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

Report on Carcinogens

Monograph on Haloacetic Acids Found as Water Disinfection By-Products: Appendices

March 2018

This Page Intentionally Left Blank

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

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

Appendices

March 30, 2018

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

This Page Intentionally Left Blank

Table of Contents

List of Tables

List of Figures

This Page Intentionally Left Blank

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. Figure A-1 illustrates the overall approach to the searches and screening and the numbers of citations identified. 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.

Figure A-1. Literature search strategy and review

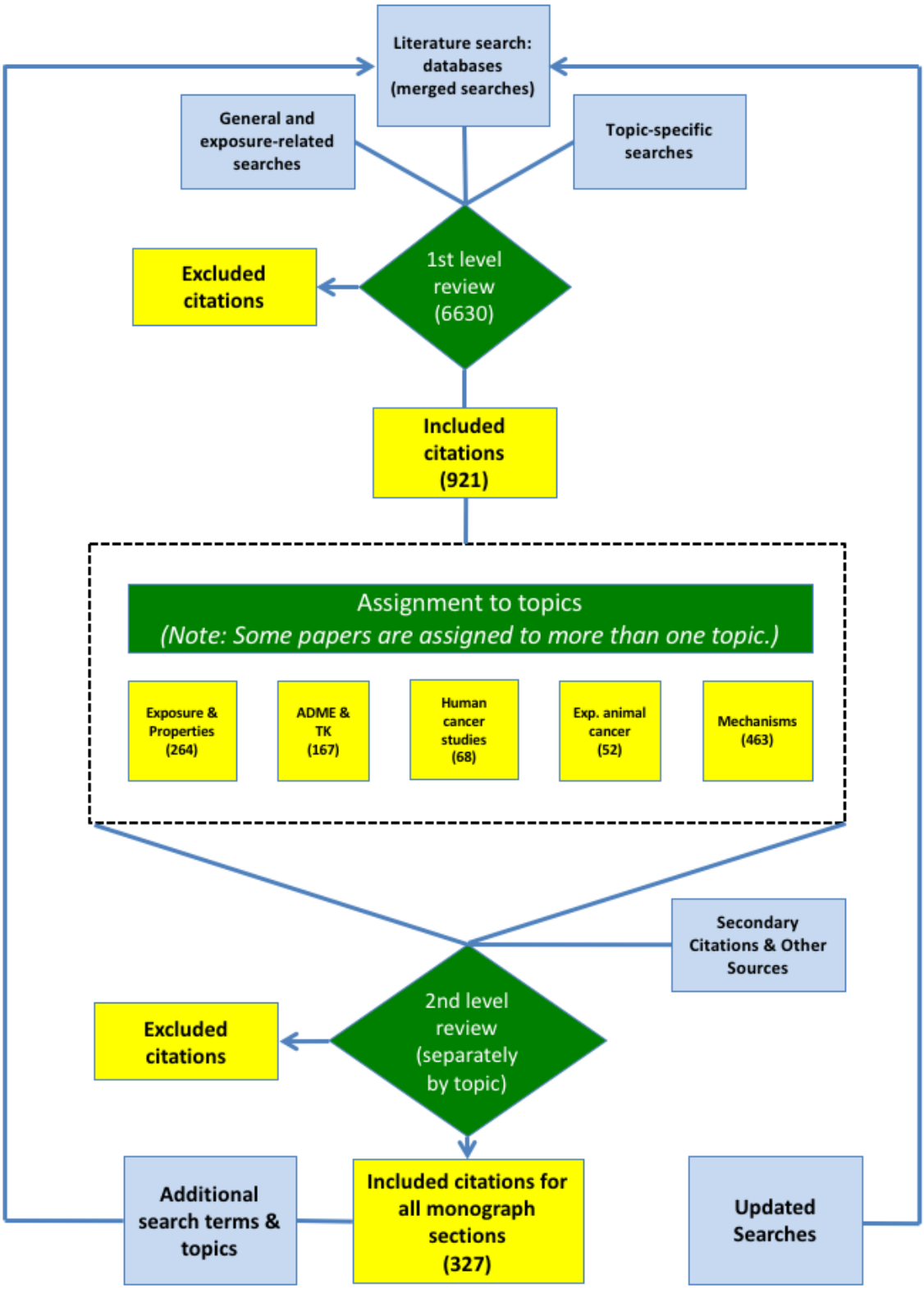

Table A-1. Major topics searched

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 Haloacetic 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 "Bichloracetic acid"[tiab] OR "Dichloracetic acid"[tiab] OR "Dichlorethanoic acid"[tiab] OR "Dichloroethanoic acid"[tiab]) OR ("Trichloroacetic Acid"[mh] OR 76-03-9[rn] OR "Trichloroacetic Acid"[tiab] OR Trichloroacetate[tiab] OR "Trichloracetic 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 "Monochloracetic acid"[tiab] OR "79-11-8"[rn] OR Chloroacetic-acid[tiab] OR "Chloroacetic acid"[nm] OR "Chloracetic 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 trichloracetic-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 diseas*"[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 "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 (TS=("Diiodoacetic acid" OR "598-89-0" OR Diiodoacetate OR "71815-43-5" OR "Bromoiodoacetic acid" OR

Bromoiodoacetate OR "Chloroiodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR Chloroiodoacetate)) 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 "Bromoiodoacetic acid" OR bromoiodoacetate OR "Chloroiodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR chloroiodoacetate)) 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 “tumorgenesis” OR “tumorgenic” OR “tumorigenesis” OR “tumorigenic” OR “tumorogenesis” OR “tumorogenic” 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 “premalignant” 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 "tumorgenesis" OR "tumorgenic" OR "tumorigenesis" OR "tumorigenic" OR "tumorogenesis" OR "tumorogenic" 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 "premalignant" 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 too 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)))

Appendix B: Disposition and Toxicokinetics

Appendix B contains information supplementing that provided in Section 3 for disposition and toxicokinetics. The three tables below contain information for pharmacokinetic or toxicokinetic paramers 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 B6C3F$_1$ mice (Table B-3).

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

HAA	Dose, mg/kg (route)	Vd (mL/kg)	AUC (mg/L•h)	Plasma T½ (h)	Clearance (mL/min•kg)	Comments	Reference
DCA	10 (i.v.) 20 (i.v.)	337 190	14.9 76.7	0.34 0.51	11.31 4.55	Two adults/dose (sex not specified). Data are mean values for two subjects.	Lukas *et al.* 1980
DCA	10 × 5 (i.v.) 25 × 5 (i.v.) 50 × 5 (i.v.)	260 270 340 290 330	increased with dose	1.06 1.84 3.14 3.50 6.23	1393.8 mg/kg/h 1089.1 mg/kg/h 881.7 mg/kg/h 950.2 mg/kg/h 723.8 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, T½ and elimination rate constants shown after each dose interval; however, the dose was not specified.	Curry *et al.* 1985
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 *et al.* 1991
DCA	46 (i.v.)	[750]	261	2.3	[5.33]	13 adults (sex not specified) with severe malaria)	Krishna *et al.* 1994
DCA.	50 (i.v.)	323	378	1.8	2.68	4 boys + 4 girls with lactic acidosis from malaria	Krishna *et al.* 1995
DCA	46 (i.v.) 46 × 2 (i.v.)	440	NR	3.4 4.4	[2.17]	8 men + 3 women with severe malaria given one dose and 9 subjects given a 2nd dose 12 h after 1st. Vd and Cl data after 2nd dose were reported as similar to those after the 1st dose.	Krishna *et al.* 1996
DCA	40 × 2 (i.v.)	618 618	NR	[7.58] [4.65]	1.0 (paleohepatic) 1.7 (neohepatic)	33 adults (sex not specified) with end-stage liver disease and liver transplant. 2nd dose 4 h after 1st dose; 2-compartment model. No clearance occurred during the anhepatic phase.	Shangraw and Fisher 1996
DCA	35 (i.v.) controls cirrhosis	 413 413	 NR	 [3.35] [6.78]	 2.14 0.78	5 men + 1 women (controls) 5 men + 2 women (cirrhosis) 2-compartment model	Shangraw and Fisher 1999
DCA	50 × 2 (i.v.)	0.51 L[a] 0.27 L[b] 0.27 L[c]	1233[a] 1863[b] NR	5.94[a] 18.15[b] 49.37[c]	[1.02][a] [0.69][b] [0.33][c]	66 men + 45 women with lactic acidosis. 2nd dose 2h after 1st . 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	Henderson *et al.* 1997

HAA	Dose, mg/kg (route)	Vd (mL/kg)	AUC (mg/L•h)	Plasma T½ (h)	Clearance (mL/min•kg)	Comments	Reference
						of these. Plasma drug clearance tended to decrease as the number of compartments required to fit the data increased.	
DCA	(50 + 50) × 2 (i.v.)	0.29 L[a] 0.28 L[b]	1954[a] 4306[b]	8.77[a] 68.63[b]	[0.42][a] [0.28][b]	15 of the 111 patients mentioned above (sex not specified) received a 2nd 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.	Henderson *et al.* 1997
DCA	Basal study 2 (oral) + 0.3 (i.v) men women Chronic study (0.02 × 14) (oral) + 2 (oral) + 0.3 (i.v) men women	 374 377 377 227	 212, 755[d] 243, 935[d] 281, 1123[d] 368, 1453[d]	 0.15 0.16 0.16 0.17	 [29.2] [26.8] [22.0] [16.0]	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 1st study. Subjects subsequently ingested low concentrations of DCA in drinking water for 14 days and on day 15 repeated protocol of 1st 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
TCA	3 (oral)	NR	NR	50.6	NR	3 men administered a single oral dose of TCA	Müller *et al.* 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 T½ 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 *et al.* 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.

[a]One-compartment model.

[b]Two-compartment model.

[c]Three-compartment model.

[d]AUC values provided for i.v. dose and oral dose, respectively.

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

Haloacetic acid	Dose/route (μmol/kg)	AUC (μM•h)	Vd_{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t½ (h)	References
Trichloro-								Schultz *et al.* 1999
Single	500 i.v.	5406 ± 144	782 ± 117	93 ± 3.0	42.1 ± 9.9	50.4 ± 11	8.0 ± 2.4	
Single	[610] oral	10,000 ± 600	485	58	NR	NR	5.8	Larson and Bull 1992
Single	[120] oral	2530 ± 70	365	36	NR	NR	7.0	
Mixture[a]	25 i.v.	1561 ± 85	287 ± 23	17.1 ± 1.4	NR	NR	12.03 ± 0.36	Saghir and Schultz 2005
GST-ζ-depleted[a]	25 i.v.	1289 ± 78	200 ± 10	19.7 ± 1.2	NR	NR	7.49 ± 0.15	
Bromodichloro-								Schultz *et al.* 1999
Single	500 i.v.	1856 ± 579	730 ± 138	286 ± 82	89 ± 2.7	197 ± 52	1.85 ± 0.30	
Single	100 i.v.	NR	573 ± 179	138 ± 41	NR	NR	3.0 ± 0.40	Saghir and Schultz 2005
Single	25 i.v.	NR	328 ± 62	279 ± 53.5	NR	NR	1.3 ± 0.25	
Mixture[a]	25 i.v.	291 ± 31	368 ± 6.0	63.9 ± 13.0	NR	NR	3.49 ± 0.14	
GST-ζ-depleted[a]	25 i.v.	306 ± 27	308 ± 21	83.9 ± 7.0	NR	NR	2.33 ± 0.10	
Chlorodibromo-								Schultz *et al.* 1999
Single	500 i.v.	1107 ± 331	636 ± 268	486 ± 153	182 ± 58	304 ± 137	1.26 ± 0.27	
Single	25 i.v.	NR	264 ± 45	128 ± 13	NR	NR	1.40 ± 0.25	Saghir and Schultz 2005
Mixture[b]	25 i.v.	246 ± 22	247 ± 25	105 ± 8	NR	NR	1.55 ± 0.21	
GST-ζ-depleted[b]	25 i.v.	199 ± 10	281 ± 12	127 ± 6	NR	NR	1.62 ± 0.13	
Tribromo-								Schultz *et al.* 1999
Single	500 i.v.	676 ± 110	449 ± 175	754 ± 116	171 ± 23	582 ± 126	0.58 ± 0.18	
Mixture[b]	25 i.v.	121 ± 36	278 ± 51	291 ± 77	NR	NR	0.76 ± 0.03	Saghir and Schultz 2005
GST-ζ-depleted[b]	25 i.v.	112 ± 5	237 ± 21	225 ± 9	NR	NR	0.85 ± 0.11	

Haloacetic acid	Dose/route (μmol/kg)	AUC (μM•h)	Vd_{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t½ (h)	References
Dichloro-								Schultz *et al.* 1999
Single	500 i.v.	2092 ± 1821	618 ± 318	267 ± 104	2.9 ± 0.5	264 ± 103	2.4 ± 0.80	Larson and Bull 1992
Single	[770] oral	750 ± 40	1000	820	NR	NR	0.9	Gonzalez-Leon *et al.* 1997
Single	[160] oral	13 ± 4	2400	2900	NR	NR	0.9	Saghir and Schultz 2002
Single	[770] i.v.	[3360 ± 1810]	618 ± 319	267 ± 105	2.9 ± 0.5	265 ± 103	2.4 ± 0.15	Saghir and Schultz 2005
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	
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	
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 *et al.* 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 *et al.* 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 (μmol/kg)	AUC (μM•h)	Vd_{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t½ (h)	References
(−)Bromochloro-								Schultz and Sylvester 2001
Single	520 i.v.	74.8 ± 9.0	380 ± 41	3712 ± 140	31.4 ± 9.5	3693 ± 155	0.07 ± 0.01	Saghir and Schultz 2005
GST-ζ-depleted	520 i.v.	584 ± 135	417 ± 139	484 ± 142	17.4 ± 10.8	468 ± 143	0.40 ± 0.02	NTP 2009
Mixture[a]	25 i.v.	1.7 ± 0.1	680 ± 103	7660 ± 478	NR	NR	0.06 ± 0.01	
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-								Schultz and Sylvester 2001
Single	520 i.v.	234 ± 25	587 ± 104	1,248 ± 132	13.2 ± 3.0	1,236 ± 127	0.40 ± 0.09	Saghir and Schultz 2005
GST-ζ-depleted	520 i.v.	487 ± 119	467 ± 168	591 ± 136	13.4 ± 7.4	580 ± 139	0.44 ± 0.04	NTP 2009
Mixture[a]	25 i.v.	7.2 ± 0.6	393 ± 34	1,773 ± 184	NR	NR	0.19 ± 0.01	
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-								Schultz *et al.* 1999
Single	500 i.v.	1,120 ± 362	400 ± 112	491 ± 116	12.9 ± 4.0	490 ± 137	0.72 ± 0.12	Saghir and Schultz 2005
Mixture[a]	25 i.v.	2.4 ± 0.1	987 ± 142	10,540 ± 312	NR	NR	0.08 ± 0.01	NTP 2007a
GST-ζ-depleted[a]	25 i.v.	13.2 ± 2.5	599 ± 68	2,390 ± 71	NR	NR	0.22 ± 0.02	
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	Saghir *et al.* 2001
	[790] i.v.	NR	1060	262	154	NR	5.40	
	[110] i.v.	NR	3033	750	546	NR	3.25	

Data in brackets indicate unit conversions: (dose mg/kg•1000 μg/mg)/(MW μg/μmol) = dose μmoles/kg, (AUC μg/mL•hr •1000 ng/μg)/(MW ng/nmol) = AUC μM•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, t½ = half life of elimination, NR = not reported.

[a]Administered as a mixture containing trichlor-, bromodichloro-, bromochloro-, and dibromoacetic acid.

[b]Administered 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 (μM•h)	Vd_{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t½ (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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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-								NTP 2007a
single[d]	[320] oral	[6.5; 5.6]	NR	NR	NR	NR	0.36; 0.33	
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 (μM•h)	Vd_{ss} (mL/kg)	Total body Cl (mL/kg/h)	Renal Cl (mL/kg/h)	Non-renal Cl (mL/kg/h)	t½ (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-								NTP 2009
Single[d]	[580] i.v.	[83.3; 97.1]	NR	[6900; 5930]	NR	NR	0.08; 0.08	

Data in brackets indicate unit conversion: Cl μg/mL•hr •1000 ng/μg)/(MW ng/nmol) = Cl μM•hr] or (AUC μg/mL•hr •1000 ng/μg)/(MW ng/nmol) = AUC μM•hr).
Cl = clearance, Vd_{ss} = apparent steady state volume of distribution, t½ = half life of elimination, NR = not reported.
[a]Animals 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.
[b]8-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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival and body weight was not significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Confounding	

Study utility domain and question	Rating and rationale
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.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Confounding	

Study utility domain and question	Rating and rationale
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.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	++ 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 (sensitivity)	+++ 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	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ 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.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	++ Two exposure levels were used that spanned a range of 2 fold, making the detection of a dose response limited.
Outcome	

Study utility domain and question	Rating and 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	++ 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 (sensitivity)	+++ 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	
Chemical characterization	+++ Chemical purity and stability were well characterized.

Study utility domain and question	Rating and rationale
Dosing regimen	++ There was a significnat 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.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	++ 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.

Study utility domain and question	Rating and rationale
Statistical power (sensitivity)	+++ Moderate numbers of animals (29-32) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ 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.
Exposure duration (sensitivity)	+++ A near-lifespan duration (104 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near-lifespan duration (104 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	Not reported.
Controls	+++ A concurrent negative control of acetic acid was used.
Historical data	No
Animal model (sensitivity)	+ The sex of the mice were not reported. The strain of mouse is unfamiliar.
Statistical power (sensitivity)	+ Small and insufficient numbers of animals (12) were used for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ There was no significant difference in survival and body weight was not reported. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ A short duration was used (27 weeks), but initiaion/promotion studies are normally short.
Dose-response (sensitivity)	+ 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 60.05, 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.
Study duration (sensitivity)	+++ A short duration was used (30 weeks), but initiation/promotion studies are usually short.
Confounding	

Study utility domain and question	Rating and rationale
Confounding	+ Disease surveillance, animal husbandry, or chemical characterization were not reported.
Reporting and analysis	
Reporting data and statistics	Not reported.
Combining lesions	+++ Only benign papillomas were reported.

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 and question	Rating and rationale
Study design	
Randomization	Not reported.
Controls	+++ Concurrent vehicle controls of sodium chloride at equal molar concentrations as the high dose were used.
Historical data	Yes
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ The exact number of animals was not clearly reported. It appears to have been between 21 to 33 for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ 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.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	++ 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.
Study duration (sensitivity)	+++ 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.
Confounding	

Study utility domain and question	Rating and rationale
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.

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 and question	Rating and rationale
Study design	
Randomization	Not reported.
Controls	+++ Concurrent controls were exposed to deionized water (vehicle). NaCl was omitted from the water of control animals becuase 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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ The exact number of animals was not clearly reported. It appears to have been between 27 to 28 for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ At sacrifice, the mean body weight of the animals (1.6 g/l) was significantly reduced to 73% of the control value.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	+ One exposure level was used, which cause toxicity, requiring dose reduction.
Outcome	

Study utility domain and question	Rating and rationale
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.
Study duration (sensitivity)	+++ A near life-span duration (103 weeks exposure period) was used.
Confounding	
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.

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): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ The number of animals was sufficient (23-29) for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ There was no clear significant difference in survival and body weight was not reported. There was a non-significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ Near life-span duration (104 weeks) were used.

Study utility domain and question	Rating and rationale
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ Near life-span and less than near life-span durations (104 weeks) were used.
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.

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-3d. DeAngelo *et al.* 1991 (M Mouse [Study 1]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Sufficient numbers of animals (27-30) were used for each group.
Exposure	

Study utility domain and question	Rating and rationale
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival and there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.
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.

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-3e. DeAngelo *et al.* 1991 (M Mouse [Study 2]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and 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.

Study utility domain and question	Rating and rationale
Historical data	No
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	+ Small and insufficient numbers of animals (10-12) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival and there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A less than life-span durations (60 weeks for the high exposure group and 75 weeks for the other exposure group) were used.
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.
Combining lesions	+++ Only hepatocellular adenomas and carcinomas were combined.

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-3f. DeAngelo *et al.* 1999 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ The numbers of animals (53-16) varied considerably for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was a significant decrease in survival and body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (90-100 weeks) was used.
Dose-response (sensitivity)	+++ 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.
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.
Study duration (sensitivity)	+++ A near life-span duration (90-100 weeks) was used.
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	Tumor types were not combined.

Overall utility: ++. Only male mice, initially confirmed pathogen free, were used with differing numbers per group (as low was 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-3g. Herren-Freund *et al.* 1987 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-26) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
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 and question	Rating and rationale
Combining lesions	+++ Tumor types were not combined.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-26) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
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 and question	Rating and rationale
Combining lesions	+++ Tumor types were not combined.

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-3i. Wood *et al.* 2015 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	Not reported.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	++ 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	
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	+++ There was no significant difference in survival, but there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ Exposure duration was short (10 weeks), though the observation duration was near life-span (94 weeks).
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (94 weeks) was used.
Confounding	
Confounding	++ Continual disease surveillance was not reported.

Study utility domain and question	Rating and rationale
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.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	Not reported.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	++ 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	
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	+++ There was no significant difference in survival, but there was a significant decrease in body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ Exposure duration was short (10 weeks), though the observation duration was near life-span (94 weeks).
Dose-response (sensitivity)	++ 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.

Study utility domain and question	Rating and rationale
Consistency between groups	+++ Groups were not reported to have been treated differently.
Study duration (sensitivity)	+++ A near life-span duration (94 weeks) was used.
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.

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-3k. Pereira 1996 (F Mouse [Study 1]): Dichloroacetic acid (DCA): Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ The number of animals (15-90) varied considerably for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (576 days) was used.
Dose-response (sensitivity)	+++ Three exposure levels were used that spanned a range of 10 fold.

Study utility domain and question	Rating and rationale
Outcome	
Pathology	++ Only the liver was histologically examined.
Consistency between groups	+++ Groups were not reported to have been treated differently.
Study duration (sensitivity)	+++ A near life-span duration (576 days) was used.
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.

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-3l. Pereira 1996 (F Mouse [Study 2]): Dichloroacetic acid (DCA): Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ The number of animals (15-90) varied considerably for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.

Study utility domain and question	Rating and rationale
Exposure duration (sensitivity)	++ A less than life-span duration (360 days) was used.
Dose-response (sensitivity)	+++ 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 duration (sensitivity)	+++ A less than life-span duration (360 days) was used.
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.

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

Study utility domain and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ Small, but sufficient numbers of animals (>20) were used for each group.
Exposure	
Chemical characterization	+ Chemical purity and stability were not reported.

Study utility domain and question	Rating and rationale
Dosing regimen	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A less than near life-span duration (48 weeks) was used, but initiation/promotion studies are normally short.
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.

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-3n. Bull *et al.* 1990 (M Mouse): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	+ Small numbers of animals (11-24) were used for each group.

Study utility domain and question	Rating and rationale
Exposure	
Chemical characterization	++ Chemical purity and stability were not verified.
Dosing regimen	+++ There were no deaths and body weight was not reported. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (52 weeks) was used.
Dose-response (sensitivity)	++ 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.
Study duration (sensitivity)	+++ A less than life-span duration (52 weeks) was used.
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.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Moderate numbers of animals (20-24) were used for each group..
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

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-3p. NTP 2007b (M & F Mouse [Study 1]): Dichloroacetic acid: Dermal

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ 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.
Exposure duration (sensitivity)	+++ While the duration was short (39 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (39 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Confounding	
Confounding	+++ The test agent was well characterized, animals were treated the same between groups, and were continually monitored for infectious diseases.

Study utility domain and question	Rating and rationale
Reporting and analysis	
Reporting data and statistics	+++ Appropriate statistical analyses were reported.
Combining lesions	+++ Tumor types were not combined.

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-3q. NTP 2007b (M Mouse [Study 2]): Dichloroacetic acid: Dermal

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ 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.
Exposure duration (sensitivity)	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.

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

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 1]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival and no significant decrease in body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ Three exposure levels were used that spanned a range of 16 fold.
Outcome	

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

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 (F Mouse [Study 1]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.

Study utility domain and question	Rating and rationale
Dosing regimen	++ 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.
Exposure duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
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.

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 (M Mouse [Study 2]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No

Study utility domain and question	Rating and rationale
Animal model (sensitivity)	+ 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 (sensitivity)	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+ There was no significant difference in survival and no significant decrease in body weight. There was no significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
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.

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 (F Mouse [Study 2]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was a significant decrease in survival and body weight. There was no significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
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.

Study utility domain and question	Rating and rationale
Combining lesions	+++ Tumor types were not combined.

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 (M Mouse [Study 3]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Exposure duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Confounding	

Study utility domain and question	Rating and 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.

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 (F Mouse [Study 3]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	+ Small and insufficient numbers of animals (10) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Exposure duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
Dose-response (sensitivity)	+++ 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.

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

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 (M Mouse [Study 4]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used..
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was no significant difference in survival, but there was a significant decrease in body weight. There was no significant difference in neoplasm incidence.
Exposure duration (sensitivity)	++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.

Study utility domain and question	Rating and rationale
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ While the duration was short (26 weeks) compared to carcinogenicity studies, it was performed in transgenic mice prone to developing cancer.
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.

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 (F Mouse [Study 4]): Dichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls of 99.9% acetone were used.
Historical data	No
Animal model (sensitivity)	+ 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 (sensitivity)	++ Small and insufficient numbers of animals (15) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.

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

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival and body weight was not significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ 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.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ 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.
Exposure duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (106 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival, but there was a significant decrease in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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.* 1997 (M Rat): Trichloroacetic acid: Drinking water

Study utility domain and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Moderate numbers of animals (29-32) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was no significant difference in survival, but there was a significant increase in body weight. There was no significant difference in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near-lifespan duration (104 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near-lifespan duration (104 weeks) was used.
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.

Overall utility: +++. A well conducted study on almost all aspects, but only involved male rats.

Table C-6b. DeAngelo 2008 (M Mouse [Study 1]): Trichloroacetic acid: Drinking water

Study utility domain and question	Rating and 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
Animal model (sensitivity)	+++ Only males of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Moderate numbers of animals (30) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ A less than life-span duration (60 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	++ A less than life-span duration (60 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Only males of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ A large number of animals (42-51) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Mice were randomly assigned to texposure groups.
Controls	+++ Concurrent vehicle controls of neutralized drinking water was used.
Historical data	No
Animal model (sensitivity)	+++ Only males of non-transgenic animals were used and the strain is in common use.
Statistical power (sensitivity)	+++ Large numbers of animals (51-53) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	++ There was no significant difference in survival or body weight and there was a significant increase in neoplasm incidence, but the highest level was low compared to the other studies by this author.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	++ Two expose 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

Overall utility: ++. 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.

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

Study utility domain and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-26) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	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
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-26) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Survival was not reported and body weight was significantly decreased. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
Dose-response (sensitivity)	+ 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.
Study duration (sensitivity)	+++ A less than life-span duration (61 weeks) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ The number of animals (15-90) varied considerably for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (576 days) was used.
Dose-response (sensitivity)	+++ 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 duration (sensitivity)	+++ A near life-span duration (576 days) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ The number of animals (15-90) varied considerably for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ Survival was not reported, but there was a significant decreased in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	++ A less than life-span duration (360 days) was used.
Dose-response (sensitivity)	+++ 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 duration (sensitivity)	+++ A less than life-span duration (360 days) was used.
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.

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 and question	Rating and 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
Animal model (sensitivity)	++ Only females of non-transgenic animals were used.
Statistical power (sensitivity)	++ Small, but sufficient numbers of animals (>20) were used for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+++ There was no significant decrease in survival or body weight and there was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ 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 (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A less than near life-span duration (48 weeks) was used, but initiation/promotion studies are normally short.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups.
Controls	+++ Concurrent vehicle controls were used.
Historical data	No
Animal model (sensitivity)	++ Only males of non-transgenic animals were used.
Statistical power (sensitivity)	+ Small numbers of animals (11-24) were used for each group.
Exposure	
Chemical characterization	++ Chemical purity and stability were not verified.
Dosing regimen	+++ There were no deaths and body weight was not reported. There was a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A less than life-span duration (52 weeks) was used.
Dose-response (sensitivity)	++ 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.
Study duration (sensitivity)	+++ A less than life-span duration (52 weeks) was used.
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.

Study utility domain and question	Rating and rationale
Combining lesions	++ Only total lesions were adequately reported to allow for quantitation of the results. These included liver hyperplasiic nodues, hepatocellular adenoma, and hepatocellular carcinoma, which entail and continuum of the same disease process, however there were 4/73 lesions types that were also included, but were not hyperplastic or neoplastic.

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 and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used. Modifying factor is age of animals at study start.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-24) were used for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+ There was no significant difference in survival and body weight was not reported. There was no significant difference in neoplasm incidence.
Exposure duration (sensitivity)	+ Two doses were administered at the beginning of the study, no additional exposure occurred throughout the study.
Dose-response (sensitivity)	++ 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.

Study utility domain and question	Rating and rationale
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.
Study duration (sensitivity)	+++ Duration was near life-span (20 months).
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.

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-6l. Von Tungeln *et al.* 2002 (M+F Mouse [Study 2]): Trichloroacetic acid: ip injection

Study utility domain and question	Rating and 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
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used. Modifying factor is age of animals at study start.
Statistical power (sensitivity)	++ Moderate numbers of animals (22-24) were used for each group.
Exposure	
Chemical characterization	Chemical purity and stability were not reported.
Dosing regimen	+ 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 utility domain and question	Rating and rationale
Exposure duration (sensitivity)	+ Two doses were administered at the beginning of the study, no additional exposure occurred throughout the study.
Dose-response (sensitivity)	++ 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.
Study duration (sensitivity)	++ Duration was less than life-span (12 months).
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was no significant decrease in survival, but there was a decrease in body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ controls adequate number
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (49-51) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was a significant decrease in survival and body weight and a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (104 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ Large numbers of animals (50-51) were used for each group.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ There was a significant decrease in survival and body weight, with a significant increase in neoplasm incidence.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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 and question	Rating and rationale
Study design	
Randomization	+++ Animals were randomly assigned to exposure groups, but were not blinded.
Controls	+++ Concurrent vehicle controls were used.
Historical data	Yes
Animal model (sensitivity)	+++ Both sexes of non-transgenic animals were used.
Statistical power (sensitivity)	+++ There was no significant decrease in survival, but there was a decrease in body weight and a significant increase in neoplasm incidence.
Exposure	
Chemical characterization	+++ Chemical purity and stability were well characterized.
Dosing regimen	+++ Three exposure levels were used that spanned a range of 4 fold.
Exposure duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
Dose-response (sensitivity)	+++ 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.
Study duration (sensitivity)	+++ A near life-span duration (105 weeks) was used.
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.

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

Studies in this section are grouped by number of halogen substitutions on the alpha carbon of acetic acid (mono- to di- to tri-haloacetic acids) followed by increasing electrophilicity of the HAA (i..e, chloro- to bromo- to iodoacetic acid; dichloro to dibromoacetic acid; trichloro- to tribromo- to bromodichloroacetic acid). This format follows the text in the monograph. Animals for a given study are grouped by male then female rats first, followed by male then female mice; monochloroacetic acid is the only gavage study and is listed first, followed by drinking water studies.

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% **Treatment:** Gavage 0 15 30 mg/kg bw 5 doses/week x 104 weeks	**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	
		30	1/53 (2%)	
		Liver – Carcinoma		
		0	0/53	
		15	1/53 (2%)	
		30	0/53	
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: =0.0.059N **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% **Treatment**: Gavage 0 50 100 mg/kg in deionized water 5 doses/week x 104 weeks	**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%)	
		100	2/60 (3%)	
		Liver – Carcinoma		
		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% **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 – 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%)	
		500	0/23	
		2,000	1/25 (4%)	
		Liver – Carcinoma		
		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		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
DeAngelo *et al.* 1996 **Animal:** Rat (Study 2) F344 M 28-30 days (59-79g bw) **Study duration:** 103 weeks	**Agent**: Dichloroacetic acid >99% **Treatment**: Drinking water 0+ 2,500++ mg/L in deionized water ad libitum x 103 weeks + NaCl in the first experiment had no effect on water consumption or tumor incidence, so was not included here ++ 1,500 mg/L at 8 weeks then 1,000 mg/L after 26 weeks due to mild transient neurotoxicity	**Liver – Adenoma** 0 2,500 **Liver – Carcinoma** 0 2,500 **Liver – Adenoma or carcinoma** 0 2,500	 0/33 3/28 (10.7%) 1/33 (3%) 6/28* (21.4%) 1/28 (3%) 8/27** (28.6%)	**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 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Richmond *et al.* 1995 **Animal:** Rat F344 M 28 days **Study duration:** 104 weeks	**Agent**: Dichloroacetic acid Not reported **Treatment**: Drinking water 0+ 50 500 2,400 mg/L in drinking water (pH 7.0) x 60++ or 104 weeks + 2,000 mg/L NaCl ++ high dose (2,400 mg/L) stopped at 60 weeks due to tumors and hind limb paralysis	**Liver – Adenoma** 0 50 500 **Liver – Carcinoma** 0 50 500	 1/23 (4%) 0/26 6/29 (21%) 0/23 0/26 3/29 (10%)	**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 significantly increased at 2,400 mg/L (all high-dose animals were sacrificed at 60 d due to hind limb paralysis and tumors.. 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). **Interim (60d) values:** Adenoma: 0/7, 0/7, 0/7, 7/27 (26%); Carcinoma: 0/7, 0/7, 0/7, 1/27 (4%) **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 **Animal:** Mouse (Study 1) B6C3F1 M 28 days **Study duration:** 60 weeks (high dose), 75 weeks (low and medium	**Agent**: Dichloroacetic acid >99% **Treatment**: Drinking water 0+ 50 500	**Liver – Adenoma** 0 50 500 5,000 **Liver – Carcinoma**	 0/28 2/29 (7%) 1/27 (4%) 24/30*** (80%)	**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

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
dose levels)	5,000++ mg/l in distilled water (pH 6.8-7.2) ad libitum x 75 weeks + 2,000 mg/l NaCl ++ Exposure duration was reduced to 60 weeks	0	2/28 (7%)	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 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.
		50	6/29 (21%)	
		500	2/27 (7%)	
		5,000	25/30*** (83%)	
		Liver – Adenoma or carcinoma		
		0	2/28 (7%)	
		50	7/29 (24%)	
		500	3/27 (11%)	
		5,000	27/30*** (90%)	
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	**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
		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	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	+ 1,500 mg/l acetic acid	3,500	12/12*** (100%)	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.
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 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)	**Liver – Adenoma** 0 500 1,000 2,000 3,500 **Liver – Carcinoma** 0 500 1,000 2,000 3,500	 5/53 (10%) 5/25 (20%) 21/41* (51.4%) 11/25* (42.9%) 7/16* (45%) 14/53 (26%) 12/25 (48%) 29/41*** (71%) 24/25*** (95%) 16/16*** (100%)	**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 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		[Trend p-value <0.001]		**Strengths and limitations**: Only male mice, initially confirmed pathogen free, were used with differing numbers per group (as low was 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 **Animal:** Mouse B6C3F1 M 28 days **Study duration:** 94 weeks	**Agent**: Dichloroacetic acid Not reported **Treatment**: Drinking water 0 1,000 2,000 3,500 mg/L deionoized water (pH 6.8-7.1) ad libitum x 10 weeks	**Liver – Adenoma** 0 1,000 2,000 3,500 Trend p-value: <0.05 **Liver – Carcinoma** 0 1,000 2,000 3,500	 5/27 (19%) 13/27 (48%) 11/27 (41%) 15/26* (58%) 8/27 (30%) 8/27 (30%) 6/27 (22%) 19/26* (73%)	**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% 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		Trend p-value: <0.01		**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.
		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 **Animal:** Mouse B6C3F1 F 28 days **Study duration:** 94 weeks	**Agent**: Dichloroacetic acid Not reported **Treatment**: Drinking water 0 1,000 2,000 mg/L in deionoized water (pH 6.8-7.1) ad libitum x 10 weeks	**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% 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
		0	0/27	
		1,000	9/26** (35%)	
		2,000	6/28 (21%)	
		Liver – Carcinoma		
		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	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		Liver – Adenoma, carcinoma, and hepatoblastoma		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	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 + 20 mmol/L NaCl ++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol +++ intermittent cycles of 24 days on, 48 days off	**Liver – Adenoma**		**Survival**: Not reported **Body weight**: The high dose level of DCA was caused signficant 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 was tested, with only their livers examined histologically. The study duration was near life-span.
		0	2/90 (2.2%)	
		260	3/50 (6%)	
		860	7/28* (25%)	
		2,600 intermittent	3/34 (8.8%)	
		2,600	16/19* (84.2%)	
		Liver – Carcinoma		
		0	2/90 (2.2%)	
		260	0/50	
		860	1/28 (3.6%)	
		2,600 intermittent	1/34 (2.9%)	
		2,600	5/19[**] (26.3%)	
		[Trend p-value: <0.001]		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Pereira 1996 **Animal:** Mouse (Study 2) B6C3F1 F 7-8 weeks **Study duration:** 360 days	**Agent**: Dichloroacetic acid (DCA) Not reported **Treatment**: Drinking water 0+ 260++ 860++ 2,600+++ 2,600++ mg/L in drinkign water ad libitum x 360 days + 20 mmol/L NaCl ++ Concentrations were reported at mmol/L and NTP converted them to mg/L based on a mw of 28.942g/mol +++ intermittent cycles of 24 days on, 48 days off	**Liver – Adenoma** 0 260 860 2,600 intermittent 2,600 **Liver – Carcinoma** 0 260 860 2,600 intermittent 2,600	 1/40 (2.5%) 0/40 3/20 (15%) 0/15 7/20* (35%) 0/40 0/40 0/20 0/15 1/20 (5%)	**Survival**: Not reported **Body weight**: The high dose level of DCA was caused signficant 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 was tested, with only their livers examined histologically. Study duration was less than life-span.
Bull *et al.* 1990 **Animal**: Mouse B6C3F1 M 5 weeks **Study duration:** 52 weeks	**Agent**: Dichloroacetic acid Analytical grade **Treatment**: Drinking water 0 1,000 2,000 mg/L in drinking	**Liver – Adenoma** 0 1,000 2,000 **Liver – Carcinoma** 0 1,000	 0/2 0/1 2/10 (20%) 0/2 0/1	**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

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	water (pH 6.8-7.2) ad libitum x 52 weeks	2,000	5/10 (50%)	evaluated. Results were reported so that incidences of specific neoplasms could not be determined, but could be estimated.
Daniel *et al.* 1992 **Animal:** Mouse B6C3F1 M 28 days **Study duration:** 104 weeks	**Agent**: Dichloroacetic acid >95% **Treatment**: Drinking water 0 0 500 500 mg/l in distilled water (pH 6.8-7.2) ad libitum x 104 weeks 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.	**Liver – Adenoma** 0 500 **Liver – Carcinoma** 0 500 **Liver – Adenoma or carcinoma** 0 500	 1/20 (5%) 10/24** (42%) 2/20 (10%) 15/24** (63%) 3/20 (15%) 18/24** (75%)	**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 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 **Animal:** Mouse B6C3F1 M 6 weeks **Study duration:** 106 weeks	**Agent**: Dibromoacetic acid >99% **Treatment**: Drinking water 0 50 500	**Liver – Adenoma[a]** 0 50 500 1,000 Trend p-value: <0.001	 18/49 (42%) 37/50***[b] (78%) 37/50***[b] (80%) 42/50***[b] (89%)	**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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	1,000 mg/L of drinking water ad libitum x 106 weeks Average daily dose: 0 - 4, 45, 87 mg/kg	**Liver – Carcinoma[a]**		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.
		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%)	
		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		

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% **Treatment**: Drinking water 0 50 500 1,000 mg/L of drinking water ad libitum x 106 weeks Average daily dose: 0 - 4, 35, 65 mg/kg	**Liver – 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. 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.
		0	19/49 (41%)	
		50	26/50 (57%)	
		500	32/50**[f] (70%)	
		1,000	35/49***[f] (76%)	
		Trend p-value: <0.001		
		Liver – Carcinoma[a]		
		0	3/49 (7%)	
		50	3/50 (7%)	
		500	12/50**[g] (27%)	
		1,000	8/49 (18%)	
		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	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2009 **Animal:** Rat F344/N M 6-7 weeks **Study duration:** 105 weeks	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	**Liver – Adenoma[a]**		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. **Strengths and limitations**: A very high quality study, with no major concerns.
		0	2/50[i] (4.6%)	
		250	0/50	
		500	3/50[i] (7.5%)	
		1,000	4/50[i] (9.5%)	
NTP 2009 **Animal:** Rat F344/N F 6-7 weeks **Study duration:** 105 weeks	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	**Liver – Adenoma[a]**		**Survival**: No significant difference: 34/50 - 31/50, 37/50, 35/50 **Body weight**: 1,000 mg/l group was <10% of controls. after 85 weeks. Significantly increased pre-neoplastic lesions: Lung alveolar epithelium hyperplasia occurred at increased incidences. **Strengths and limitations**: A very high quality study, with no major concerns.
		0	0/50	
		250	0/50	
		500	0/50	
		1,000	3/50[j] (6.6%)	
		Trend p-value: =0.012		
NTP 2009 **Animal:** Mouse B6C3F1 M 6-7 weeks **Study duration:** 105 weeks	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	**Liver – Adenoma[a]**		**Survival**: 38/50 - 35/50, 30/50, 21/50 **Body weight**: 1,000 mg/l group was 12% lower than controls after 97 weeks. **Strengths and limitations**: A very high quality study, with no major concerns.
		0	27/50 (58.7%)	
		250	40/50**[k] (83.6%)	
		500	40/50**[k] (83.7%)	
		1,000	31/50 (67.4%)	
		Liver – Carcinoma[a]		
		0	19/50 (39.6%)	
		250	25/50[l] (52.5%)	
		500	36/50***[l] (76.9%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		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%)	
		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 **Animal:** Mouse B6C3F1 F 6-7 weeks **Study duration:**	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0	**Liver – Adenoma[a]**		**Survival**: 36/50 - 42/50, 32/50, 40/50 **Body weight**: No significant difference. **Strengths and limitations**: A very high quality study, with no major concerns.
		0	27/50 (59.4%)	
		250	48/50***[o] (96%)	
		500	44/50***[o] (90.9%)	
		1,000	46/50***[o] (95.2%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
105 weeks	250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	Trend p-value: <0.001		
		Liver – Carcinoma[a]		
		0	14/50[p] (31.1%)	
		250	23/50[p] (48.3%)	
		500	26/50*[p] (56.1%)	
		1,000	20/50[p] (42.3%)	
		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) **Animal:** Rat F344/N M 28-30 days **Study duration:** 104 weeks	**Agent**: Trichloroacetic acid >99% **Treatment**: Drinking water 0+ 2,500++ mg/L in drinking water (pH 6.9-7.1) ad libitum x 104 weeks + 31-32 mM NaCl (~isomolar to 5,000 mg/l of TCA) ++ 1,500 mg/L x 8 weeks then 1,000 mg/L	**Liver – Adenoma**		**Survival**: No significant difference in survival: 23/29 - 24/32, 19/32, 22/29 **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).
		0	1/23 (4%)	
		50	1/24 (4%)	
		500	3/20 (15%)	
		5,000	1/22 (5%)	
		Liver – Carcinoma		
		0	0/23	
		50	0/24	
		500	0/20	
		5,000	1/22 (5%)	
		Liver – Adenoma or carcinoma		
		0	1/23 (4%)	
		50	1/24 (4%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	after 24 weeks, because of significant differences in body weight gain	500	3/20 (15%)	**Strengths and limitations**: A well conducted study on almost all aspects, but only involved male rats.
		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 99% **Treatment**: Drinking water 0+ 50 500 5,000 mg/L in drinking water ad libitum x 60 weeks + 2,000 mg/L NaCl	**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.
		0	2/30 (7%)	
		50	4/27 (15%)	
		500	6/29 (21%)	
		5,000	11/29[r] (38%)	
		Liver – Carcinoma		
		0	2/30 (7%)	
		50	1/27 (4%)	
		500	6/29 (21%)	
		5,000	11/29[r] (38%)	
		Liver – Adenoma or carcinoma		
		0	4/30 (13%)	
		50	4/27 (15%)	
		500	11/29[r] (38%)	
		5,000	16/29[r] (55%)	
DeAngelo *et al.* 2008 **Animal:** Mouse (Study 2) B6C3F1 M 28-30 days **Study duration:** 104 weeks	**Agent**: Trichloroacetic acid 99% **Treatment**: Drinking water 0+ 4,500 mg/L in drinking water ad libitum x 104 weeks	**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
		0	0/25	
		4,500	21/36[r] (59%)	
		Liver – Carcinoma		
		0	3/25 (12%)	
		4,500	28/36[r] (78%)	
		Liver – Adenoma or carcinoma		
		0	3/25 (12%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	+ 1.5 g/L of neutralized acetic acid	4,500	32/36[r] (89%)	animals. Most organs were histologically evaluated and evaluations were confirmed by an independent pathologist.
DeAngelo *et al.* 2008 **Animal:** Mouse (Study 3) B6C3F1 M 28-30 days **Study duration:** 104 weeks	**Agent**: Trichloroacetic acid 99% **Treatment**: Drinking water 0 50 500 mg/L in neutralized drinking water ad libitum x 104 weeks	**Liver – Adenoma**		**Survival**: No difference in survival: 34/51 - 29/53, 27/51 **Body weight**: No reported. 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. Other comments: Denominators of incidences are based on surviving mice. No difference in water consumption occurred. **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.
		0	9/42 (21%)	
		50	8/35 (23%)	
		500	19/37[r] (51%)	
		Liver – Carcinoma		
		0	23/42 (55%)	
		50	14/35 (40%)	
		500	29/37[r] (78%)	
		[Trend p value: <0.01]		
		Liver – Adenoma or carcinoma		
		0	27/42 (64%)	
		50	20/35 (57%)	
		500	32/37[r] (87%)	
Herren-Freund *et al.* 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%)	
		5,000	8/22** (36%)	
		Liver – Carcinoma		
		0	0/22	
		5,000	7/22** (32%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
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** 0 330 1,100 3,300 **Liver – Carcinoma** 0 330 1,100 3,300 [Trend p-value: <0.001]	 2/90 (2.2%) 4/53 (7.6%) 3/27 (11.1%) 7/18* (38.9%) 2/90 (2.2%) 0/53 5/27** (18.5%) 5/18** (27.8%)	**Survival**: Not reported **Body weight**: The high dose level of DCA was caused signficant 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.
Pereira 1996 **Animal:** Mouse (Study 2) B6C3F1 F 7-8 weeks	**Agent**: Trichloroacetic acid (TCA) Not reported **Treatment**:	**Liver – Adenoma** 0 330 1,100	 1/40 (2.5%) 3/40 (7.5%) 3/19 (15.8%)	**Survival**: Not reported **Body weight**: The high dose level of DCA was caused signficant weight loss after 35 weeks and beyond, while the high dose of TCA caused it at 51 weeks, with near significant decreases beyond.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Study duration: 360 days	Drinking water 0+ 330++ 1,100++ 3,300++mg/L in filtered and deionized water, pH 6.5-7.5 ad libitum x 360 days + 20 mmol/L NaCl ++ Concentrations were reported as mmol/L and were calculated to mg/L based on a mw of 163.3869g/mol	3,300	2/20 (10%)	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.
		Liver – Carcinoma		
		0	0/40	
		330	0/40	
		1,100	0/19	
		3,300	5/20* (25%)	
Bull *et al.* 1990 **Animal:** Mouse B6C3F1 M 5 weeks **Study duration**: 52 weeks	**Agent**: Trichloroacetic acid Analytical grade **Treatment**: Drinking water 0 1,000 2,000 mg/L in drinking water (pH 6.8-7.2) ad libitum x 52 wk	**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.
		0	0/2	
		1,000	2/5 (40%)	
		2,000	1/11 (9%)	
		Liver – Carcinoma		
		0	0/2	
		1,000	2/5 (40%)	
		2,000	4/11 (36.4%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Von Tungeln *et al.* 2002 **Animal:** Mouse (Study 1) B6C3F1 M 8 days (neonatal) **Study duration:** 20 months	**Agent**: Trichloroacetic acid Purity not reported **Treatment**: ip injection 0+ 1,000 nmol 1/3 of the dose was injected at age 8 days and 2/3 at age 15 days + DMSO	**Liver – Adenoma** 0 1,000 **Liver – Carcinoma** 0 1,000 **Liver – Adenoma or carcinoma** 0 1,000	 0/23 4/23 (17%) 0/23 1/23 (4%) 0/23 5/23[*] (22%)	**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 1) B6C3F1 F 8 days (neonatal) **Study duration:** 20 months	**Agent**: Trichloroacetic acid Purity not reported **Treatment**: ip injection 0+ 1,000 nmol 1/3 of the dose was injected at age 8 days and 2/3 at age 15 days + DMSO	**Liver – Adenoma** **0** **1,000** **Liver – Carcinoma** **0** **1,000** **Liver – Adenoma or carcinoma** **0** **1,000**	 **0/23** **0/23** **0/23** **0/23** **0/23** **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:**	**Agent**: Trichloroacetic acid Purity not reported **Treatment**: ip injection 0+ 2,000 nmol	**Liver – Adenoma** 0 2,000 **Liver – Carcinoma** 0 2,000	 0/24 4/24 (17%) 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

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
12 months	3/7 of the dose was injected at age 8 days and 4/7 at age 15 days. + DMSO	**Liver – Adenoma or carcinoma**		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.
		0	0/24	
		2,000	4/24 (17%)	
Von Tungeln *et al.* 2002 **Animal:** Mouse (Study 2) B6C3F1 F 8 days (neonatal) **Study duration:** 12 months	**Agent**: Trichloroacetic acid Purity not reported **Treatment**: ip injection 0+ 2,000 nmol 3/7 of the dose was injected at age 8 days and 4/7 at age 15 days. + DMSO	**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.
		0	**0/24**	
		2,000	**0/24**	
		Liver – Carcinoma		
		0	**0/24**	
		2,000	**0/24**	
		Liver – Adenoma or carcinoma		
		0	**0/24**	
		2,000	**0/24**	
NTP 2015 **Animal:** Mouse B6C3F1/N 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	**Liver – Adenoma[a]**		**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.
		0	39/50 (87%)	
		250	41/50 (90%)	
		500	42/49 (91%)	
		1,000	40/51 (91%)	
		Liver – Carcinoma[a]		
		0	12/50 (29%)	
		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]		

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 **Animal:** Mouse B6C3F1/N F 5-6 wk **Study duration:**	**Agent**: Bromodichloroacetic acid 97% **Treatment**:	**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
		0	33/49[v] (75%)	
		250	42/50*[v] (91%)	
		500	42/49*[v] (93%)	

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	

* < 0.05; ** < 0.01; *** < 0.001 *P*-value.
[ ] = *P*-value calculated by NTP using Fisher's Exact Test for pair-wise compairsons or Cochran-Armitage Trend 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% **Treatment:** Gavage 0 50 100 mg/kg bw in deionized water 5 doses/week x 104 weeks	**All organs – Tumor NOS** 0 50 100	 None None None	**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.
NTP (1992) **Animal**: Mouse B6C3F1 F 7-8 weeks Study duration: 104 weeks	**Agent:** Monochloroacetic acid 99% **Treatment:** Gavage 0 50 100 mg/kg bw in deionized water 5 doses/week x 104 weeks	**All organs – Tumor NOS** 0 50 100	 None None None	**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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2007a **Animal:** Rat F344/N M 6 weeks Study duration: 106 weeks	**Agent:** Dibromoacetic acid >99% **Treatment:** Drinking water 0 50 500 1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks Mean daily doses (0 - 2, 20, 40 mg/kg bw)	**Whole body – Malignant mesothelioma[a]**		**Survival**: Survival was similar in all groups. 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. **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.
		0	3/50 (7%)	
		50	1/50 (2%)	
		500	0/50	
		1,000	10/50*[b] (23%)	
		Trend p-value: <0.001		
		Whole body – Mononuclear cell leukemia[a]		
		0	17/50[c] (37%)	
		50	31/50**[c] (66%)	
		500	24/50[c] (56%)	
		1,000	13/50 (30%)	
		Lung – Adenoma[a]		
		0	2/50 (4.6%)	
		50	0/50	
		500	4/50 (10.1%)	
		1,000	2/50 (4.9%)	
		Lung – Adenoma or carcinoma[a]		
		0	2/50 (4.6%)	
		50	1/50 (2.4%)	
		500	5/50 (12.6%)	
		1,000	2/50 (4.9%)	
NTP 2007a **Animal:** Rat F344/N F 6 weeks Study duration:	**Agent:** Dibromoacetic acid >99% **Treatment:** Drinking water	**Whole body – Malignant mesothelioma**		**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
		0	0/50	
		50	0/50	
		500	1/50 (2%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
106 weeks	0 50 500 1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks Mean daily doses (0 - 2, 25, 45 mg/kg bw)	1,000	0/50	increases in the incidence of lung per-neoplasia (alveolar epithelium hyperplasia) occurred at 500 and 1,000 mg/L. 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.
		Whole body – Mononuclear cell leukemia[a]		
		0	11/50 (24%)	
		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 **Animal**: Mouse B6C3F1 M 6 weeks Study duration: 106 weeks	**Agent:** Dibromoacetic acid >99% **Treatment:** Drinking water 0 50 500 1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks Average daily dose: 0 - 4, 45, 87 mg/kg	**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.
		0	7/49 (16%)	
		50	5/50 (11%)	
		500	17/50*[f] (38%)	
		1,000	12/50[g] (27%)	
		Trend p-value: =0.019		
		Lung – Carcinoma		
		0	5/49 (10%)	
		50	8/50[h] (16%)	
		500	8/50[h] (16%)	
		1,000	7/50[h] (14%)	
		Lung – Adenoma or carcinoma[a]		
		0	12/49[i] (28%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		50	12/50 (26%)	
		500	22/50*[j] (49%)	
		1,000	17/50[i] (37%)	
NTP 2007a **Animal:** Mouse B6C3F1 F 6 weeks Study duration: 106 weeks	**Agent**: Dibromoacetic acid >99% **Treatment**: Drinking water 0 50 500 1,000 mg/L of drinking water (pH 5) ad libitum x 106 weeks Average daily dose: 0 - 4, 35, 65 mg/kg	**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.
		0	1/50 (2%)	
		50	3/50 (7%)	
		500	3/50 (7%)	
		1,000	6/50[k] (13%)	
		Trend p-value: = 0.044		
		Lung – Carcinoma		
		0	1/50 (2%)	
		50	2/50 (4%)	
		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% **Treatment**: Drinking water 0 250 500	**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
		0	3/50 (6%)	
		250	4/50 (8%)	
		500	3/50 (6%)	
		1,000	4/50 (8%)	
		Lung – Adenoma or carcinoma[a]		

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
	1,000 mg/L of drinking water x 105 weeks	0	3/50 (7%)	no major concerns.
		250	1/50 (2.5%)	
		500	0/50	
		1,000	3/50 (7.1%)	
		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 **Animal:** Rat F344/N F 6-7 weeks Study duration:	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0	**Mammary gland – Fibroadenoma[a]**		**Survival**: No significant difference: 34/50 - 31/50, 37/50, 35/50 **Body weight**: 1,000 mg/l group was <10% of controls. after 85 weeks. Significantly increased pre-neoplastic lesions: The incidence of lung pre-neoplasia (alveolar epithelium hyperplasia) was
		0	43/50 (92%)	
		250	43/50 (90%)	
		500	47/50 (96.9%)	
		1,000	46/50 (96.9%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
105 weeks	250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	**Mammary gland – Fibroadenoma (multiple only)[a]**		significantly increased at 1,000 mg/L. **Strengths and limitations**: A very high quality study, with no major concerns.
		0	22/50 (44%)	
		250	24/50 (48%)	
		500	43/50** (86%)	
		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 **Animal:** Mouse B6C3F1 M 6-7 weeks Study duration:	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water	**Harderian gland – Adenoma[a]**		**Survival**: 38/50 - 35/50, 30/50, 21/50 **Body weight**: 1,000 mg/l group was 12% lower than controls after 97 weeks. **Strengths and limitations**: A very high quality study, with
		0	5/50 (11.1%)	
		250	9/50 (20%)	
		500	9/50 (20.7%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
105 weeks	0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	1,000	8/50 (18.5%)	no major concerns.
NTP 2009 **Animal:** Mouse B6C3F1 F 6-7 weeks Study duration: 105 weeks	**Agent**: Bromochloroacetic acid 96% **Treatment**: Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	**Harderian gland – Adenoma[a]** 0 250 500 1,000	 1/50 (2.2%) 7/50* (14.5%) 1/50 (2.2%) 7/50* (14.7%)	**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.
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]** 0 250 500 1,000 Trend p-value: <0.001 **Mammary gland – Fibroadenoma[a]** 0 250 500 1,000	 1/50 (3%) 12/50*** (28%) 18/50*** (41%) 37/50*** (78%) 0/50 2/50 (5%) 3/50 (7%) 1/50 (3%)	**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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		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%)	
		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	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		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]		
		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	**Agent**: Bromodichloroacetic acid >97%	**Brain – Glioma or oligodendroglioma (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***
		0	1/50 (2.2%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Study duration: up to 104 wk	**Treatment**: Drinking water 0 250 500 1000 mg/L of drinking water ad libitum x 104 weeks	250	0/50	**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. **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.
		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%)	
		1000	39/50*** (89.6%)	
		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%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		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]		
		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%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
		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 Animal: Mouse B6C3F1/N 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	**Harderian gland – Adenoma[a]**		Survival: Significant decrease in survival Body weight: Significant decrease in body weight after 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.
		0	6/50 (15%)	
		250	11/50 (26%)	
		500	14/49* (38%)	
		1,000	19/51*** (49%)	
		Trend p-value: <0.001		
		Harderian gland – Carcinoma[a]		
		0	0/50	
		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:**	**Agent:** Bromodichloroacetic	**Harderian gland – Adenoma or carcinoma[a]**		**Survival**: No significant change in survival. **Body weight**: Significant decrease in body weight after 73
		0	5/50 (12%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Mouse B6C3F1/N F 5-6 wk Study duration: 105 wk	acid 97% **Treatment:** Drinking water 0 250 500 1,000 mg/L of drinking water ad libitum x 105 weeks	250 500 1,000	4/50 (9%) 7/50 (16%) 6/50 (14%)	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.

* < 0.05, ** < 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2007b **Animal:** Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha-ras)Led) M 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 1/15 (7%) 2/15 (13%)	**Survival**: Survival was similar to untreated controls: 13/15 - 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	**Agent:** Dichloroacetic acid 98.5%-99%	**Lung – Adenoma** 0 500	 1/10 (10%) 2/10 (20%)	**Survival:** Survival was similar to untreated controls: 9/10 - 9/10, 10/10, 10/10 **Body weight**: Body weights were significantly greater than

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Tg.AC hemizygous (FVB/N-TgN(v-Ha-ras)Led) M 6 weeks Study duration: 41 weeks	**Treatment:** Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	1,000	7/10** (70%)	untreated controls at 500 mg/l after 17 weeks and 1,000 mg/l after 21 weeks. 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.
		2,000	3/10 (30%)	
		Forestomach – Squamous cell papilloma		
		0	5/10 (50%)	
		500	9/10 (90%)	
		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% **Treatment**: Drinking water 0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	**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.
		0	0/10	
		500	0/10	
		1,000	0/10	
		2,000	2/10 (20%)	
		Forestomach – Squamous cell papilloma		
		0	6/10 (60%)	
		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%)	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2007b **Animal:** Mouse (Study 2) FVB Tg.AC hemizygous (FVB/N-TgN(v-Ha-ras)Led) 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 – 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. 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.
		0	0/15	
		500	0/15	
		1,000	1/15 (7%)	
		2,000	0/15	
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**		**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.
		0	0/15	
		500	1/15 (7%)	
		1,000	0/15	
		2,000	1/15 (7%)	
NTP 2007b **Animal:** Mouse (Study 3) p53 Haploinsufficient M 6 weeks	**Agent**: Dichloroacetic acid 98.5%-99% **Treatment**: Drinking water	**Lung – Adenoma or carcinoma**		**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.
		0	0/10	
		500	0/10	
		1,000	0/10	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Study duration: 41 weeks	0 500 1,000 2,000 mg/L in drinking water ad libitum x 26 weeks	2,000	0/10	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 3) p53 Haploinsufficient F 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: 10/10 - 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
NTP 2007b **Animal:** Mouse (Study 4) p53 Haploinsufficient 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 – 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, 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. 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 Not reported **Treatment**: Dermal Initiator: 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone: single dose 0.15% in 3 ml Promotor: Iodoacetic acid in acetone: start 21 days after DMBA 1.4% (2/wk x 12wk, then 1/wk x 15wk)	**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.
		0 (acetone)	1/12 (8.3%)	
		0 (acetic acid)	1/16 (6.25%)	
		1.4	8/10[@@][##] (80%)	
Herren-Freund *et al.* (1987) **Animal:** Mouse B6C3F1 M 4 weeks Study duration: 61 weeks	**Agent**: Dichloroacetic acid >99% **Treatment**: Drinking water Initiator: Ethylnitrosourea (ENU): ip injection at 15 days old 0+ 2.5 µg/g bw Promotor: DCA: in drinking water	**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. **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.
		0/0	2/22 (9%)	
		0/5,000	25/26** (96%)	
		2.5/0	1/22 (5%)	
		2.5/2,000	22/29** (76%)	
		2.5/5,000	31/32** (97%)	
		Liver – Carcinoma		
		0/0	0/22	
		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 None 7.8 DCA None 15.6 DCA None 25 DCA None **Liver – Carcinoma** 0 DCA/TCA 0/29 7.8 DCA 0/17 15.6 DCA 0/19 25 DCA 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.

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
Herren-Freund *et al.* (1987) **Animal:** Mouse B6C3F1 M 15 days Study duration: 61 weeks	**Agent**: Trichloroacetic acid >99% **Treatment**: Drinking water Initiator: Ethylnitrosourea (ENU): ip injection at 15 days old 0+ 2.5 10 µg/g bw Promotor: TCA: in drinking water (pH 6.5-7.5) 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 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. **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.
		0/0	2/22 (9%)	
		0/5,000	8/22** (36%)	
		2.5/0	1/22 (5%)	
		2.5/2,000	11/33** (33%)	
		2.5/5,000	6/23** (26%)	
		10/0	9/23 (39%)	
		10/5,000	11/28 (39%)	
		Liver – Carcinoma		
		0/0	0/22	
		0/5,000	7/22** (32%)	
		2.5/0	1/22 (5%)	
		2.5/2,000	16/33** (48%)	
		2.5/5,000	11/23** (48%)	
		10/0	9/23 (39%)	
		10/5,000	15/28 (54%)	
Pereira 1997 **Animal:** Mouse B6C3F1 F Initiator: 15 d; Promotor: 6 wks Study duration:	**Agent**: Trichloroacetic acid Vendor, but not purity given **Treatment**: Drinking water Initiator: Methylnitrosourea	**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
		0 DCA/TCA	None	
		6 TCA	None	
		25 TCA	None	
		Liver – Carcinoma		
		0 DCA/TCA	0/29	

Reference and study design	Exposure	Dose levels	Tumor incidence (n/N) (%)	Comments
50 wks	(MNU): single ip dose in 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	6 TCA 25 TCA	0/20 4/29 (13.8%)	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 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				
	CA	BA	IA	DCA	DBA	BCA	CIA	BIA	TCA	TBA	BDCA	CDBA	Reference
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 *et al.* 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 *et al.* 2016
ARE-bla: human hepatocellular carcinoma cell line (HepG2) (1/mM, IR = 1.5)	14	141	256										Procházka *et al.* 2015
ARE-bla: human hepatocellular carcinoma cell line (HepG2) (1/mM, IR = 1.5)	116	510	1,010										Pals *et al.* 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 *et al.* 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 *et al.* 1996
Oxidative DNA damage; male mice, 21 day water– 0.5 and 2 g/L (8-OHdG/10^5 dG liver)				– –	1.4[a] 1.8[a]	1.2[a] 1.6[a]			– –				Parrish *et al.* 1996

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, 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) Female mice (micronucleus)	– –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0004-0000-5
Dichloro-	Male mice (micronucleus) Female mice (micronucleus)	– –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0006-0000-7
Dichloro-	Male mice (micronucleus) Female mice (micronucleus)	– –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0007-0000-8
Dichloro-	Male mice (micronucleus) Female mice (micronucleus)	– +	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02007-0005-0000-6
Dibromo-	Ames	+	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01996-0006-0000-3
Dibromo-	Male mice (micronucleus) Female mice (micronucleus)	+ –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01996-0005-0000-2
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) Female mice (micronucleus)	– –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01740-0004-0000-8
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) Female mice (micronucleus)	– –	https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01742-0003-0000-9

– = 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 *et al.* 2016
− S9 (1/M, IR = 1.5)	–[a]	–[a]	–[a]	–	2,564	2,941	5,263	9,091	–	142,860	9,091	9,091	
+ 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 *et al.* 2016
− S9 (1/M, IR = 2)	(+)	1,107	8,696	83	89				(+)				
− 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 *et al.* 1991
− S9 (β-galactosidase activity)[c]	–			neg					0.72				
+ S9 (β-galactosidase activity)[c]	–			1.5					1.18				
Ames preincubation: TA100													Plewa *et al.* 2000, Plewa *et al.* 2004b
− S9 (revertants/µmol)	27	5,465	14,129		148					–			
Ames preincubation: TA100													Kargalioglu *et al.* 2002
− S9 (revertants/µmol)[d]	44	6,588		36	183				–	–			
+ S9 (revertants/µmol)[d]	63	2,642		13	165				–	–			
Ames preincubation: TA98													Kargalioglu *et al.* 2002
− S9 (revertants/µmol)[d]	6	351		2	16				–	–			
+ S9 (revertants/µmol)[d]	–	179		–	12				–	–			
Ames preincubation: RSJ100													Kargalioglu *et al.* 2002
− S9 (revertants/µmol)[d]	–	–		17	–				–	–			
+ S9 (revertants/µmol)[d]	–	–		–	–				–	–			
SOS chromotest: *E. coli* PQ37													Giller *et al.* 1997
− S9 (1/(mg/mL), IR = 1.5)	–	–		2	5				–	1.3			
+ S9 (1/(mg/mL), IR = 1.5)	–	–		–	10				–	10			
Ames fluctuation: TA100													Giller *et al.* 1997
− S9 (1/[mg/mL])	–	–		10	100				0.57	0.5			
+ 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 *et al.* 2016
SCGE: CHO AS52 cells													Plewa *et al.* 2002, Plewa *et al.* 2004b, Richardson *et al.* 2008, Plewa *et al.* 2010
LGC (1/M)	3,333	76,920	200,000	–	1,333	333	(1,000)	400	–	333	–	71	
GP (1/M)	2,439	58,820	114,900	–	556	333	(500)	313	–	400	–	71	
SCGE: CHO AS52 cells (tail moment units/µmol)		68,900			887					254			Plewa *et al.* 2000
HGPRT mutations: CHO-K1 cells (mutant frequency/mM)	8.7	14.6	835.9	2.8	66.2				–				Zhang *et al.* 2010
SCGE assay: HepG2 cells MEC (1/µM)	–	10	100	0.1	1				0.01				Zhang *et al.* 2012
p53-bla: human colon carcinoma cell line HCT-116 (1/M, IR = 1.5)	10,000	98,039	156,250										Procházka *et al.* 2015
SCGE: human lymphocytes GP (1/mM)	1.2	83	96										Escobar-Hoyos *et al.* 2013
Mitotic index: human lymphocytes EC_{50} (1/mM)	1.4	12.7	20.3										Escobar-Hoyos *et al.* 2013
SCGE: human small intestine epithelial cells EC_{50} (1/mM)	0.29	17.7	45.7										Attene-Ramos *et al.* 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

				Hypomethylation			
Species (sex)	**Tissue**	**HAA**	**Conc (g/L)**	**DNA (% reduction)[a]**	**Genes**	**Comments**	**Reference**
Mice (M,F)	Kidney	DCA TCA	3.2 4.0	40 65	*c-myc*	Mice treated for 7 days. Unlike the liver, effects in kidney only in males. *c-myc* hypomethylation prevented by treatment with methionine.	Tao *et al.* 2005
Mice (M) Rats (M)	Kidney	DBA	1–2	40–60 45–56	*c-myc*	High dose caused reduction at 7 days and 28 days; at low dose, significant reduction only after 28 days.	Tao *et al.* 2005
Mice (F) Rats (M)	Liver	DBA	1–2	45–70 33–52	*c-myc*, *IGF-II*	Both doses caused significant reduction after 7 and 28 days. mRNA expression of *c-myc* and IGF-II genes significantly increased in mice and c-myc expression increased in rats.	Tao *et al.* 2004a
Mice (F)	Liver Liver tumors	DCA TCA	2.6[b] 3.3[b]	27–85 27–85	*IGF-II*	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 *et al.* 2004b
Mice (F)	Liver	DCA TCA	500 mg/kg 500 mg/kg	NR	*c-jun*, *c-myc*	Single gavage dose administered daily for 5 days. Treatment with methionine prevented hypomethylation.	Tao *et al.* 2000a
Mice (F)	Liver Liver tumors	DCA TCA	2.6[b] 3.3[b]	NR	*c-jun*, *c-myc*	mRNA expression and protein levels of *c-myc* and *c-jun* 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 *et al.* 2000b

				Hypomethylation			
Species (sex)	Tissue	HAA	Conc (g/L)	DNA (% reduction)[a]	Genes	Comments	Reference
Mice (F)	Liver Liver tumors	DCA TCA	3.2[b] 4.0[b]	36[c] 40–51[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 *et al.* 1998
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 *et al.* 2004a
Mice (F)	Liver Kidney	DCA TCA	500 mg/kg 500 mg/kg	NR	*c-myc*	Single gavage dose administered daily for 5 days. Both DCA and TCA induced hypomethylation and expression of *c-myc* 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 *et al.* 2001
Mice (F)	Liver Kidney Bladder	DCA TCA	500 mg/kg 500 mg/kg	NR	*c-myc*	Hypomethylation of the promoter region of *c-myc* 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 *et al.* 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 *et al.* 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 *et al.* 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 *c-myc* and *c-H-ras* compared to surrounding tissue or controls in both preneoplastic nodules and carcinomas. *Myc* expression was similar in DCA-induced nodules and carcinomas. However, *myc* 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. *c-H-ras* expression was higher in carcinomas from both treatments than in hyperplastic nodules or normal liver, thus, was closely associated with malignancy.	Nelson *et al.* 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 *et al.* 2016

Haloacetic acid	Test system	Results	Reference
Bromodichloro-	Mouse: normal liver tissue and tumors (chronic exposure)	**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. **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. **Hepatoblastomas:** Markedly different from adjacent hepatocellular carcinomas and normal liver with upregulation of oncogenic signaling pathways (especially *Wnt/Ctnnb1*-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.	NTP 2015
Bromodichloro-	Rat: normal mammary tissue and tumors (chronic exposure)	**Mammary adenocarcinomas:** Eight genes significantly upregulated. Five associated with *Tgfß* pathway signaling, including its effects on matrix remodeling, mammary gland cancer progression, angiogenesis, tumor invasion, and metastasis.	NTP 2015
Bromochloro-	Rat: peritoneal mesothelioma (chronic exposure)	**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/ß-catenin, and integrin signaling and are similar to mesotheliomas in humans.	Kim *et al.* 2006
Bromochloro-	Mouse sperm (daily treatment for 14 days)	**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.	Tully *et al.* 2005

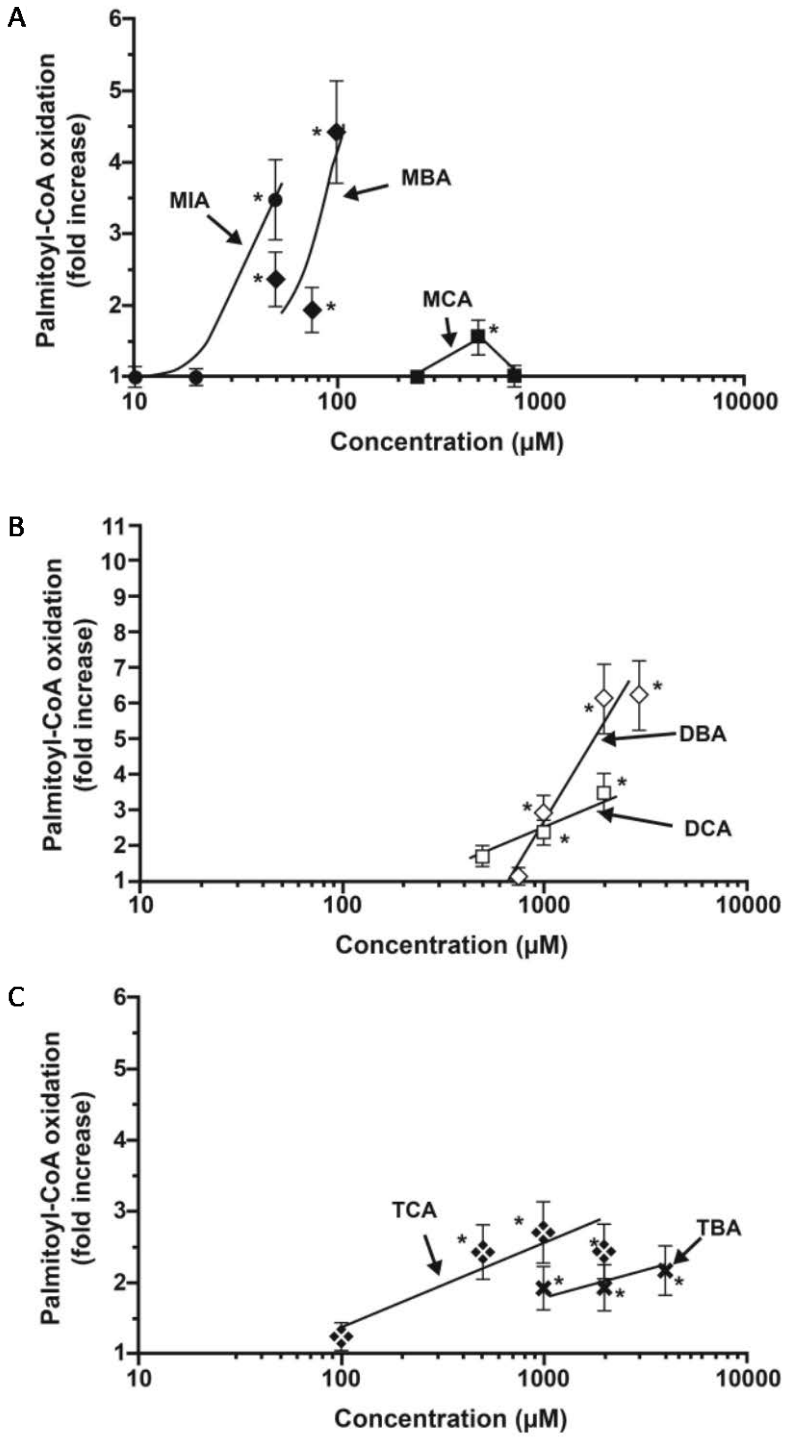

Figure D-1. Palmitoyl-CoA oxidation in cultured rat hepatocytes exposed to haloacetic acids

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).

This Page Intentionally Left Blank

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-14
Research Triangle Park, NC 27709

ISSN# 2331-267X