



National Toxicology Program
U.S. Department of Health and Human Services

**Draft Report on Carcinogens Monograph on
Haloacetic Acids Found as Water Disinfection
By-Products:
Appendices**

Peer-Review Draft

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Office of the Report on Carcinogens
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
U.S. Department of Health and Human Services

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Appendix A: Literature Search Strategy

Introduction

The objective of the literature search approach is to identify published literature that is relevant for evaluating the potential carcinogenicity of the haloacetic acids of interest. As discussed in the Concept Document for haloacetic acids

(https://ntp.niehs.nih.gov/ntp/about_ntp/bsc/2016/april/haa_508.pdf), the monograph relies on the IARC monograph and studies published since the monograph (new studies). The literature search strategy was used to identify new human cancer studies and recent reviews of mechanistic data.

A.1 General approach

Database searching encompasses selecting databases and search terms and conducting the searches. Searches of several citation databases are generally conducted using search terms for the individual haloacetic acids, combined with search terms for cancer and/or specific topics, including epidemiological and mechanistic studies. A critical step in the process involves consultation with an information specialist to develop relevant search terms. These terms are used to search bibliographic databases. IARC volume 101 used literature found in PubMed before December 2012, so any searches limited by date sought new information published since 2011. The body of literature for haloacetic acids of interest was searched using narrowing terms for the relevant major topics within the bibliographic databases. The results were then processed in EndNote to remove duplicates before being transferred to Health Assessment Workplace Collaborative (HAWC) for screening. Table A-1 highlights the general concepts searched and databases consulted. To review all the terms used, please refer to the full search strings below.

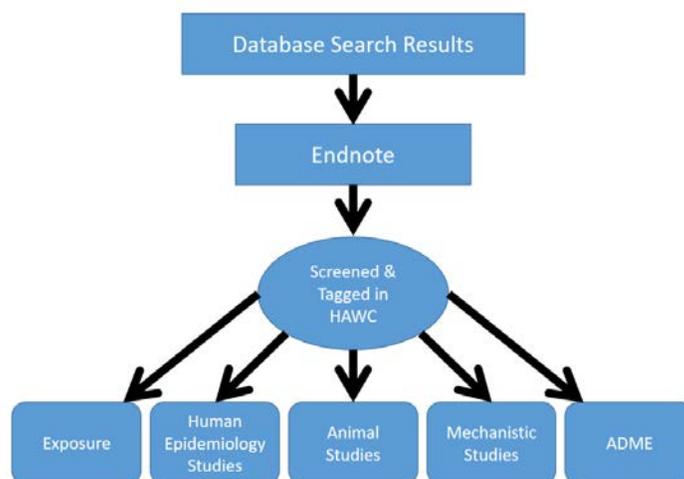


Table A-1. Major topics searched

Topic	Search Method	Databases searched
Exposure	13 HAAs String AND occur*[tiab]	PubMed
Human Studies	13 HAAs String AND OROc Epidemiological (Human) Studies Search AND OROc Cancer Search	PubMed, Scopus, Web of Science
Animal Studies	13 HAAs String AND Experimental Animals Studies Search AND OROc Cancer Search	PubMed, Scopus, Web of Science
Mechanism and Genetox	13 HAAs String AND OROc Characteristics of Carcinogens Search	PubMed, Scopus, Web of Science

In addition to the standard search concepts described above, three special targeted searches were run to seek additional detail for the Human Cancer and Mechanistic sections of the monograph. To confirm full coverage on bladder cancer a targeted search was run for that endpoint alone. The primary use for Halo-acetic acids in the treatment and disinfection of water suggests that and research conducted on the subject of water treatment and disinfection methods in general may contain data of interest to the evaluation. A search was conducted to collect human studies referring to the general methods rather than the specific HAAs. Finally, in an effort to categorize the HAAs for possible read-across efforts and specific search was conducted on metabolic and mechanistic concepts thought to be common between some of the HAAs. Table A-2 highlights the general concepts searched and databases consulted for these special searches. To review all the terms used, please refer to the full search strings below in the section [Supplementary Searches](#).

Table A-2. Supplementary searches

Topic	Search Method	Databases searched
Bladder Cancer	Supplementary Bladder Cancer Search (See detailed description below)	PubMed, Scopus, Web of Science
Human Studies on water treatment or disinfection	DBPs OR disinfection OR water treatment OR treated water AND OROc Cancer Search (See detailed description below)	PubMed, Scopus, Web of Science
Read Across	13 HAAs String AND terms for select metabolic concepts (see detailed description below for Mechanism Special Search)	PubMed, Scopus, Web of Science

A.2. Standard Searches

A.2.1 13 HAAs

A search of primary and universal terms for the 13 haloacetic acids chemicals and cancer. Limiting terms have been applied to reduce the number of irrelevant results returned that are associated with chemical peel treatments, wart removal methods, and trichloroacetic acid (TCA) precipitation.

This search was used in most cases to characterize the haloacetic acids and was combined with narrowing terms for cancer, animal studies, human epidemiology studies, mechanistic literature (characteristics of carcinogens) and more.

PubMed:

((Haloacetic-acid*[tiab]) OR Dihaloacetic-acid*[tiab]) OR Trihaloacetic-acid*[tiab] OR ("dichloroacetic acid"[nm] OR 79-43-6[rn] OR dichloroacetate[tiab] OR "dichloroacetic acid"[tiab] OR "Bichloroacetic acid"[tiab] OR "Dichloroacetic acid"[tiab] OR "Dichloroethanoic acid"[tiab] OR "Dichloroethanoic acid"[tiab]) OR ("Trichloroacetic Acid"[mh] OR 76-03-9[rn] OR "Trichloroacetic Acid"[tiab] OR Trichloroacetate[tiab] OR "Trichloroacetic acid"[tiab]) OR ("Dibromoacetic acid"[nm] OR 631-64-1[rn] OR dibromoacetate[tiab] OR "Dibromoacetic acid"[tiab]) OR ("tribromoacetic acid"[nm] OR 75-96-7[rn] OR tribromoacetate[tiab] OR "tribromoacetic acid"[tiab]) OR ("Dichlorobromoacetic acid"[tiab] OR Bromodichloroacetate[tiab] OR "bromodichloroacetic acid"[nm] OR bromodichloroacetic-acid[tiab] OR 71133-14-7[rn]) OR ("Dibromochloroacetic acid"[tiab] OR 5278-95-5[rn] OR bromochloroacetate[tiab]) OR ("bromochloroacetic acid"[nm] OR 5589-96-8[rn] OR "bromochloroacetic acid"[tiab] OR bromochloroacetate[tiab] OR "Chlorobromoacetic acid"[tiab]) OR (Diiodoacetic-acid[tiab] OR "598-89-0"[tiab] OR Diiodoacetate[tiab]) OR (71815-43-5[rn] OR Bromoiodoacetic-acid[tiab] OR Bromoiodoacetate[tiab]) OR (Chloroiodoacetic-acid[tiab] OR "Chloro(iodo)acetic acid"[tiab] OR 53715-09-6[rn] OR "2-Chloro-2-iodoacetic acid"[tiab] OR "Acetic acid, 2-chloro-2-iodo-"[tiab] OR "chloro-iodoacetic acid"[tiab] OR Chloroiodoacetate[tiab]) OR ("Monochloroacetic acid"[tiab] OR "Monochloroacetic acid"[tiab] OR "79-11-8"[rn] OR Chloroacetic-acid[tiab] OR "Chloroacetic acid"[nm] OR "Chloroacetic acid"[tiab]) OR (Bromoacetic-acid[tiab] OR Bromoacetate[tiab] OR Monobromoacetic-acid[tiab] OR 79-08-3[rn]) OR ("Iodoacetic acid"[mh] OR 64-69-7[rn] OR Monoiodoacetic-acid[tiab] OR Monoiodoacetate[tiab] OR Monoiodine-acetate[tiab] OR Iodoacetate[tiab] OR Iodoacetic-acid[tiab]) NOT (trichloro-acetic-acid-peel*[tiab] OR trichloroacetic-acid-peel*[tiab] OR trichloroacetic-acid-peel*[tiab] OR Trichloroacetic-Acid-solub* OR Trichloroacetic-Acid-insolub* OR Trichloroacetic-Acid-precipit* OR TCA-solub* OR TCA-insolub* OR TCA-precipit* OR anogenital-wart*[tiab] OR genital-wart*[tiab] OR "Condylomata Acuminata"[Mh] OR "Human papillomavirus"[tiab] OR "Human papillomavirus 31"[mh] OR "Sexually transmitted diseases"[tiab])

Web of Science:

(TS=("Haloacetic acid*" OR "Dihaloacetic acid*" OR "Trihaloacetic acid*")) OR (TS=("dichloroacetic acid" OR "79-43-6" OR dichloroacetate OR "dichloroacetic acid" OR "Bichloroacetic acid" OR "Dichloroacetic acid" OR "Dichloroethanoic acid" OR "Dichloroethanoic acid" OR "76-03-9" OR "Trichloroacetic Acid" OR Trichloroacetate OR "Trichloroacetic acid" OR "Dibromoacetic acid" OR "631-64-1" OR dibromoacetate OR "Dibromoacetic acid" OR "tribromoacetic acid" OR "75-96-7" OR tribromoacetate OR "tribromoacetic acid" OR "Dichlorobromoacetic acid" OR Bromodichloroacetate OR "bromodichloroacetic acid" OR "bromodichloroacetic-acid" OR "71133-14-7" OR "Dibromochloroacetic acid" OR "5278-95-5" OR bromochloroacetate OR "bromochloroacetic acid" OR "5589-96-8" OR "bromochloroacetic acid" OR bromochloroacetate OR "Chlorobromoacetic acid")) OR (TS=("Diiodoacetic acid" OR "598-89-0" OR Diiodoacetate OR "71815-43-5" OR "Bromoiodoacetic acid" OR

Bromiodoacetate OR "Chloriodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR Chloriodoacetate)) OR (TS=("Monochloroacetic acid" OR "Monochloracetic acid" OR "79-11-8" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Chloracetic acid" OR "Iodoacetic acid" OR "64-69-7" OR "Monoiodoacetic acid" OR Monoiodoacetate OR "Monoiodine acetate" OR Iodoacetate OR "Bromoacetic acid" OR Bromoacetate OR "Monobromoacetic acid" OR "79-08-3")) NOT (TS=("trichloro-acetic acid peel*" OR "trichloroacetic acid peel*" OR "trichloracetic acid peel*" OR "Trichloroacetic-Acid solub*" OR "Trichloroacetic Acid insolub*" OR "Trichloroacetic Acid precipit*" OR "TCA solub*" OR "TCA insolub*" OR "TCA precipit*" OR "anogenital wart*" OR "genital wart*" OR "Condylomata Acuminata" OR "Human papillomavirus*" OR "Sexually transmitted diseas*"))

Scopus:

((TITLE-ABS-KEY ("Haloacetic acid*" OR "Dihaloacetic acid*" OR "Trihaloacetic acid*")) OR (TITLE-ABS-KEY ("dichloroacetic acid" OR "79-43-6" OR dichloroacetate OR "dichloroacetic acid" OR "Bichloracetic acid" OR "Dichloracetic acid" OR "Dichlorethanoic acid" OR "Dichloroethanoic acid" OR "76-03-9" OR "Trichloroacetic Acid" OR trichloroacetate OR "Trichloracetic acid" OR "Dibromoacetic acid" OR "631-64-1" OR dibromoacetate OR "Dibromoacetic acid" OR "tribromoacetic acid" OR "75-96-7" OR tribromoacetate OR "tribromoacetic acid" OR "Dichlorobromoacetic acid" OR bromodichloroacetate OR "bromodichloroacetic acid" OR bromodichloroacetic-acid OR "71133-14-7" OR "Dibromochloroacetic acid" OR "5278-95-5" OR bromochloroacetate OR "bromochloroacetic acid" OR "5589-96-8" OR "bromochloroacetic acid" OR bromochloroacetate OR "Chlorobromoacetic acid")) OR (TITLE-ABS-KEY ("Diiodoacetic acid" OR "598-89-0" OR diiodoacetate OR "594-68-3" OR "71815-43-5" OR "Bromiodoacetic acid" OR bromiodoacetate OR "Chloriodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR chloriodoacetate)) OR (TITLE-ABS-KEY ("Monochloroacetic acid" OR "Monochloracetic acid" OR "79-11-8" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Chloracetic acid" OR "Iodoacetic acid" OR "64-69-7" OR "Monoiodoacetic acid" OR monoiodoacetate OR "Monoiodine acetate" OR iodoacetate OR "Bromoacetic acid" OR bromoacetate OR "Monobromoacetic acid" OR "79-08-3")) AND NOT (TITLE-ABS-KEY("trichloro-acetic acid peel*" OR "trichloroacetic acid peel*" OR "trichloracetic acid peel*" OR "Trichloroacetic-Acid solub*" OR "Trichloroacetic Acid insolub*" OR "Trichloroacetic Acid precipit*" OR "TCA solub*" OR "TCA insolub*" OR "TCA precipit*" OR "anogenital wart*" OR "genital wart*" OR "Condylomata Acuminata" OR "Human papillomavirus*" OR "Sexually transmitted diseas*"))

A.2.2. RoC Cancer String:

The PubMed String is the same as described in the Handbook Appendix (https://ntp.niehs.nih.gov/ntp/roc/handbook/rochandbookappendix_508.pdf), however additional options for wildcard use and truncation have allowed the same string to be shortened for WOS and Scopus. The altered strings are presented here.

Web of Science:

(TS=(**broma OR *bromas OR *doma OR *domas OR *droma OR *dromas OR *eoma OR *eomas OR *goma OR *gomas OR *ioma OR *iomas OR *loma OR *lomas OR *moma OR *momas OR *noma OR *nomas OR *omatosis OR *phoma OR *phomas OR *poma OR *pomas OR *phroma OR *phromas OR *sarcoma OR *sarcomas OR *scoma OR *scomas OR *thecoma OR *thecomas OR *thoma OR *thomas OR *toma OR *tomas OR *uroma OR *uromas OR *xoma OR *xomas OR *yoma OR *yomas OR *kaemia OR *kaemia OR *kemia OR *kemia OR *plakia OR *plakias*)) OR (TS=(*"cancer" OR "cancerous" OR "cancers" OR "carcinogen" OR "carcinogenesis" OR "carcinogenic" OR "carcinogens" OR "carcinoid" OR "carcinomatosis" OR "cocarcinogenesis" OR "metaplasia" OR "anaplasia" OR "neoplasia" OR "neoplasia" OR "neoplasm" OR "neoplasms" OR "neoplastic" OR "tumor" OR "tumorigenesis" OR "tumorigenic" OR "tumorigenesis" OR "tumorigenic" OR "tumorigenesis" OR "tumorigenic" OR "tumors" OR "tumour" OR "tumours" OR "nonhodgkin" OR "nonhodgkins" OR "Hodgkin" OR "hodgkins"*))) OR (TS=(*"acrochordon" OR "acrochordons" OR "acrospiroma" OR "acrospiromas" OR "adenomatous" OR "adenosis" OR "Buschke-Lowenstein" OR "chloroma" OR "chloromas" OR "CIN" OR "CLL" OR "dermoid" OR "dysmyelopoiesis" OR "epidermoid" OR "essential thrombocythemia" OR "exostosis" OR "fibroid" OR "fibroids" OR "lymphoproliferation" OR "lymphoproliferations" OR "lymphoproliferative" OR "macroglobulinemia" OR "macroglobulinemias" OR "malignancies" OR "malignancy" OR "malignant" OR "mastocytosis" OR "meigs syndrome" OR "micrometastases" OR "micrometastasis" OR "mycosis fungoides" OR "myelofibrosis" OR "myeloproliferation" OR "myeloproliferations" OR "myeloproliferative" OR "NSCLC" OR "papillomata" OR "papillomatosis" OR "pilomatricoma" OR "pilomatricomas" OR "polyposis" OR "poroma" OR "poromas" OR "pre malignant" OR "preneoplastic" OR "seminomatous" OR "sezary syndrome" OR "struma ovarii" OR "waldenstrom" OR "waldenstroms" OR "oncogene fusion" OR "5q syndrome" OR "aberrant crypt foci" OR "Aberrant crypt focus" OR "carney complex" OR "denys drash" OR "leukostasis" OR "zollinger ellison"*))) OR ((TS=(*"sentinel lymph node" NOT "biopsy"*))) OR (TS=(*"ASCO" NOT "fungi"*))) OR (TS=(*"WAGR" AND "syndrome"*)))

Scopus:

(TITLE-ABS (**broma OR *bromas OR *doma OR *domas OR *droma OR *dromas OR *eoma OR *eomas OR *goma OR *gomas OR *ioma OR *iomas OR *loma OR *lomas OR *moma OR *momas OR *noma OR *nomas OR *omatosis OR *phoma OR *phomas OR *poma OR *pomas OR *phroma OR *phromas OR *sarcoma OR *sarcomas OR *scoma OR *scomas OR *thecoma OR *thecomas OR *thoma OR *thomas OR *toma OR *tomas OR *uroma OR *uromas OR *xoma OR *xomas OR *yoma OR *yomas OR *kaemia OR *kaemia OR *kemia OR *kemia OR *plakia OR *plakias*)) OR (TITLE-ABS (*"cancer" OR "cancerous" OR "cancers" OR "carcinogen" OR "carcinogenesis" OR "carcinogenic" OR "carcinogens" OR "carcinoid" OR "carcinomatosis" OR "cocarcinogenesis" OR "metaplasia" OR "anaplasia" OR "neoplasia" OR "neoplasia" OR "neoplasm" OR "neoplasms" OR "neoplastic" OR "tumor" OR "tumorigenesis" OR "tumorigenic" OR "tumorigenesis" OR "tumorigenic" OR "tumorigenesis" OR "tumorigenic" OR "tumors" OR "tumour" OR "tumours" OR "nonhodgkin" OR "nonhodgkins" OR "non-hodgkin" OR "non-hodgkins" OR "Hodgkin" OR "hodgkins"*))) OR (TITLE-ABS(*"acrochordon" OR "acrochordons" OR "acrospiroma" OR "acrospiromas" OR*

"adenomatous" OR "adenosis" OR "Buschke-Lowenstein" OR "chloroma" OR "chloromas" OR "CIN" OR "CLL" OR "dermoid" OR "dysmyelopoiesis" OR "epidermoid" OR "essential thrombocythemia" OR "exostosis" OR "fibroid" OR "fibroids" OR "lymphoproliferation" OR "lymphoproliferations" OR "lymphoproliferative" OR "macroglobulinemia" OR "macroglobulinemias" OR "malignancies" OR "malignancy" OR "malignant" OR "mastocytosis" OR "meigs syndrome" OR "micrometastases" OR "micrometastasis" OR "mycosis fungoides" OR "myelofibrosis" OR "myeloproliferation" OR "myeloproliferations" OR "myeloproliferative" OR "NSCLC" OR "papillomata" OR "papillomatosis" OR pilomatricoma OR pilomatricomas OR polyposis OR poroma OR poromas OR "pre-malignant" OR "preneoplastic" OR "seminomatous" OR "sezary syndrome" OR "struma ovarii" OR "waldenstrom" OR "waldenstroms" OR "oncogene fusion" OR "5q syndrome" OR "aberrant crypt foci" OR "Aberrant crypt focus" OR "carney complex" OR "denys drash" OR leukostasis OR "zollinger ellison")) OR ((TITLE-ABS ("ASCO" AND NOT "fungi")) OR (TITLE-ABS ("WAGR" AND "syndrome"))))

A.3. Supplementary Searches

A.3.1 Disinfection by-products, water disinfection, water treatment:

This search was used to run supplemental cancer searches to capture references for studies evaluating general categories of chemicals that could include the selected HAAs. The search was limited to the years 2011 and forward because the IARC evaluation was used as a source for earlier studies of this type.

PubMed:

(Disinfection-ByProduct*[tiab] OR Disinfection-By-Product*[tiab]) OR water-disinfect*[tiab] OR disinfected-water[tiab] OR ("treated water"*[tiab] OR "water treatment"*[tiab])

Web of Science:

((TS=("Disinfection ByProduct*" OR "Disinfection By-Product*")) OR (TS=(water n/2 disinfect*))) OR (TS= ("treated water" OR (water NEAR/2 treatment*)))

Scopus:

((TITLE-ABS-KEY ("Disinfection ByProduct*" OR "Disinfection By-Product*")) OR (TITLE-ABS-KEY (water W/2 disinfect*))) OR ((TITLE-ABS-KEY (water W/2 treatment* OR "treated water")))

A.3.2 Supplementary bladder cancer search:

While bladder cancer is represented in the RoC Cancer search string, a supplemental search was conducted to collect all bladder cancer literature, in case there were studies that discussed the relevant chemicals in the body of the paper but not in the title abstracts or keyword.

PubMed:

As the most productive source of medical literature a broader search was conducted in PubMed than the other two databases. Therefore the search was not limited by any haloacetic acid terms. However, the search was limited to the years 2011 and forward because the IARC evaluation

was used as a source for earlier studies of this type. The other two databases included many non-medical “bladder” concepts so the HAA terms were needed to focus the search.

(bladder[tiab] OR "Urinary Bladder"[Mh])

AND

RoC Cancer String

Web of Science:

(TS=(bladder))

AND

13 HAAs Search (as described [above](#))

Scopus:

(TITLE-ABS-KEY (bladder*))

AND

13 AAs Search (as described [above](#))

A.3.4 Mechanism special search:

A search of select terms focused on mechanistic and metabolic concepts to help identify viable justifications for grouping chemicals for read-across. The specific concepts search were pyruvate dehydrogenase (excluding dichloroacetic acid terms), Glutathione Transferase Zeta, and cell transformation. These strings were combined with the 13 HAAs string.

PubMed:

((GAPDH[tiab] OR Glyceraldehyde-3-Phosphate-Dehydrogenas*[tiab] OR "Glyceraldehyde-3-Phosphate Dehydrogenases"[mh]) OR (tumorigenic-transformation*[tiab] OR Cell-transformation*[tiab] OR "Cell Transformation, Neoplastic"[Mh]) OR ("Glutathione Transferase*" [tiab] OR "Glutathione Transferase"[mh] OR glutathione-S-transferase*[tiab] OR GST-zeta[tiab] OR GSTz[tiab]))

Web of Science:

((TS=("Glyceraldehyde 3 Phosphate Dehydrogenas**" OR "GAPDH")) OR (TS=("tumorigenic transformation*" OR "Cell transformation*")) OR (TS=("Glutathione Transferase*" OR "glutathione-S-transferase*" OR "GST zeta" OR gstz)))

Scopus:

((TITLE-ABS-KEY ("Glyceraldehyde 3 Phosphate Dehydrogenas**" OR "GAPDH")) OR (TITLE-ABS-KEY ("tumorigenic transformation*" OR "Cell transformation*")) OR (TITLE-ABS-KEY ("Glutathione Transferase*" OR "glutathione-S-transferase*" OR "GST zeta" OR gstz)))

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Appendix B: Disposition and Toxicokinetics

Appendix B contains information that supplements that provided in Section 3 for disposition and toxicokinetics. The three tables below contain information for pharmacokinetic or toxicokinetic parameters of haloacetic acids in humans (Table B-1), toxicokinetic parameters of haloacetic acids in rats (Table B-2), and toxicokinetic parameters of haloacetic acids in B6C3F₁ mice (Table B-3).

Table B-1. Pharmacokinetic or toxicokinetic parameters of haloacetic acids in humans

HAA	Dose, mg/kg (route)	Vd (mL/kg)	AUC (mg/L•h)	Plasma T _{1/2} (h)	Clearance (mL/min•kg)	Comments	Reference
DCA	10 (i.v.)	337	14.9	0.34	11.31	Two adults/dose (sex not specified). Data are mean values for two subjects.	Lukas <i>et al.</i> 1980
	20 (i.v.)	190	76.7	0.51	4.55		
DCA	10 × 5 (i.v.)	260	increased	1.06	1393.8 mg/kg/h	7 men + 4 women, 5 doses administered at 2-h intervals; 3 subjects at low dose, 5 subjects at mid dose (including one from low dose group) and 4 subjects at high dose. Vd, T _{1/2} and elimination rate constants shown after each dose interval; however, the dose was not specified.	Curry <i>et al.</i> 1985
	25 × 5 (i.v.)	270	with dose	1.84	1089.1 mg/kg/h		
	50 × 5 (i.v.)	340		3.14	881.7 mg/kg/h		
		290		3.50	950.2 mg/kg/h		
		330		6.23	723.8 mg/kg/h		
DCA	50 (i.v.) + 50 × 2 (oral)	19.9 L	609	2.65	102.1 mL/min	8 men + 4 women; one i.v. and 2 oral doses given 4 days apart (randomized)	Curry <i>et al.</i> 1991
DCA	46 (i.v.)	[750]	261	2.3	[5.33]	13 adults (sex not specified) with severe malaria)	Krishna <i>et al.</i> 1994
DCA.	50 (i.v.)	323	378	1.8	2.68	4 boys + 4 girls with lactic acidosis from malaria	Krishna <i>et al.</i> 1995
DCA	46 (i.v.)	440	NR	3.4	[2.17]	8 men + 3 women with severe malaria given one dose and 9 subjects given a 2 nd dose 12 h after 1 st . Vd and Cl data after 2 nd dose were reported as similar to those after the 1 st dose.	Krishna <i>et al.</i> 1996
	46 × 2 (i.v.)			4.4			
DCA	40 × 2 (i.v.)	618	NR	[7.58]	1.0 (paleohepatic)	33 adults (sex not specified) with end-stage liver disease and liver transplant. 2 nd dose 4 h after 1 st dose; 2-compartment model. No clearance occurred during the anhepatic phase.	Shangraw and Fisher 1996
		618		[4.65]	1.7 (neohepatic)		
DCA	35 (i.v.)					5 men + 1 women (controls)	Shangraw and Fisher 1999
	controls	413	NR	[3.35]	2.14	5 men + 2 women (cirrhosis)	
	cirrhosis	413		[6.78]	0.78	2-compartment model	
DCA	50 × 2 (i.v.)	0.51 L ^a	1233 ^a	5.94 ^a	[1.02] ^a	66 men + 45 women with lactic acidosis. 2 nd dose 2h after 1 st . Pharmacokinetics in acidosis patients were complex and differed from those in healthy volunteers. Data for most patients fitted a one or two-compartment model but a few fitted a three-compartment model or none of these. Plasma drug clearance tended to decrease as the number of compartments required to fit the data increased.	Henderson <i>et al.</i> 1997
		0.27 L ^b	1863 ^b	18.15 ^b	[0.69] ^b		
		0.27 L ^c	NR	49.37 ^c	[0.33] ^c		
DCA	(50 + 50) × 2	0.29 L ^a	1954 ^a	8.77 ^a	[0.42] ^a	15 of the 111 patients mentioned above (sex not specified)	Henderson <i>et al.</i>

HAA	Dose, mg/kg (route)	Vd (mL/kg)	AUC (mg/L•h)	Plasma T _{1/2} (h)	Clearance (mL/min•kg)	Comments	Reference
	(i.v.)	0.28 L ^b	4306 ^b	68.63 ^b	[0.28] ^b	received a 2 nd treatment. No significant differences compared to patients that received a single treatment but drug clearance tended to decrease with increase in number of drug treatments.	1997
DCA	<u>Basal study</u> 2 (oral) + 0.3 (i.v.)	374	212, 755 ^d	0.15	[29.2]	8 men + 8 women: For basal study all subjects consumed HAA-free bottled water for 14 days then given unlabeled DCA in 500 mL bottled water and 5 minutes later given i.v. ¹³ C-labeled DCA; chronic study used same subjects and began 1 day after the 1 st study. Subjects subsequently ingested low concentrations of DCA in drinking water for 14 days and on day 15 repeated protocol of 1 st study. Only women were significantly affected by chronic DCA exposure compared to basal study with significantly increased AUC and reduced clearance and Vd	Schultz and Shangraw 2006
	men	377	243, 935 ^d	0.16	[26.8]		
	women						
	<u>Chronic study</u> (0.02 × 14) (oral) + 2 (oral) + 0.3 (i.v.)	377	281, 1123 ^d	0.16	[22.0]		
	men	227	368, 1453 ^d	0.17	[16.0]		
	women						
TCA	3 (oral)	NR	NR	50.6	NR	3 men administered a single oral dose of TCA	Müller <i>et al.</i> 1974
TCA	2.3–73 µg/day (drinking water)	NR	NR	55.2-88.1 (2.3-3.7 d)	NR	8 men + 2 women (elimination T _{1/2} data based on 3 subjects). Monitored TCA levels in tap water and urine during a 12-day study period. Dose range reflects mean intake over 12 days.	Froese <i>et al.</i> 2002

Data in [brackets] indicate unit conversion of data reported in the study.

HAA = haloacetic acid, Vd = apparent volume of distribution, AUC = area under the concentration-time curve, DCA = dichloroacetic acid, TCA = trichloroacetic acid, NR = not reported.

^aOne-compartment model.

^bTwo-compartment model.

^cThree-compartment model.

^dAUC values provided for i.v. dose and oral dose, respectively.

Table B-2. Toxicokinetic parameters of haloacetic acids in rats

Haloacetic acid	Dose/route ($\mu\text{mol/kg}$)	AUC ($\mu\text{M}\cdot\text{h}$)	Vd _{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t _{1/2} (h)	References
Trichloro-								
Single	500 i.v.	5406 \pm 144	782 \pm 117	93 \pm 3.0	42.1 \pm 9.9	50.4 \pm 11	8.0 \pm 2.4	Schultz <i>et al.</i> 1999
Single	[610] oral	10,000 \pm 600	485	58	NR	NR	5.8	Larson and Bull 1992
Single	[120] oral	2530 \pm 70	365	36	NR	NR	7.0	Saghir and Schultz 2005
Mixture ^a	25 i.v.	1561 \pm 85	287 \pm 23	17.1 \pm 1.4	NR	NR	12.03 \pm 0.36	
GST- ζ -depleted ^a	25 i.v.	1289 \pm 78	200 \pm 10	19.7 \pm 1.2	NR	NR	7.49 \pm 0.15	
Bromodichloro-								
Single	500 i.v.	1856 \pm 579	730 \pm 138	286 \pm 82	89 \pm 2.7	197 \pm 52	1.85 \pm 0.30	Schultz <i>et al.</i> 1999
Single	100 i.v.	NR	573 \pm 179	138 \pm 41	NR	NR	3.0 \pm 0.40	Saghir and Schultz 2005
Single	25 i.v.	NR	328 \pm 62	279 \pm 53.5	NR	NR	1.3 \pm 0.25	
Mixture ^a	25 i.v.	291 \pm 31	368 \pm 6.0	63.9 \pm 13.0	NR	NR	3.49 \pm 0.14	
GST- ζ -depleted ^a	25 i.v.	306 \pm 27	308 \pm 21	83.9 \pm 7.0	NR	NR	2.33 \pm 0.10	
Chlorodibromo-								
Single	500 i.v.	1107 \pm 331	636 \pm 268	486 \pm 153	182 \pm 58	304 \pm 137	1.26 \pm 0.27	Schultz <i>et al.</i> 1999
Single	25 i.v.	NR	264 \pm 45	128 \pm 13	NR	NR	1.40 \pm 0.25	Saghir and Schultz 2005
Mixture ^b	25 i.v.	246 \pm 22	247 \pm 25	105 \pm 8	NR	NR	1.55 \pm 0.21	
GST- ζ -depleted ^b	25 i.v.	199 \pm 10	281 \pm 12	127 \pm 6	NR	NR	1.62 \pm 0.13	
Tribromo-								
Single	500 i.v.	676 \pm 110	449 \pm 175	754 \pm 116	171 \pm 23	582 \pm 126	0.58 \pm 0.18	Schultz <i>et al.</i> 1999
Mixture ^b	25 i.v.	121 \pm 36	278 \pm 51	291 \pm 77	NR	NR	0.76 \pm 0.03	Saghir and Schultz 2005
GST- ζ -depleted ^b	25 i.v.	112 \pm 5	237 \pm 21	225 \pm 9	NR	NR	0.85 \pm 0.11	

Haloacetic acid	Dose/route ($\mu\text{mol/kg}$)	AUC ($\mu\text{M}\cdot\text{h}$)	Vd _{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t _{1/2} (h)	References
Dichloro-								Schultz <i>et al.</i> 1999
Single	500 i.v.	2092 ± 1821	618 ± 318	267 ± 104	2.9 ± 0.5	264 ± 103	2.4 ± 0.80	Larson and Bull
Single	[770] oral	750 ± 40	1000	820	NR	NR	0.9	1992
Single	[160] oral	13 ± 4	2400	2900	NR	NR	0.9	Gonzalez-Leon <i>et al.</i> 1997
Single	[770] i.v.	[3360 ± 1810]	618 ± 319	267 ± 105	2.9 ± 0.5	265 ± 103	2.4 ± 0.15	
GST- ζ -depleted	[770] i.v.	[18,700 ± 3100]	582 ± 146	42.7 ± 8.2	8.9 ± 3.3	33.8 ± 4.9	10.8 ± 2.0	Saghir and Schultz 2002
Single	[160] i.v.	[110 ± 6.6]	223 ± 111	1571 ± 97	NR	NR	0.15 ± 0.01	
GST- ζ -depleted	[160] i.v.	[1060 ± 26]	513 ± 18.5	168 ± 22	NR	NR	1.81 ± 0.09	Saghir and Schultz 2005
Single	[40] i.v.	[9.6 ± 0.4]	415 ± 47.2	5265 ± 636	NR	NR	0.08 ± 0.003	
GST- ζ -depleted	[40] i.v.	[64 ± 4]	392 ± 31.4	614 ± 39	NR	NR	0.50 ± 0.03	
Single	[8] i.v.	[1.2 ± 0.08]	508 ± 68.6	6554 ± 356	NR	NR	0.07 ± 0.001	
GST- ζ -depleted	[8] i.v.	[4.7 ± 0.16]	261 ± 13.6	1640 ± 57	NR	NR	0.20 ± 0.05	
Mixture ^b	25 i.v.	8.8 ± 0.09	405 ± 82.0	2980 ± 332	NR	NR	0.15 ± 0.04	
GST- ζ -depleted ^b	25 i.v.	145 ± 33	668 ± 128	199 ± 42	NR	NR	2.30 ± 0.29	
Dichloro-								James <i>et al.</i> 1998
Young (3-4 mo)	[400] oral	[91 ± 13]	680 ± 70	NR	NR	NR	0.11 ± 0.02	
Young (3-4 mo)	[400 × 2] oral	[1,870 ± 580]	390 ± 140				5.4 ± 0.76	
Aged (16 mo)	[400 × 2] oral	[11,700 ± 1920]	140 ± 20				9.7 ± 0.97	
Bromochloro-								Schultz <i>et al.</i> 1999
Single	500 i.v.	576 ± 286	881 ± 373	1,037 ± 453	36.9 ± 20.8	1014 ± 443	3.93 ± 1.5	NTP 2009
Single ^c	[58] i.v.	[16.4; 19.8]	NR	[3510; 2920]	NR	NR	0.10; 0.09	
Single ^c	[58] (oral)	[2.7; 2.8]	NR	[21,240; 20,640]	NR	NR	0.25; 0.21	
Single ^c	[230] (oral)	[94; 137]	NR	[2460; 1670]	NR	NR	0.62; 0.53	
Single ^c	[580] (oral)	[450; 678]	NR	[1280; 852]	NR	NR	0.71; 0.67	
GST- ζ -depleted ^c	[16] (oral)	[26; 2.8]	NR	[6420; 5720]	NR	NR	0.05; 0.07	
GST- ζ -depleted ^c	[160] (oral)	[117; 133]	NR	[1420; 1190]	NR	NR	0.16; 0.11	
GST- ζ -depleted ^c	[320] (oral)	[356; 375]	NR	[940; 850]	NR	NR	0.08; 0.10	

Haloacetic acid	Dose/route ($\mu\text{mol/kg}$)	AUC ($\mu\text{M}\cdot\text{h}$)	Vd _{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t _{1/2} (h)	References
(-)Bromochloro-								
Single	520 i.v.	74.8 ± 9.0	380 ± 41	3712 ± 140	31.4 ± 9.5	3693 ± 155	0.07 ± 0.01	Schultz and Sylvester 2001
GST- ζ -depleted	520 i.v.	584 ± 135	417 ± 139	484 ± 142	17.4 ± 10.8	468 ± 143	0.40 ± 0.02	Saghir and Schultz 2005
Mixture ^a	25 i.v.	1.7 ± 0.1	680 ± 103	7660 ± 478	NR	NR	0.06 ± 0.01	NTP 2009
GST- ζ -depleted ^a	25 i.v.	7.3 ± 1.1	361 ± 53	1997 ± 42	NR	NR	0.19 ± 0.03	
Single ^c	[58] i.v.	[7.6; 4.7]	NR	[7560; 12,400]	NR	NR	NR	
(+)Bromochloro-								
Single	520 i.v.	234 ± 25	587 ± 104	1,248 ± 132	13.2 ± 3.0	1,236 ± 127	0.40 ± 0.09	Schultz and Sylvester 2001
GST- ζ -depleted	520 i.v.	487 ± 119	467 ± 168	591 ± 136	13.4 ± 7.4	580 ± 139	0.44 ± 0.04	Saghir and Schultz 2005
Mixture ^a	25 i.v.	7.2 ± 0.6	393 ± 34	1,773 ± 184	NR	NR	0.19 ± 0.01	NTP 2009
GST- ζ -depleted ^a	25 i.v.	28.9 ± 3.5	246 ± 25	466 ± 56	NR	NR	0.40 ± 0.02	
Single ^c	[58] i.v.	[13.4; 10.4]	NR	[4310; 5570]	NR	NR	NR	
Dibromo-								
Single	500 i.v.	1,120 ± 362	400 ± 112	491 ± 116	12.9 ± 4.0	490 ± 137	0.72 ± 0.12	Schultz <i>et al.</i> 1999
Mixture ^a	25 i.v.	2.4 ± 0.1	987 ± 142	10,540 ± 312	NR	NR	0.08 ± 0.01	Saghir and Schultz 2005
GST- ζ -depleted ^a	25 i.v.	13.2 ± 2.5	599 ± 68	2,390 ± 71	NR	NR	0.22 ± 0.02	NTP 2007
Single ^c	[115] oral	[36; 50]	NR	NR	NR	NR	0.8; 0.87	
Single ^c	[230] oral	[95; 121]	NR	NR	NR	NR	0.95; 0.77	
Single ^c	[570] oral	[251; 353]	NR	NR	NR	NR	1.2; 0.98	
Monochloro-								
	[2400] oral	[3120 ± 23]	NR	558 ± 2.4	NR	NR	2.19 ± 0.79	Saghir and Rozman 2003
	[110] oral	[105 ± 0.8]	NR	769 ± 3.8	NR	NR	1.89 ± 0.11	
	[790] i.v.	NR	1060	262	154	NR	5.40	Saghir <i>et al.</i> 2001
	[110] i.v.	NR	3033	750	546	NR	3.25	

Data in brackets indicate unit conversions: (dose mg/kg•1000 $\mu\text{g}/\text{mg}$)/(MW $\mu\text{g}/\mu\text{mol}$) = dose $\mu\text{moles}/\text{kg}$, (AUC $\mu\text{g}/\text{mL}\cdot\text{hr}$ •1000 ng/ μg)/(MW ng/nmol) = AUC $\mu\text{M}\cdot\text{hr}$, or (Cl mL/min/kg •60 min/h) = Cl mL/kg/h) where MW of trichloroacetic acid = 163.4, dichloroacetic acid = 128.9, bromochloroacetic acid = 173.4, dibromoacetic acid = 217.86, and monochloroacetic acid = 94.5.

Cl = clearance, Vd_{ss} = apparent steady state volume of distribution, t_{1/2} = half life of elimination, NR = not reported.

^aAdministered as a mixture containing trichloro-, bromodichloro-, bromochloro-, and dibromoacetic acid.

^bAdministered as a mixture containing chlorodibromo- tribromo- and dichloroacetic acid.

^c Males; Females

Table B-3. Toxicokinetic parameters of haloacetic acids in male B6C3F1 mice

Haloacetic acid	Dose/route (mg/kg)	AUC ($\mu\text{M}\cdot\text{h}$)	Vd _{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t _{1/2} (h)	Reference
Trichloro-								
Control	100 i.v.	[19,500 ± 2240]	571 ± 91	40.1 ± 4.6	28.1 ± 9.1	12	10.0 ± 2.0	Gonzalez-Leon <i>et al.</i> 1999
TCA-pretreat ^a	100 i.v.	[23,000 ± 3240]	483 ± 42	37.2 ± 5.2	22.0 ± 3.4	15	9.40 ± 0.7	
DCA-pretreat ^a	100 i.v.	[23,100 ± 1,980]	521 ± 15	34.0 ± 3.0	20.2 ± 1.9	14	10.7 ± 1.0	
Trichloro-	100 oral	7180 ± 210	555	66	NR	NR	5.8	Larson and Bull 1992
	20 oral	2020 ± 60	335	55			4.2	
Bromodichloro-	100 i.v.	3127 ± 231	518 ± 21	156 ± 10	3.7	152.3	2.05 ± 0.10	Merdink <i>et al.</i> 2001
	20 i.v.	709 ± 255	380 ± 25	217 ± 76	0	217	1.94 ± 0.56	
	5 i.v.	119 ± 19	383 ± 26	222 ± 33	0	222	1.33 ± 0.15	
Dichloro-	100 oral	30 ± 0	32,500	14,300	NR	NR	1.6	Larson and Bull 1992
	20 oral	8 ± 2	34,800	16,000			1.5	
Dichloro-								
Control	100 i.v.	[690 ± 93]	548 ± 96	1188 ± 147	1.61 ± 0.69	1186	0.35 ± 0.1	Gonzalez-Leon <i>et al.</i> 1999
TCA-pretreat ^a	100 i.v.	[2310 ± 396]	534 ± 53	387 ± 100	3.13 ± 1.8	384	1.14 ± 0.2	
DCA-pretreat ^a	100 i.v.	[950 ± 39]	475 ± 26	813 ± 37	2.20 ± 0.61	811	0.40 ± 0.3	
Dichloro-								
Controls ^b	20 i.v.	[22 ± 4.7]	497 ± 160	7420 ± 1,460	NR	NR	0.053 ± 0.02	Schultz <i>et al.</i> 2002
6 h	20 i.v.	[143 ± 10]	437 ± 29	1085 ± 179			0.30 ± 0.04	
16 h	20 i.v.	[123 ± 36]	691 ± 27	1051 ± 204			0.33 ± 0.03	
36 h	20 i.v.	[60 ± 12]	334 ± 43	2408 ± 392			0.11 ± 0.01	
48 h	20 i.v.	[32 ± 5.4]	467 ± 62	4887 ± 740			0.086 ± 0.01	
aged controls ^c	20 i.v.	[98 ± 46]	459 ± 160	1903 ± 850			0.23 ± 0.09	
≤ 16 h	20 i.v.	[75 ± 2.6]	597 ± 68	2296 ± 852			0.24 ± 0.05	
Dibromo-								
single^d	[320] oral	[6.5; 5.6]	NR	NR	NR	NR	0.36; 0.33	NTP 2007a
single^d	[800] oral	[36.9; 34.1]	NR	NR	NR	NR	0.80; 0.67	
single^d	[1600] oral	[112; 113]	NR	NR	NR	NR	1.75; 1.99	

Haloacetic acid	Dose/route (mg/kg)	AUC ($\mu\text{M}\cdot\text{h}$)	Vd _{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t _{1/2} (h)	Reference
Bromochloro-								NTP 2009
Single ^d	[580] i.v.	[135; 128]	NR	[4280; 4520]	NR	NR	0.089; 0.062	
Single ^d	[580] (oral)	[25; 14.9]	NR	[23,100; 38,760]	NR	NR	0.15; 0.18	
Single ^d	[1150] (oral)	[134; 74]	NR	[8640; 15;600]	NR	NR	0.25; 0.20	
Single ^d	[2300] (oral)	[445; 331]	NR	[5180; 6960]	NR	NR	0.32; 0.25	
GST- ζ -depleted ^d	[520] (oral)	[18.6; 134]	NR	[24,900; 4320]	NR	NR	0.10; 0.22	
GST- ζ -depleted ^d	[1040] (oral)	[271; 327]	NR	[3400; 3530]	NR	NR	0.22; 0.21	
(-)Bromochloro-								NTP 2009
Single ^d	[580] i.v.	[50.8; 54.9]	NR	[11,340; 10;500]	NR	NR	0.03; 0.03	
(+)Bromochloro-								
Single ^d	[580] i.v.	[83.3; 97.1]	NR	[6900; 5930]	NR	NR	0.08; 0.08	

Data in brackets indicate unit conversion: $\text{Cl } \mu\text{g}/\text{mL}\cdot\text{hr} \cdot 1000 \text{ ng}/\mu\text{g}/(\text{MW ng}/\text{nmol}) = \text{Cl } \mu\text{M}\cdot\text{hr}$ or $(\text{AUC } \mu\text{g}/\text{mL}\cdot\text{hr} \cdot 1000 \text{ ng}/\mu\text{g})/(\text{MW ng}/\text{nmol}) = \text{AUC } \mu\text{M}\cdot\text{hr}$.

Cl = clearance, Vd_{ss} = apparent steady state volume of distribution, t_{1/2} = half life of elimination, NR = not reported.

^aAnimals were pretreated with 2 g/L DCA or TCA in drinking water for 14 days then administered a challenge dose of 100 mg/kg of TCA or DCA 16 h later.

^b8-week old mice exposed to 2 g/L DCA in drinking water for 14 days and i.v. dose administered 6, 16, 36, or 48 h after removal of DCA from drinking water.

^c 4-week old mice given 2 g/L DCA in drinking water for 56 weeks and i.v. dose administered within 16 h after removal of DCA from the drinking water

^d Males; Females

Appendix C: Animal Studies

Appendix C contains information on study quality for animal studies in mice and rats exposed to haloacetic acids. Tables C-1a through C-1e report ratings for studies on monochloroacetic acid; Table C-2a for iodoacetic acid; Tables C-3a through C-3z for dichloroacetic acid; Tables C-4a through C-4d for dibromoacetic acid; Tables C-5a through C-5d for bromochloroacetic acid; Tables C-6a through C-6l for trichloroacetic acid; Tables C-7a through C-7d for bromodichloroacetic acid. Table C-8 reports results for liver tumors; Table C-9 for all other tumors; Table C-10 for transgenic studies; and Table C-11 for initiation-promotion studies.

C.1 Monochloroacetic acid: Study quality for animal studies

Table C-1a. NTP 1992 (M Mouse): Monochloroacetic acid: Gavage

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold, making the detection of a dose response limited.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	

Study utility domain Question	Rating Rationale
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Large numbers of animals (60) were used for each group.
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	++ There was a significant decrease in survival, but not in body weight. There was no significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: +++.

The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.

Table C-1b. NTP 1992 (F Mouse): Monochloroacetic acid: Gavage

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold, making the detection of a dose response limited.
Outcome	

Study utility domain Question	Rating Rationale
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Large numbers of animals (60) were used for each group.
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	++ There was no significant decrease in survival, but there was a significant decrease in body weight. There was no significant increase in neoplasm incidence, but there was a significant increase in nonneoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: +++.

The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.

Table C-1c. NTP 1992 (M Rat): Monochloroacetic acid: Gavage

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.

Study utility domain Question	Rating Rationale
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold, making the detection of a dose response limited.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	++ Both sexes of non-transgenic animals were used and the strain is in common use. Rats were more sensitive to monochloroacetic acid non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
Statistical power	+++ The numbers of animals (50 at 104 weeks and 10 at 6 and 15 months) varied considerably for each group depending on the time of sacrifice.
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	++ There was a significant decrease in survival, but not body weight. There was no significant increase in neoplasm incidence. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: ++.

The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.

Table C-1d. NTP 1992 (F Rat): Monochloroacetic acid: Gavage

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold, making the detection of a dose response limited.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	++ Both sexes of non-transgenic animals were used and the strain is in common use. Rats were more sensitive to monochloroacetic acid non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
Statistical power	+++ The numbers of animals (50 at 104 weeks and 10 at 6 and 15 months) varied considerably for each group depending on the time of sacrifice.
Exposure duration	+++ A near life-span duration (104 weeks) was used.

Study utility domain Question	Rating Rationale
Dose/response	++ There was a significant decrease in survival, but not in body weight. There was no significant increase in neoplasm incidence, but there was a significant increase in preneoplasm incidence. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: ++.

The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.

Table C-1e. DeAngelo *et al.* 1997 (M Rat): Monochloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as the trichloroacetic acid high dose group were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 100 fold.
Outcome	
Pathology	+++ Full necropsies with histological evaluations were performed.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	+++ The rats were confirmed pathogen free and the chemical purity and stability were confirmed.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver hyperplasia, adenomas, and carcinomas. The authors felt the hyperplasia were preneoplastic.

Study utility domain Question	Rating Rationale
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	+++ Moderate numbers of animals (29-32) were used for each group.
Exposure duration	+++ A near-lifespan duration (104 weeks) was used.
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight that required dose reduction. There was no significant difference in neoplasm incidence.
Study duration	+++ A near-lifespan duration (104 weeks) was used.

Overall utility: +++.

A well conducted study on almost all aspects, but only involved male rats.

C.2 Monoiodoacetic acid: Study quality for animal studies

Table C-2a. Gwynn and Salaman 1953 (NR Mouse): Iodoacetic acid: Dermal

Study utility domain Question	Rating Rationale
Study design	
Randomization	0 Not reported.
Controls	+++ A concurrent negative control of acetic acid was used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+ One exposure level was used. Preliminary tests showed this to be the maximumly tolerated dose without causing crusting or ulceration. dose was the MTD, not to cause crusting or ulceration. [1.4% (average) was calculated by NTP from a reported M/20-M/10 solution, i.e., 1/20 to 1/10 of a molar solution. Based on a MW of 185.95, it comes to 0.92975%-1.8595%, averaging to 1.4%.] Vehicle control (acetone): 21 days after 0.15% DMBA, acetone was administered (2/wk x 12wk; 1/wk x 15wk) Acetic acid negative control (0.9% acetic acid): 39 days after 0.1% DMBA, 0.9% acetic acid was administered (1/wk x 20 wk). Reported at M/10-M/5 solution, i.e., 1/10 to 1/5 of a molar solution. Based on a MW of , it comes to 0.6%-1.2%, averaging to 0.9%.
Outcome	
Pathology	+ Not reported and necropsies were not likely to have occurred beyond histological examination of the skin tumors.
Consistency between groups	Not reported Not reported.
Confounding	
Confounding	+ Disease surveillance, animal husbandry, or chemical characterization were not reported.
Reporting and analysis	
Reporting data and statistics	0 Not reported.
Combining lesions	+++ Only benign papillomas were reported.
Sensitivity	

Study utility domain Question	Rating Rationale
Animal model	0 The sex of the mice were not reported. The strain of mouse is unfamiliar.
Statistical power	+ Small and insufficient numbers of animals (12) were used for each group.
Exposure duration	++ A short duration was used (27 weeks), but initiation/promotion studies are normally short.
Dose/response	+++ There was no significant difference in survival and body weight was not reported. There was a significant increase in neoplasm incidence.
Study duration	+++ A short duration was used (30 weeks), but initiation/promotion studies are usually short.

Overall utility: +

The chemicals were not characterized and purity wasn't reported. The sex of the animals were not reported and only a single dose level was tested on a very low number of animals per group. Histology of the neoplasms were carried out, but the skin tumors were classified as benign papillomas based on their appearance "macroscopically". Statistical significance was not calculated.

C.3 Dichloroacetic acid: Study quality for animal studies

Table C-3a. DeAngelo *et al.* 1996 (M Rat (Study 1)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as the high dose were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ Two exposure levels were effectively used that spanned a range of 10 fold. The original high dose was 100 fold higher than the low exposure level, but caused toxicity, requiring dose reduction and were not reported.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	++ The high dose group had their dose reduced three times due to irreversible peripheral neuropathy and they were all scarified at 60 weeks, while other dose groups were scarified at 100 weeks. They also have 5 rats undergo full necropsies, with histological evaluations of all major organs. However, the high dose group results were not reported.
Confounding	
Confounding	++ Animals started out viral free, but continual disease surveillance wasn't reported. The high dose group was treated differently, but results from that group were not reported.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver hyperplasia, adenomas, and carcinomas. The authors felt the hyperplasia were preneoplastic.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ The exact number of animals was not clearly reported. It appears to have been between 21 to 33 for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+++ A near life-span duration (100 weeks) was used. The high dose group was stopped at 60 weeks due to peripheral neuropathy and wasn't included in the study results.
Dose/response	+++ There were no significant differences in survival, but the high dose group was sacrificed early because of peripheral neuropathy and body weight was not significantly different. There was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (100 weeks) was used. The high dose group was stopped at 60 weeks due to peripheral neuropathy and wasn't included in the study results.

Overall utility: ++.

Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only two exposed dose levels were effectively reported and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.

Table C-3b. DeAngelo *et al.* 1996 (M Rat (Study 2)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent controls were exposed to deionized water (vehicle). NaCl was omitted from the water of control animals because no significant effects such as differences in tumor incidence or altered water consumption was noted in the previous bioassay at 100 weeks exposure.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ One exposure level was used, which cause toxicity, requiring dose reduction.
Outcome	
Pathology	++ No indication that a full necropsy was done.
Consistency between groups	++ 103 wk bioassay grossly evaluated liver, kidneys, spleen, testes, thyroid, stomach, rectum, duodenum, ileum, jejunum, colon, and urinary bladder and microscopically evaluated grossly detected lesions.
Confounding	

Study utility domain Question	Rating Rationale
Confounding	++ Animals started out viral free, but continual disease surveillance wasn't reported. The 100 week and the 103 week DeAngelo 1996 studies were done in two different laboratories but were reported in the same publication; it is unclear if the same animal care procedures were performed at both locations.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Liver lesions were reported, which included liver hyperplasia, adenomas, and carcinomas.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ The exact number of animals was not clearly reported. It appears to have been between 27 to 28 for each group.
Exposure duration	+++ A near life-span duration (103 weeks exposure period) was used. The high dose group (2.5 g/l) DCA was lowered to 1.5 g/l at eight weeks exposure and to 1.0 g/l at 26 weeks exposure. A mean daily exposure concentration was reported as 1.6 g/l.
Dose/response	+++ At sacrifice, the mean body weight of the animals (1.6 g/l) was significantly reduced to 73% of the control value.
Study duration	+++ A near life-span duration (103 weeks exposure period) was used.

Overall utility: ++.

Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only one exposed dose level was tested and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.

Table C-3c. Richmond *et al.* 1995 (M Rat (Study 1)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.

Study utility domain Question	Rating Rationale
Dosing regimen	+++ Three exposure levels were used that spanned a range of 50 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	++ All groups were treated the same except for the last time point for sacrifice was 60 week for the 2.4 g/l group and 104 weeks for all other groups. This was because of toxicity, causing tumors and hind limb paralysis.
Confounding	
Confounding	+ Neither disease surveillance, nor chemical purity or characterization were reported. Neither survival nor body weight were clearly reported.
Reporting and analysis	
Reporting data and statistics	++ Statistical significance was reported for neoplasm incidence, though no analysis of body weight changes or differences in survival were reported and body weights were not reported. Survival was not clearly reported, but was estimated by NTP to not have been greatly different.
Combining lesions	+++ Total proliferative lesions were reported which included liver hyperplastic nodules, hepatocellular adenomas, and hepatocellular carcinomas.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ The number of animals (7-27) varied considerably for each group.
Exposure duration	++ Less than near life-span duration (60 weeks) was used.
Dose/response	+++ There was no clear significant difference in survival and body weight was not reported. There was a significant increase in neoplasm incidence.
Study duration	+++ Near life-span and less than near life-span durations (60 to 104 weeks) were used.

Overall utility: ++.

The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was less than near-lifespan. Survival and body weights were not clearly reported and only the liver was histologically evaluated.

Table C-3d. Richmond *et al.* 1995 (M Rat (Study 2)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	

Study utility domain Question	Rating Rationale
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 50 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	++ All groups were treated the same except for the last time point for sacrifice was 60 week for the 2.4 g/l group and 104 weeks for all other groups. This was because of toxicity, causing tumors and hind limb paralysis.
Confounding	
Confounding	+ Neither disease surveillance, nor chemical purity or characterization was reported. Neither survival nor body weight was clearly reported.
Reporting and analysis	
Reporting data and statistics	++ Statistical significance was reported for neoplasm incidence, though no analysis of body weight changes or differences in survival were reported and body weights were not reported at all and survival was not clearly reported, but was estimated by NTP to not have been greatly different.
Combining lesions	+++ Total proliferative lesions were reported which included liver hyperplastic nodules, hepatocellular adenomas, and hepatocellular carcinomas.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ The number of animals was sufficient (23-29) for each group.
Exposure duration	+++ Near life-span duration (104 weeks) were used.
Dose/response	+++ There was no clear significant difference in survival and body weight was not reported. There was a significant increase in neoplasm incidence.
Study duration	+++ Near life-span and less than near life-span durations (60 to 104 weeks) were used.

Overall utility: ++.

The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was near-lifespan for all but the high dose group. Survival and body weights were not clearly reported and only the liver was histologically evaluated.

Table C-3e. DeAngelo *et al.* 1991 (M Mouse (Study 1)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride at equal molar concentrations as the high exposure dose level were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 100 fold.
Outcome	
Pathology	++ Only a select number of organs were examined and only lesions or tissues with lesions were histologically evaluated.
Consistency between groups	++ Different numbers of animals in each treatment group were tested at either 60 weeks or 75 weeks of exposure.
Confounding	
Confounding	++ The chemical was well characterized, but disease surveillance was not reported and different numbers of animals were sacrificed at different times. Further, only a select few organs were involved in the necropsy. The high dose group drank 60% less water than controls near the last third of the study and so the mean daily dose was decreased from 573 mg/kg/day to 387. The medium and low dose had not difference in water intake compared to controls.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Sufficient numbers of animals (27-30) were used for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.
Dose/response	+++ There was no significant difference in survival and there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.

Overall utility: +++.

Three dose levels, which spanned a 100 fold range were tested in only males for up to 75 weeks. Only a few select organs were necropsied.

Table C-3f. DeAngelo *et al.* 1991 (M Mouse (Study 2): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 1.5 g/l of acetic acid at equal molar concentrations as the high exposure dose level were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ One exposure level was used.
Outcome	
Pathology	++ Only a select number of organs were examined and only lesions or tissues with gross lesions were histologically evaluated.
Consistency between groups	+++ Exposed and controls were treated the same.
Confounding	
Confounding	++ The chemical was well characterized, but disease surveillance was not reported. Further, only a select few organs were involved in the necropsy. The water intake was significantly lower than controls (69% of that in controls), though the DCA concentration was 7% higher than expected.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.

Study utility domain Question	Rating Rationale
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	+ Small and insufficient numbers of animals (10-12) were used for each group.
Exposure duration	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.
Dose/response	+++ There was no significant difference in survival and there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.
Overall utility: ++. One dose level was tested in a small number of males for 60 weeks. Only a few select organs were necropsied.	

Table C-3g. DeAngelo et al. 1999 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Two sets of concurrent vehicle controls were used, one for the 500, 1,000, 2,000, and 3,500 mg/L groups and one started a month later for the 50 mg/L group.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Four exposure levels were used that spanned a range of 7 fold.
Outcome	
Pathology	++ Only gross lesions were histologically examined and normal tissue from all major organs was only histologically examined in 5 mice of the high dose group.

Study utility domain Question	Rating Rationale
Consistency between groups	++ Only 5 mice from the high dose group had undergone histological examinations of all major organs, while the other groups only had histological examinations of gross lesions.
Confounding	
Confounding	++ The mice were initially viral, bacteria, and parasite free, but continual disease surveillance was not reported. All major organs from only 5 mice from the high dose group were histologically evaluated, only gross lesions were histologically evaluated in the other groups.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	Not reported Tumor types were not combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ The numbers of animals (53-16) varied considerably for each group.
Exposure duration	+++ A near life-span duration (90-100 weeks) was used.
Dose/response	+++ There was a significant decrease in survival and body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (90-100 weeks) was used.

Overall utility: ++.

Only male mice, initially confirmed pathogen free, were used with differing numbers per group (as low as 16 in the high dose group). Continual disease surveillance was not reported. Multiple dose levels ranging 100 fold, were tested for a near life-span duration. However, only 5 high dose group mice received histological evaluations of all major organs, with histological evaluations of only gross lesions in the other groups.

Table C-3h. Herren-Freund *et al.* 1987 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride to match that found in exposed groups and a positive control of phenobarbital at 500 mg/l (positive controls are not reported here) were used.
Historical data	No
Exposure	

Study utility domain Question	Rating Rationale
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ One exposure level was used, so dose response relationships could not be measured.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ Disease surveillance was not reported, but chemical purity and stability were tested.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Moderate numbers of animals (22-26) were used for each group.
Exposure duration	+++ A less than life-span duration (61 weeks) was used.
Dose/response	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (61 weeks) was used.
Overall utility: ++. The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.	

Table C-3i. Herren-Freund *et al.* 1987 (M Mouse): Dichloroacetic acid: Drinking water (Initiation-promotion)

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.

Study utility domain Question	Rating Rationale
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride to match that found in exposed groups and a positive control of phenobarbital at 500 mg/l (positive controls are not reported here) were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ Two exposure levels were used which spanned a range of 2.5 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ Disease surveillance was not reported, but chemical purity and stability were tested.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Moderate numbers of animals (22-26) were used for each group.
Exposure duration	+++ A less than life-span duration (61 weeks) was used.
Dose/response	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (61 weeks) was used.
Overall utility: ++. The duration was less than near life-span. Only males were tested at two narrow dose levels and only livers were histologically evaluated.	
Table C-3j. Wood <i>et al.</i> 2015 (M Mouse): Dichloroacetic acid: Drinking water	
Study utility domain Question	Rating Rationale

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Exposure	
Chemical characterization	++ Purity was not reported. Stability had been shown previously in stock drinking water over 8-12 days, while bottles were changed twice a week.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 3.5 fold. These were based on previous chronic studies in the same strain of male mice which were estimated to be 20,000-30,000 times greater than those in normal tap water.
Outcome	
Pathology	+ Only livers were examined. Even gross examination of other organs were not reported.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance was not reported.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Hepatocellular adenomas, hepatocellular carcinomas, and hepatocellular blastomas were combined as neoplasms which is appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	++ Moderate numbers of animals (27) were used for each group, though the original number of animals at the start of the study were not reported.
Exposure duration	++ Exposure duration was short (10 weeks), though the observation duration was near life-span (94 weeks).
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.

Study utility domain Question	Rating Rationale
Study duration	+++ A near life-span duration (94 weeks) was used.

Overall utility: ++.

Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Three dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.

Table C-3k. Wood *et al.* 2015 (F Mouse): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Exposure	
Chemical characterization	++ Purity was not reported. Stability had been shown previously in stock drinking water over 8-12 days, while bottles were changed twice a week.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold. These were based on previous chronic studies in the same strain of male mice which were estimated to be 20,000-30,000 times greater than those in normal tap water.
Outcome	
Pathology	+ Only livers were examined. Even gross examination of other organs were not reported.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance was not reported.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Hepatocellular adenomas, hepatocellular carcinomas, and hepatocellular blastomas were combined as neoplasms which is appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.

Study utility domain Question	Rating Rationale
Statistical power	++ Moderate numbers of animals (27) were used for each group, though the original number of animals at the start of the study were not reported.
Exposure duration	++ Exposure duration was short (10 weeks), though the observation duration was near life-span (94 weeks).
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (94 weeks) was used.

Overall utility: ++.

Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Two dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.

Table C-3l. Pereira 1996 (F Mouse (Study 1)): Dichloroacetic acid (DCA): Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ It was not reported if animals were randomly assigned to exposure groups, but they were necropsied blinded and the mice were randomly assigned an ID number, which suggests they were randomly assigned to treatment groups, but it is not known for sure.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as in the high dose group were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 10 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance and survival were not reported. The high exposure group had a significant decrease in water consumption.
Reporting and analysis	

Study utility domain Question	Rating Rationale
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ The number of animals (15-90) varied considerably for each group.
Exposure duration	+++ A near life-span duration (576 days) was used.
Dose/response	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (576 days) was used.

Overall utility: ++.

The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. The study duration was near life-span.

Table C-3m. Pereira 1996 (F Mouse (Study 2)): Dichloroacetic acid (DCA): Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ It was not reported if animals were randomly assigned to exposure groups, but they were necropsied blinded and the mice were randomly assigned an ID number, which suggests they were randomly assigned to treatment groups, but it is not known for sure.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as in the high dose group were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 10 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.

Study utility domain Question	Rating Rationale
Confounding	
Confounding	++ Continual disease surveillance and survival were not reported. The high exposure group had a significant decrease in water consumption.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ The number of animals (15-90) varied considerably for each group.
Exposure duration	++ A less than life-span duration (360 days) was used.
Dose/response	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (360 days) was used.

Overall utility: ++.

The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. Study duration was less than life-span.

Table C-3n. Pereira *et al.* 1997 (F Mouse): Dichloroacetic acid (DCA): Drinking water (I/P)

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent controls were appropriate for an initiation/promotion study in which all groups received the initiator (NMU) and the negative control group was given only the initiator and promotor vehicle.
Historical data	No
Exposure	
Chemical characterization	+ Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 3 fold.

Study utility domain Question	Rating Rationale
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance and survival were not reported.
Reporting and analysis	
Reporting data and statistics	++ Significance levels were reported, but the statistical test was not reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ Small, but sufficient numbers of animals (>20) were used for each group.
Exposure duration	+++ Animals were exposed for 44 weeks, starting 4 weeks after a single injection of the initiator at 15 d of age. This is short for carcinogenicity studies, but initiation/promotion studies are normally shorter and tumors were significantly induced.
Dose/response	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Study duration	+++ A less than near life-span duration (48 weeks) was used, but initiation/promotion studies are normally short.

Overall utility: +

The chemicals were not characterized, not even purity was reported. Disease surveillance was not reported. A low number of only females were tested, with only their livers examined histologically. The statistical methods were not reported.

Table C-30. Bull *et al.* 1990 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Exposure	

Study utility domain Question	Rating Rationale
Chemical characterization	++ Chemical purity and stability were not verified.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold.
Outcome	
Pathology	+ Only livers from some animals were histologically evaluated. Results only reported overall lesions, which include hyperplasia. Liver lesions that were histologically evaluated were randomly selected and blindly evaluated.
Consistency between groups	+ All untreated control lesions were histologically evaluated, but only some of the exposed groups were evaluated.
Confounding	
Confounding	+ Disease surveillance was not reported. Only samples of liver lesions were histologically evaluated and inappropriately reported.
Reporting and analysis	
Reporting data and statistics	+ Statistical evaluations were rarely reported and incidences that could be significant by Fisher's Exact test (calculated by NTP) were not reported as significant, so NTP calculations were included.
Combining lesions	++ Only total lesions were adequately reported to allow for quantitation of the results. These included liver hyperplasia, hepatocellular adenoma, and hepatocellular carcinoma, which entail a continuum of the same disease process, however there were 4/73 lesion types that were also included, but were neither hyperplastic nor neoplastic.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	+ Small numbers of animals (11-24) were used for each group.
Exposure duration	+++ A less than life-span duration (52 weeks) was used.
Dose/response	+++ There were no deaths and body weight was not reported. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (52 weeks) was used.

Overall utility: +

The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.

Table C-3p. Daniel *et al.* 1992 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ One exposure level was used, which was low compared to other similar studies from the same lab.
Outcome	
Pathology	+ Histological evaluations were only done on liver, kidney, testes, spleen, and gross lesions.
Consistency between groups	++ Only 5 mice from the high dose group had undergone histological examinations of all major organs, while the other groups only had histological examinations of gross lesions.
Confounding	
Confounding	++ Continual disease surveillance was not reported. Only some mice received a full histological evaluation.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver hyperplasia, adenomas, and carcinomas. The authors felt the hyperplasia were preneoplastic.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Moderate numbers of animals (20-24) were used for each group..
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: ++.

Continual disease surveillance was not reported. Only males were tested and only at one dose level. Histological evaluations from all major organs only occurred in five mice per group, with histological evaluations in the other mice only occurring on tissues with gross lesions.

Table C-3q. NTP 2007b (F Mouse (Study 1)): Dichloroacetic acid: Dermal

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be reported here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	+ Small and insufficient numbers of animals (10) were used for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+++ While the duration was short (39 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant increase in neoplasm incidence, but there was in preneoplasm incidence.
Study duration	+++ While the duration was short (39 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

Table C-3r. NTP 2007b (M Mouse (Study 2)): Dichloroacetic acid: Dermal

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be reported here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.

Study utility domain Question	Rating Rationale
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure duration	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival or body weight. There was no significant increase in neoplasm incidence, but there was a significant increase in preneoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Overall utility: + The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.	

Table C-3s. NTP 2007b (M Mouse (Study 1)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	

Study utility domain Question	Rating Rationale
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	+++ There was no significant difference in survival and no significant decrease in body weight. There was a significant increase in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Overall utility: + The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.	

Table C-3t. NTP 2007b (F Mouse [Study 1]): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.

Study utility domain Question	Rating Rationale
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant increase in neoplasm incidence, but there was a significant increase in preneoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

Table C-3u. NTP 2007b (M Mouse [Study 2]): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.

Study utility domain Question	Rating Rationale
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be reported here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure duration	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	+ There was no significant difference in survival and no significant decrease in body weight. There was no significant increase in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

Table C-3v. NTP 2007b (F Mouse (Study 2)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop squamous papillomas or carcinomas of the skin or forestomach, were used. However, the transgenic strain is sensitive to skin injury and will develop papillomas, suggesting it may overestimate a chemical's carcinogenic potential.
Statistical power	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure duration	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was a significant decrease in survival and body weight. There was no significant increase in neoplasm incidence.

Study utility domain Question	Rating Rationale
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

Table C-3w. NTP 2007b (M Mouse (Study 3)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop lymphomas or sarcomas, were used. However, the transgenic strain may overestimate a chemical's carcinogenic potential.
Statistical power	+ Small and insufficient numbers of animals (10) were used for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

Table C-3x. NTP 2007b (F Mouse (Study 3)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.

Study utility domain Question	Rating Rationale
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop lymphomas or sarcomas, were used. However, the transgenic strain may overestimate a chemical's carcinogenic potential.
Statistical power	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Overall utility: + The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.	

Table C-3y. NTP 2007b (M Mouse (Study 4)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used..
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	

Study utility domain Question	Rating Rationale
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop lymphomas or sarcomas, were used. However, the transgenic strain may overestimate a chemical's carcinogenic potential.
Statistical power	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure duration	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Overall utility: + The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.	

Table C-3z. NTP 2007b (F Mouse (Study 4)): Dichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	

Study utility domain Question	Rating Rationale
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same except that positive controls underwent a complete necropsy. However, positive controls will not be considered here.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	+ Both sexes of transgenic animals, that are sensitive to carcinogens with a propensity to develop lymphomas or sarcomas, were used. However, the transgenic strain may overestimate a chemical's carcinogenic potential.
Statistical power	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure duration	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Study duration	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Overall utility: +

The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

C.4 Dibromoacetic acid: Study quality for animal studies

Table C-4a. NTP 2007a (M Mouse): Dibromoacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 20 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	++ Drinking water contained 44.7 ug/l of dihaloacetic acids and 3.8 ug/l of dibromoacetic acid.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (106 weeks) was used.
Dose/response	+++ There was no significant difference in survival and body weight was not significantly decreased. There was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (106 weeks) was used.

Overall utility: +++.

Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.

Table C-4b. NTP 2007a (F Mouse): Dibromoacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 20 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	++ Drinking water contained 44.7 ug/l of dihaloacetic acids and 3.8 ug/l of dibromoacetic acid.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (106 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight. There was a significant increase in neoplasm incidence.

Study utility domain Question	Rating Rationale
Study duration	+++ A near life-span duration (106 weeks) was used.

Overall utility: +++.

Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.

Table C-4c. NTP 2007a (M Rat): Dibromoacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 20 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	++ Drinking water contained 44.7 ug/l of dihaloacetic acids and 3.8 ug/l of dibromoacetic acid.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (106 weeks) was used.

Study utility domain Question	Rating Rationale
Dose/response	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant increase in neoplasm incidence, but was a significant increase in preneoplasm incidence.
Study duration	+++ A near life-span duration (106 weeks) was used.

Overall utility: +++.

Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.

Table C-4d. NTP 2007a (F Rat): Dibromoacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 20 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	++ Drinking water contained 44.7 ug/l of dihaloacetic acids and 3.8 ug/l of dibromoacetic acid.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.

Study utility domain Question	Rating Rationale
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (106 weeks) was used.
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant increase in neoplasm incidence, but was a significant increase in preneoplasm incidence.
Study duration	+++ A near life-span duration (106 weeks) was used.

Overall utility: +++.

Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.

C.5 Bromochloroacetic acid: Study quality for animal studies

Table C-5a. NTP 2009 (M Rat): Bromochloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

A very high quality study, with no major concerns.

Table C-5b. NTP 2009 (F Rat): Bromochloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.

Study utility domain Question	Rating Rationale
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

A very high quality study, with no major concerns.

Table C-5c. NTP 2009 (M Mouse): Bromochloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.

Study utility domain Question	Rating Rationale
Dose/response	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (105 weeks) was used.
Overall utility: +++. A very high quality study, with no major concerns.	

Table C-5d. NTP 2009 (F Mouse): Bromochloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used and the strain is in common use.

Study utility domain Question	Rating Rationale
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

A very high quality study, with no major concerns.

C.6 Trichloroacetic acid: Study quality for animal studies

Table C-6a. DeAngelo *et al.* (1, 1997 #143) (M Rat): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as the trichloroacetic acid high dose group were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 100 fold.
Outcome	
Pathology	+++ Full necropsies with histological evaluations were performed.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	+++ The rats were confirmed pathogen free and the chemical purity and stability were confirmed.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver hyperplasia, adenomas, and carcinomas. The authors felt the hyperplasia were preneoplastic.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	+++ Moderate numbers of animals (29-32) were used for each group.
Exposure duration	+++ A near-lifespan duration (104 weeks) was used.
Dose/response	++ There was no significant difference in survival, but there was a significant increase in body weight. There was no significant difference in neoplasm incidence.
Study duration	+++ A near-lifespan duration (104 weeks) was used.

Overall utility: +++.

A well conducted study on almost all aspects, but only involved male rats.

Table C-6b. DeAngelo (, 2008 #145) (M Mouse (Study 1)): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposed dose levels were used, which increased by 10 fold each time, covering a wide dose range.
Outcome	
Pathology	+++ A complete necropsy with histological examine was performed, including verification by an independent pathologist.
Consistency between groups	++ All tissues were evaluated from 5 mice of the high dose and negative control groups, while other mice had most organs and all organs with gross lesions histologically evaluated.
Confounding	
Confounding	+++ Infectious disease detection was used and the vehicle control was appropriate.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	+++ Only males of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ Moderate numbers of animals (30) were used for each group.
Exposure duration	+++ A less than life-span duration (60 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.

Study utility domain Question	Rating Rationale
Study duration	++ A less than life-span duration (60 weeks) was used.

Overall utility: +++.

Three dose levels were used that spanned a 100 fold range. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.

Table C-6c. DeAngelo (2008) (M Mouse (Study 2)): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 1.5 g/l of neutralized acetic acid in drinking water was used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ Only one exposure level was used.
Outcome	
Pathology	+++ A complete necropsy with histological examine was performed, including verification by an independent pathologist.
Consistency between groups	+++ All treatment groups appear to have been treated similarly.
Confounding	
Confounding	+++ Infectious disease detection was used and husbandry was reported. Vehicle control was appropriate, though not ideal.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	+++ Only males of non-transgenic animals were used and the strain is in common use.
Statistical power	+++ A large number of animals (42-51) were used for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: ++.

Only one dose level was tested, but was given for a near life-span of a large number of animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.

Table C-6d. DeAngelo (2008) (M Mouse (Study 3)): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Mice were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of neutralized drinking water was used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ Two exposure levels were used that spanned a range of 10 fold.
Outcome	
Pathology	+++ A complete necropsy with histological examine was performed, including verification by an independent pathologist.
Consistency between groups	+++ All exposure groups appear to have been treated similarly.
Confounding	
Confounding	+++ Infectious disease detection was used and the vehicle control was appropriate.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	+++ Only males of non-transgenic animals were used and the strain is in common use.

Study utility domain Question	Rating Rationale
Statistical power	+++ Large numbers of animals (51-53) were used for each group.
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: +++.

Only two dose level were tested, but were given for near a life-span of time to a large number of animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.

Table C-6e. Herren-Freund *et al.* ({, 1987 #229}) (M Mouse): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride to match that found in exposed groups and a positive control of phenobarbital at 500 mg/l (positive controls are not reported here) were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ One exposure level was used, so dose response relationships could not be measured.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ Disease surveillance was not reported, but chemical purity and stability were tested.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.

Study utility domain Question	Rating Rationale
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Moderate numbers of animals (22-26) were used for each group.
Exposure duration	+++ A less than life-span duration (61 weeks) was used.
Dose/response	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (61 weeks) was used.

Overall utility: ++.

The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.

Table C-6f. Herren-Freund *et al.* (1987) (M Mouse): Trichloroacetic acid: Drinking water (I/P)

Study utility domain Question	Rating Rationale
Study design	
Randomization	Not reported Not reported.
Controls	+++ Concurrent vehicle controls of 2 g/l of sodium chloride to match that found in exposed groups and a positive control of phenobarbital at 500 mg/l (positive controls are not reported here) were used.
Historical data	No
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ Two exposure levels were used which spanned a range of 2.5 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ Disease surveillance was not reported, but chemical purity and stability were tested.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.

Study utility domain Question	Rating Rationale
Combining lesions	+++ Tumor types were not combined.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	++ Moderate numbers of animals (22-26) were used for each group.
Exposure duration	+++ A less than life-span duration (61 weeks) was used.
Dose/response	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (61 weeks) was used.
Overall utility: ++. The duration was less than near life-span. Only males were tested at two narrow dose levels and only livers were histologically evaluated.	

Table C-6g. Pereira 1996 (F Mouse (Study 1)): Trichloroacetic acid (TCA): Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ It was not reported if animals were randomly assigned to exposure groups, but they were necropsied blinded and the mice were randomly assigned an ID number, which suggests they were randomly assigned to treatment groups, but it is not known for sure.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as in the high dose group were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 10 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	

Study utility domain Question	Rating Rationale
Confounding	++ Continual disease surveillance and survival were not reported. The high exposure group had a significant decrease in water consumption.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ The number of animals (15-90) varied considerably for each group.
Exposure duration	+++ A near life-span duration (576 days) was used.
Dose/response	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (576 days) was used.
Overall utility: ++. The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.	

Table C-6h. Pereira (1996) (F Mouse (Study 2)): Trichloroacetic acid (TCA): Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ It was not reported if animals were randomly assigned to exposure groups, but they were necropsied blinded and the mice were randomly assigned an ID number, which suggests they were randomly assigned to treatment groups, but it is not known for sure.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as in the high dose group were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 10 fold.
Outcome	

Study utility domain Question	Rating Rationale
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance and survival were not reported. The high exposure group had a significant decrease in water consumption.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ The number of animals (15-90) varied considerably for each group.
Exposure duration	++ A less than life-span duration (360 days) was used.
Dose/response	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (360 days) was used.
Overall utility: ++.	
The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.	

Table C-6i. Pereira et al 1997 (F Mouse): TCA: Drinking water (I/P)

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups with initial body weights the same for each group.
Controls	+++ Concurrent controls were appropriate for an initiation/promotion study in which all groups received the initiator (NMU) and the negative control group was given only the initiator and promotor vehicle.
Historical data	No
Exposure	

Study utility domain Question	Rating Rationale
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 3 fold.
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Confounding	
Confounding	++ Continual disease surveillance and survival were not reported.
Reporting and analysis	
Reporting data and statistics	++ Significance levels were reported, but the statistical test was not reported.
Combining lesions	+++ Lesions were reported, which included liver foci, adenomas, and carcinomas. The authors felt the foci were preneoplastic.
Sensitivity	
Animal model	++ Only females of non-transgenic animals were used.
Statistical power	++ Small, but sufficient numbers of animals (>20) were used for each group.
Exposure duration	+++ Animals were exposed for 44 weeks, starting 4 weeks after a single injection of the initiator. This is short for carcinogenicity studies, but initiation/promotion studies are normally shorter and tumors were significantly induced.
Dose/response	+++ There was no significant decrease in survival or body weight and there was a significant increase in neoplasm incidence.
Study duration	+++ A less than near life-span duration (48 weeks) was used, but initiation/promotion studies are normally short.
Overall utility: + The chemicals were not characterized, not even purity was reported. Disease surveillance was not reported. A low number of only females were tested, with only their livers examined histologically. The statistical methods were not reported.	

Table C-6j. Bull *et al.* 1990 (M Mouse): Trichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.

Study utility domain Question	Rating Rationale
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Exposure	
Chemical characterization	++ Chemical purity and stability were not verified.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold.
Outcome	
Pathology	+ Only livers from some animals were histologically evaluated. Results only reported overall lesions, which include hyperplasia. Liver lesions that were histologically evaluated were randomly selected and blindly evaluated.
Consistency between groups	+ All untreated control lesions were histologically evaluated, but only some of the exposed groups were.
Confounding	
Confounding	+ Disease surveillance was not reported. Only samples of liver lesions were histologically evaluated and inappropriately reported.
Reporting and analysis	
Reporting data and statistics	+ Statistical evaluations were rarely reported and incidences that could be significant by Fisher's Exact test (calculated by NTP) were not reported as significant, so no NTP calculations were included.
Combining lesions	++ Only total lesions were adequately reported to allow for quantitation of the results. These included liver hyperplastic nodules, hepatocellular adenoma, and hepatocellular carcinoma, which entail a continuum of the same disease process, however there were 4/73 lesion types that were also included, but were not hyperplastic or neoplastic.
Sensitivity	
Animal model	++ Only males of non-transgenic animals were used.
Statistical power	+ Small numbers of animals (11-24) were used for each group.
Exposure duration	+++ A less than life-span duration (52 weeks) was used.
Dose/response	+++ There were no deaths and body weight was not reported. There was a significant increase in neoplasm incidence.
Study duration	+++ A less than life-span duration (52 weeks) was used.
Overall utility: + The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less	

than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.

Table C-6k. Von Tungeln *et al.* 2002 (M+F Mouse (Study 1)): Trichloroacetic acid: ip injection

Study utility domain Question	Rating Rationale
Study design	
Randomization	+ Randomization was not reported. However, survival during the first 28 days of age ranged from 100% to 71% (data not reported). After 28 days of age, surviving mice were allocated to each treatment group, after which survival was 92% or higher.
Controls	+++ Concurrent vehicle controls of DMSO and 4-aminobiphenyl positive controls were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold.
Outcome	
Pathology	++ Necropsies and histological evaluations were stated to have occurred, but only incidences of liver tumors were reported.
Consistency between groups	++ Before the mice reached 28 days of age, mortality was as high as 29% and then a set number of the survivors were used for the remainder of the study. No information was reported about the early mortality.
Confounding	
Confounding	++ Mice were reported to be specific pathogen free, but mortality was high before the age of 28 days and this early mortality wasn't reported.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	++ Both sexes of non-transgenic animals were used. Modifying factor is age of animals at study start.
Statistical power	++ Moderate numbers of animals (22-24) were used for each group.

Study utility domain Question	Rating Rationale
Exposure duration	+ Two doses were administered at the beginning of the study, no additional exposure occurred throughout the study.
Dose/response	+ There was no significant difference in survival and body weight was not reported. There was no significant difference in neoplasm incidence.
Study duration	+++ Duration was near life-span (20 months).

Overall utility: ++.

The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.

Table C-6I. Von Tungeln *et al.* (2002) (M+F Mouse (Study 2)): Trichloroacetic acid: ip injection

Study utility domain Question	Rating Rationale
Study design	
Randomization	+ Randomization was not reported. However, survival during the first 28 days of age ranged from 100% to 71% (data not reported). After 28 days of age, surviving mice were allocated to each treatment group, after which survival was 92% or higher.
Controls	+++ Concurrent vehicle controls of DMSO and 4-aminobiphenyl positive controls were used.
Historical data	No
Exposure	
Chemical characterization	Not reported Chemical purity and stability were not reported.
Dosing regimen	++ Two exposure levels were used that spanned a range of 2 fold.
Outcome	
Pathology	++ Necropsies and histological evaluations were stated to have occurred, but only incidences of liver tumors were reported.
Consistency between groups	++ Before the mice reached 28 days of age, mortality was as high as 29% and then a set number of the survivors were used for the remainder of the study. No information was reported about the early mortality.
Confounding	
Confounding	++ Mice were reported to be specific pathogen free, but mortality was high before the age of 28 days and this early mortality wasn't reported.
Reporting and analysis	

Study utility domain Question	Rating Rationale
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.
Sensitivity	
Animal model	++ Both sexes of non-transgenic animals were used. Modifying factor is age of animals at study start.
Statistical power	++ Moderate numbers of animals (22-24) were used for each group.
Exposure duration	+ Two doses were administered at the beginning of the study, no additional exposure occurred throughout the study.
Dose/response	+ There was no significant difference in survival and body weight was not reported. There was no significant difference in neoplasm incidence after exposure of neonatal mouse to TCA.
Study duration	++ Duration was less than life-span (12 months).

Overall utility: ++.

The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.

C.7 Bromodichloroacetic acid: Study quality for animal studies

Table C-7a. NTP 2015 (M Rat): Bromodichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (50) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ There was no significant decrease in survival, but there was a decrease in body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.

Table C-7b. NTP (2015) (F Rat): Bromodichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ controls adequate number
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (49-51) were used for each group.
Exposure duration	+++ A near life-span duration (104 weeks) was used.
Dose/response	+++ There was a significant decrease in survival and body weight and a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (104 weeks) was used.

Overall utility: +++.

Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.

Table C-7c. NTP (2015) (M Mouse): Bromodichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ Large numbers of animals (50-51) were used for each group.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ There was a significant decrease in survival and body weight, with a significant increase in neoplasm incidence.
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.

Table C-7d. NTP (2015) (F Mouse): Bromodichloroacetic acid: Drinking water

Study utility domain Question	Rating Rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Outcome	
Pathology	+++ All major organs and gross lesions were histologically evaluated and findings were verified by an independent pathologist.
Consistency between groups	+++ All groups were treated the same.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor combinations were appropriate.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic animals were used.
Statistical power	+++ There was no significant decrease in survival, but there was a decrease in body weight and a significant increase in neoplasm incidence.
Exposure duration	+++ A near life-span duration (105 weeks) was used.
Dose/response	+++ Three exposure levels were used that spanned a range of 4 fold.
Study duration	+++ A near life-span duration (105 weeks) was used.

Overall utility: +++.

Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.

C.8 Animal studies for haloacetic acids: Results by tumor

Table C-8. Liver tumors

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 1992 Animal: Rat F344/N M 6-7 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	Liver – Adenoma		Survival: After 104 weeks, the survival of the 30 mg/kg group was lower than controls and there was a significant trend: 27/53*(trend=0.011) - 21/53, 16/53*(=0.015) Body weight: Body weights were similar to controls during the 6 and 15 month interim evaluations as well as the 2 year study. Other comments: No neoplasms were found at the 6 month evaluation and no treatment related neoplasms were found at the 15 month interim evaluation or at the end of the study. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
		0	1/53 (2%)	
	Treatment: Gavage	15	0/53	
		Liver – Carcinoma		
		0	0/53	
		15	1/53 (2%)	
30	0/53			
NTP 1992 Animal: Rat F344/N	Agent: Monochloroacetic acid 99%	Liver – Adenoma		
		0	1/53 (2%)	
		15	0/53	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
F 6-7 weeks Study duration: 104 weeks	Treatment: Gavage 0 15 30 mg/kg bw 5 doses/week x 104 weeks	30	0/53	26/53*(=0.046) Body weight: Body weights were similar to controls during the 6 and 15 month interim evaluations as well as the 2 year study. Other comments: No neoplasms were found at the 6 month evaluation and no treatment related neoplasms were found at the 15 month interim evaluation or at the end of the study. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
NTP 1992 Animal: Mouse B6C3F1 M 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99% Treatment: Gavage 0 50 100 mg/kg in deionized water 5 doses/week x 104 weeks	Liver – Adenoma^a 0 50 100 Trend p-value: =0.059N Liver – Carcinoma^a 0 50 100 Trend p-value: =0.440N Liver – Adenoma or carcinoma^a 0 50 100	6/60 (12.7%) 6/59 (14.8%) 1/59 (4.2%) 6/60 (11.7%) 2/59 (4.7%) 5/59 (19.9%) 12/60 (23.6%) 8/59 (19.1%) 6/59 (23.3%)	Survival: The 100 mg/kg group was lower than controls and there was a significant trend: 46/60***(trend <0.001) - 39/60, 21/60***(<0.001) Body weight: Body weights were similar to controls. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Trend p-value: =0.082N				
NTP 1992 Animal: Mouse B6C3F1 F 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	Liver – Adenoma		Survival: Survival of the exposed groups were similar to controls: 42/60 - 40/60, 44/60 Body weight: Body weights of the low dose group were similar to controls, but after a year the high dose group had significantly lower body weight. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.
		0	1/60 (2%)	
		50	1/60 (2%)	
	Treatment: Gavage 0 50 100 mg/kg in deionized water 5 doses/week x 104 weeks	Liver – Carcinoma		
		100	2/60 (3%)	
		0	0/60	
		50	1/59 (2%)	
		100	0/60	
DeAngelo et al. 1997 Animal: Rat F344/N M 28-30 days Study duration: 104 weeks	Agent: Monochloroacetic acid >99%	Liver – Adenoma		Survival: No significant difference in survival: 23/29 - 24/32, 23/32, 25/29 Body weight: The 2.0 g/l MCA was decreased to 1.5 g/l after 8 weeks and to 1 g/l after 24 weeks because of significant differences in body weight gain. The 0.5 g/l group had 13% lower body weight than untreated controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were reported, but were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Amount of water consumed was similar among groups (76.9 ml/kg/d - 70.5, ml/kg/d, 55.6 ml/kg/d, 55.5 ml/kg/d). Strengths and limitations: A well conducted study on almost all aspects, but only involved male rats.
		0	1/23 (4%)	
		50	2/25 (8%)	
	Treatment: Drinking water 0+ 50 500 2,000++ mg/L in drinking water ad libitum x 104 weeks + 31-32 mM NaCl (~isomolar to 5,000 mg/L TCA) ++ 2,000 mg/L x 8 wk, then 1,500 mg/L to 24 weeks, then 1,000 mg/L; averaging 1,100 mg/L throughout the study	Liver – Carcinoma		
		500	0/23	
		2,000	1/25 (4%)	
		Liver – Adenoma or carcinoma		
		0	1/23 (4%)	
		50	2/25 (8%)	
		500	0/23	
		2,000	1/25 (4%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
DeAngelo et al. 1996 Animal: Rat (Study 1) F344 M 28-30 days (59-79g bw) Study duration: 100 weeks	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: There were no significant differences in survival in the 0.05 or 0.5 g/l groups. The 5 g/l group rats had irreversible peripheral neuropathy and were sacrificed at 60 weeks and were excluded from the study analysis. Body weight: Body weights did not differ after 100 weeks of treatment. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: The exact value for N at the beginning of the study is not confirmed as the paper didn't clearly report them. Water consumption didn't differ among groups. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. All non-hepatic neoplasms were considered spontaneous and not treatment related and included, testicular cancer (97% - 100%, 100%) and leukemia (24% - 20%, 43%). Strengths and limitations: Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only two exposed dose levels were effectively reported and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.
	>99%	0	1/23 (4.4%)	
	Treatment: Drinking water	50	0/26	
	0+	500	5/29 (17.2%)	
	50	Trend p-value: <0.05		
	500	Liver – Carcinoma		
	5,000++ mg/L in drinking water ad libitum x 100 weeks	0	0/23	
		50	0/26	
	+ 2 g/L NaCl (~isomolar to 5,000 mg/l DCA)	500	3/29 (10.3%)	
		Trend p-value: <0.05		
	Liver – Adenoma or carcinoma			
++ 2,500 mg/L at 9 weeks then 2,000 mg/L after 23 wks then 1,000 mg/L after 52 wks and stopped at 60 weeks due to peripheral neuropathy and wasn't included in the study results.	0	1/23 (4.4%)		
	50	0/21		
	500	6/23* (24.1%)		
	Trend p-value: <0.01			
DeAngelo et al. 1996 Animal:	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: There were no significant differences in survival in the 0.05 or 0.5 g/l groups. The 5 g/l group rats had
		0	0/33	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Rat (Study 2) F344 M 28-30 days (59-79g bw) Study duration: 103 weeks	>99%	2,500	3/28 (10.7%)	irreversible peripheral neuropathy and were sacrificed at 60 weeks and were excluded from the study analysis. Body weight: Body weights in the exposed group were significantly less than (73%) those in the untreated control group after 103 weeks of treatment. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: The exact value for N at the beginning of the study is not confirmed as the paper didn't clearly report them. Water consumption didn't differ among groups. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. All non-hepatic neoplasms were considered spontaneous and not treatment related and included, testicular cancer (97% - 100%) and leukemia (9% - 11%). Strengths and limitations: Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only one exposed dose level was tested and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.
	Treatment: Drinking water	Liver – Carcinoma		
	0+	0	1/33 (3%)	
	2,500++ mg/L in deionized water ad libitum x 103 weeks	2,500	6/28* (21.4%)	
	Liver – Adenoma or carcinoma			
0	0	1/28 (3%)		
+ NaCl in the first experiment had no effect on water consumption or tumor incidence, so was not included here	2,500	8/27** (28.6%)		
++ 1,500 mg/L at 8 weeks then 1,000 mg/L after 26 weeks due to mild transient neurotoxicity				
Richmond et al. 1995	Agent:	Liver – Adenoma		Survival: Survival wasn't clearly reported, but based on 7 animals sacrificed at 15, 30, 45, and for all but the high dose group, 60 week time points survival was estimated from the
Animal:	Dichloroacetic acid	0	0/7	
Rat (Study 1) F344	Not reported	50	0/7	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
M 28 days Study duration: 60 weeks	Treatment: Drinking water	500	0/7	60 animals per group at the beginning of the study to be: 51/60 - 54/60, 57/60, 51/60 Body weight: Not reported. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 2,400 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was less than near-lifespan. Survival and body weights were not clearly reported and only the liver was histologically evaluated.
	0+	2,400	7/27 (26%)	
	50	Liver – Carcinoma		
	500	0	0/7	
	2,400 mg/L in drinking water(pH 7.0) x 60++ or	50	0/7	
	104 weeks	500	0/7	
	+ 2 g/L NaCl	2,400	1/27 (4%)	
	++ High dose stopped at 60 weeks due to tumors and hind limb paralysis			
Richmond et al. 1995	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: Survival wasn't clearly reported, but based on 7 animals sacrificed at 15, 30, 45, and for all but the high dose group, 60 week time points survival was estimated from the 60 animals per group at the beginning of the study to be: 51/60 - 54/60, 57/60, 51/60 Body weight: Not reported. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was
Animal: Rat (Study 2) F344	Not reported	0	1/23 (4%)	
M 28 days	Treatment: Drinking water	50	0/26	
Study duration: 104 weeks	0+	Liver – Carcinoma		
	50	0	0/23	
	500	0	0/23	
	2,400 mg/L in drinking	50	0/26	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	water (pH 7.0) x 60++ or 104 weeks + 2,000 mg/L NaCl ++ high dose stopped at 60 weeks due to tumors and hind limb paralysis	500	3/29 (10%)	reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was near-lifespan for all but the high dose group. Survival and body weights were not clearly reported and only the liver was histologically evaluated.
DeAngelo et al. 1991	Agent: Dichloroacetic acid Animal: Mouse (Study 1) B6C3F1 M 28 days Study duration: 60 weeks (high dose), 75 weeks (low and medium dose levels)	Liver – Adenoma 0 50 500 5,000 Liver – Carcinoma 0 50 500 5,000 Liver – Adenoma or carcinoma 0 50 500	Liver – Adenoma 0/28 2/29 (7%) 1/27 (4%) 24/30*** (80%) Liver – Carcinoma 2/28 (7%) 6/29 (21%) 2/27 (7%) 25/30*** (83%) Liver – Adenoma or carcinoma 2/28 (7%) 7/29 (24%) 3/27 (11%)	Survival: No difference. Body weight: The high dose group had significantly lower body weight than controls (17% lower, p<0.001). Body weights of the medium and low doses didn't differ from controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 5,000 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Only the high dose group was quantitatively reported (as a percentage - fractional incidence was back calculated from percent), the other groups were extrapolated from a graph. Nine animals from each group

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		5,000	27/30*** (90%)	were scarified at 60 weeks, with the remainder sacrificed at 75 weeks. All high dose animals were sacrificed at 60 weeks. Strengths and limitations: Three dose levels, which spanned a 100 fold range were tested in only males for up to 75 weeks. Only a few select organs were necropsied.
DeAngelo et al. 1991	Agent:	Liver – Adenoma		Survival: No difference. Body weight: The high dose group had significantly lower body weight than controls (13% lower, p<0.001). Body weights of the medium and low doses didn't differ from controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 3,500 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Incidences were only reported as a percentage - fractional incidence was back calculated from percent. All animals were sacrificed at 60 weeks. Strengths and limitations: One dose level was tested in a small number of males for 60 weeks. Only a few select organs were necropsied.
Animal:	Dichloroacetic acid	0	0/10	
Mouse (Study 2)	>99%	3,500	12/12*** (100%)	
B6C3F1	Treatment:	Liver – Carcinoma		
M 28 days	Drinking water	0	0/10	
Study duration:	0+	3,500	8/12*** (67%)	
60 weeks	3,500 mg/l in distilled water (pH 6.8-7.2) ad libitum x 60 weeks	Liver – Adenoma or carcinoma		
	+ 1,500 mg/l acetic acid	0	0/10	
		3,500	12/12*** (100%)	
DeAngelo et al. 1999	Agent:	Liver – Adenoma		
Animal:	Dichloroacetic acid	0	5/53 (10%)	
Mouse B6C3F1	>99%	500	5/25 (20%)	
M 28-30 days (18-21 g bw)	Treatment:	1,000	21/41* (51.4%)	
	Drinking water	2,000	11/25* (42.9%)	
Study duration:	0+	3,500	7/16* (45%)	
90-100 weeks	50+	Liver – Carcinoma		
	500			
	1,000			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	2,000 3,500 mg/L in drinking water (pH 6.9-7.1) ad libitum x 90 or 100 weeks + started 1 month after the other groups (10 mice from each group were sacrificed at 26, 52, and 78 weeks - data not reported here)	0 500 1,000 2,000 3,500	14/53 (26%) 12/25 (48%) 29/41* (71%) 24/25* (95%) 16/16* (100%)	levels 500 to 3,500 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Water consumption was lower in the high dose group. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. Strengths and limitations: Only male mice, initially confirmed pathogen free, were used with differing numbers per group (as low as 16 in the high dose group). Continual disease surveillance was not reported. Multiple dose levels ranging 100 fold, were tested for a near life-span duration. However, only 5 high dose group mice received histological evaluations of all major organs, with histological evaluations of only gross lesions in the other groups.
Herren-Freund et al. 1987 Animal: Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	Agent: Dichloroacetic acid >99% Treatment: Drinking water 0+ 5,000 mg/l in drinking water (pH 6.5-7.5) ad libitum x 61 weeks + 2,000 mg/l of NaCl	Liver – Adenoma 0 5,000 Liver – Carcinoma 0 5,000	2/22 (9%) 25/26** (96%) 0/22 21/26** (81%)	Survival: Not reported. Body weight: Body weights were significantly decreased (p<0.001). Calculations were done by one-way analysis of variance with a Tukey's comparison. Significantly increased pre-neoplastic lesions: Not reported. Strengths and limitations: The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.
Wood et al. 2015	Agent:	Liver – Adenoma		Survival: Survival was similar in all groups.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse B6C3F1 M 28 days Study duration: 94 weeks	Dichloroacetic acid	0	5/27 (19%)	Body weight: Body weight of the high dose group, after DCA exposure had stopped, was decreased by 12% compared to controls. Other comments: The original number of mice used were reported as those given a specific dose of DCA and were not differentiated by those given phenobarbital and those that weren't. The incidence denominator was differentiated by co-administration of phenobarbital and only represents those animals given only dichloroacetic acid. Water consumption was decreased at the medium and high dose groups, which limited the daily intake to (target dose mg/kg/d: 0 - 168, 315, 429; Measured dose mg/kg/d: 0 - 136, 232, 297), so the high dose was not nearly as high as expected. Strengths and limitations: Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Three dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.
	Not reported	1,000	13/27 (48%)	
	Treatment: Drinking water	2,000	11/27 (41%)	
	0	3,500	15/26* (58%)	
	1,000	Trend p-value: <0.05		
	2,000	Liver – Carcinoma		
	3,500 mg/L deionized water (pH 6.8-7.1) ad libitum x 10 weeks	0	8/27 (30%)	
		1,000	8/27 (30%)	
		2,000	6/27 (22%)	
		3,500	19/26* (73%)	
		Trend p-value: <0.01		
		Liver – Hepatoblastoma		
		0	0/27	
		1,000	1/27 (4%)	
		2,000	0/27	
	3,500	0/26		
	Liver – Adenoma, carcinoma, and hepatoblastoma			
	0	12/27 (44%)		
	1,000	15/27 (56%)		
	2,000	14/27 (52%)		
	3,500	24/26** (92%)		
	Trend p-value: <0.01			
Wood et al. 2015	Agent:	Liver – Adenoma		
Animal: Mouse B6C3F1	Dichloroacetic acid	0	0/27	
	Not reported	1,000	9/26** (35%)	
			Survival: Survival was similar in all groups.	
			Body weight: Body weight of the high dose group, after DCA exposure had stopped, was decreased by 12%	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
F 28 days Study duration: 94 weeks	Treatment: Drinking water 0 1,000 2,000 mg/L in deionized water (pH 6.8-7.1) ad libitum x 10 weeks	2,000	6/28 (21%)	compared to controls.
		Liver – Carcinoma		Other comments: The original number of mice used were reported as those given a specific dose of DCA and were not differentiated by those given phenobarbital and those that weren't. The incidence denominator was differentiated by co-administration of phenobarbital and only represents those animals given only dichloroacetic acid. Water consumption was decreased at the medium and high dose groups, which limited the daily intake to (target dose mg/kg/d: 0 - 168, 315, 429; Measured dose mg/kg/d: 0 - 136, 232, 297), so the high dose was not nearly as high as expected. Strengths and limitations: Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Two dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.
		0	0/27	
		1,000	2/26 (8%)	
		2,000	3/28 (11%)	
		Liver – Hepatoblastoma		
		0	0/27	
		1,000	0/26	
		2,000	0/28	
		Liver – Adenoma, carcinoma, and hepatoblastoma		
		0	0/27	
		1,000	10/26** (38%)	
2,000	9/28** (32%)			
Trend p-value: <0.01				
Pereira 1996 Animal: Mouse (Study 1) B6C3F1 F 7-8 weeks Study duration: 576 days	Agent: Dichloroacetic acid (DCA) Not reported Treatment: Drinking water 0+ 260++ 860++ 2,600+++ 2,600++ mg/L in drinking water ad libitum x 360 days	Liver – Adenoma		Survival: Not reported Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface
		0	2/90 (2.2%)	
		260	3/50 (6%)	
		860	7/28* (25%)	
		2,600	3/34 (8.8%)	
		intermittent		
		2,600	16/19* (84.2%)	
		Liver – Carcinoma		
		0	2/90 (2.2%)	
		260	0/50	
860	1/28 (3.6%)			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	+ 20 mmol/L NaCl	2,600 intermittent	1/34 (2.9%)	(DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. The study duration was near life-span.
	++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol	2,600	5/19* (26.3%)	
	+++ intermittent cycles of 24 days on, 48 days off			
Pereira 1996	Agent:	Liver – Adenoma		Survival: Not reported
Animal:	Dichloroacetic acid (DCA)	0	1/40 (2.5%)	Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemicals were not
Mouse (Study 2)	(DCA)	260	0/40	
B6C3F1	Not reported	860	3/20 (15%)	
F 7-8 weeks	Treatment:	2,600	0/15	
Study duration:	Drinking water	intermittent		
360 days	0+	2,600	7/20* (35%)	
	260++			
	860++			
	2,600+++	Liver – Carcinoma		
	2,600++ mg/L in drinkign water ad libitum x 360 days	0	0/40	
		260	0/40	
		860	0/20	
	+ 20 mmol/L NaCl	2,600	0/15	
		intermittent		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol	2,600	1/20 (5%)	characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. Study duration was less than life-span.
	+++ intermittent cycles of 24 days on, 48 days off			
Bull et al. 1990	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: All mice survived. Significantly increased pre-neoplastic lesions: A non-significant increase in hyperplasia was reported. Strengths and limitations: The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.
Animal: Mouse B6C3F1	Analytical grade	0	0/2	
M 5 weeks	Treatment:	1,000	0/1	
Study duration: 52 weeks	Drinking water	2,000	2/10 (20%)	
	0	Liver – Carcinoma		
	1,000	0	0/2	
	2,000 mg/L in drinking water (pH 6.8-7.2) ad libitum x 52 weeks	1,000	0/1	
		2,000	5/10 (50%)	
Daniel et al. 1992	Agent:	Liver – Adenoma		
Animal: Mouse B6C3F1	Dichloroacetic acid	0	1/20 (5%)	
M 28 days	>95%	500	10/24** (42%)	
Study duration: 104 weeks	Drinking water	Liver – Carcinoma		
	0	0	2/20 (10%)	
	0	500	15/24** (63%)	
	500	Liver – Adenoma or carcinoma		
	500 mg/l in distilled water (pH 6.8-7.2) ad	0	3/20 (15%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	libitum x 104 weeks	500	18/24** (75%)	adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: All tumors occurred at necropsy and did not cause premature mortality. Both groups of controls and exposed mice were combined into one group each. Fractional incidence was based on surviving animals as the denominator and percent incidence was also reported. Strengths and limitations: Continual disease surveillance was not reported. Only males were tested and only at one dose level. Histological evaluations from all major organs only occurred in five mice per group, with histological evaluations in the other mice only occurring on tissues with gross lesions.
	Two sets of animals were used as they were born, both groups are the same, just started at different times. Except for the body weights, the two groups were statistically analyzed as one combined group.			
NTP 2007a	Agent: Dibromoacetic acid	Liver – Adenoma^a		Survival: Survival was similar in all groups. 31/50 - 38/50, 34/50, 31/50 Body weight: Body weights were greater in the 50 and 500 mg/l groups compared to the untreated controls after 85 weeks. Significantly increased pre-neoplastic lesions: Spleen hematopoiesis 18/49 - 20/50, 28/50, 38/50 Other comments: Water consumption was similar to controls. Onset was reported in days. Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
Animal: Mouse B6C3F1	>99%	0	18/49 (42%)	
Study duration: M 6 weeks	Treatment: Drinking water	50	37/50*** ^b (78%)	
106 weeks	0	500	37/50*** ^b (80%)	
	50	1,000	42/50*** ^b (89%)	
	500	Trend p-value: <0.001		
	1,000 mg/L of drinking water ad libitum x 106 weeks	Liver – Carcinoma^a		
		0	14/49 (31%)	
		50	9/50 (19%)	
	Average daily dose: 0 - 4, 45, 87 mg/kg	500	19/50 (41%)	
		1,000	26/50* ^c (55%)	
		Trend p-value: <0.001		
		Liver – Adenoma or carcinoma^a		
		0	28/49 (61%)	
		50	41/50*** ^d (86%)	
		500	42/50*** ^d (88%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		1,000	47/50*** ^d (96%)	
		Trend p-value: <0.001		
		Liver – Hepatoblastoma^a		
		0	0/49	
		50	4/50 (9%)	
		500	6/50* (13%)	
		1,000	18/50*** ^e (39%)	
		Trend p-value: <0.001		
		Liver – Adenoma, carcinoma or hepatoblastoma^a		
		0	28/49 (61%)	
		50	41/50** (86%)	
		500	43/50*** (90%)	
		1,000	48/50*** (97%)	
		Trend p-value: <0.001		
NTP 2007a	Agent:	Liver – Adenoma^a		Survival: Survival was similar in all groups.
Animal:	Dibromoacetic acid	0	19/49 (41%)	38/50 - 35/50, 32/50, 32/50
Mouse B6C3F1	>99%	50	26/50 (57%)	Body weight: Body weights were similar to the untreated controls.
F 6 weeks	Treatment:	500	32/50*** ^f (70%)	Other comments: Water consumption was similar to controls.
Study duration:	Drinking water	1,000	35/49*** ^f (76%)	Onset was reported in days.
106 weeks	0	Trend p-value: <0.001		Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
	50	Liver – Carcinoma^a		
	500	0	3/49 (7%)	
	1,000 mg/L of drinking water ad libitum x 106 weeks	50	3/50 (7%)	
	Average daily dose: 0 - 4, 35, 65 mg/kg	500	12/50*** ^g (27%)	
		1,000	8/49 (18%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
			Trend p-value: =0.019	
			Liver – Adenoma or carcinoma^a	
		0	22/49 (48%)	
		50	28/50 (61%)	
		500	37/50*** ^h (80%)	
		1,000	37/49*** ^h (80%)	
			Trend p-value: <0.001	
			Liver – Hepatoblastoma	
		0	1/49 (2%)	
		50	0/50	
		500	1/50 (2%)	
		1,000	0/49	
NTP 2009	Agent:	Liver – Adenoma^a		Survival: No significant difference:
Animal:	Bromochloroacetic acid	0	2/50 ⁱ (4.6%)	31/50 - 26/50, 25/50, 29/50
Rat F344/N	96%	250	0/50	Body weight: 1,000 mg/l group was 10% less than controls after 69 weeks.
M 6-7 weeks	Treatment:	500	3/50 ⁱ (7.5%)	Strengths and limitations: A very high quality study, with no major concerns.
Study duration:	Drinking water	1,000	4/50 ⁱ (9.5%)	
105 weeks	0			
	250			
	500			
	1,000 mg/L of drinking water ad libitum x 105 weeks			
NTP 2009	Agent:	Liver – Adenoma^a		Survival: No significant difference:
Animal:	Bromochloroacetic acid	0	0/50	34/50 - 31/50, 37/50, 35/50
Rat F344/N	96%	250	0/50	Body weight: 1,000 mg/l group was <10% of controls. after 85 weeks.
F 6-7 weeks	Treatment:	500	0/50	Significantly increased pre-neoplastic lesions: Lung alveolar epithelium hyperplasia occurred at increased incidences.
Study duration:	Drinking water	1,000	3/50 ⁱ (6.6%)	
105 weeks	0			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	250 500 1,000 mg/L of drinking water ad libitum x 105 weeks		Trend p-value: =0.012	Strengths and limitations: A very high quality study, with no major concerns.
NTP 2009	Agent: Bromochloroacetic acid 96%	Liver – Adenoma^a		Survival: 38/50 - 35/50, 30/50, 21/50
Animal: Mouse B6C3F1 M 6-7 weeks	Treatment: Drinking water	0	27/50 (58.7%)	Body weight: 1,000 mg/l group was 12% lower than controls after 97 weeks.
Study duration: 105 weeks	0	250	40/50***k (83.6%)	Strengths and limitations: A very high quality study, with no major concerns.
	250	500	40/50***k (83.7%)	
	500	1,000	31/50 (67.4%)	
	1,000 mg/L of drinking water ad libitum x 105 weeks	Liver – Carcinoma^a		
		0	19/50 (39.6%)	
		250	25/50 ^l (52.5%)	
		500	36/50*** ^l (76.9%)	
		1,000	45/50*** ^l (92.7%)	
			Trend p-value: <0.001	
		Liver – Adenoma or carcinoma^a		
		0	34/50 (70.6%)	
		250	44/50* ^m (89.7%)	
		500	49/50*** ^m (99.9%)	
		1,000	49/50*** ^m (98.6%)	
			Trend p-value: <0.001	
		Liver – Hepatoblastoma^a		
		0	4/50 (8.8%)	
		250	11/50* (23.8%)	
		500	28/50*** ⁿ (61.3%)	
		1,000	34/50*** ⁿ (73.7%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
			Trend p-value: <0.001	
			Liver – Adenoma, carcinoma, or hepatoblastoma^a	
		0	21/50 (43.8%)	
		250	32/50* (66.3%)	
		500	43/50*** (90.7%)	
		1,000	49/50*** (98%)	
			Trend p-value: <0.001	
NTP 2009	Agent:	Liver – Adenoma^a		Survival: 36/50 - 42/50, 32/50, 40/50
Animal:	Bromochloroacetic acid	0	27/50 (59.4%)	Body weight: No significant difference.
Mouse B6C3F1	96%	250	48/50*** ^o (96%)	Strengths and limitations: A very high quality study, with no major concerns.
F 6-7 weeks	Treatment:	500	44/50*** ^o (90.9%)	
Study duration:	Drinking water	1,000	46/50*** ^o (95.2%)	
105 weeks	0		Trend p-value: <0.001	
	250		Liver – Carcinoma^a	
	500		0	14/50 ^p (31.1%)
	1,000 mg/L of drinking water ad libitum x 105 weeks		250	23/50 ^p (48.3%)
			500	26/50* ^p (56.1%)
			1,000	20/50 ^p (42.3%)
				Trend p-value: <0.001
			Liver – Adenoma or carcinoma^a	
			0	31/50 ^q (67.6%)
			250	49/50*** ^q (98%)
			500	46/50*** ^q (94.6%)
			1,000	46/50*** ^q (95.2%)
			Trend p-value: <0.001	
DeAngelo et al. (1997)	Agent:	Liver – Adenoma		Survival: No significant difference in survival:

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Rat F344/N M 28-30 days Study duration: 104 weeks	Trichloroacetic acid	0	1/23 (4%)	23/29 - 24/32, 19/32, 22/29
	>99%	50	1/24 (4%)	Body weight: Body weights were similar among the 0.05 and 0.5 g/l groups, but decreased more than 10% compared to controls in the 5 g/l group.
	Treatment: Drinking water	500	3/20 (15%)	
	0+	5,000	1/22 (5%)	Significantly increased pre-neoplastic lesions: Hyperplastic nodules were reported, but were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008).
	2,500++ mg/L in drinking water (pH 6.9-7.1) ad libitum x 104 weeks	Liver – Carcinoma		
		0	0/23	Other comments: Amount of water consumed was similar among groups (76.9 ml/kg/d - 71.2, ml/kg/d, 70.6 ml/kg/d, 74,2 ml/kg/d).
		50	0/24	
	+ 31-32 mM NaCl (~isomolar to 5,000 mg/l of TCA)	500	0/20	Strengths and limitations: A well conducted study on almost all aspects, but only involved male rats.
		5,000	1/22 (5%)	
		Liver – Adenoma or carcinoma		
		0	1/23 (4%)	
		50	1/24 (4%)	
	500	3/20 (15%)		
	5,000	1/22 (5%)		
DeAngelo et al. 2008 Animal: Mouse (Study 1) B6C3F1 M 28-30 days Study duration: 60 weeks	Agent: Trichloroacetic acid	Liver – Adenoma		Survival: No difference in survival: 30/30 - 27/30, 29/30, 29/30 Body weight: Not reported.
	99%	0	2/30 (7%)	
	Treatment: Drinking water	50	4/27 (15%)	Significantly increased pre-neoplastic lesions: Large foci of cellular alteration were significantly increased at 5,000 mg/L (p<005). Large foci of cellular alteration were considered pre-neoplastic.
	0+	500	6/29 (21%)	
	50	5,000	11/29 ^f (38%)	Other comments: Denominators of incidences are based on surviving mice. Water consumption decreased in 0.5 and 5.0 g/l groups.
	500	Liver – Carcinoma		
	5,000 mg/L in drinking water ad libitum x 60 weeks	0	2/30 (7%)	Strengths and limitations: Three dose levels were used that spanned a 100 fold range. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.
		50	1/27 (4%)	
		500	6/29 (21%)	
	+ 2,000 mg/L NaCl	5,000	11/29 ^f (38%)	
		Liver – Adenoma or carcinoma		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		0	4/30 (13%)	
		50	4/27 (15%)	
		500	11/29 ^f (38%)	
		5,000	16/29 ^f (55%)	
DeAngelo et al. 2008	Agent:	Liver – Adenoma		Survival: No difference in survival: 34/51 - 30/42 Body weight: No reported. Significantly increased pre-neoplastic lesions: Large foci of cellular alteration were not significantly increased. Large foci of cellular alteration were considered pre-neoplastic. Other comments: Denominators of incidences are based on surviving mice. Water consumption decreased in 0.5 and 5.0 g/l groups. Strengths and limitations: Only one dose level was tested, but was given for a near life-span of a large number of animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.
Animal:	Trichloroacetic acid	0	0/25	
Mouse (Study 2)	99%	4,500	21/36 ^f (59%)	
B6C3F1	Treatment:	Liver – Carcinoma		
M 28-30 days	Drinking water	0	3/25 (12%)	
Study duration:	0+	4,500	28/36 ^f (78%)	
104 weeks	4,500 mg/L in drinking water ad libitum x 104 weeks	Liver – Adenoma or carcinoma		
	+ 1.5 g/L of neutralized acetic acid	0	3/25 (12%)	
		4,500	32/36 ^f (89%)	
DeAngelo et al. 2008	Agent:	Liver – Adenoma		
Animal:	Trichloroacetic acid	0	9/42 (21%)	
Mouse (Study 3)	99%	50	8/35 (23%)	
B6C3F1	Treatment:	Liver – Carcinoma		
M 28-30 days	Drinking water	500	19/37 ^f (51%)	
Study duration:	0	Liver – Adenoma or carcinoma		
104 weeks	50	0	23/42 (55%)	
	500 mg/L in neutralized drinking water ad libitum x 104 weeks	50	14/35 (40%)	
		500	29/37 ^f (78%)	
		Liver – Adenoma or carcinoma		
		0	27/42 (64%)	
		50	20/35 (57%)	
		500	32/37 ^f (87%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Herren-Freund <i>et al.</i> 1987 Animal: Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	Agent: Trichloroacetic acid >99% Treatment: Drinking water 0+ 5,000 mg/L in drinking water (pH 6.5-7.5) ad libitum x 61 weeks + 2,000 mg/L NaCl	Liver – Adenoma		Survival: Not reported. Body weight: Body weights were significantly decreased (p<0.001). Calculations were done by one-way analysis of variance with a Tukey's comparison. Significantly increased pre-neoplastic lesions: Not reported. Strengths and limitations: The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.
		0	2/22 (9%)	
		Liver – Carcinoma		
		5,000	8/22** (36%)	
Pereira 1996 Animal: Mouse (Study 1) B6C3F1 F 7-8 weeks Study duration: 576 days	Agent: Trichloroacetic acid (TCA) Not reported Treatment: Drinking water 0+ 330++ 1,100++ 3,300++ mg/L in filtered and deionized water, pH 6.5-7.5 ad libitum x 576 days + 20.0 mmol/L NaCl ++ Concentrations were reported as mmol/L and calculated to mg/L based on a mw of 163.3869 g/mol	Liver – Adenoma		Survival: Not reported Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of its surface (DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.
		0	2/90 (2.2%)	
		Liver – Carcinoma		
		330	4/53 (7.6%)	
		1,100	3/27 (11.1%)	
		3,300	7/18* (38.9%)	
		Liver – Adenoma		
		0	2/90 (2.2%)	
		Liver – Carcinoma		
		330	0/53	
		1,100	5/27* (18.5%)	
		3,300	5/18* (27.8%)	
Pereira 1996	Agent:	Liver – Adenoma		Survival: Not reported

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments	
Animal: Mouse (Study 2) B6C3F1 F 7-8 weeks Study duration: 360 days	Trichloroacetic acid (TCA)	0	1/40 (2.5%)	Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.	
	Not reported	330	3/40 (7.5%)		
	Treatment: Drinking water	1,100	3/19 (15.8%)		
	0+	3,300	2/20 (10%)		
	330++	Liver – Carcinoma			
	1,100++	0	0/40		
	3,300++mg/L in filtered and deionized water, pH 6.5-7.5 ad libitum x 360 days	330	0/40		
		1,100	0/19		
		3,300	5/20* (25%)		
		+ 20 mmol/L NaCl			
	++ Concentrations were reported as mmol/L and were calculated to mg/L based on a mw of 163.3869g/mol				
Bull et al. 1990	Agent:	Liver – Adenoma		Survival: All mice survived. Significantly increased pre-neoplastic lesions: A non-significant increase in hyperplasia was reported. Strengths and limitations: The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.	
Animal: Mouse B6C3F1 M 5 weeks	Trichloroacetic acid Analytical grade	0	0/2		
Study duration: 52 weeks	Treatment: Drinking water	1,000	2/5 (40%)		
	0	2,000	1/11 (9%)		
	Liver – Carcinoma				
	1,000	0	0/2		
	2,000 mg/L in drinking water (pH 6.8-7.2) ad libitum x 52 wk	1,000	2/5 (40%)		
		2,000	4/11 (36.4%)		
Von Tungeln et al. 2002	Agent:	Liver – Adenoma			Survival: One mouse died after the age of 28 days in the 20

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse (Study 1) B6C3F1 M 8 days (neonatal) Study duration: 20 months	Trichloroacetic acid	0	0/23	month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
	Purity not reported	1,000	4/23 (17%) ^s	
	Treatment: ip injection	Liver – Carcinoma		
	0+	0	0/23	
	1,000 nmol	1,000	1/23 (4%)	
	1/3 of the dose was injected at age 8 days and 2/3 at age 15 days	Liver – Adenoma or carcinoma		
+ DMSO	0	0/23		
		1,000	5/23[*] (22%)	
Von Tungeln et al. 2002	Agent:	Liver – Adenoma		Survival: One mouse died after the age of 28 days in the 20 month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
Animal: Mouse (Study 1) B6C3F1	Trichloroacetic acid	0	0/23	
F 8 days (neonatal)	Purity not reported	1,000	0/23	
Study duration: 20 months	Treatment: ip injection	Liver – Carcinoma		
	0+	0	0/23	
	1,000 nmol	1,000	0/23	
	1/3 of the dose was injected at age 8 days and 2/3 at age 15 days	Liver – Adenoma or carcinoma		
		0	0/23	
		1,000	0/23	
	+ DMSO			
Von Tungeln et al. 2002	Agent:	Liver – Adenoma		Survival: One mouse died after the age of 28 days in the 20 month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
Animal: Mouse (Study 2) B6C3F1	Trichloroacetic acid	0	0/24	
M 8 days (neonatal)	Purity not reported	2,000	4/24 (17%)	
Study duration: 12 months	Treatment: ip injection	Liver – Carcinoma		
	0+	0	0/24	
	2,000 nmol	2,000	0/24	
	3/7 of the dose was	Liver – Adenoma or carcinoma		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	injected at age 8 days and 4/7 at age 15 days. + DMSO	0 2,000	0/24 4/24 (17%)	used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
Von Tungeln <i>et al.</i> 2002	Agent: Trichloroacetic acid Animal: Mouse (Study 2) B6C3F1 F 8 days (neonatal) Study duration: 12 months	Liver – Adenoma 0 2,000 Liver – Carcinoma 0 2,000 Liver – Adenoma or carcinoma 0 2,000	0/24 0/24 0/24 0/24 0/24 0/24	Survival: One mouse died after the age of 28 days in the 20 month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
NTP 2015	Agent: Bromodichloroacetic acid Animal: Mouse B6C3F1/N M 5-6 wk Study duration: 105 wk	Liver – Adenoma^a 0 250 500 1,000 Liver – Carcinoma^a 0 250 500 1,000 Trend p-value: <0.001 Liver – Adenoma or carcinoma^a	39/50 (87%) 41/50 (90%) 42/49 (91%) 40/51 (91%) 12/50 (29%) 22/50* (50%) 27/49*** [†] (66%) 39/51*** [†] (87%)	Survival: Significant decrease in survival. Body weight: Significant decreased in body weight after 57 weeks at 1,000 mg/l and after 73 weeks at 500 mg/l. Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		0	42/50 (91%)	
		250	47/50 (98%)	
		500	46/49 (97%)	
		1,000	48/51 (98%)	
		Liver – Hepatoblastoma^a		
		0	4/50 (10%)	
		250	24/50*** ^u (54%)	
		500	40/49*** ^u (87%)	
		1,000	34/51*** ^u (78%)	
		Trend p-value: <0.001		
		Liver – Adenoma, carcinoma, or hepatoblastoma^a		
		0	42/50 (91%)	
		250	50/50* (100%)	
		500	48/49 (98%)	
		1,000	49/51* (99%)	
		Trend p-value: =0.036		
		Liver – Hemangiosarcoma^a		
		0	1/50 (3%)	
		250	4/50 (10%)	
		500	2/49 (6%)	
		1,000	4/51 (12%)	
NTP 2015	Agent:	Liver – Adenoma^a		Survival: No effect on survival.
Animal:	Bromodichloroacetic	0	33/49 ^v (75%)	30/50 - 33/50, 29/50, 27/50
Mouse B6C3F1/N	acid	250	42/50* ^v (91%)	Body weight: Significant decrease in body weight after 73 weeks at 1,000 mg/l and after 89 weeks at 250 mg/L.
F 5-6 wk	97%	500	42/49* ^v (93%)	Strengths and limitations: Well reported and designed
Study duration:	Treatment:			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
105 wk	Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	1,000	44/50**v (93%)	study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
		Trend p-value: =0.009		
		Liver – Carcinoma^a		
		0	9/49 (21%)	
		250	17/50 ^w (38%)	
		500	22/49***x (50%)	
		1,000	26/50***x (59%)	
		Trend p-value: <0.001		
		Liver – Adenoma or carcinoma^a		
		0	36/49 (81.1%)	
		250	44/50* (93.7%)	
		500	43/49* (94.7%)	
		1,000	46/50* (95.5%)	
		Trend p-value: =0.013		
		Liver – Hepatoblastoma^a		
		0	0/49	
		250	1/50 (2%)	
		500	4/49 (9%)	
		1,000	6/50* ^y (14%)	
		Trend p-value: =0.003		
Liver – Hemangiosarcoma^a				
0	2/49 (5%)			
250	4/50 (9%)			
500	4/49 (9%)			
1,000	8/50* (19%)			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Trend p-value: =0.026				
NTP 1992 Animal: Rat F344/N M 6-7 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	Liver – Adenoma		Survival: After 104 weeks, the survival of the 30 mg/kg group was lower than controls and there was a significant trend: 27/53*(trend=0.011) - 21/53, 16/53*(=0.015) Body weight: Body weights were similar to controls during the 6 and 15 month interim evaluations as well as the 2 year study. Other comments: No neoplasms were found at the 6 month evaluation and no treatment related neoplasms were found at the 15 month interim evaluation or at the end of the study. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
		0	1/53 (2%)	
		15	0/53	
	Treatment: Gavage	Liver – Carcinoma		
		0	0/53	
		15	1/53 (2%)	
30	1/53 (2%)			
NTP 1992 Animal: Rat F344/N	Agent: Monochloroacetic acid 99%	Liver – Adenoma		Survival: After 104 weeks, the survival of the 30 mg/kg group was lower than controls and there was a significant trend: 37/53*(Trend=0.043) - 19/53***(=0.001),
		0	1/53 (2%)	
		15	0/53	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
F 6-7 weeks Study duration: 104 weeks	Treatment: Gavage 0 15 30 mg/kg bw 5 doses/week x 104 weeks	30	0/53	26/53*(=0.046) Body weight: Body weights were similar to controls during the 6 and 15 month interim evaluations as well as the 2 year study. Other comments: No neoplasms were found at the 6 month evaluation and no treatment related neoplasms were found at the 15 month interim evaluation or at the end of the study. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships. Rats were more sensitive to non-neoplastic effects, causing death and cardiomyopathy during the short term studies, compared to mice and so their dose levels were much lower.
NTP 1992 Animal: Mouse B6C3F1 M 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99% Treatment: Gavage 0 50 100 mg/kg in deionized water 5 doses/week x 104 weeks	Liver – Adenoma^a 0 50 100 Trend p-value: =0.059N Liver – Carcinoma^a 0 50 100 Trend p-value: =0.440N Liver – Adenoma or carcinoma^a 0 50 100	6/60 (12.7%) 6/59 (14.8%) 1/59 (4.2%) 6/60 (11.7%) 2/59 (4.7%) 5/59 (19.9%) 12/60 (23.6%) 8/59 (19.1%) 6/59 (23.3%)	Survival: The 100 mg/kg group was lower than controls and there was a significant trend: 46/60***(trend <0.001) - 39/60, 21/60***(<0.001) Body weight: Body weights were similar to controls. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Trend p-value: =0.082N				
NTP 1992 Animal: Mouse B6C3F1 F 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	Liver – Adenoma		Survival: Survival of the exposed groups were similar to controls: 42/60 - 40/60, 44/60 Body weight: Body weights of the low dose group were similar to controls, but after a year the high dose group had significantly lower body weight. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.
		0	1/60 (2%)	
		50	1/60 (2%)	
	Treatment: Gavage 0 50 100 mg/kg in deionized water 5 doses/week x 104 weeks	Liver – Carcinoma		
		100	2/60 (3%)	
		0	0/60	
		50	1/59 (2%)	
		100	0/60	
		Liver – Adenoma		
		0	1/23 (4%)	
DeAngelo et al. 1997 Animal: Rat F344/N M 28-30 days Study duration: 104 weeks	Agent: Monochloroacetic acid >99%	Liver – Adenoma		Survival: No significant difference in survival: 23/29 - 24/32, 23/32, 25/29 Body weight: The 2.0 g/l MCA was decreased to 1.5 g/l after 8 weeks and to 1 g/l after 24 weeks because of significant differences in body weight gain. The 0.5 g/l group had 13% lower body weight than untreated controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were reported, but were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Amount of water consumed was similar among groups (76.9 ml/kg/d - 70.5, ml/kg/d, 55.6 ml/kg/d, 55.5 ml/kg/d). Strengths and limitations: A well conducted study on almost all aspects, but only involved male rats.
		50	2/25 (8%)	
		500	0/23	
	Treatment: Drinking water 0+ 50 500 2,000++ mg/L in drinking water ad libitum x 104 weeks + 31-32 mM NaCl (~isomolar to 5,000 mg/L TCA) ++ 2,000 mg/L x 8 wk, then 1,500 mg/L to 24 weeks, then 1,000 mg/L; averaging 1,100 mg/L throughout the study	Liver – Carcinoma		
		2,000	1/25 (4%)	
		0	0/23	
		50	0/25	
		500	0/23	
		2,000	0/25	
		Liver – Adenoma or carcinoma		
0	1/23 (4%)			
50	2/25 (8%)			
500	0/23			
2,000	1/25 (4%)			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
DeAngelo et al. 1996 Animal: Rat (Study 1) F344 M 28-30 days (59-79g bw) Study duration: 100 weeks	Agent: Dichloroacetic acid >99% Treatment: Drinking water 0+ 50 500 5,000++ mg/L in drinking water ad libitum x 100 weeks + 2 g/L NaCl (~isomolar to 5,000 mg/l DCA) ++ 2,500 mg/L at 9 weeks then 2,000 mg/L after 23 wks then 1,000 mg/L after 52 wks and stopped at 60 weeks due to peripheral neuropathy and wasn't included in the study results.	Liver – Adenoma		Survival: There were no significant differences in survival in the 0.05 or 0.5 g/l groups. The 5 g/l group rats had irreversible peripheral neuropathy and were sacrificed at 60 weeks and were excluded from the study analysis. Body weight: Body weights did not differ after 100 weeks of treatment. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: The exact value for N at the beginning of the study is not confirmed as the paper didn't clearly report them. Water consumption didn't differ among groups. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. All non-hepatic neoplasms were considered spontaneous and not treatment related and included, testicular cancer (97% - 100%, 100%) and leukemia (24% - 20%, 43%). Strengths and limitations: Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only two exposed dose levels were effectively reported and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.
		0	1/23 (4.4%)	
		50	0/26	
		500	5/29 (17.2%)	
		Trend p-value: <0.05		
		Liver – Carcinoma		
		0	0/23	
		50	0/26	
		500	3/29 (10.3%)	
		Trend p-value: <0.05		
Liver – Adenoma or carcinoma				
0	1/23 (4.4%)			
50	0/21			
500	6/23* (24.1%)			
Trend p-value: <0.01				
DeAngelo et al. 1996 Animal:	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: There were no significant differences in survival in the 0.05 or 0.5 g/l groups. The 5 g/l group rats had
		0	0/33	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Rat (Study 2) F344 M 28-30 days (59-79g bw) Study duration: 103 weeks	>99%	2,500	3/28 (10.7%)	irreversible peripheral neuropathy and were sacrificed at 60 weeks and were excluded from the study analysis. Body weight: Body weights in the exposed group were significantly less than (73%) those in the untreated control group after 103 weeks of treatment. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: The exact value for N at the beginning of the study is not confirmed as the paper didn't clearly report them. Water consumption didn't differ among groups. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. All non-hepatic neoplasms were considered spontaneous and not treatment related and included, testicular cancer (97% - 100%) and leukemia (9% - 11%). Strengths and limitations: Animals were certified pathogen free, though were not reported to be continuously monitored for disease. Only one exposed dose level was tested and only males were tested. The duration was near life-span and historical controls were considered during data analysis. However, only liver lesion incidences were reported.
	Treatment: Drinking water	Liver – Carcinoma		
	0+	0	1/33 (3%)	
	2,500++ mg/L in deionized water ad libitum x 103 weeks	2,500	6/28* (21.4%)	
	Liver – Adenoma or carcinoma			
	0	1/28 (3%)		
	+ NaCl in the first experiment had no effect on water consumption or tumor incidence, so was not included here	2,500	8/27** (28.6%)	
	++ 1,500 mg/L at 8 weeks then 1,000 mg/L after 26 weeks due to mild transient neurotoxicity			
Richmond et al. 1995	Agent:	Liver – Adenoma		Survival: Survival wasn't clearly reported, but based on 7 animals sacrificed at 15, 30, 45, and for all but the high dose group, 60 week time points survival was estimated from the
Animal:	Dichloroacetic acid	0	0/7	
Rat (Study 1) F344	Not reported	50	0/7	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
M 28 days Study duration: 60 weeks	Treatment: Drinking water	500	0/7	60 animals per group at the beginning of the study to be: 51/60 - 54/60, 57/60, 51/60 Body weight: Not reported. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 2,400 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was less than near-lifespan. Survival and body weights were not clearly reported and only the liver was histologically evaluated.
	0+	2,400	7/27 (26%)	
	50	Liver – Carcinoma		
	500	0	0/7	
	2,400 mg/L in drinking water(pH 7.0) x 60++ or	50	0/7	
	104 weeks	500	0/7	
	+ 2 g/L NaCl	2,400	1/27 (4%)	
	++ High dose stopped at 60 weeks due to tumors and hind limb paralysis			
Richmond et al. 1995 Animal: Rat (Study 2) F344 M 28 days Study duration: 104 weeks	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: Survival wasn't clearly reported, but based on 7 animals sacrificed at 15, 30, 45, and for all but the high dose group, 60 week time points survival was estimated from the 60 animals per group at the beginning of the study to be: 51/60 - 54/60, 57/60, 51/60 Body weight: Not reported. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was
	Not reported	0	1/23 (4%)	
	Treatment: Drinking water	50	0/26	
	0+	500	6/29 (21%)	
	50	Liver – Carcinoma		
	500	0	0/23	
2,400 mg/L in drinking	50	0/26		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	water (pH 7.0) x 60++ or 104 weeks + 2,000 mg/L NaCl ++ high dose stopped at 60 weeks due to tumors and hind limb paralysis	500	3/29 (10%)	reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemical and animal husbandry conditions were not characterized and only a low to moderate number of males rats were tested. However, they were tested at three dose levels spanning a 50 fold range and the exposure duration was near-lifespan for all but the high dose group. Survival and body weights were not clearly reported and only the liver was histologically evaluated.
DeAngelo et al. 1991	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: No difference.
Animal: Mouse (Study 1) B6C3F1 M 28 days	>99%	0	0/28	Body weight: The high dose group had significantly lower body weight than controls (17% lower, p<0.001). Body weights of the medium and low doses didn't differ from controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 5,000 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Only the high dose group was quantitatively reported (as a percentage - fractional incidence was back calculated from percent), the other groups were extrapolated from a graph. Nine animals from each group
Study duration: 60 weeks (high dose), 75 weeks (low and medium dose levels)	Treatment: Drinking water	50	2/29 (7%)	
	0+	500	1/27 (4%)	
	50	5,000	24/30*** (80%)	
	500	Liver – Carcinoma		
	5,000++ mg/l in distilled water (pH 6.8-7.2) ad libitum x 75 weeks	0	2/28 (7%)	
		50	6/29 (21%)	
		500	2/27 (7%)	
	+ 2,000 mg/l NaCl	5,000	25/30*** (83%)	
	++ Exposure duration was reduced to 60 weeks	Liver – Adenoma or carcinoma		
		0	2/28 (7%)	
		50	7/29 (24%)	
		500	3/27 (11%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		5,000	27/30*** (90%)	were scarified at 60 weeks, with the remainder sacrificed at 75 weeks. All high dose animals were sacrificed at 60 weeks. Strengths and limitations: Three dose levels, which spanned a 100 fold range were tested in only males for up to 75 weeks. Only a few select organs were necropsied.
DeAngelo et al. 1991 Animal: Mouse (Study 2) B6C3F1 M 28 days Study duration: 60 weeks	Agent: Dichloroacetic acid >99% Treatment: Drinking water 0+ 3,500 mg/l in distilled water (pH 6.8-7.2) ad libitum x 60 weeks + 1,500 mg/l acetic acid	Liver – Adenoma		Survival: No difference. Body weight: The high dose group had significantly lower body weight than controls (13% lower, p<0.001). Body weights of the medium and low doses didn't differ from controls. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were significantly increased at 3,500 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Incidences were only reported as a percentage - fractional incidence was back calculated from percent. All animals were sacrificed at 60 weeks. Strengths and limitations: One dose level was tested in a small number of males for 60 weeks. Only a few select organs were necropsied.
		0	0/10	
		3,500	12/12*** (100%)	
		Liver – Carcinoma		
		0	0/10	
		3,500	8/12*** (67%)	
Liver – Adenoma or carcinoma				
0	0/10			
3,500	12/12*** (100%)			
DeAngelo et al. 1999 Animal: Mouse B6C3F1 M 28-30 days (18-21 g bw) Study duration: 90-100 weeks	Agent: Dichloroacetic acid >99% Treatment: Drinking water 0+ 50+ 500 1,000	Liver – Adenoma		Survival: Significant decrease in survival at the two highest doses and a significant trend (<0.05): 50/53 - 33/35, 24/25, 32/41, 14/25*, 8/16* Body weight: The high dose group (3.5 g/l) had significantly lower body weight after 52 weeks and continued throughout the study, while the 2 g/l group was significantly lower after 100 weeks. Significantly increased pre-neoplastic lesions: Hyperplastic nodule multiplicity was significantly increased at all exposed
		0	5/53 (10%)	
		500	5/25 (20%)	
		1,000	21/41* (51.4%)	
		2,000	11/25* (42.9%)	
		3,500	7/16* (45%)	
Liver – Carcinoma				

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	2,000 3,500 mg/L in drinking water (pH 6.9-7.1) ad libitum x 90 or 100 weeks + started 1 month after the other groups (10 mice from each group were sacrificed at 26, 52, and 78 weeks - data not reported here)	0 500 1,000 2,000 3,500	14/53 (26%) 12/25 (48%) 29/41* (71%) 24/25* (95%) 16/16* (100%)	levels 500 to 3,500 mg/L. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Water consumption was lower in the high dose group. The percent incidence was reported and fractional incidence was extrapolated from that and the original number of animals per group, however these calculations did not exactly match the percent incidence. Strengths and limitations: Only male mice, initially confirmed pathogen free, were used with differing numbers per group (as low as 16 in the high dose group). Continual disease surveillance was not reported. Multiple dose levels ranging 100 fold, were tested for a near life-span duration. However, only 5 high dose group mice received histological evaluations of all major organs, with histological evaluations of only gross lesions in the other groups.
Herren-Freund <i>et al.</i> (1987) Animal: Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	Agent: Dichloroacetic acid >99% Treatment: Drinking water 0+ 5,000 mg/l in drinking water (pH 6.5-7.5) ad libitum x 61 weeks + 2,000 mg/l of NaCl	Liver – Adenoma 0 5,000 Liver – Carcinoma 0 5,000	2/22 (9%) 25/26** (96%) 0/22 21/26** (81%)	Survival: Not reported. Body weight: Body weights were significantly decreased ($p < 0.001$). Calculations were done by one-way analysis of variance with a Tukey's comparison. Significantly increased pre-neoplastic lesions: Not reported. Strengths and limitations: The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.
Wood <i>et al.</i> (2015)	Agent:	Liver – Adenoma		Survival: Survival was similar in all groups.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse B6C3F1 M 28 days Study duration: 94 weeks	Dichloroacetic acid	0	5/27 (19%)	Body weight: Body weight of the high dose group, after DCA exposure had stopped, was decreased by 12% compared to controls. Other comments: The original number of mice used were reported as those given a specific dose of DCA and were not differentiated by those given phenobarbital and those that weren't. The incidence denominator was differentiated by co-administration of phenobarbital and only represents those animals given only dichloroacetic acid. Water consumption was decreased at the medium and high dose groups, which limited the daily intake to (target dose mg/kg/d: 0 - 168, 315, 429; Measured dose mg/kg/d: 0 - 136, 232, 297), so the high dose was not nearly as high as expected. Strengths and limitations: Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Three dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.
	Not reported	1,000	13/27 (48%)	
	Treatment: Drinking water	2,000	11/27 (41%)	
	0	3,500	15/26* (58%)	
	1,000	Trend p-value: <0.05		
	2,000	Liver – Carcinoma		
	3,500 mg/L deionized water (pH 6.8-7.1) ad libitum x 10 weeks	0	8/27 (30%)	
		1,000	8/27 (30%)	
		2,000	6/27 (22%)	
		3,500	19/26* (73%)	
		Trend p-value: <0.01		
		Liver – Hepatoblastoma		
		0	0/27	
		1,000	1/27 (4%)	
		2,000	0/27	
	3,500	0/26		
	Liver – Adenoma, carcinoma, and hepatoblastoma			
	0	12/27 (44%)		
	1,000	15/27 (56%)		
	2,000	14/27 (52%)		
	3,500	24/26** (92%)		
	Trend p-value: <0.01			
Wood et al. (2015)	Agent:	Liver – Adenoma		Survival: Survival was similar in all groups. Body weight: Body weight of the high dose group, after DCA exposure had stopped, was decreased by 12%
Animal: Mouse B6C3F1	Dichloroacetic acid	0	0/27	
	Not reported	1,000	9/26** (35%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
F 28 days Study duration: 94 weeks	Treatment: Drinking water 0 1,000 2,000 mg/L in deionized water (pH 6.8-7.1) ad libitum x 10 weeks	2,000	6/28 (21%)	compared to controls.
		Liver – Carcinoma		Other comments: The original number of mice used were reported as those given a specific dose of DCA and were not differentiated by those given phenobarbital and those that weren't. The incidence denominator was differentiated by co-administration of phenobarbital and only represents those animals given only dichloroacetic acid. Water consumption was decreased at the medium and high dose groups, which limited the daily intake to (target dose mg/kg/d: 0 - 168, 315, 429; Measured dose mg/kg/d: 0 - 136, 232, 297), so the high dose was not nearly as high as expected. Strengths and limitations: Chemical stability was reported and target concentrations were verified, but purity was not reported. Disease surveillance was not reported. Two dose levels, previously shown to be carcinogenic were used. The exposure duration was short, but the observation duration was near life-span. Only livers were examined during necropsy.
		0	0/27	
		1,000	2/26 (8%)	
		2,000	3/28 (11%)	
		Liver – Hepatoblastoma		
		0	0/27	
		1,000	0/26	
		2,000	0/28	
		Liver – Adenoma, carcinoma, and hepatoblastoma		
		0	0/27	
		1,000	10/26** (38%)	
		2,000	9/28** (32%)	
Trend p-value: <0.01				
Pereira 1996 Animal: Mouse (Study 1) B6C3F1 F 7-8 weeks Study duration: 576 days	Agent: Dichloroacetic acid (DCA) Not reported Treatment: Drinking water 0+ 260++ 860++ 2,600+++ 2,600++ mg/L in drinking water ad libitum x 360 days	Liver – Adenoma		Survival: Not reported Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface
		0	2/90 (2.2%)	
		260	3/50 (6%)	
		860	7/28* (25%)	
		2,600	3/34 (8.8%)	
		intermittent		
		2,600	16/19* (84.2%)	
		Liver – Carcinoma		
		0	2/90 (2.2%)	
		260	0/50	
860	1/28 (3.6%)			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	+ 20 mmol/L NaCl	2,600 intermittent	1/34 (2.9%)	(DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. The study duration was near life-span.
	++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol	2,600	5/19* (26.3%)	
	+++ intermittent cycles of 24 days on, 48 days off			
Pereira 1996	Agent:	Liver – Adenoma		Survival: Not reported
Animal:	Dichloroacetic acid (DCA)	0	1/40 (2.5%)	Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond.
Mouse (Study 2)	Not reported	260	0/40	Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008).
B6C3F1	Treatment:	860	3/20 (15%)	
F 7-8 weeks	Drinking water	2,600 intermittent	0/15	
Study duration:	0+	2,600	7/20* (35%)	
360 days	260++	Liver – Carcinoma		
	860++	0	0/40	
	2,600+++	260	0/40	
	2,600++ mg/L in drinkign water ad libitum x 360 days	860	0/20	
	+ 20 mmol/L NaCl	2,600 intermittent	0/15	Strengths and limitations: The chemicals were not

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol	2,600	1/20 (5%)	characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females was tested, with only their livers examined histologically. Study duration was less than life-span.
	+++ intermittent cycles of 24 days on, 48 days off			
Bull et al. 1990	Agent:	Liver – Adenoma		Survival: All mice survived. Significantly increased pre-neoplastic lesions: A non-significant increase in hyperplasia was reported. Strengths and limitations: The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.
Animal:	Dichloroacetic acid	0	0/2	
Mouse B6C3F1	Analytical grade	1,000	0/1	
M 5 weeks	Treatment:	Liver – Carcinoma		
Study duration:	Drinking water	2,000	2/10 (20%)	
52 weeks	0	0	0	
	1,000	0	0/2	
	2,000 mg/L in drinking water (pH 6.8-7.2) ad libitum x 52 weeks	1,000	0/1	
		2,000	5/10 (50%)	
		Liver – Adenoma or carcinoma		
		0	3/20 (15%)	
Daniel et al. (1992)	Agent:	Liver – Adenoma		Survival: Survival was not significantly different: 13/10, 10/10 - 16/18, 8/10 Body weight: No significant differences in body weight. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from
Animal:	Dichloroacetic acid	0	1/20 (5%)	
Mouse B6C3F1	>95%	500	10/24** (42%)	
M 28 days	Treatment:	Liver – Carcinoma		
Study duration:	Drinking water	0	2/20 (10%)	
104 weeks	0	0	0	
	0	500	15/24** (63%)	
	500	500	15/24** (63%)	
	500 mg/l in distilled water (pH 6.8-7.2) ad	Liver – Adenoma or carcinoma		
		0	3/20 (15%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	libitum x 104 weeks	500	18/24** (75%)	adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: All tumors occurred at necropsy and did not cause premature mortality. Both groups of controls and exposed mice were combined into one group each. Fractional incidence was based on surviving animals as the denominator and percent incidence was also reported. Strengths and limitations: Continual disease surveillance was not reported. Only males were tested and only at one dose level. Histological evaluations from all major organs only occurred in five mice per group, with histological evaluations in the other mice only occurring on tissues with gross lesions.
NTP 2007a	Agent: Dibromoacetic acid	Liver – Adenoma^a		Survival: Survival was similar in all groups. 31/50 - 38/50, 34/50, 31/50
Animal: Mouse B6C3F1	>99%	0	18/49 (42%)	Body weight: Body weights were greater in the 50 and 500 mg/l groups compared to the untreated controls after 85 weeks.
M 6 weeks	Treatment: Drinking water	50	37/50*** ^b (78%)	Significantly increased pre-neoplastic lesions: Spleen hematopoiesis 18/49 - 20/50, 28/50, 38/50
Study duration: 106 weeks	0	500	37/50*** ^b (80%)	Other comments: Water consumption was similar to controls.
	50	1,000	42/50*** ^b (89%)	Onset was reported in days.
	1,000 mg/L of drinking water ad libitum x 106 weeks	Trend p-value: <0.001		Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
	Average daily dose: 0 - 4, 45, 87 mg/kg	Liver – Carcinoma^a		
		0	14/49 (31%)	
		50	9/50 (19%)	
		500	19/50 (41%)	
		1,000	26/50* ^c (55%)	
		Trend p-value: <0.001		
		Liver – Adenoma or carcinoma^a		
		0	28/49 (61%)	
		50	41/50*** ^d (86%)	
		500	42/50*** ^d (88%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		1,000	47/50*** ^d (96%)	
		Trend p-value: <0.001		
		Liver – Hepatoblastoma^a		
		0	0/49	
		50	4/50 (9%)	
		500	6/50* (13%)	
		1,000	18/50*** ^e (39%)	
		Trend p-value: <0.001		
		Liver – Adenoma, carcinoma or hepatoblastoma^a		
		0	28/49 (61%)	
		50	41/50** (86%)	
		500	43/50*** (90%)	
		1,000	48/50*** (97%)	
		Trend p-value: <0.001		
NTP 2007a	Agent:	Liver – Adenoma^a		Survival: Survival was similar in all groups.
Animal:	Dibromoacetic acid	0	19/49 (41%)	38/50 - 35/50, 32/50, 32/50
Mouse B6C3F1	>99%	50	26/50 (57%)	Body weight: Body weights were similar to the untreated controls.
F 6 weeks	Treatment:	500	32/50*** ^f (70%)	Other comments: Water consumption was similar to controls.
Study duration:	Drinking water	1,000	35/49*** ^f (76%)	Onset was reported in days.
106 weeks	0	Trend p-value: <0.001		Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
	50	Liver – Carcinoma^a		
	500	0	3/49 (7%)	
	1,000 mg/L of drinking water ad libitum x 106 weeks	50	3/50 (7%)	
	Average daily dose: 0 - 4, 35, 65 mg/kg	500	12/50*** ^g (27%)	
		1,000	8/49 (18%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
			Trend p-value: =0.019	
			Liver – Adenoma or carcinoma^a	
		0	22/49 (48%)	
		50	28/50 (61%)	
		500	37/50*** ^h (80%)	
		1,000	37/49*** ^h (80%)	
			Trend p-value: <0.001	
			Liver – Hepatoblastoma	
		0	1/49 (2%)	
		50	0/50	
		500	1/50 (2%)	
		1,000	0/49	
NTP 2009	Agent:	Liver – Adenoma^a		Survival: No significant difference:
Animal:	Bromochloroacetic acid	0	2/50 ⁱ (4.6%)	31/50 - 26/50, 25/50, 29/50
Rat F344/N	96%	250	0/50	Body weight: 1,000 mg/l group was 10% less than controls after 69 weeks.
M 6-7 weeks	Treatment:	500	3/50 ⁱ (7.5%)	Strengths and limitations: A very high quality study, with no major concerns.
Study duration:	Drinking water	1,000	4/50 ⁱ (9.5%)	
105 weeks	0			
	250			
	500			
	1,000 mg/L of drinking water ad libitum x 105 weeks			
NTP 2009	Agent:	Liver – Adenoma^a		Survival: No significant difference:
Animal:	Bromochloroacetic acid	0	0/50	34/50 - 31/50, 37/50, 35/50
Rat F344/N	96%	250	0/50	Body weight: 1,000 mg/l group was <10% of controls. after 85 weeks.
F 6-7 weeks	Treatment:	500	0/50	Significantly increased pre-neoplastic lesions: Lung alveolar epithelium hyperplasia occurred at increased incidences.
Study duration:	Drinking water	1,000	3/50 ⁱ (6.6%)	
105 weeks	0			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	250 500 1,000 mg/L of drinking water ad libitum x 105 weeks		Trend p-value: =0.012	Strengths and limitations: A very high quality study, with no major concerns.
NTP 2009	Agent:	Liver – Adenoma^a		Survival: 38/50 - 35/50, 30/50, 21/50
Animal:	Bromochloroacetic acid	0	27/50 (58.7%)	Body weight: 1,000 mg/l group was 12% lower than controls after 97 weeks.
Mouse B6C3F1	96%	250	40/50 ^{**k} (83.6%)	Strengths and limitations: A very high quality study, with no major concerns.
M 6-7 weeks	Treatment:	500	40/50 ^{**k} (83.7%)	
Study duration:	Drinking water	1,000	31/50 (67.4%)	
105 weeks	0	Liver – Carcinoma^a		
	250	0	19/50 (39.6%)	
	500	250	25/50 ^l (52.5%)	
	1,000 mg/L of drinking water ad libitum x 105 weeks	500	36/50 ^{**^l} (76.9%)	
		1,000	45/50 ^{**^l} (92.7%)	
		Trend p-value: <0.001		
		Liver – Adenoma or carcinoma^a		
		0	34/50 (70.6%)	
		250	44/50 ^{*m} (89.7%)	
		500	49/50 ^{**^m} (99.9%)	
		1,000	49/50 ^{**^m} (98.6%)	
		Trend p-value: <0.001		
		Liver – Hepatoblastoma^a		
		0	4/50 (8.8%)	
		250	11/50 [*] (23.8%)	
		500	28/50 ^{**ⁿ} (61.3%)	
		1,000	34/50 ^{**ⁿ} (73.7%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
			Trend p-value: <0.001	
			Liver – Adenoma, carcinoma, or hepatoblastoma^a	
		0	21/50 (43.8%)	
		250	32/50* (66.3%)	
		500	43/50*** (90.7%)	
		1,000	49/50*** (98%)	
			Trend p-value: <0.001	
NTP 2009	Agent:	Liver – Adenoma^a		Survival: 36/50 - 42/50, 32/50, 40/50
Animal:	Bromochloroacetic acid	0	27/50 (59.4%)	Body weight: No significant difference.
Mouse B6C3F1	96%	250	48/50*** ^o (96%)	Strengths and limitations: A very high quality study, with no major concerns.
F 6-7 weeks	Treatment:	500	44/50*** ^o (90.9%)	
Study duration:	Drinking water	1,000	46/50*** ^o (95.2%)	
105 weeks	0		Trend p-value: <0.001	
	250	Liver – Carcinoma^a		
	500	0	14/50 ^p (31.1%)	
	1,000 mg/L of drinking water ad libitum x 105 weeks	250	23/50 ^p (48.3%)	
		500	26/50* ^p (56.1%)	
		1,000	20/50 ^p (42.3%)	
			Trend p-value: <0.001	
		Liver – Adenoma or carcinoma^a		
		0	31/50 ^q (67.6%)	
		250	49/50*** ^q (98%)	
		500	46/50*** ^q (94.6%)	
		1,000	46/50*** ^q (95.2%)	
			Trend p-value: <0.001	
DeAngelo et al. (1997)	Agent:	Liver – Adenoma		Survival: No significant difference in survival:

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Rat F344/N M 28-30 days Study duration: 104 weeks	Trichloroacetic acid	0	1/23 (4%)	23/29 - 24/32, 19/32, 22/29
	>99%	50	1/24 (4%)	Body weight: Body weights were similar among the 0.05 and 0.5 g/l groups, but decreased more than 10% compared to controls in the 5 g/l group. Significantly increased pre-neoplastic lesions: Hyperplastic nodules were reported, but were not significantly increased. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Other comments: Amount of water consumed was similar among groups (76.9 ml/kg/d - 71.2, ml/kg/d, 70.6 ml/kg/d, 74,2 ml/kg/d). Strengths and limitations: A well conducted study on almost all aspects, but only involved male rats.
	Treatment: Drinking water	500	3/20 (15%)	
	0+	5,000	1/22 (5%)	
	Liver – Carcinoma			
	2,500++ mg/L in drinking water (pH 6.9-7.1) ad libitum x 104 weeks	0	0/23	
		50	0/24	
	+ 31-32 mM NaCl	500	0/20	
	(~isomolar to 5,000 mg/l of TCA)	5,000	1/22 (5%)	
	Liver – Adenoma or carcinoma			
		0	1/23 (4%)	
	++ 1,500 mg/L x 8 weeks then 1,000 mg/L after 24 weeks, because of significant differences in body weight gain	50	1/24 (4%)	
	500	3/20 (15%)		
	5,000	1/22 (5%)		
DeAngelo 2008 Animal: Mouse (Study 1) B6C3F1 M 28-30 days Study duration: 60 weeks	Agent: Trichloroacetic acid	Liver – Adenoma		Survival: No difference in survival: 30/30 - 27/30, 29/30, 29/30 Body weight: Not reported. Significantly increased pre-neoplastic lesions: Large foci of cellular alteration were significantly increased at 5,000 mg/L (p<005). Large foci of cellular alteration were considered pre-neoplastic. Other comments: Denominators of incidences are based on surviving mice. Water consumption decreased in 0.5 and 5.0 g/l groups. Strengths and limitations: Three dose levels were used that spanned a 100 fold range. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.
	99%	0	2/30 (7%)	
	Treatment: Drinking water	50	4/27 (15%)	
	0+	500	6/29 (21%)	
	50	5,000	11/29 ^f (38%)	
	Liver – Carcinoma			
	500	0	2/30 (7%)	
	5,000 mg/L in drinking water ad libitum x 60 weeks	50	1/27 (4%)	
		500	6/29 (21%)	
	+ 2,000 mg/L NaCl	5,000	11/29 ^f (38%)	
	Liver – Adenoma or carcinoma			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments	
		0	4/30 (13%)		
		50	4/27 (15%)		
		500	11/29 ^f (38%)		
		5,000	16/29 ^f (55%)		
DeAngelo 2008	Agent:	Liver – Adenoma		<p>Survival: No difference in survival: 34/51 - 30/42</p> <p>Body weight: No reported.</p> <p>Significantly increased pre-neoplastic lesions: Large foci of cellular alteration were not significantly increased. Large foci of cellular alteration were considered pre-neoplastic.</p> <p>Other comments: Denominators of incidences are based on surviving mice. Water consumption decreased in 0.5 and 5.0 g/l groups.</p> <p>Strengths and limitations: Only one dose level was tested, but was given for a near life-span of a large number of animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.</p>	
Animal:	Trichloroacetic acid	0	0/25		
Mouse (Study 2)	99%	4,500	21/36 ^f (59%)		
B6C3F1	Treatment:	Liver – Carcinoma			
M 28-30 days	Drinking water	0	3/25 (12%)		
Study duration:	0+	4,500	28/36 ^f (78%)		
104 weeks	4,500 mg/L in drinking water ad libitum x 104 weeks	Liver – Adenoma or carcinoma			
	+ 1.5 g/L of neutralized acetic acid	0	3/25 (12%)		
		4,500	32/36 ^f (89%)		
DeAngelo 2008	Agent:	Liver – Adenoma			<p>Survival: No difference in survival: 34/51 - 29/53, 27/51</p> <p>Body weight: No reported.</p> <p>Significantly increased pre-neoplastic lesions: Large foci of cellular alteration were significantly increased at 5,00 mg/L (p<005). Large foci of cellular alteration were considered pre-neoplastic.</p> <p>Other comments: Denominators of incidences are based on surviving mice. No difference in water consumption occurred.</p> <p>Strengths and limitations: Only two dose level were tested, but were low compared to other studies and were given for near a life-span of time to a large number of animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.</p>
Animal:	Trichloroacetic acid	0	9/42 (21%)		
Mouse (Study 3)	99%	50	8/35 (23%)		
B6C3F1	Treatment:	Liver – Carcinoma			
M 28-30 days	Drinking water	500	19/37 ^f (51%)		
Study duration:	0	Liver – Adenoma or carcinoma			
104 weeks	50	0	23/42 (55%)		
	500 mg/L in neutralized drinking water ad libitum x 104 weeks	50	14/35 (40%)		
		500	29/37 ^f (78%)		
		Liver – Adenoma or carcinoma			
		0	27/42 (64%)		
		50	20/35 (57%)		
		500	32/37 ^f (87%)		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Herren-Freund <i>et al.</i> (1987) Animal: Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	Agent: Trichloroacetic acid >99% Treatment: Drinking water 0+ 5,000 mg/L in drinking water (pH 6.5-7.5) ad libitum x 61 weeks + 2,000 mg/L NaCl	Liver – Adenoma		Survival: Not reported. Body weight: Body weights were significantly decreased (p<0.001). Calculations were done by one-way analysis of variance with a Tukey's comparison. Significantly increased pre-neoplastic lesions: Not reported. Strengths and limitations: The durations was less than near life-span. Only males were tested at a single dose level and only livers were histologically evaluated.
		0	2/22 (9%)	
		Liver – Carcinoma		
		5,000	8/22** (36%)	
Pereira 1996 Animal: Mouse (Study 1) B6C3F1 F 7-8 weeks Study duration: 576 days	Agent: Trichloroacetic acid (TCA) Not reported Treatment: Drinking water 0+ 330++ 1,100++ 3,300++ mg/L in filtered and deionized water, pH 6.5-7.5 ad libitum x 576 days + 20.0 mmol/L NaCl ++ Concentrations were reported as mmol/L and calculated to mg/L based on a mw of 163.3869 g/mol	Liver – Adenoma		Survival: Not reported Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.
		0	2/90 (2.2%)	
		Liver – Carcinoma		
		330	4/53 (7.6%)	
		1,100	3/27 (11.1%)	
		3,300	7/18* (38.9%)	
		Liver – Adenoma		
		0	2/90 (2.2%)	
		Liver – Carcinoma		
		330	0/53	
		1,100	5/27* (18.5%)	
		3,300	5/18* (27.8%)	
Pereira 1996	Agent:	Liver – Adenoma		Survival: Not reported

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse (Study 2) B6C3F1 F 7-8 weeks Study duration: 360 days	Trichloroacetic acid (TCA)	0	1/40 (2.5%)	Body weight: The high dose level of DCA was caused significant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond. Significantly increased pre-neoplastic lesions: The foci of altered hepatocytes were reported combined with neoplasms, but not separately. They are likely considered part of the continuum towards neoplasia as it was reported combined with adenomas and carcinomas as proliferative lesions and other publications by this author under similar studies reported that a similar lesion (large foci of cellular alteration) was only distinguishable from adenomas because the nodules caused compression at less than 80% of it's surface (DeAngelo 2008). Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not continually monitored. A variable number of only females were tested, with only their livers examined histologically.
	Not reported	330	3/40 (7.5%)	
	Treatment: Drinking water	1,100	3/19 (15.8%)	
	0+	3,300	2/20 (10%)	
	330++	Liver – Carcinoma		
	1,100++	0	0/40	
	3,300++mg/L in filtered and deionized water, pH 6.5-7.5 ad libitum x 360 days	330	0/40	
	1,100	0/19		
	3,300	5/20* (25%)		
	+ 20 mmol/L NaCl			
	++ Concentrations were reported as mmol/L and were calculated to mg/L based on a mw of 163.3869g/mol			
Bull et al. 1990 Animal: Mouse B6C3F1 M 5 weeks Study duration: 52 weeks	Agent: Trichloroacetic acid	Liver – Adenoma		Survival: All mice survived. Significantly increased pre-neoplastic lesions: A non-significant increase in hyperplasia was reported. Strengths and limitations: The chemical wasn't characterized, disease surveillance wasn't reported. A low number of mice per group were exposed for a less than near life-span duration and only males had results reported. Only livers were histologically examined. Not all lesions were histologically evaluated, but instead samples of lesions were evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.
	Analytical grade	0	0/2	
	Treatment: Drinking water	1,000	2/5 (40%)	
	0	2,000	1/11 (9%)	
	1,000	Liver – Carcinoma		
	2,000 mg/L in drinking water (pH 6.8-7.2) ad libitum x 52 wk	0	0/2	
	1,000	2/5 (40%)		
	2,000	4/11 (36.4%)		
Von Tungeln et al.	Agent:	Liver – Adenoma		Survival: One mouse died after the age of 28 days in the 20

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
(2002) Animal: Mouse (Study 1) B6C3F1 M 8 days (neonatal) Study duration: 20 months	Trichloroacetic acid	0 (20 mo)	0/23	month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.
	Purity not reported	1,000 (20 mo)	4/23 (17%) ^s	
	Treatment: ip injection	Liver – Carcinoma		
	0+	0 (20 mo)	0/23	
	1,000 nmol	1,000 (20 mo)	1/23 (4%)	
	1/3 of the dose was injected at age 8 days and 2/3 at age 15 days	Liver – Adenoma or carcinoma		
+ DMSO	0 (20 mo)	0/23	Survival: One mouse died after the age of 28 days in the 20 month vehicle control group. All other groups had no mortality after 28 days of age. Body weight: Not reported. Other comments: Early mortality, before 28 days of age was not reported, but was as high as 29% in some groups (which may have included testing of other chemicals). Strengths and limitations: The study used both positive and negative controls, but did not characterize the chemicals and used a small, number of male mice per group. Only two doses were administered at two narrow dose levels, though the duration of observation was almost near life-span. Early mortality wasn't reported.	
Von Tungeln et al. (2002) Animal: Mouse (Study 2) B6C3F1 M 8 days (neonatal) Study duration: 12 months	Agent: Trichloroacetic acid	Liver – Adenoma		
	Purity not reported	0 (12 mo)		0/24
	Treatment: ip injection	2,000 (12 mo)		4/24 (17%)
	0+	Liver – Carcinoma		
	2,000 nmol	0 (12 mo)		0/24
	3/7 of the dose was injected at age 8 days and 4/7 at age 15 days.	2,000 (12 mo)	0/24	
+ DMSO	Liver – Adenoma or carcinoma		Survival: Significant decrease in survival. Body weight: Significant decreased in body weight after 57 weeks at 1,000 mg/l and after 73 weeks at 500 mg/l. Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.	
NTP 2015 Animal: Mouse B6C3F1/N M 5-6 wk Study duration: 105 wk	Agent: Bromodichloroacetic acid	Liver – Adenoma^a		
	97%	0		39/50 (87%)
	Treatment: Drinking water	250		41/50 (90%)
	0	500		42/49 (91%)
	250	1,000		40/51 (91%)
	500	Liver – Carcinoma^a		
	0	12/50 (29%)		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments	
	1,000 mg/L of drinking water ad libitum x 105 weeks	250	22/50* (50%)		
		500	27/49*** ^t (66%)		
		1,000	39/51*** ^t (87%)		
		Trend p-value: <0.001			
		Liver – Adenoma or carcinoma^a			
		0	42/50 (91%)		
		250	47/50 (98%)		
		500	46/49 (97%)		
		1,000	48/51 (98%)		
		Liver – Hepatoblastoma^a			
		0	4/50 (10%)		
		250	24/50*** ^u (54%)		
		500	40/49*** ^u (87%)		
		1,000	34/51*** ^u (78%)		
		Trend p-value: <0.001			
Liver – Adenoma, carcinoma, or hepatoblastoma^a					
0	42/50 (91%)				
250	50/50* (100%)				
500	48/49 (98%)				
1,000	49/51* (99%)				
Trend p-value: =0.036					
Liver – Hemangiosarcoma^a					
0	1/50 (3%)				
250	4/50 (10%)				
500	2/49 (6%)				

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		1,000	4/51 (12%)	
NTP 2015 Animal: Mouse B6C3F1/N F 5-6 wk Study duration: 105 wk	Agent: Bromodichloroacetic acid 97% Treatment: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	Liver – Adenoma^a		Survival: No effect on survival. 30/50 - 33/50, 29/50, 27/50 Body weight: Significant decrease in body weight after 73 weeks at 1,000 mg/l and after 89 weeks at 250 mg/L. Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
		0	33/49 ^v (75%)	
		250	42/50* ^v (91%)	
		500	42/49* ^v (93%)	
		1,000	44/50** ^v (93%)	
		Trend p-value: =0.009		
		Liver – Carcinoma^a		
		0	9/49 (21%)	
		250	17/50 ^w (38%)	
		500	22/49** ^x (50%)	
		1,000	26/50*** ^x (59%)	
		Trend p-value: <0.001		
		Liver – Adenoma or carcinoma^a		
		0	36/49 (81.1%)	
		250	44/50* (93.7%)	
500	43/49* (94.7%)			
1,000	46/50* (95.5%)			
Trend p-value: =0.013				
Liver – Hepatoblastoma^a				
0	0/49			
250	1/50 (2%)			
500	4/49 (9%)			
1,000	6/50* ^y (14%)			
Trend p-value: =0.003				

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		Liver – Hemangiosarcoma^a		
		0	2/49 (5%)	
		250	4/50 (9%)	
		500	4/49 (9%)	
		1,000	8/50* (19%)	
		Trend p-value: =0.026		

* < 0.05; ** < 0.01; *** < 0.001 *P*-value.

[] = *P*-value calculated by NTP using Fisher's Exact Test.

^a Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

^b Exceeds historical controls from drinking water studies: 84/197 (range 34%–63%); exceeds historical controls from studies of all routes: 490/1,506 (range 12%–63%).

^c Exceeds historical controls from drinking water studies: 57/197 (range 18%–42%); exceeds historical controls from studies of all routes: 344/1,506 (range 8%–46%).

^d Exceeds historical controls from drinking water studies: 122/197 (range 48%–85%); exceeds historical controls from studies of all routes: 745/1,506 (range 20%–85%).

^e Exceeds historical controls from drinking water studies: 11/197 (range 0%–13%); exceeds historical controls from studies of all routes: 22/1,506 (range 0%–13%).

^f Exceeds historical controls from drinking water studies: 93/248 (range 18%–61%); exceeds historical controls from studies of all routes: 312/1,549 (range 6%–61%).

^g Exceeds historical controls from drinking water studies: 28/248 (range 4%–26%); exceeds historical controls from studies of all routes: 128/1,549 (range 0%–26%).

^h Exceeds historical controls from drinking water studies: 110/248 (range 20%–63%); exceeds historical controls from studies of all routes: 408/1,549 (range 8%–63%).

ⁱ Exceeds historical controls from drinking water studies: 4/300 (range 0%–4%); exceeds historical controls from studies of all routes: 10/1,199 (range 0%–4%).

^j Exceeds historical controls from drinking water studies: 3/250 (range 0%–4%).

^k Exceeds historical controls from drinking water studies: 140/247 (range 37%–72%); exceeds historical controls from studies of all routes: 544/1,146 (range 14%–72%).

^l Exceeds historical controls from drinking water studies: 91/247 (range 28%–48%); exceeds historical controls from studies of all routes: 317/1,146 (range 8%–48%).

^m Exceeds historical controls from drinking water studies: 182/247 (range 57%–85%); exceeds historical controls from studies of all routes: 729/1,146 (range 20%–85%).

ⁿ Exceeds historical controls from drinking water studies: 28/247 (range 0%–34%); exceeds historical controls from studies of all routes: 43/1,146 (range 0%–34%).

^o Exceeds historical controls from drinking water studies: 133/297 (range 29%–61%); exceeds historical controls from studies of all routes: 345/1,245 (range 6%–62%).

^p Exceeds historical controls from drinking water studies: 51/297 (range 6%–28%); exceeds historical controls from studies of all routes: 131/1,245 (range 0%–28%).

^q Exceeds historical controls from drinking water studies: 158/297 (range 35%–63%); exceeds historical controls from studies of all routes: 419/1,245 (range 8%–64%).

^r *P* < 0.03.

^s [*P* = 0.054].

^t Exceeds historical controls from drinking water studies: 38/100 (range 24%–52%); exceeds historical controls from studies of all routes: 348/949 (range 22%–56%).

^u Exceeds historical controls from drinking water studies: 10/100 (range 8%–12%); exceeds historical controls from studies of all routes: 40/949 (range 0%–12%).

^v Exceeds historical controls from drinking water studies: 71/98 (range 67%–78%); exceeds historical controls from studies of all routes: 378/948 (range 14%–78%).

^w Exceeds historical controls from drinking water studies: 20/98 (range 18%–22%).

^x Exceeds historical controls from drinking water studies: 20/98 (range 18%–22%); exceeds historical controls from studies of all routes: 152/948 (range 4%–46%).

^y Exceeds historical controls from drinking water studies: 10/98 (range 8%–12%); exceeds historical controls from studies of all routes: 40/948 (range 0%–12%).

Table C-9. All other tumors

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP (1992) Animal: Mouse B6C3F1 M 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	All organs – Tumor NOS		Survival: The 100 mg/kg group was lower than controls and there was a significant trend: 46/60***(trend <0.001) - 39/60, 21/60***(<0.001) Body weight: Body weights were similar to controls. Significantly increased pre-neoplastic lesions: The incidence of forestomach pre-neoplasia (squamous cell hyperplasia) was significantly increased at 100 mg/kg. The incidence of nasal cavity pre-neoplasia (olfactory epithelium metaplasia) was not significantly increased. Metaplasia was from olfactory epithelium to ciliated columnar respiratory epithelium. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.
	Treatment: Gavage	0	None	
		50	None	
		100	None	
NTP (1992) Animal: Mouse B6C3F1 F 7-8 weeks Study duration: 104 weeks	Agent: Monochloroacetic acid 99%	All organs – Tumor NOS		Survival: Survival of the exposed groups were similar to controls: 42/60 - 40/60, 44/60 Body weight: Body weights of the low dose group were similar to controls, but after a year the high dose group had significantly lower body weight. Significantly increased pre-neoplastic lesions: The incidences of pre-neoplasia in the forestomach (squamous cell hyperplasia) and nasal cavity were significantly increased at 100 mg/kg. Hyperplasia included diffuse, focal, and multifocal lesions. Metaplasia was from olfactory epithelium to ciliated columnar respiratory epithelium. Strengths and limitations: The study was well conducted to rule out confounding and with a strong power to detect tumor induction. However, only two exposed dose levels were tested, which limit the detection of dose response relationships.
	Treatment: Gavage	0	None	
		50	None	
		100	None	
NTP 2007a	Agent:	Whole body – Malignant mesothelioma^a		Survival: Survival was similar in all groups.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Rat F344/N M 6 weeks Study duration: 106 weeks	Dibromoacetic acid >99% Treatment: Drinking water	0 50 500 1,000	3/50 (7%) 1/50 (2%) 0/50 10/50* ^b (23%)	34/50 - 24/50, 30/50, 28/50 Body weight: Body weights were lower in the 500 (after 57 weeks) and 1,000 (after 29 weeks) mg/l groups compared to the untreated controls. Significantly increased pre-neoplastic lesions: Liver cystic degeneration (3/50 - 9/50*, 11/50*, 15/50**) Other comments: Water consumption was reduced in the 1,000 mg/l group after 2 years.
	1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks	0 50 500 1,000	17/50 ^c (37%) 31/50** ^c (66%) 24/50 ^c (56%) 13/50 (30%)	Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
	Mean daily doses (0 - 2, 20, 40 mg/kg bw)	0 50 500 1,000	2/50 (4.6%) 0/50 4/50 (10.1%) 2/50 (4.9%)	
			Lung – Adenoma^a	
			0 50 500 1,000	
			Lung – Adenoma or carcinoma^a	
			0 50 500 1,000	
NTP 2007a Animal: Rat F344/N F 6 weeks Study duration: 106 weeks	Agent: Dibromoacetic acid >99% Treatment: Drinking water	0 50 500 1,000 50	Whole body – Malignant mesothelioma 0/50 0/50 1/50 (2%) 0/50	Survival: Survival was similar in all groups. 34/50 - 39/50, 35/50, 32/50 Body weight: Body weights were lower in the 1,000 (after 49 weeks) mg/l groups compared to the untreated controls. Significantly increased pre-neoplastic lesions: Significant increases in the incidence of lung per-neoplasia (alveolar epithelium hyperplasia) occurred at 500 and 1,000 mg/L.
			Whole body – Mononuclear cell leukemia^a	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	500 1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks Mean daily doses (0 - 2, 25, 45 mg/kg bw)	0	11/50 (24%)	Kidney nephropathy (18/50 - 32/50**, 37/50**, 40/50**) Other comments: Water consumption was reduced in the 1,000 mg/l group after 2 years. Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
		50	13/50 (27%)	
		500	16/50 ^d (35%)	
		1,000	22/50* ^d (47%)	
		Trend p-value: =0.006		
		Lung – Adenoma or carcinoma		
		0	2/50 (4%)	
		50	3/50 (8%)	
		500	2/50 (4%)	
		1,000	5/50 ^e (10%)	
NTP 2007a	Agent:	Lung – Adenoma^a		Survival: Survival was similar in all groups. 31/50 - 38/50, 34/50, 31/50 Body weight: Body weights were greater in the 50 and 500 mg/l groups compared to the untreated controls after 85 weeks. Significantly increased pre-neoplastic lesions: The incidence of lung pre-neoplasia (Alveolar epithelium hyperplasia) was not significantly increased compared to controls. Spleen hematopoiesis occurred at an significant increased incidences at 500 and 1,000 mg/L. Other comments: Water consumption was similar to controls. Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.
Animal:	Dibromoacetic acid	0	7/49 (16%)	
Mouse B6C3F1	>99%	50	5/50 (11%)	
M 6 weeks	Treatment:	500	17/50* ^f (38%)	
Study duration:	Drinking water	1,000	12/50 ^g (27%)	
106 weeks	0	Trend p-value: =0.019		
	50	Lung – Carcinoma		
	1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks	0	5/49 (10%)	
		50	8/50 ^h (16%)	
	Average daily dose: 0 - 4, 45, 87 mg/kg	500	8/50 ^h (16%)	
		1,000	7/50 ^h (14%)	
		Lung – Adenoma or carcinoma^a		
		0	12/49 ⁱ (28%)	
		50	12/50 (26%)	
		500	22/50* ^{ji} (49%)	
		1,000	17/50 ⁱ (37%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments	
NTP 2007a Animal: Mouse B6C3F1 F 6 weeks Study duration: 106 weeks	Agent: Dibromoacetic acid >99%	Lung – Adenoma^a		Survival: Survival was similar in all groups. 38/50 - 35/50, 32/50, 32/50 Body weight: Body weights were similar to the untreated controls. Other comments: Water consumption was similar to controls. Strengths and limitations: Large numbers of animals per group were used in both sexes and were continuously monitored for disease. Three dose levels spanning a range of 200 fold were used. Lesions and all major organs were histologically evaluated and statistics were clearly reported.	
	Treatment: Drinking water	0	1/50 (2%)		
		50	3/50 (7%)		
		500	3/50 (7%)		
		0	1,000		6/50 ^k (13%)
		50	Trend p-value: = 0.044		
		500	Lung – Carcinoma		
	1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks	0	1/50 (2%)		
		50	2/50 (4%)		
	Average daily dose: 0 - 4, 35, 65 mg/kg	500	2/50 (4%)		
		1,000	2/50 (4%)		
		Lung – Adenoma or carcinoma^a			
		0	2/50 (4%)		
		50	5/50 (11%)		
	500	5/50 (11%)			
	1,000	7/50 ^l (15%)			
NTP 2009 Animal: Rat F344/N M 6-7 weeks Study duration: 105 weeks	Agent: Bromochloroacetic acid 96%	Mammary gland – Fibroadenoma		Survival: No significant difference: 31/50 - 26/50, 25/50, 29/50 Body weight: 1,000 mg/l group was 10% less than controls after 69 weeks. Significantly increased pre-neoplastic lesions: The incidence of lung pre-neoplasia (alveolar epithelium hyperplasia) was not significantly increased. Strengths and limitations: A very high quality study, with no major concerns.	
	Treatment: Drinking water	0	3/50 (6%)		
		250	4/50 (8%)		
		500	3/50 (6%)		
		1,000	4/50 (8%)		
		250	Lung – Adenoma or carcinoma^a		
		500	0		3/50 (7%)
	1,000 mg/L of drinking water x 105 weeks	250	1/50 (2.5%)		
		500	0/50		
		1,000	3/50 (7.1%)		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Pancreatic islets – Adenoma^a				
		0	3/50 (7%)	
		250	4/50 (9.4%)	
		500	9/50* (21.6%)	
		1,000	3/50 (7.1%)	
All organs – Malignant mesothelioma^a				
		0	1/50 (2.3%)	
		250	5/50 (11.7%)	
		500	10/50** (23.7%)	
		1,000	6/50 (14%)	
Large intestine – Adenoma^a				
		0	0/50	
		250	2/50 (4.8%)	
		500	0/50	
		1,000	4/50 (9.5%)	
Trend p-value: =0.031				
NTP 2009	Agent:	Mammary gland – Fibroadenoma^a		Survival: No significant difference:
Animal:	Bromochloroacetic acid	0	43/50 (92%)	34/50 - 31/50, 37/50, 35/50
Rat F344/N	96%	250	43/50 (90%)	Body weight: 1,000 mg/l group was <10% of controls. after 85 weeks.
F 6-7 weeks	Treatment:	500	47/50 (96.9%)	Significantly increased pre-neoplastic lesions: The incidence of lung pre-neoplasia (alveolar epithelium hyperplasia) was significantly increased at 1,000 mg/L.
Study duration:	Drinking water	1,000	46/50 (96.9%)	Strengths and limitations: A very high quality study, with no major concerns.
105 weeks	0	Mammary gland – Fibroadenoma (multiple only)^a		
	250	0	22/50 (44%)	
	500	250	24/50 (48%)	
	1,000 mg/L of drinking water ad libitum x 105 weeks	500	43/50** (86%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		1,000	38/50** (76%)	
		Lung – Adenoma or carcinoma		
		0	1/50 (2%)	
		250	1/50 (2%)	
		500	2/50 (4%)	
		1,000	2/50 (4%)	
		Pancreatic islets – Adenoma^a		
		0	3/49 (7%)	
		250	1/50 (2.3%)	
		500	1/50 (2.2%)	
		1,000	2/50 (4.4%)	
		Large intestine – Adenoma^a		
		0	0/50	
		250	0/50	
		500	3/50 (6.6%)	
		1,000	7/50** (15.5%)	
		Trend p-value: <0.001		
NTP 2009	Agent:	Harderian gland – Adenoma^a		Survival: 38/50 - 35/50, 30/50, 21/50
Animal:	Bromochloroacetic acid	0	5/50 (11.1%)	Body weight: 1,000 mg/l group was 12% lower than controls after 97 weeks.
Mouse B6C3F1	96%	250	9/50 (20%)	Strengths and limitations: A very high quality study, with no major concerns.
M 6-7 weeks	Treatment:	500	9/50 (20.7%)	
Study duration:	Drinking water	1,000	8/50 (18.5%)	
105 weeks	0			
	250			
	500			
	1,000 mg/L of drinking water ad libitum x 105 weeks			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2009 Animal: Mouse B6C3F1 F 6-7 weeks Study duration: 105 weeks	Agent:	Harderian gland – Adenoma^a		Survival: 36/50 - 42/50, 32/50, 40/50 Body weight: No significant difference. Significantly increased pre-neoplastic lesions: Harderian gland focal hyperplasia was also significantly increased in the 250 mg/l group. Other comments: The significance was possibly found because of the low incidence of the untreated controls, which were at the bottom end of the historical control range. Strengths and limitations: A very high quality study, with no major concerns.
	Bromochloroacetic acid	0	1/50 (2.2%)	
	96%	250	7/50* (14.5%)	
	Treatment: Drinking water	500	1/50 (2.2%)	
	0	1,000	7/50* (14.7%)	
NTP 2015 Animal: Rat F344/NTac M 5-6 wk Study duration: 105 wk	Agent: Bromodichloroacetic acid 97% Treatment: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	Whole body – Malignant mesothelioma^a		Survival: No effect on survival. 19/50 - 21/50, 25/50, 19/50 Body weight: Significant decrease of body weight after 89 weeks with 1,000 mg/l, associated with a 10% in water consumption. Other comments: Large intestine includes cecum, colon, and rectum. Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
		0	1/50 (3%)	
		250	12/50*** (28%)	
		500	18/50*** (41%)	
		1,000	37/50*** (78%)	
		Trend p-value: <0.001		
		Mammary gland – Fibroadenoma^a		
		0	0/50	
		250	2/50 (5%)	
		500	3/50 (7%)	
		1,000	1/50 (3%)	
		Brain – Glioma or oligodendroglioma (original evaluation and extended evaluations)^a		
		0	1/50 (3%)	
		250	1/50 (3%)	
		500	4/50 (10%)	
1,000	3/50 (8%)			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		Skin – Fibroma^a		
		0	4/50 (10%)	
		250	6/50 (15%)	
		500	10/50 (23%)	
		1,000	15/50** (36%)	
		Trend p-value: <0.001		
		Skin – Keratoacanthoma^a		
		0	7/50 (17%)	
		250	3/50 (8%)	
		500	10/50 (23%)	
		1,000	15/50* (37%)	
		Trend p-value: =0.003		
		Skin – Squamous cell papilloma^a		
		0	3/50 (8%)	
		250	1/50 (3%)	
		500	0/50	
		1,000	1/50 (3%)	
		Skin – Basal cell adenoma^a		
		0	0/50	
		250	0/50	
		500	4/50 (9%)	
		1,000	4/50 (10%)	
		Trend p-value: =0.012		
		Skin – Squamous cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma^a		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		0	9/50 (22%)	
		250	7/50 (17%)	
		500	15/50 (34%)	
		1,000	21/50** (50%)	
		Trend p-value: <0.001		
		Large intestine – Adenoma		
		0	0/50	
		250	0/50	
		500	2/50 (4%)	
		1,000	2/50 (4%)	
		Oral cavity – Squamous cell papilloma or squamous cell carcinoma^a		
		0	1/50 (3%)	
		250	0/50	
		500	3/50 (7%)	
		1,000	3/50 (8%)	
NTP 2015 Animal: Rat F344/NTac F 5-6 wk Study duration: up to 104 wk	Agent: Bromodichloroacetic acid >97% Treatment: Drinking water 0 250 500 1000 mg/L of drinking water ad libitum x 104 weeks	Brain – Glioma or oligodendrogloma (original evaluation and extended evaluations)^a	Survival: Survival significantly decrease for the 500 and 1,000 mg/l groups and there was a significant negative trend of survival with exposure level. 34/50 - 26/50, 7/50***, 2/50*** Body weight: Significant body weight loss compared to controls (10% lower than control) from 1,000 mg/l after 13 weeks and (20% lower than control) after 52 weeks. Water consumption was decreased durign the first year, but similar to controls during the second year. Body weight loss was not related to decreased water consumption. Other comments: Large intestine includes the colon and rectum. The cecum was not reported, suggesting an incidence of zero.	
		0	1/50 (2.2%)	
		250	0/50	
		500	3/50 (9%)	
		1000	1/50 (3.5%)	
		Mammary gland – Fibroadenoma^a		
		0	28/50 (60.1%)	
		250	47/50*** (96.6%)	
		500	47/50*** (99.1%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		1000	39/50*** (89.6%)	Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
		Trend p-value: <0.001		
		Mammary gland – Adenoma		
		0	1/50 (2%)	
		250	2/50 (4%)	
		500	3/50 (6%)	
		1000	1/50 (2%)	
		Mammary gland – Carcinoma^a		
		0	0/50	
		250	1/50 (2.3%)	
		500	3/50 (9.1%)	
		1000	8/50*** (25.8%)	
		Trend p-value: <0.001		
		Mammary gland – Adenoma or carcinoma		
		0	1/50 (2%)	
		250	3/50 (6%)	
		500	6/50* (12%)	
		1000	9/50** (18%)	
		Mammary gland – Adenoma, carcinoma, or fibroadenoma^a		
		0	28/50 (60.1%)	
		250	47/50*** (96.6%)	
		500	48/50*** (99.4%)	
		1000	42/50*** (92.5%)	
		Trend p-value: < 0.001		
		Skin – Fibroma^a		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		0	2/50 (4.4%)	
		250	0/50	
		500	3/50 (8.9%)	
		1000	2/50 (6.9%)	
		Skin – Basal cell adenoma		
		0	0/50	
		250	0/50	
		500	0/50	
		1000	1/50 (2%)	
		Large intestine – Adenoma^a		
		0	1/50 (2.2%)	
		250	0/50	
		500	1/50 (3.1%)	
		1000	2/50 (7%)	
		Oral cavity – Squamous cell papilloma or squamous cell carcinoma^a		
		0	0/50	
		250	2/50 (4.6%)	
		500	1/50 (3.1%)	
		1000	2/50 (6.9%)	
NTP 2015	Agent:	Harderian gland – Adenoma^a		Survival: Significant decrease in survival.
Animal:	Bromodichloroacetic acid	0	6/50 (15%)	Body weight: Significant decreased in body weight after 57 weeks at 1,000 mg/l and after 73 weeks at 500 mg/lL
Mouse B6C3F1/N	97%	250	11/50 (26%)	Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
M 5-6 wk	Treatment:	500	14/49* (38%)	
Study duration:	Drinking water	1,000	19/51*** (49%)	
105 wk	0	Trend p-value: <0.001		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	250	Harderian gland – Carcinoma^a		
	500	0	0/50	
	1,000 mg/L of drinking water ad libitum x 105 weeks	250	0/50	
		500	0/49	
		1,000	3/51 (9%)	
		Trend p-value: =0.008		
		Harderian gland – Adenoma or carcinoma^a		
		0	6/50 (15%)	
		250	11/50 (26%)	
		500	14/49* (38%)	
		1,000	20/51*** (51%)	
		Trend p-value: <0.001		
NTP 2015 Animal: Mouse B6C3F1/N F 5-6 wk Study duration: 105 wk	Agent: Bromodichloroacetic acid 97% Treatment: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	Harderian gland – Adenoma or carcinoma^a		Survival: No significant change in survival. Body weight: Significant decrease in body weight after 73 weeks at 1,000 mg/l and after 89 weeks at 250 mg/L. Strengths and limitations: Well reported and designed study, with a large number of animals of both sexes exposed for near life-span at three exposure levels.
		0	5/50 (12%)	
		250	4/50 (9%)	
		500	7/50 (16%)	
		1,000	6/50 (14%)	

* < 0.05, ** < 0.01, *** < 0.001 p-value

^a Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

^b Exceeds historical controls from drinking water studies: 15/250 (range 0%–12%); exceeds historical controls from studies of all routes: 57/1,459 (range 0%–12%).

^c Exceeds historical controls from drinking water studies: 79/250 (range 26%–34%); exceeds historical controls from studies of all routes: 622/1,459 (range 22–68%).

^d Exceeds historical controls from drinking water studies: 47/200 (range 20%–30%); exceeds historical controls from studies of all routes: 383/1,459 (range 12–52%).

^e Exceeds historical controls from drinking water studies: 8/200 (range 2%–6%).

^f Exceeds historical controls from drinking water studies: 26/199 (range 6%–20%); exceeds historical controls from studies of all routes: 258/1,507 (range 4–28%).

^g Exceeds historical controls from drinking water studies: 26/199 (range 6%–20%).

^h Exceeds historical controls from drinking water studies: 16/199 (range 6%–10%).

ⁱ Exceeds historical controls from drinking water studies: 41/199 (range 12%–26%).

^j Exceeds historical controls from drinking water studies: 41/199 (range 12%–26%); exceeds historical controls from studies of all routes: 385/1,507 (range 12–44%).

^k Exceeds historical controls from drinking water studies: 13/250 (range 2%–12%); exceeds historical controls from studies of all routes: 80/1,552 (range 0%–12%).

^l Exceeds historical controls from drinking water studies: 16/250 (range 2%–12%); exceeds historical controls from studies of all routes: 117/1,552 (range 0%–14%).

Table C-10. Transgenic studies

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2007b Animal: Mouse (Study 1) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha- ras)Led) M 6 weeks Study duration: 39 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Dermal 0 31.25 125 500 mg/kg bw in Water:Acetone (1:2) (pH 6-8) 5 doses/week x 39 weeks	Skin – Squamous cell papilloma 0 31.25 125 500	0/10 0/10 2/10 (20%) 8/10** (80%)	Survival: Survival was similar to untreated controls: 9/10 - 6/10, 8/10, 7/10 Body weight: Body weights were significantly lower in 31.25 mg/kg group after 22 weeks, 500 mg/kg after 21 weeks, and 125 mg/kg temporarily was lower from weeks 28 to 38, but were the same as controls by the end of the study. Significantly increased pre-neoplastic lesions: The incidence of pre-neoplasia of the skin (epidermis hyperplasia) was significantly increased at 125 and 500 mg/kg. Other comments: Only reporting neoplasms at the site of application. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 1) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha- ras)Led) F 6 weeks Study duration: 39 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Dermal 0 31.25 125 500 mg/kg bw in Water:Acetone (1:2) (pH 6-8) 5 doses/week x 39 weeks	Skin – Squamous cell papilloma 0 31.25 125 500	0/10 0/10 0/10 6/10** (60%)	Survival: Survival was similar to untreated controls: 8/10 - 5/10, 6/10, 8/10 Body weight: Body weights of 31.25 and 125 mg/kg groups were greater than controls at the end of the study, and 500 mg/kg were greater after 17 weeks. Significantly increased pre-neoplastic lesions: The incidence of pre-neoplasia of the skin (epidermis hyperplasia) was significantly increased at 500 mg/kg. Other comments: Only reporting neoplasms at the site of application. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b	Agent:	Skin – Squamous cell papilloma		Survival: Survival was similar to untreated controls: 13/15 -

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha- ras)Led) M 6 weeks Study duration: 26 weeks	Dichloroacetic acid 98.5%-99% Treatment: Dermal 0 31.25 125 500 mg/kg bw in Water:Acetone (1:2) (pH 6-8) 5 doses/week x 26 weeks	0 31.25 125 500	0/15 0/15 1/15 (7%) 2/15 (13%)	14/15, 14/15, 12/15 Body weight: Body weights were similar to untreated controls. Significantly increased pre-neoplastic lesions: The incidence of pre-neoplasia of the skin (epidermis hyperplasia) was significantly increased at 125 and 500 mg/kg. The severity grades of the hyperplasia in all cases were minimal. Other comments: Only reporting neoplasms at the site of application. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha- ras)Led) F 6 weeks Study duration: 26 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Dermal 0 31.25 125 500 mg/kg bw in Water:Acetone (1:2) (pH 6-8) 5 doses/week x 26 weeks	Skin – Squamous cell papilloma 0 31.25 125 500	0/15 0/15 0/15 2/15 (13%)	Survival: Survival was similar to untreated controls: 11/15 - 12/15, 14/15, 15/15 Body weight: Body weights were similar to untreated controls. Significantly increased pre-neoplastic lesions: The incidence of pre-neoplasia of the skin (epidermis hyperplasia) was significantly increased at 125 and 500 mg/kg. The severity grades of the hyperplasia in all cases were minimal. Other comments: Only reporting neoplasms at the site of application. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 1) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha- ras)Led)	Agent: Dichloroacetic acid 98.5%-99% Treatment: Drinking water	Lung – Adenoma 0 500 1,000	1/10 (10%) 2/10 (20%) 7/10** (70%)	Survival: Survival was similar to untreated controls: 9/10 - 9/10, 10/10, 10/10 Body weight: Body weights were significantly greater than untreated controls at 500 mg/l after 17 weeks and 1,000 mg/l after 21 weeks.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
ras)Led) M 6 weeks Study duration: 41 weeks	0	2,000	3/10 (30%)	Other comments: Water consumption at 2,000 mg/l was less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
	500	Forestomach – Squamous cell papilloma		
	1,000	0	5/10 (50%)	
	2,000 mg/L in drinking water	500	9/10 (90%)	
	ad libitum x 26 weeks	1,000	6/10 (60%)	
		2,000	7/10 (70%)	
NTP 2007b Animal: Mouse (Study 1) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha-ras)Led) F 6 weeks Study duration: 41 weeks	Agent: Dichloroacetic acid 98.5%-99%	Lung – Adenoma		Survival: Survival was similar to untreated controls: 7/10 - 9/10, 7/10, 8/10 Body weight: Body weights of 1,000 and 2,000 mg/l were significantly lower than untreated controls after 15 and 15 weeks respectively. Other comments: Water consumption at 2,000 mg/l was less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
	Treatment: Drinking water	0	0/10	
	0	500	0/10	
	500	1,000	0/10	
	1,000	2,000	2/10 (20%)	
		Forestomach – Squamous cell papilloma		
	2,000 mg/L in drinking water	0	6/10 (60%)	
	ad libitum x 26 weeks	500	7/10 (70%)	
		1,000	7/10 (70%)	
		2,000	6/10 (60%)	
		Forestomach – Squamous cell papilloma (multiple only)		
	0	1/10 (10%)		
	500	6/10* (60%)		
	1,000	4/10 (40%)		
	2,000	4/10 (40%)		
NTP 2007b Animal: Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha-	Agent: Dichloroacetic acid 98.5%-99%	Lung – Carcinoma		Survival: Survival was similar to untreated controls: 14/15 - 13/15, 11/15, 14/15 Body weight: Body weights were significantly greater than untreated controls at 500 mg/l after 17 weeks and 1,000 mg/l after 21 weeks.
	Treatment: Drinking water	0	0/15	
	500	500	0/15	
	1,000	1,000	1/15 (7%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
ras)Led) M 6 weeks Study duration: 26 weeks	0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	2,000	0/15	Other comments: Water consumption at 2,000 mg/l was less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha-ras)Led) F 6 weeks Study duration: 26 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	Lung – Carcinoma 0 500 1,000 2,000	0/15 1/15 (7%) 0/15 1/15 (7%)	Survival: Survival was significantly lower than untreated controls at 500 and 2,000 mg/l: 15/15 - 8/15*(=0.009), 13/15, 10/15*(=0.05) Body weight: Body weights of 1,000 and 2,000 mg/l were significantly lower than untreated controls after 15 and 15 weeks respectively. Other comments: Water consumption at 2,000 mg/l was less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 3) p53 Haploinsufficient M 6 weeks Study duration: 41 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	Lung – Adenoma or carcinoma 0 500 1,000 2,000	0/10 0/10 0/10 0/10	Survival: Survival was similar to untreated controls: 9/10 - 10/10, 9/10, 10/10 Body weight: Body weights of 500, 1,000, and 2,000 mg/l were significantly lower than untreated controls after 4, 3, and 1 weeks respectively. Other comments: Water consumption at 1,000 and 2,000 mg/l were less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b	Agent:	Lung – Adenoma or carcinoma		Survival: Survival was similar to untreated controls: 10/10 -

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Animal: Mouse (Study 3) p53 Haploinsufficient F 6 weeks Study duration: 41 weeks	Dichloroacetic acid 98.5%-99% Treatment: Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	0 500 1,000 2,000	0/10 0/10 0/10 0/10	9/10, 10/10, 9/10 Body weight: Body weights of 500, 1,000 and 2,000 mg/l were significantly lower than untreated controls after 27, 9, and 9 weeks respectively. Other comments: Water consumption at 1,000 and 2,000 mg/l were less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 4) p53 Haploinsufficient M 6 weeks Study duration: 26 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	Lung – Adenoma or carcinoma 0 500 1,000 2,000	0/15 0/15 0/15 0/15	Survival: Survival was similar to untreated controls: 15/15 - 15/15, 15/15, 15/15 Body weight: Body weights of 1,000 and 2,000 mg/l were significantly lower than untreated controls after 4 and 2 weeks respectively. Other comments: Water consumption at 1,000 and 2,000 mg/l were less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.
NTP 2007b Animal: Mouse (Study 4) p53 Haploinsufficient F 6 weeks	Agent: Dichloroacetic acid 98.5%-99% Treatment: Drinking water	Lung – Adenoma or carcinoma 0 500 1,000	0/15 0/15 0/15	Survival: Survival was similar to untreated controls: 15/15 - 15/15, 14/15, 14/15 Body weight: Body weights of 1,000 and 2,000 mg/l were significantly lower than untreated controls after 11 and 10 weeks respectively.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Study duration: 26 weeks	0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	2,000	0/15	Other comments: Water consumption at 1,000 and 2,000 mg/l were less than controls. Strengths and limitations: The study was well conducted except for a low number of transgenic animals per group, which may overestimate carcinogenic potential. The ability to translate hazards in this model to non-transgenic mice is limited.

* $P < 0.05$; ** $P < 0.01$.

Table C-11. Initiation-promotion studies

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
GWYNN and SALAMAN (1953) Animal: Mouse Stock albino "S" strain NR NR Study duration: 30 weeks	Agent: Monoiodoacetic acid	Skin – Papilloma		Survival: Only two of the exposed mice died. Body weight: Not reported. Strengths and limitations: The chemicals were not characterized and purity wasn't reported. The sex of the animals were not reported and only a single dose level was tested on a very low number of animals per group. Histology of the neoplasms were carried out, but the skin tumors were classified as benign papillomas based on their appearance "macroscopically". Statistical significance was not calculated.
	Not reported	0 (acetone)	1/12 (8.3%)	
Treatment: Dermal		0 (acetic acid)	1/16 (6.25%)	
	Initiator: 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone: single dose 0.15% in 3 ml	1.4	8/10[[@]][^{##}] (80%)	
	Promotor: Iodoacetic acid in acetone: start 21 days after DMBA 1.4% (2/wk x 12wk, then 1/wk x 15wk)			
Herren-Freund <i>et al.</i> (1987) Animal: Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	Agent: Dichloroacetic acid	Liver – Adenoma		Survival: Not reported. Body weight: Body weights were significantly decreased (p<0.001). Calculations were done by one-way analysis of variance with a Tukey's comparison.
	>99%	0/0	2/22 (9%)	
	Treatment: Drinking water	0/5,000	25/26** (96%)	Strengths and limitations: The duration was less than near life-span. Only males were tested at two narrow dose levels and only livers were histologically evaluated.
	Initiator: Ethylnitrosourea (ENU): ip injection at 15 days old	2.5/0	1/22 (5%)	
		2.5/2,000	22/29** (76%)	Liver – Carcinoma
		2.5/5,000	31/32** (97%)	
		0+ 2.5 µg/g bw	0/0	0/22
	Promotor: DCA: in drinking water	0/5,000	21/26** (81%)	
		2.5/0	1/22 (5%)	
		2.5/2,000	19/29** (66%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	at 4 weeks old 0++ 2,000 5,000 mg/L in drinking water (pH 6.5-7.5) x 61 weeks + 2 µg/g bw of 0.1 M sodium acetate ++ 2,000 mg/l of sodium chloride	2.5/5,000	25/32** (72%)	
Pereira 1997 Animal: Mouse B6C3F1 F Initiator: 15 d; Promotor: 6 wks Study duration: 50 wks	Agent: Dichloroacetic acid NR Treatment: Drinking water Initiator: Methylnitrosourea (MNU): single ip dose 25 mg/kg bw in sterile saline Promotor: DCA: in drinking water, ad libitum x 44 weeks, starting 4 weeks after MNU 0 25.0 15.6 7.8 mmol/L in filtered and deionized water, pH 6.5-7.5	Liver – Adenoma 0 DCA/TCA 7.8 DCA 15.6 DCA 25 DCA Liver – Carcinoma 0 DCA/TCA 7.8 DCA 15.6 DCA 25 DCA	None None None None 0/29 0/17 0/19 3/29 (10.3%)	Survival: No significant difference in survival: 29/30, 19/20, 17/20, 29/30 (# of mice at scarified/# of mice as the start of promotion). Body weight: There was a decrease in body weight of less than 10% of the control weight in the groups receiving TCA and top two highest levels of DCA. Significantly increased pre-neoplastic lesions: The liver foci of altered hepatocytes were distinguished from adenomas by compression at less than 80% of it's boarder. This suggests that it was considered pre-neoplastic. The multiplicity of these pre-neoplastic lesions was significantly increased at 25 mmol/L of DCA. Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not reported. A low number of only females were tested, with only their livers examined histologically. The statistical methods were not reported.
Herren-Freund <i>et al.</i>	Agent:	Liver – Adenoma		Survival: Not reported.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
(1987) Animal: Mouse B6C3F1 M 15 days Study duration: 61 weeks	Trichloroacetic acid	0/0	2/22 (9%)	Body weight: Body weights were significantly decreased (p<0.001). Calculations were done by one-way analysis of variance with a Tukey's comparison. Strengths and limitations: The duration was less than near life-span. Only males were tested at two narrow dose levels and only livers were histologically evaluated.
	>99%	0/5,000	8/22** (36%)	
	Treatment:	2.5/0	1/22 (5%)	
	Drinking water	2.5/2,000	11/33** (33%)	
	Initiator:	2.5/5,000	6/23** (26%)	
	Ethylnitrosourea (ENU): ip injection at 15 days	10/0	9/23 (39%)	
	old	10/5,000	11/28 (39%)	
	0+	Liver – Carcinoma		
	2.5	0/0	0/22	
	10 µg/g bw	0/5,000	7/22** (32%)	
	Promotor:	2.5/0	1/22 (5%)	
	TCA: in drinking water (pH 6.5-7.5) at 4 weeks	2.5/2,000	16/33** (48%)	
	old	2.5/5,000	11/23** (48%)	
	0++	10/0	9/23 (39%)	
	2,000	10/5,000	15/28 (54%)	
5,000 mg/L in drinking water (pH 6.5-7.5) x 61 weeks				
+ 2 µg/g bw of 0.1 M sodium acetate				
++ 2,000 mg/L NaCl				
Pereira 1997	Agent:	Liver – Adenoma		Survival: No significant difference in survival: 29/30, 20/20, 29/30 (# of mice at scarified/# of mice as the start of promotion). Body weight: There was a decrease in body weight of less than 10% of the control weight in the groups receiving TCA and top two highest levels of DCA. Significantly increased pre-neoplastic lesions: The liver foci of altered hepatocytes were distinguished from adenomas by compression at less than 80% of it's boarder. This suggests
Animal:	Trichloroacetic acid	0 DCA/TCA	None	
Mouse B6C3F1	Vendor, but not purity	6 TCA	None	
F Initiator: 15 d;	given	25 TCA	None	
Promotor: 6 wks	Treatment:	Liver – Carcinoma		
Study duration:	Drinking water	0 DCA/TCA	0/29	
50 wks	Initiator:	6 TCA	0/20	
	Methylnitrosourea (MNU): single ip dose in			

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	sterile saline 25 mg/kg Promotor: TCA: in drinking water ad libitum x 44 weeks, starting 4 wk after MNU 0 25.0 6.0 mmol/L in filtered and deionized water, pH 6.5-7.5	25 TCA	4/29 (13.8%)	that it was considered pre-neoplastic. The multiplicity of these pre-neoplastic lesions was not significantly increased over controls. Strengths and limitations: The chemicals were not characterized, not even purity was reported. Disease surveillance was not reported. A low number of only females were tested, with only their livers examined histologically. The statistical methods were not reported.

** < 0.01 (compared to the control group without a promotor), @ < 0.01 (compared to acetone), ## < 0.001 (compared to acetic acid) p-value

[] = p-value calculated by NTP using Fisher's Exact Test.

Appendix D: Oxidative stress and genotoxic potency data for haloacetic acids

The three tables on the following pages contain data discussed in Section 5.3 “Induces oxidative stress” and Section 5.4 “Is genotoxic and/or alters DNA repair” for haloacetic acids. Data are reported for *in vitro* and *in vivo* haloacetic acid-induced oxidative stress (Table D-1), Mutagenic/genotoxic potency estimates of haloacetic acids in bacteria (Table D-2), and Mutagenic/genotoxic potency estimates of haloacetic acids in mammalian cells (Table D-3)

Table D-1. *In vitro* and *in vivo* haloacetic acid-induced oxidative stress

Test system (potency measurement, units)	Monohaloacetic acids			Dihaloacetic acids					Trihaloacetic acids				Reference
	CA	BA	IA	DCA	DBA	BCA	CIA	BIA	TCA	TBA	BDCA	CDBA	
AREc32: human breast cancer cell line (MCF7) (1/mM, IR = 1.5)	3.7	192	278	0.17	8.3	7.1	45.5	38.5	–	2.3	0.5	0.2	Stalter <i>et al.</i> 2016
ARE-bla: human hepatocellular carcinoma cell line (HepG2) (1/mM, IR = 1.5)	4.0	90.9	196	0.06	4.0	2.2	10	18.9	–	1.5	0.25	0.46	Stalter <i>et al.</i> 2016
ARE-bla: human hepatocellular carcinoma cell line (HepG2) (1/mM, IR = 1.5)	14	141	256										Procházka <i>et al.</i> 2015
ARE-bla: human hepatocellular carcinoma cell line (HepG2) (1/mM, IR = 1.5)	116	510	1,010										Pals <i>et al.</i> 2013
Lipid peroxidation; male mice, single 300 mg/kg oral dose TBARS (nmol/g liver, wet wt)				129	250 ^a	290 ^a			67		240 ^a		Larson and Bull 1992, Austin <i>et al.</i> 1996
Oxidative DNA damage; male mice, single 300 mg/kg oral dose (8-OHdG/10 ⁵ dG liver)				1.4 ^a	2.9 ^a	2.9 ^a			1.2 ^a		1.7 ^a		Austin <i>et al.</i> 1996
Oxidative DNA damage; male mice, 21 day water– 0.5 and 2 g/L (8-OHdG/10 ⁵ dG liver)				–	1.4 ^a	1.2 ^a			–				Parrish <i>et al.</i> 1996

CA = chloro-, BA = bromo-, IA = iodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, CIA = chloriodo-, BIA = bromiodo-, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloro-, CDBA = chlorodibromoacetic acid, mM = millimolar, IR = induction ratio, TBARS = thiobarbituric acid-reactive substances, 8-OHdG = 8-hydroxydeoxyguanosine, – = negative, blank cell = not tested.

^a Values estimated from figures using WebPlot Digitizer @ <http://arohatgi.info/WebPlotDigitizer/app/>.

Table D-2. Summary of genetic toxicology results of haloacetic acids in CEBs

Haloacetic acid	Study type	Results	URL
Chloro-	Ames	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0003-0000-4
Chloro-	Ames	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0004-0000-5
Chloro-	Mammalian cell cytogenetics (CA)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0002-0000-3
Chloro-	Mammalian cell cytogenetics (SCE)	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0001-0000-2
Chloro-	Mammalian cell mutagenicity	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0010-0000-2
Chloro-	Drosophila germ cell mutagenicity	E	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02485-0009-0000-0
Bromo-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01737-0001-0000-1
Bromo-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01737-0002-0000-2
Iodo-	Ames	E	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02289-0001-0000-4
Iodo-	Ames	(+)	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02289-0002-0000-5
Iodo-	Drosophila germ cell mutagenicity	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02289-0003-0000-6
Dichloro-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0008-0000-9
Dichloro-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0009-0000-0
Dichloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0004-0000-5
	Female mice (micronucleus)	–	
Dichloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0006-0000-7
	Female mice (micronucleus)	–	
Dichloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0007-0000-8
	Female mice (micronucleus)	–	
Dichloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0005-0000-6
	Female mice (micronucleus)	+	
Dibromo-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01996-0006-0000-3
Dibromo-	Male mice (micronucleus)	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01996-0005-0000-2
	Female mice (micronucleus)	–	
Bromochloro-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01740-0005-0000-9
Bromochloro-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01740-0006-0000-0

Haloacetic acid	Study type	Results	URL
Bromochloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01740-0004-0000-8
	Female mice (micronucleus)	–	
Trichloro-	Ames	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02942-0001-0000-0
Tribromo-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02933-0002-0000-1
Bromodichloro-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01742-0018-0000-5
Bromodichloro-	Ames	(+)	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01742-0004-0000-0
Bromodichloro-	Male mice (micronucleus)	–	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01742-0003-0000-9
	Female mice (micronucleus)	–	

– = negative, (+) = weak positive, + = positive, E = equivocal

Table D-3. Mutagenic/genotoxic potency estimates of haloacetic acids in bacteria

Test system (potency measurement, units)	Monohaloacetic acids			Dihaloacetic acids				Trihaloacetic acids				Reference	
	CA	BA	IA	DCA	DBA	BCA	CIA	BIA	TCA	TBA	BDCA		CDBA
SOS-umuC: TA1535/pSK1002													Stalter <i>et al.</i>
– S9 (1/M, IR = 1.5)	– ^a	– ^a	– ^a	–	2,564	2,941	5,263	9,091	–	142,860	9,091	9,091	2016
+ S9 (1/M, IR = 1.5)	– ^a	– ^a	– ^a	–	1,493	1,064	2,083	11,490	–	13,890	455	625	
SOS-umuC: TA1535/pSK1002													Zhang <i>et al.</i>
– S9 (1/M, IR = 2)	(+)	1,107	8,696	83	89				(+)				2016
– S9 (1/M, IR = 1.5)	60 ^b	2,400 ^b	15,400 ^b	180 ^b	760 ^b				60 ^b				
SOS-umuC: TA1535/pSK1002													Ono <i>et al.</i> 1991
– S9 (β-galactosidase activity) ^c	–			neg					0.72				
+ S9 (β-galactosidase activity) ^c	–			1.5					1.18				
Ames preincubation: TA100													Plewa <i>et al.</i>
– S9 (revertants/μmol)													2000, Plewa <i>et al.</i> 2004b
	27	5,465	14,129		148								Kargalioglu <i>et al.</i> 2002
Ames preincubation: TA100													Kargalioglu <i>et al.</i> 2002
– S9 (revertants/μmol) ^d	44	6,588		36	183				–	–			
+ S9 (revertants/μmol) ^d	63	2,642		13	165				–	–			
Ames preincubation: TA98													Kargalioglu <i>et al.</i> 2002
– S9 (revertants/μmol) ^d	6	351		2	16				–	–			
+ S9 (revertants/μmol) ^d	–	179		–	12				–	–			
Ames preincubation: RSJ100													Kargalioglu <i>et al.</i> 2002
– S9 (revertants/μmol) ^d	–	–		17	–				–	–			
+ S9 (revertants/μmol) ^d	–	–		–	–				–	–			
SOS chromotest: <i>E. coli</i> PQ37													Giller <i>et al.</i>
– S9 (1/(mg/mL), IR = 1.5)	–	–		2	5				–	1.3			1997
+ S9 (1/(mg/mL), IR = 1.5)	–	–		–	10				–	10			
Ames fluctuation: TA100													Giller <i>et al.</i>
– S9 (1/[mg/mL])	–	–		10	100				0.57	0.5			1997
+ S9 (1/[mg/mL])	–	50		0.67	33				0.33	0.2			

CA= chloro-, BA = bromo-, IA = iodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, CIA = chloroiodo-, BIA = bromoiodo-, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloro-, CDBA = chlorodibromoacetic acid, M = molar, IR = induction ratio, –S9 = without metabolic activation, +S9 = with metabolic activation, – = negative, (+) = weak positive but no potency value reported, blank cell = not tested.

^a Likely a false negative due to cytotoxicity.

^b Potency values at an induction ratio of 1.5 were estimated from figures using WebPlot Digitizer @ <http://arohatgi.info/WebPlotDigitizer/app/>.

^c Calculated as [(A-B)/B] where A = the β-galactosidase activity of the test agent and B is the baseline activity (<0.5, negative; >0.5-1.0, weak positive; >1.0-2.0, positive).

^d Values adjusted for cytotoxicity.

Table D-4. Mutagenic/genotoxic potency estimates of haloacetic acids in mammalian cells

Test system (potency measurement, units)	Monohaloacetic acids				Dihaloacetic acids				Trihaloacetic acids				Reference
	CA	BA	IA	DCA	DBA	BCA	CIA or (DIA)	BIA	TCA	TBA	BDCA	CDBA	
p53-bla: human colon carcinoma cell line HCT-116 (1/M, IR = 1.5)	5,882	105,260	212,770	–	3,846	4,348	9,091	9,091	–	–	–	–	Stalter <i>et al.</i> 2016
SCGE: CHO AS52 cells													
LGC (1/M)	3,333	76,920	200,000	–	1,333	333	(1,000)	400	–	333	–	71	Plewa <i>et al.</i> 2002, Plewa <i>et al.</i> 2004b,
GP (1/M)	2,439	58,820	114,900	–	556	333	(500)	313	–	400	–	71	Richardson <i>et al.</i> 2008, Plewa <i>et al.</i> 2010
SCGE: CHO AS52 cells (tail moment units/μmol)		68,900			887					254			Plewa <i>et al.</i> 2000
HGPRT mutations: CHO-K1 cells (mutant frequency/mM)	8.7	14.6	835.9	2.8	66.2				–				Zhang <i>et al.</i> 2010
SCGE assay: HepG2 cells MEC (1/μM)	–	10	100	0.1	1				0.01				Zhang <i>et al.</i> 2012
p53-bla: human colon carcinoma cell line HCT-116 (1/M, IR = 1.5)	10,000	98,039	156,250										Procházka <i>et al.</i> 2015
SCGE: human lymphocytes GP (1/mM)	1.2	83	96										Escobar-Hoyos <i>et al.</i> 2013
Mitotic index: human lymphocytes EC ₅₀ (1/mM)	1.4	12.7	20.3										Escobar-Hoyos <i>et al.</i> 2013
SCGE: human small intestine epithelial cells EC ₅₀ (1/mM)	0.29	17.7	45.7										Attene-Ramos <i>et al.</i> 2010

CA= chloro-, BA = bromo-, IA = iodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, CIA = chloroiodo-, DIA = diiodo-, BIA = bromoiodo-, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloro-, CDBA = chlorodibromoacetic acid, M = molar, IR = induction ratio, SCGE = single cell gel electrophoresis or comet assay, – = negative, LGC = lowest genotoxic concentration, GP = genotoxic potency (calculated using regression analysis as the midpoint of the curve within the concentration range that expressed above 70% cell viability), MEC = minimum effective concentration, EC₅₀ = effective concentration that reduced the mitotic index by 50% or induced average SCGE damage of 50% tail DNA, blank cell = not tested.

Table D-5. Epigenetic effects of haloacetic acids in mouse and rat tissues

Species (sex)	Tissue	HAA	Conc (g/L)	Hypomethylation		Comments	Reference
				DNA (% reduction) ^a	Genes		
Mice (M,F)	Kidney	DCA	3.2	40	<i>c-myc</i>	Mice treated for 7 days. Unlike the liver, effects in kidney only in males. <i>c-myc</i> hypomethylation prevented by treatment with methionine.	Tao <i>et al.</i> 2005
		TCA	4.0	65			
Mice (M)	Kidney	DBA	1–2	40–60	<i>c-myc</i>	High dose caused reduction at 7 days and 28 days; at low dose, significant reduction only after 28 days.	Tao <i>et al.</i> 2005
Rats (M)				45–56			
Mice (F)	Liver	DBA	1–2	45–70	<i>c-myc</i> , <i>IGF-II</i>	Both doses caused significant reduction after 7 and 28 days. mRNA expression of <i>c-myc</i> and IGF-II genes significantly increased in mice and <i>c-myc</i> expression increased in rats.	Tao <i>et al.</i> 2004a
Rats (M)				33–52			
Mice (F)	Liver	DCA	2.6 ^b	27–85	<i>IGF-II</i>	Hypomethylation status in DCA- and TCA-promoted liver tumors that were initiated by MNU and in normal liver. Both compounds caused the same reduction in liver and liver tumor DNA (estimated from a figure) but there was significantly greater reduction in liver tumors compared to normal liver tissue. 79.3% of 28 CpG sites in the promoter region of the IGF-II gene were methylated in control mouse liver compared to 46.4% and 58% in normal liver and 8.7% and 10.7% in liver tumors of DCA- and TCA-treated mice, respectively. IGF-II expression was increased 4.5- to 5.1-fold in tumors compared to normal liver.	Tao <i>et al.</i> 2004b
	Liver tumors	TCA	3.3 ^b	27–85			
Mice (F)	Liver	DCA	500 mg/kg	NR	<i>c-jun</i> , <i>c-myc</i>	Single gavage dose administered daily for 5 days. Treatment with methionine prevented hypomethylation.	Tao <i>et al.</i> 2000a
		TCA	500 mg/kg				
Mice (F)	Liver	DCA	2.6 ^b	NR	<i>c-jun</i> , <i>c-myc</i>	mRNA expression and protein levels of <i>c-myc</i> and <i>c-jun</i> were increased in DCA- and TCA-promoted liver tumors but not normal tissue. DNA methyltransferase (MTase) activity increased in liver tumors promoted by DCA and TCA (greater effect for DCA) but decreased in normal liver. Increased MTase activity is associated with silencing tumor suppressor genes.	Tao <i>et al.</i> 2000b
	Liver tumors	TCA	3.3 ^b				

Species (sex)	Tissue	HAA	Conc (g/L)	Hypomethylation		Comments	Reference
				DNA (% reduction) ^a	Genes		
Mice (F)	Liver	DCA	3.2 ^b	36 ^c	NR	Hypomethylation status was measured in DCA- and TCA-promoted liver tumors that were initiated by MNU and in normal liver. Hypomethylation in normal liver was observed after 11 days but not after 44 weeks. Methylation status in adenomas also returned to normal with termination of DCA exposure 1 week prior to sacrifice but not with TCA.	Tao <i>et al.</i> 1998
	Liver tumors	TCA	4.0 ^b	40–51 ^c			
Mice (F)	Liver Liver tumors	DCA	3.2	55	NR	Mice sacrificed after 8 or 44 weeks of exposure. Methionine treatment prevented hypomethylation, reduced DCA-induced tumor multiplicity, and slowed the progression of foci to tumors. (Hypomethylation estimated from a figure in the paper with results after 8-weeks exposure.)	Pereira <i>et al.</i> 2004a
Mice (F)	Liver Kidney	DCA TCA	500 mg/kg 500 mg/kg	NR	<i>c-myc</i>	Single gavage dose administered daily for 5 days. Both DCA and TCA induced hypomethylation and expression of <i>c-myc</i> in liver. Co-administration of chloroform prevented DCA- but not TCA-induced hypomethylation. In a second experiment, chronic administration of TCA in the drinking water promoted kidney tumors. DCA also promoted kidney tumors when co-administered with chloroform.	Pereira <i>et al.</i> 2001
Mice (F)	Liver Kidney Bladder	DCA TCA	500 mg/kg 500 mg/kg	NR	<i>c-myc</i>	Hypomethylation of the promoter region of <i>c-myc</i> in liver, kidney, and urinary bladder occurred after 72 and 96 hours (but not at earlier time points) after a single gavage dose of either DCA or TCA. Enhanced cell proliferation in the liver also reported after 72 and 96 hours.	Ge <i>et al.</i> 2001

HAA = haloacetic acid, DCA = dichloroacetic acid, DBA = dibromoacetic acid, TCA = trichloroacetic acid, MNU = *N*-methyl-*N*-nitrosourea, IGF-II = insulin-like growth factor-II, NR = not reported.

^a % Reduction in 5-methylcytosine compared to control DNA.

^b Converted from 20 mmol/L or 25 mmol/L, MW DCA = 128.9, TCA = 163.4, administered in drinking water for 44 to 46 weeks beginning at 6 weeks of age.

^c Reduction in liver tumors compared to normal liver tissue from the same animal. TCA promoted both adenomas and carcinomas, DCA only adenomas.

Table D-6. Gene expression studies of di- and trihaloacetic acids in yeast and rodent tissue

Haloacetic acid	Test system	Results	Reference
Dichloro-	Mouse normal liver (4 wk treatment) and liver tumors induced by dichloroacetic acid after 93 weeks	Normal liver: 15 genes differentially expressed (14 were suppressed) in the functional categories of cell growth, tissue remodeling, apoptosis, cancer progression, fatty acid metabolism, and xenobiotic metabolism. Hepatocellular carcinomas: 11 of the same 15 genes showed a similar expression pattern as in normal liver from exposed mice.	Thai <i>et al.</i> 2003
Dichloro-	Mouse normal liver (4 wk treatment) and liver tumors induced by dichloroacetic acid after 93 weeks	Normal liver: Six differentially expressed genes (5 were suppressed; one gene was induced) involved in fatty acid metabolism, tissue remodeling and tumor invasion. No altered genes identified that are involved in genotoxicity pathways. Hepatocellular carcinomas: Four genes showed similar expression pattern as in normal liver from exposed mice.	Thai <i>et al.</i> 2001
Dichloro-	Rat normal liver (i.p. injection)	One gene with 50% homology to a mouse fibroblast growth factor mRNA identified and might be involved in hepatocellular proliferation and DNA synthesis.	Choi and Park 1996
Dichloro- Trichloro-	Mouse liver tumors (chronic exposure)	Hyperplastic nodules and hepatocellular carcinomas: Increased expression of <i>c-myc</i> and <i>c-H-ras</i> compared to surrounding tissue or controls in both preneoplastic nodules and carcinomas. <i>Myc</i> expression was similar in DCA-induced nodules and carcinomas. However, <i>myc</i> expression in TCA-induced tumors was significantly higher than in DCA-induced nodules and tumors and appeared to be related to the early progression of TCA-induced tumors to a malignant state. <i>c-H-ras</i> expression was higher in carcinomas from both treatments than in hyperplastic nodules or normal liver, thus, was closely associated with malignancy.	Nelson <i>et al.</i> 1990
Trichloro-	Green-fluorescent protein (GFP)-tagged yeast reporter strains	Activated DNA repair pathways suggested strong base damage, mismatches and double-strand breaks. Damage was consistent with oxidative DNA damage.	Lan <i>et al.</i> 2016

Haloacetic acid	Test system	Results	Reference
Bromodichloro-	Mouse: normal liver tissue and tumors (chronic exposure)	<p>Nontumor liver tissue from treated mice: Altered oncogenic, metabolic, and hepatic function-related pathways. The top differentially expressed genes were involved in cell growth and proliferation, neoplasia, and transcriptional regulation.</p> <p>Hepatocellular carcinomas: Dysregulated metabolic and cancer-related pathways including cell movement, growth, development, and proliferation; cancer signaling, fatty acid metabolism, cell cycle regulation, apoptosis signaling, upregulation of oncogenes, and downregulation of tumor suppressor genes.</p> <p>Hepatoblastomas: Markedly different from adjacent hepatocellular carcinomas and normal liver with upregulation of oncogenic signaling pathways (especially <i>Wnt/Ctnnb1</i>-pathway related genes), reduced hepatic metabolic function, upregulation of stem/pluripotent progenitor cell genes and stem cell-related target genes, and upregulation of genomic imprinting genes.</p>	NTP 2015
Bromodichloro-	Rat: normal mammary tissue and tumors (chronic exposure)	<p>Mammary adenocarcinomas: Eight genes significantly upregulated. Five associated with <i>Tgfb</i> pathway signaling, including its effects on matrix remodeling, mammary gland cancer progression, angiogenesis, tumor invasion, and metastasis.</p>	NTP 2015
Bromochloro-	Rat: peritoneal mesothelioma (chronic exposure)	<p>Mesotheliomas: 169 cancer-related genes differentially expressed and were categorized into binding activity, cell growth and proliferation, cell cycle progression, apoptosis, invasion, and metastasis. Important carcinogenic pathways involved in rat peritoneal mesothelioma development included insulin-like growth factor-1 (IGF-1), p38 MAPK, Wnt/β-catenin, and integrin signaling and are similar to mesotheliomas in humans.</p>	Kim <i>et al.</i> 2006
Bromochloro-	Mouse sperm (daily treatment for 14 days)	<p>Testes-expressed genes: 40 genes with altered expression involved in cell communication and adhesion, cell cycle and cell proliferation, metabolism, signal transduction, stress response, spermatogenesis, and male fertility.</p>	Tully <i>et al.</i> 2005

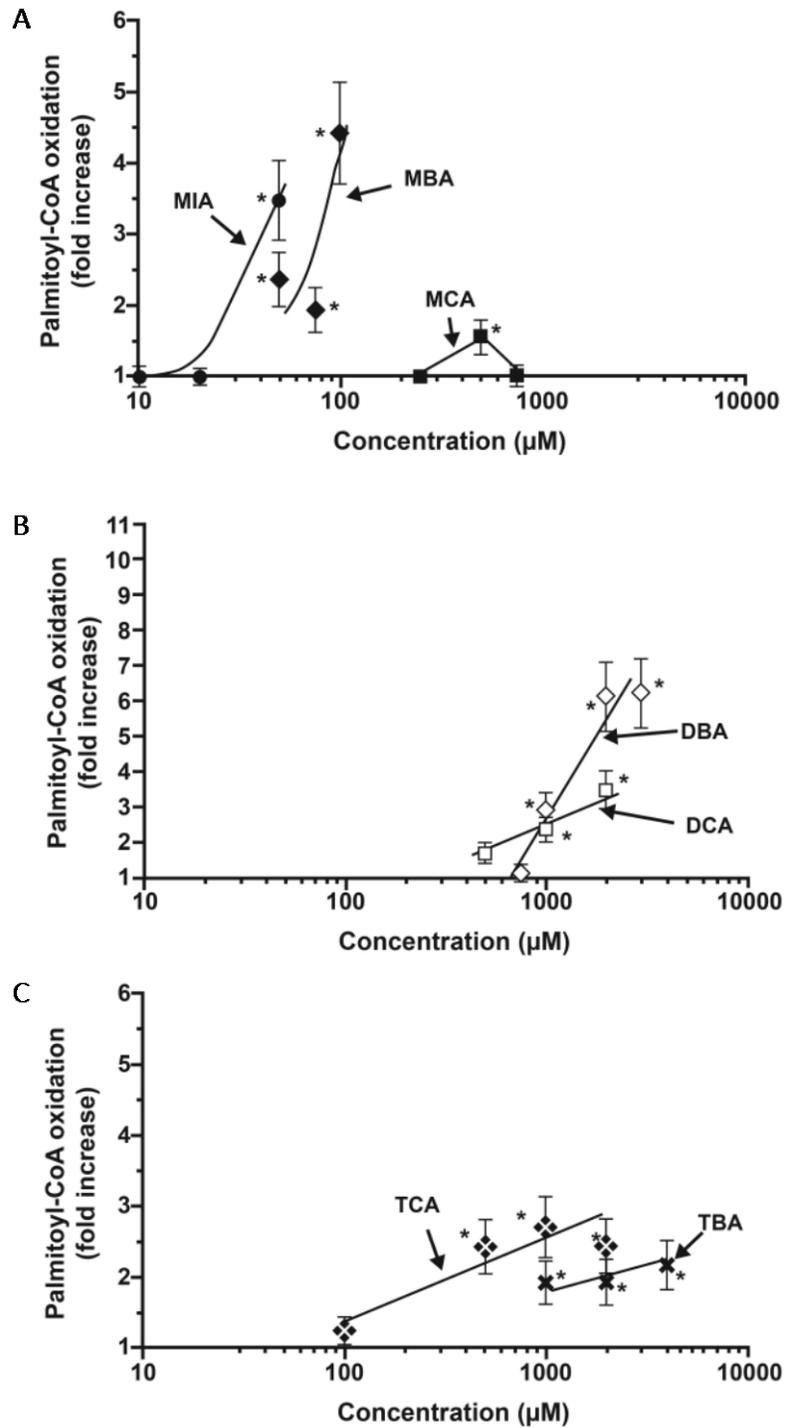


Figure D-1. Palmitoyl-CoA oxidation in cultured rat hepatocytes exposed to haloacetic acids

Source: Walgren *et al.* 2004 (used by permission from Elsevier Ireland Ltd., License No. 4061470820696).

A = monohaloacetic acids: monoiodo- (MIA), monobromo- (MBA), and monochloroacetic acid (MCA); B = dihaloacetic acids: dibromo- (DBA) and dichloroacetic acid (DCA); C = trihaloacetic acids: trichloro- (TCA) and tribromoacetic acid (TBA).

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