.S.
	Combined Hepatocellular Adenoma and Carcinoma	16/48	25/48 ^c	23/47 ^b	22/48	N.S.
Dietary- Controlled	Hepatocellular Adenoma	9/48	7/48	10/48	10/48	N.S.
	Hepatocellular Carcinoma	2/48	5/48	4/48	8/48 ^c	p = 0.029
	Combined Hepatocellular Adenoma and Carcinoma	11/48	11/48	14/48	18/48 ^b	p = 0.041

^a Exact test for linear trend. N.S. = not significant.

^b Pairwise comparison with controls (Poly-3 test): 0.05 < p < 0.1

^c Significant increase above controls by pairwise comparison (Poly-3 test, p < 0.05).



Tumors among Male B6C3F₁ Mice Treated by Oral Gavage with a Single Dose of Chloral Hydrate at 15 Days of Age, and Then Held Until Sacrifice at Two Years (“Regimen E”; NTP, 2002a)

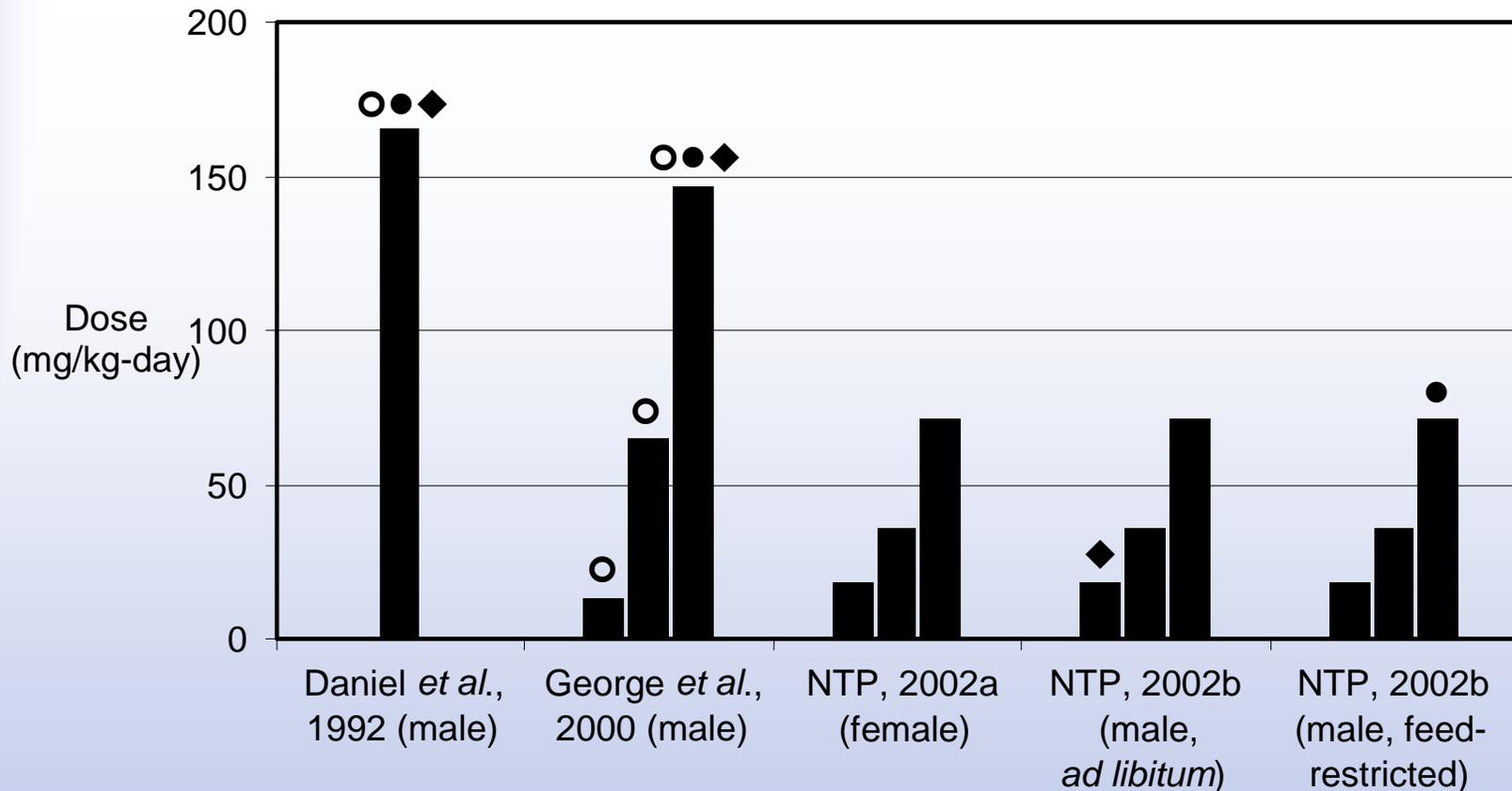
Tumor Type	Dose (mg/kg)				Trend ^a
	0	10	25	50	
Hepatocellular Adenoma	18/48	8/48 ^b	12/48	11/48	N.S.
Hepatocellular Carcinoma	10/48	10/48	6/48	12/48	N.S.
Hepatocellular Adenoma or Carcinoma	24/48	17/48	18/48	21/48	N.S.

^a P-value associated with trend test (NTP, 2002). N.S. = not significant.

^b Significantly decreased below controls by pairwise comparison (Poly-3 test, $p < 0.05$).



Average Daily Dose of Chloral Hydrate Administered in Long-Term Studies in Mice. Identification of Dose Groups with Increased Incidence of Liver Tumors



Vertical bars denote different dose groups within a given study. Symbols above bars indicate the endpoints for which statistically significant increases were observed (○=hepatocellular adenomas; ●=hepatocellular carcinomas; ◆=combined hepatocellular adenomas and carcinomas).



Dose Considerations

- **Survival**
 - little to no change in survival across studies
- **Toxicity**
 - little evidence of non-cancer toxicity
 - Heart, kidney organ weight changes in NTP male mice
 - Mild hepatocellular necrosis in Daniel *et al.* study



Long-term Studies in Rats

- Drinking water study in males and females for 124/128 weeks (Leuschner and Beuscher, 1998)
 - No increase in tumors
- Drinking water study in male Fischer rats for two years (George *et al.*, 2000)



Liver Tumors Among Male F344/N Rats Treated with Chloral Hydrate in Drinking Water for Two Years and Surviving to 78 Weeks (George *et al.*, 2000)

Tumor Type	Dose (mg/kg-day)			
	0	7.4	37.4	162.6
Hepatocellular Adenoma	0/42	3/42	1/44	2/44
Hepatocellular Carcinoma	1/42	3/42	0/44	1/44
Combined Hepatocellular Adenoma and Carcinoma	1/42	6/42 ^a	1/44	3/44

^a Marginally significant increase above controls in pairwise comparison ($p = 0.055$, by Fisher's exact test).



Liver Tumors among Male and Female B6C3F₁ Mice Treated Intraperitoneally as Neonates with Chloral Hydrate in DMSO (Von Tungeln *et al.*, 2002)

Tumor Type	Sex	Control (DMSO)	Chloral Hydrate	4-Amino-biphenyl
<i>Assay A (12 Months): 2000 nmol chloral hydrate</i>				
Hepatocellular Adenomas	M	1/24	5/24 ^a	23/24 ^b
	F	0/24	0/21	0/24
Hepatocellular Carcinomas	M	0/24	0/24	13/24 ^b
	F	0/24	0/21	0/24
Combined Hepatocellular Adenomas and Carcinomas	M	1/24	5/24 ^a	24/24 ^b
	F	0/24	0/21	0/24
<i>Assay B (20 Months): 1000 nmol chloral hydrate</i>				
Hepatocellular Adenomas	M	6/23	9/23	22/22 ^b
	F	0/23	0/22	9/23 ^b
Hepatocellular Carcinomas	M		2/23	14/22 ^b
	F	0/23	0/22	0/23
Combined Hepatocellular Adenomas and Carcinomas	M ^{2/23}		10/23	22/22 ^b
	F	0/23	0/22	9/23 ^b

^a Pairwise comparison with controls (Fisher's exact test): $\bar{p} = 0.094$.

^b Significant increase in incidence relative to controls by pairwise comparison (Fisher's exact test, $p < 0.05$).



Other Studies

- Skin painting study in mice
 - Roe and Salaman (1955): Inconclusive



Genotoxicity of Chloral Hydrate

- *In vitro* studies
 - Cytogenetic changes
 - *Aspergillus nidulans*
 - *Saccharomyces cerevisiae*
 - Gene mutations
 - *Salmonella typhimurium*
 - *Aspergillus nidulans*
 - *Saccharomyces cerevisiae*



Genotoxicity of Chloral Hydrate (cont.)

- *In vitro* studies
 - Sister chromatid exchanges, aneuploidy, micronuclei (human lymphocytes)
 - Micronuclei, aneuploidy (hamster fibroblasts and ovary cells, mouse lymphoma cells)

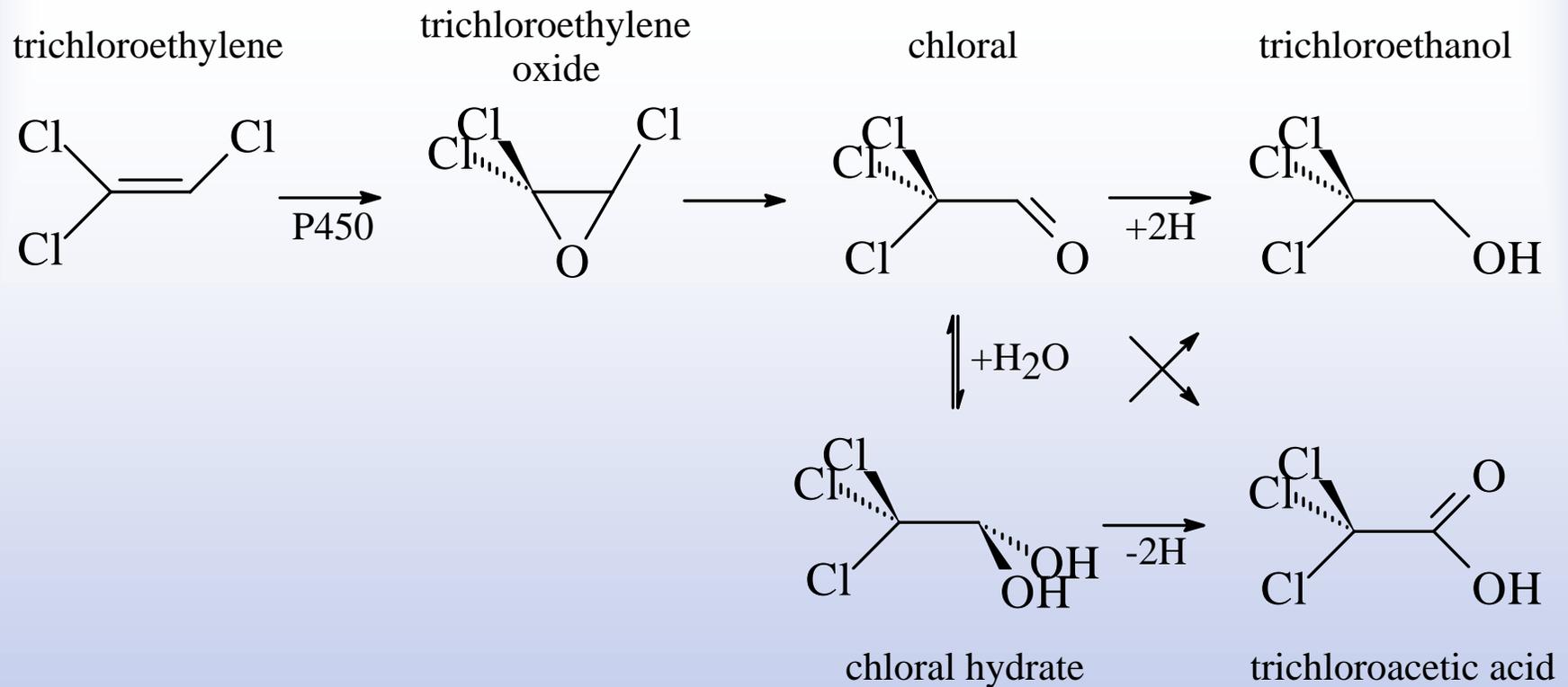


Genotoxicity of Chloral Hydrate (cont.)

- *In vivo* studies in mice and rats
 - Aneuploidy in spermatocytes (i.p.)
 - DNA strand breaks (oral)
 - Induction of micronuclei in bone marrow (i.p.)



Metabolism of Trichloroethylene and Chloral Hydrate



Mechanism of Carcinogenicity

- **Genotoxicity**
 - High dose effect?
- **Cytotoxicity**
 - Inconsistent observations
- **Peroxisome proliferation**
 - Inconsistent observations
 - Related to other compounds showing PP activity
 - Trichloroacetic acid, dichloroacetic acid



Summary of Evidence

- **Animal evidence**
 - Liver tumors in male mice
 - Two long-term drinking water studies
 - One single-exposure study
 - Conflicts with repeat study
 - Other studies in male mice more ambiguous
 - Benign pituitary tumors in female mice
 - Malignant lymphoma in female mice



Summary of Evidence (cont.)

- Other relevant evidence
 - Genotoxicity
 - Active metabolite of carcinogen?



Authoritative Body Activity

- **NTP (2002)**
 - “some evidence” in male mice (liver tumors)
 - “equivocal evidence” in female mice (pituitary adenomas)
- **IARC (1995; 2002)**
 - “limited evidence” in experimental animals
 - “inadequate evidence” in humans
 - Group 3 – “not classifiable”
- **U.S. EPA (2000)**
 - “chloral hydrate shows suggestive evidence of human carcinogenicity by the oral route of exposure”

