Article

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High discordance in development and organ site distribution of tumors in rats and mice in NTP two-year inhalation studies

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Abstract

The National Toxicology Program (NTP) reports 60 two-year inhalation studies in both mice and rats on single agents or closely related agents. "Cadmium and cadmium compounds" and "diesel exhaust particulates" were omitted from this analysis due to lack of results regarding a particular compound. No Ames test data were available for antimony trioxide, nickel sulfate hexahydrate, and indium phosphide. For antimony trioxide, a comet assay was used as a surrogate for the Ames test. To eliminate selection bias, all positive Ames assay test results and any statistically significant increase in lung tumor incidence over background in an NTP two-year inhalation study were accepted at face value. For the 58 compounds tested via inhalation by NTP, there is a high degree of discordance between mice and rats in the susceptibility to develop lung tumors. The causation of tumors at anatomical sites outside the lung via the inhalation route is also discordant in mice and rats, for example, 11/58 (19%) of agents tested in the NTP inhalation studies using mice and rats were negative in the Ames assay test and showed lung tumors in mice only. The ability to form lung tumors in mice in the absence of genotoxicity demonstrates that other mechanisms, for example, cytotoxicity followed by reparative cellular proliferation, might be involved. Mouse and rat data are discordant regarding the ability to induce tumors at organ sites outside the lungs—0/58 as compared with 16/58, respectively. Mice and rats display distinctly different patterns of both lung tumor development and development of tumors outside the lungs.

Keywords

NTP, rats, mice, lung tumors, discordance, inhalation

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Introduction

The National Toxicology Program (NTP) is a branch of the United States Department of Health and Human Services. One of NTP's major current programs is "The Toxicology in the 21st Century: The Role of the National Toxicology Progra7m."[1] On their website, NTP describes this program as follows:

> The Role of the National Toxicology Program is to support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. The intent of NTP vision is to expand the scientific basis for making public health decisions on the potential toxicity of environmental agents.

One of the major "disease-specific models" employed by NTP is the 2-year rodent inhalation bioassay.

The NTP National Institute of Environmental Health Science (NIEHS) website references 60 two-year inhalation studies conducted in both rats and mice on single agents or closely related agents.[2] Two of the 60 inhalation

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Table 1. Summary analysis of the 58 different agents tested in two-year inhalation studies conducted on both rats and mice by the NTP.

(a)

Species	Fraction (%) of 58 agents causing lung tumors only[a]	Fraction of 58 agents causing non-lung tumors only[b]	Fraction of Ames assay test negative agents causing lung tumors only[c]	Fraction of Ames assay test negative agents causing non-lung tumors only[d]	Fraction of Ames assay test positive agents causing lung tumors only[e]	Fraction of Ames assay test positive agents causing non-lung tumors only[f]	Fraction of agents causing non-lung tumors in one species only[g]
Rats	7/58 (12.1%)	34/58 (58.6%)	3/58 (5.2%)	25/58 (43.1%)	1/58 (1.7%)	14/58 (24.1%)	16/58 (27.6%)
Mice	15/58 (25.9%)	10/58 (17.2%)	11/58 (19.0%)	10/58 (17.2%)	6/58 (10.3%)	1/58 (17.2%)	0/58

(b)

Ames assay test result	Fraction of agents causing lung tumors in both rats and mice[h]	Fraction of agents not causing lung tumors in either rats or mice[i]
Positive	9/58 (15.5%)	1/58 (1.7%)
Negative	5/58 (8.6%)	22/58 (37.9%)

(c)

Species	Fraction of agents not causing tumors at any site[j]	Fraction of agents causing tumors at any site[k]
Rats	9/58 (15.5%)	49/58 (84.5%)
Mice	17/58 (29.3%)	41/58 (70.7%)

NTP: National Toxicology Program.
[a]Borderline at 95% significant difference between rats and mice at $p\text{-value}_1 = 0.0588$; $p\text{-value}_2 = 0.0549$.
[b]Statistically significant difference between rats and mice at $p\text{-value}_1 < 0.0001$; $p\text{-value}_2 < 0.0001$.
[c]Statistically significant difference between rats and mice at $p\text{-value}_1 = 0.0226$; $p\text{-value}_2 = 0.0198$.
[d]Statistically significant difference between rats and mice at $p\text{-value}_1 = 0.0024$; $p\text{-value}_2 = 0.0016$.
[e]Statistically significant difference between rats and mice at $p\text{-value}_1 = 0.0512$; $p\text{-value}_2 = 0.0477$.
[f]Statistically significant difference between rats and mice at $p\text{-value}_1 = 0.0003$; $p\text{-value}_2 < 0.0001$.
[g]Statistically significant difference between rats and mice at $p\text{-value}_1 = < 0.0001$; $p\text{-value}_2 < 0.0001$.
[h]No significant difference in Ames assay positive and Ames assay negative agents at $p\text{-value}_1 = 0.2543$; $p\text{-value}_2 = 0.2501$.
[i]Statistically significant difference in Ames assay positive and Ames assay negative agents at $p\text{-value}_1 < 0.0001$; $p\text{-value}_2 < 0.0001$.
[j]No significant difference between rats and mice at $p\text{-value}_1 = 0.0751$; $p\text{-value}_2 = 0.0703$.
[k]No significant difference between rats and mice at $p\text{-value}_1 = 0.0751$; $p\text{-value}_2 = 0.0703$.

studies were conducted on "cadmium and cadmium compounds"[3] and on "diesel exhaust particulates."[4] These two studies were omitted from this analysis due to lack of results regarding a particular compound. In each of the 58 two-year rodent inhalation studies analyzed herein, the mouse strain $B6C3F_1$ was used. In 55/58 studies, the rat strain employed was F344/N. Wistar Han rats were used for the inhalation studies on Trim® VX and antimony trioxide. Osborne-Mendel rats were used for the inhalation study of allyl glycidyl ether. For three compounds, no Ames assay test data were available: antimony trioxide, nickel sulfate hexahydrate, and indium phosphide. Antimony trioxide had a positive comet assay, so that result was considered equivalent to a positive Ames assay test.

In the vast majority of cases where a benign adenoma in the rodent lung was seen, a malignant bronchioloalveolar carcinoma was also detected. Since benign adenomas in rodent lungs are precursors to the development of malignant bronchioloalveolar carcinomas,[5] the practice of considering the tumor types as interchangeable for counting purposes was followed. In a limited number of cases, the only anatomical site outside the lung that developed tumors was the nasal passages. In this analysis, the nasal passages are considered separately from the lungs, but it could also be argued that tumorigenicity of the lungs and nasal passages could be combined although there are microanatomical and physiological differences between the two anatomic locations.[6]

Table 2. 11/58 Total NTP inhalation studies conducted in rats and mice are negative in the Ames assay and had lung tumors for mice only.

Chemical	Reference	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Nitrobenzene CAS No. 98-95-3	RoC 13th edition	Negative	Positive in chromosome aberrations in humans. SCEs negative in vitro	Slightly soluble in water, soluble in organic solvents. Log $p = 1.85$	Inhalation	Positive in male mice; negative in female mice and rats	Incidences of alveolar/bronchiolar hyperplasia (a presumed preneoplastic lesion was significantly increased in male mice at the mid and high doses and in female mice at the mid dose)
Trichloro-ethylene CAS No. 79-01-6	RoC 13th edition	Negative	Probably negative, but did cause SCE	Slightly soluble in water, soluble in ethanol, acetone, diethyl ether, and chloroform, and miscible in oil. Log $p = 2.61$	Inhalation	Clear evidence in male and female mice; negative in rats	
Vinylidene chloride CAS No. 75-35-4	NTP TR 582, August, 2015	Negative	Negative in micronucleus	Clear volatile liquid, insoluble in water but miscible with most organic solvents. Log $p = 2.13$	Inhalation	Incidence of alveolar/ bronchiolar carcinoma significantly increased in 12.5 ppm female mice; negative in male mice and in rats	Respiratory epithelium, hyperplasia
1-Bromopropane CAS No. 106-94-5	NTP TR 564, August 2011	Negative	Positive in chromosome aberrations	Slightly soluble in water, soluble in most organic solvents. Log $p = 2.10$	Inhalation	Clear evidence in female mice; No evidence in male mice and rats	Bronchiole, regeneration
Cumene CAS No. 98-82-8	NTP TR 542, February 2009	Negative	Probably negative but did cause small increase micronucleus	Alkylated benzene volatile at room temperature. Log $p = 3.66$	Inhalation	Clear evidence in male and female mice; negative in rats	Bronchiolar hyperplasia and alveolar epithelial bronchiolar metaplasia significantly increased in mice of both sexes
Divinylbenzene-HP CAS No. 1321-74-0	NTP TR 534, Nov 2006	Negative	Negative in micronucleus	Insoluble in water and soluble in methanol and ether. Log $p = 3.8$	Inhalation	Equivocal evidence of carcinogenic activity in female mice; negative male mice, male and female rats	Bronchiolar, hyperplasia, atypical, alveolar epithelium, hyperplasia
Naphthalene CAS No. 91-20-3	NTP TR 500, December 2000; NTP TR edition	Negative	Positive in chromosome aberrations and SCE	Not soluble in water, soluble in organic solvents. Log $p = 3.3$	Inhalation	Significantly increased incidence of benign lung tumors (adenoma) in female B6C3F1 mice	Nonneoplastic lesions attributed to naphthalene exposure were observed in the nose and lungs of mice of both sexes. In the nose, naphthalene exposure was associated with an increase in the incidence and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of respiratory epithelium. Chronic inflammation in the lung was associated with chemical exposure
Chloroprene CAS No. 126-99-8	NTP TR 467, September 1998	Negative	Negative in chromosome aberrations, SCEs, or micronucleus	Practically insoluble in water, soluble in alcohol, and miscible with acetone, benzene, and ethyl ether. Log $p = 2.53$	Inhalation	Positive in male and female mice; negative rats	Increased incidences of bronchiolar hyperplasia and histiocytic cell infiltration in the lung

(continued)

Table 2. (continued)

Chemical	Reference	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Ethylbenzene CAS No. 100-41-4	NTP TR 466, January 1999	Negative	Negative for SCE or chromosome aberrations	Practically insoluble in water but soluble in most organic solvents. Log $p = 3.15$	Inhalation	Some evidence of carcinogenic activity in male mice; negative in female mice and rats	Alveolar epithelial metaplasia
Nitromethane CAS No. 75-52-5	NTP TR 461, February 1997	Negative	Negative in chromosome aberrations, SCE, and micronucleus	Soluble in water, alcohol, ether, acetone, and dimethylformamide. Log $p = 0.17$	Inhalation	Positive in male and female mice. Negative in rats	Hyaline degeneration of respiratory epithelium
Isoprene CAS No. 78-79-5	NTP TR 486, July 1999	Negative	Negative for SCE or chromosome aberrations in vitro. Positive in vivo in mice for SCE and micronucleus	Log $p = 2.42$	Inhalation	Positive in male and female mice; negative in rats	Alveolar epithelial hyperplasia

NTP: National Toxicology Program.
Ten of the 11 chemicals in this table are insoluble or slightly soluble in water, soluble in organic solvents, and have hydrophobic octanol–water partition coefficients of 0.17, 1.85, 2.10, 2.13, 2.42, 2.53, 2.61, 3.15, 3.30, 3.66, and 3.80. These chemicals induce hyperplasia in the airways of mice.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 3. 3/58 Total NTP inhalation studies conducted in rats and mice that are negative in the Ames assay test and show lung tumors for rats only.

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Gallium arsenide CAS No. 1303-00-0	NTP TR 492 September 2000	Negative	Negative micronucleus assay	Insoluble in water. Particulate aerosols with MMAD 1 μm	Inhalation	No evidence in male rats; *clear evidence in female rats*; no evidence in male and female mice	Atypical epithelial hyperplasia, chronic active inflammation, metaplasia in lung
Nickel subsulfide CAS No. 12035-72-2	NTP TR 453, July 1996	Negative	Positive in chromosome aberrations and micronucleus	Black powder, insoluble in water, soluble in acid. MMAD 2.0–2.2 μm	Inhalation	*Clear evidence in male and female rats*; no evidence in male or female mice	Chronic active inflammation, focal alveolar hyperplasia
Talc containing no asbestos fibers CAS No. 14807-96-6	NTP TR 421, September 1993	Negative	Negative	Insoluble in water. Finely powdered hydrous magnesium silicate, MMAD 2.7–3.2 μm for rats, and MMAD 3.3 μm for mice	Inhalation	*Clear evidence in female rats*; no evidence in male rats, or male or female mice	Chronic granulomatous inflammation, alveolar epithelial hyperplasia, epithelial squamous metaplasia

NTP: National Toxicology Program; MMAD: mass mean aerodynamic diameter.
The three agents in this table contain metals, consist of particles, and are not soluble in water.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 4. 5/58 Total NTP inhalation studies conducted in rats and mice are negative in the Ames assay test and show lung tumors in both rats and mice.

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Trim VX	NTP 591, Scheduled Peer Review Date: February 16, 2016	Negative	Negative in micronucleus	Forms a chemical emulsion with water. Metalworking fluid used as a lubricant and coolant liquid	Inhalation	There was *equivocal evidence of carcinogenic activity* of Trim VX in male Wistar Han rats based on the combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *equivocal evidence of carcinogenic activity* of Trim VX in female Wistar Han rats based on the occurrences of alveolar/bronchiolar adenoma of the lung. There was *clear evidence of carcinogenic activity* of Trim VX in male B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *clear evidence of carcinogenic activity* of Trim VX in female B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung	*Lung male mice:* alveolar/bronchiolar epithelium, hyperplasia (3/50, 7/50, 15/49, 50/50); infiltration cellular, histiocyte (5/50, 9/50, 15/49, 49/50); inflammation, chronic (5/50, 12/50, 16/49, 50/50); alveolar epithelium, hyperplasia (3/50, 3/50, 7/49, 47/50); fibrosis (0/50, 2/50, 5/49, 45/50) *Lung female mice:* alveolar/bronchiolar epithelium, hyperplasia (0/50, 3/50, 8/50, 45/50); infiltration cellular, histiocyte (1/50, 4/50, 15/50, 48/50); inflammation, chronic (1/50, 6/50, 26/50, 47/50); alveolar epithelium, hyperplasia (0/50, 0/50, 2/50, 43/50); fibrosis (0/50, 0/50, 2/50, 42/50)
Vanadium pentoxide CAS No. 1314-62-1	NTP TR 507, December 2002	Negative	Negative in micronucleus	Dosed as a particulate aerosol; an odorless, yellow to reddish brown orthorhombic crystal; insoluble in alcohol; is slightly soluble in water with a water solubility of 1 g/125 mL, and is soluble in concentrated acid, alkalies (forming vanadates), and acetone.	Inhalation	Under the conditions of this 2-year inhalation study, there was some evidence of carcinogenic activity[a] of vanadium pentoxide in male F344/N rats and equivocal evidence of carcinogenic activity of vanadium pentoxide in female F344/N rats based on the occurrence of alveolar/bronchiolar neoplasms. There was clear evidence of carcinogenic activity of vanadium pentoxide in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms	Exposure to vanadium pentoxide caused a spectrum of nonneoplastic lesions in the respiratory tract (nose, larynx, and lung) including alveolar and bronchiolar epithelial hyperplasia, inflammation, fibrosis, and alveolar histiocytosis of the lung in male and female rats and mice and an unusual squamous metaplasia of the lung in male and female rats. Hyperplasia of the bronchial lymph node occurred in female mice

(continued)

Table 4. (continued)

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Cobalt sulfate CAS No. 10124-43-3 cobalt sulfate heptahydrate	NTP TR 471, August 1998	Negative	Negative	Cobalt sulfate is a reddish, crystalline, water-soluble powder (Smith and Carson, 1981).[7]	Inhalation	Some evidence in male rats; clear evidence in female rats, male mice, female mice	Exposure to cobalt sulfate heptahydrate caused a spectrum of inflammatory, fibrotic, and proliferative lesions in the respiratory tract of male and female rats and mice
Molybdenum trioxide CAS No. 1313-27-5	NTP TR 462, April 1997	Negative	Negative	Molybdenum trioxide is a white or slightly yellow to slightly bluish powder with a boiling point of 1155°C, a melting point of 795°C, and a specific gravity of 4.50 at 19.5°C. It is soluble in water (0.49 g/L at 28°C), concentrated mineral acids, and solutions of alkali hydroxides, ammonia, and potassium bitartrate. Its vapor pressure is less than 103 mm Hg at 600°C (Merck Index, 1989)	Inhalation	Equivocal evidence in male rats, some evidence of carcinogenic activity in male mice, some evidence in female mice	Exposure of male and female rats to molybdenum trioxide by inhalation resulted in increased incidences of chronic alveolar inflammation, hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), and squamous metaplasia of the epiglottis; exposure of male and female mice to molybdenum trioxide by inhalation resulted in increased incidences of metaplasia of the alveolar epithelium, histiocyte cellular infiltration (males), hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), squamous metaplasia of the epiglottis, and hyperplasia of the larynx

(continued)

Table 4. (continued)

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Nickel oxide CAS No. 1313-99-1	NTP TR 451, July 1996	Negative	Negative in micronucleus; negative in chromosome aberrations	Nickel oxide (high temperature green nickel oxide, oxidized at 870–900°C and heated to 1350°C; Boldt, 1967)[8] is an olive gray powder with a melting point of 2090°C and a density of 7.45 g/cm^3. It is insoluble in water and soluble in acids (Merck Index, 1989).[9] The mean values for the mass median aerodynamic diameter at each exposure concentration of nickel oxide used in these 2-year studies ranged from 2.2 to 2.6/μm. The nickel oxide used in these studies is only one form of nickel oxide within a larger family of "oxidic" nickels	Inhalation	Some evidence in male and female rats; equivocal evidence mice; negative in male mice	Exposure of rats to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung, lymphoid hyperplasia and pigmentation in the bronchial lymph nodes, and hyperplasia of the adrenal medulla (females). Exposure of mice to nickel oxide by inhalation for 2 years resulted in bronchialization, proteinosis, inflammation, and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes

NTP: National Toxicology Program.
Four of the five agents in this table are powdered metals. One of the five, Trim VX is a water-soluble oil that forms a chemical emulsion. Each of these five agents caused inflammation and hyperplasia in the lungs.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 5. 9/58 Total NTP inhalation studies conducted in rats and mice are positive in the Ames assay test and show lung tumors in both rats and mice.

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Antimony trioxide CAS No. 1309-64-4	NTP TR 590 (February, 2016)	Positive Comet Assay in mouse lung tissue samples	Positive micronucleus	Slightly soluble in water, dilute sulfuric acid, and dilute nitric acid	Inhalation	*Some evidence of carcinogenic activity* of antimony trioxide in male and female Wistar Han rats based on increased combined incidences of alveolar/bronchiolar adenoma or carcinoma in the lung. Clear evidence in male and female mice	Antimony trioxide dust and fumes have been shown to cause irritation of the respiratory tract and mucous membranes
Cobalt metal CAS No. 7440-48-4	TR 581, December 2014	Positive	Negative in micronucleus	Soluble in dilute acids	Inhalation	Clear evidence in male rats, female rats, male mice, female mice	Inflammation and hyperplasia in all four rodent types
Isobutyl nitrite CAS No. 542-56-3	NTP TR 448, July 1996	Positive	Positive in SCE and chromosome aberrations	Slightly soluble in water	Inhalation	There was clear evidence of carcinogenic activity of isobutyl nitrite in male and female F344/N rats based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was some evidence of carcinogenic activity of isobutyl nitrite in male and female $B6C3F_1$ mice based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males and females	Bronchiolar and alveolar hyperplasia
Tetranitromethane CAS No. 509-14-8	NTP TR 386, March 1990	Positive	Positive chromosome aberrations and SCEs	Log K_{ow}-2.05. Soluble in ethanol, ether, and CCl_4. Soluble in water. Freely soluble in alcoholic KOH	Inhalation	Clear evidence of carcinogenic activity[a] of tetranitromethane for male and female F344/N rats and male and female B6C3F1 mice, based on increased incidences of alveolarbronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats	Alveolar hyperplasia. Hyperplastic and squamous metaplasia or respiratory epithelium

(continued)

Table 5. (continued)

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Allyl glycidyl ether CAS No. 106-92-3	NTP TR 376, January 1990	Positive	Positive in SCE and chromosome aberrations	Soluble in water	Inhalation	Equivocal evidence of carcinogenic activity in male Osborne-Mendel rats, no evidence in female rats, some evidence in male mice, equivocal evidence in female mice	Male mice had dysplasia, both sexes had focal basal cell hyperplasia of respiratory epithelium in nasal passages
Bromoethane (ethyl bromide) CAS No. 74-96-4	NTP TR 363, October 1989	Positive	Positive for SCEs and negative for chromosome aberrations	Log K_{ow} 1.61. Soluble in water, alcohol, ether, chloroform, and organic solvents	Inhalation	Some evidence in male rats, equivocal evidence in female rats, equivocal evidence in male mice. Negative in female mice	Alveolar and nasal epithelial hyperplasia
1,2-Dibromoethane CAS No. 106-93-4	TR-210, March 1982	Positive–direct acting mutagen	Positive in SCE and DNA binding	Log K_{ow} 1.61. Soluble in water and most organic solvents	Inhalation	Positive in male and female mice and in female rats; negative in male rats	Epithelial hyperplasia, squamous metaplasia, and suppurative inflammation
Chromium hexavalent compounds CAS No. 18540-29-9	TR-546 (May, 2007), 13th RoC	Positive	Positive	Not applicable	Inhalation	Exposure to chromium(VI) compounds (calcium chromate, chromium trioxide, or sodium dichromate) via inhalation or intratracheal or intrabronchial implantation caused benign and/or malignant lung tumors in rats and/or mice	
Bis(chloromethyl) ether and technical grade chloromethyl methyl ether CAS Nos. 542-88-1 and 107-30-2	12th RoC	Positive	Positive	K_{ow} 1.04. Soluble in water and many organic solvents	Inhalation	Exposure to BCME by inhalation caused lung tumors in rats and mice	

NTP: National Toxicology Program.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 6. 6/58 Total NTP inhalation studies conducted in rats and mice are positive in the Ames assay test and show lung tumors in mice only.

Chemical	References	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
CIMSTAR 3800	NTP TR 586, September 2015	Direct mutagen in *Escherichia coli* but negative in TA98 and TA100; weakly positive	Negative in micronucleus in vivo	Semi-synthetic metal-working fluid, complex mixture of chemicals	Inhalation	Some evidence of carcinogenic activity in *female mice*. Negative in male mice and rats	Increased bronchiole hyperplasia, alveolar epithelium hyperplasia, histiocytic cellular infiltration
Ozone (CAS No. 10028-15-6)	NTP TR 440	Positive—direct mutagen in TA102	Negative in Chinese hamster ovary cells	Calculated Log $p = -0.87$	Inhalation	There was equivocal evidence of carcinogenic activity of ozone in *male* B6C3F$_1$ *mice* based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was some evidence of carcinogenic activity of ozone in *female* B6C3F$_1$ *mice* based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. Negative in rats	Increased incidences of metaplasia occurred in the nose and lung of mice exposed to 0.5 or 1.0 ppm ozone. The metaplasia in the nose consisted of increased thickening and extension of the squamous epithelium in the anterior portion of the nasal passage. The metaplasia in the lung consisted of extension of the bronchial epithelium into the alveoli of the centriacinar region
1,3-Butadiene CAS No. 106-99-0	NTP TR 434, May 1993	Metabolites are direct acting mutagens in TA100, 1535 with S9 activation	Positive in chromosome aberrations	Log $p = 1.99$. Soluble in water and some organic solvents	Inhalation	Clear evidence in male and female mice; negative in rats	Alveolar epithelial hyperplasia in mice
Chloroethane (ethyl chloride) CAS No. 75-00-3	NTP TR 346, October 1989	Positive to TA1535 without S9 activating agent	Positive chromosomal aberrations	Log $p = 1.43$. Chloroethane is 0.57% (w/v) soluble in water at 20°C, 48% soluble in ethyl alcohol at 21°C, and miscible with ethyl ether	Inhalation	Positive in *male mice*; negative in female mice and rats	None
Dichloromethane (methylene chloride) CAS No. 75-09-2	NTP TR 306, January 1986	Positive—direct acting Ames assay mutagen TA98 and TA100	Positive in chromosomal aberrations	Log $p = 1.25$. Soluble in water.	Inhalation	Clear evidence of carcinogenicity in male and female mice; negative in rats	Female rats showed squamous metaplasia of nasal cavity in high-dose group
1,2-Dibromo-3-chloropropane CAS No. 96-12-8	NTP TR 206, March 1982	Positive to TA1535 without S9	Positive in mouse local lymph node assay	Log $p = 2.96$. Soluble in miscellaneous aliphatic and aromatic hydrocarbons; soluble in water	Inhalation	Positive in male and female mice; negative in rats	Multifocal epithelial hyperplasia

NTP: National Toxicology Program.
All six of the chemicals that are positive in the Ames assay test and that cause lung tumors in mice only are direct acting Ames assay mutagens that do not require metabolic activation by hepatic S9 fraction.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

NTP considers results from the Ames assay test to be very important in its deliberations as illustrated by the following statement from a recent Report on Carcinogens.[10]

> DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[11] A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).[12,13] Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone . . .

To eliminate the introduction of selection bias into this analysis, all positive Ames assay *Salmonella* bacterial mutagenicity test results reported in the literature and any statistically significant increase in lung tumor incidence over background in an NTP two-year inhalation study were accepted at face value.

Statistical methods

The following tests were applied to assess the statistical significance of the differences in proportions.[14]

Pooled test:

$$H_0 : p_1 - p_2 = 0$$

$$z = \frac{(\hat{p}_1 - \hat{p}_2)}{\hat{p}(1-\hat{p})\frac{1}{n_1} + \frac{1}{n_2}}$$

$$\hat{p} = \frac{x_1 + x_2}{n_1 + n_2}$$

Unpooled test:

$$H_0 : p_1 - p_2 = 0$$

$$z = \frac{(\hat{p}_1 - \hat{p}_2)}{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}$$

Results

Table 1 presents a summary of the proportions of the 58 compounds tested by NTP in both rats and mice in two-year inhalation studies. The fraction of agents that only cause lung tumors was 7/58 (12.1%) in rats and 15/58 (25.9%) in mice. At 95% confidence, the difference in these proportions was borderline significant at $p\text{-value}_1 = 0.0588$ (pooled test) and $p\text{-value}_2 = 0.0549$ (unpooled test). The fraction of agents that only cause tumors outside the lung was 34/58 (58.6%) in rats and 10/58 (17.2%) in mice ($p\text{-value}_1 < 0.0001$; $p\text{-value}_2 < 0.0001$). The fraction of agents that are both negative in the Ames assay test and only cause lung tumors was 3/58 (5.2%) in rats and 11/58 (19.0%) in mice ($p\text{-value}_1 = 0.0226$; $p\text{-value}_2 = 0.0198$). The fraction of agents that are both positive in the Ames assay test and

Table 7. 1/58 Total NTP inhalation studies conducted in rats and mice are positive in the Ames assay test and show lung tumors in rats only.

Chemical	Date	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
1,2-epoxybutane CAS No. 106-88-7	March 1988	Positive direct-acting alkylating agent	Positive in SCE and chromosome aberrations	Colorless liquid, soluble in water, ethanol, and most organic solvents	Inhalation	Clear evidence in male rats; no evidence in female rats, or male or female mice. The highest exposure concentration selected for the 2-year studies in rats was 400 ppm. The highest concentration selected for the 2-year studies in mice was 100 ppm because the nasal lesions seen at 200 and 400 ppm were considered to be potentially life-threatening	1,2-Epoxybutane exposure was associated with adenomatous hyperplasia and inflammatory lesions of the nasal cavity in rats and inflammatory lesions of the nasal cavity in mice

NTP: National Toxicology Program.
The negative result in mice is confounded by the maximum dose in mice being ¼ the maximum dose in rats.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 8. 22/58 Total NTP inhalation studies conducted in rats and mice are negative in the Ames assay test and show lung tumors in neither rats nor mice.

Chemical	Date	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Diethylamine CAS No. 109-89-7	NTP TR 566, October 2011	Negative	Negative in micronucleus	Log $p = 0.58$. Soluble in water, ether, CCl_4, and chloroform	Inhalation	Negative in rats and mice	Not applicable
Tetralin CAS No. 119-64-2	NTP TR 561, April 2011	Negative	Negative in micronucleus	Log $p = 1.67$. Soluble in ether and aniline	Inhalation	Negative in rats and mice	Not applicable
Propargyl alcohol CAS No. 107-19-7	NTP TR 552, September 2008	Negative	Negative in vivo micronucleus	Log $p = -0.38$. Miscible in water and many organic solvents	Inhalation	Negative in rats and mice	Not applicable
α-Methylstyrene CAS No. 98-83-9	NTP TR 543, November 2007	Negative	Negative chromosome aberrations, positive SCEs, positive micronucleus	Log $p = 3.48$. Miscible in water and many organic solvents	Inhalation	Negative in rats and mice	Not applicable
Methyl isobutyl ketone CAS No. 108-10-1	NTP TR 538, February 2007	Negative	Negative in mouse lymphoma assay	Log $p = 1.31$. Miscible in water and many organic solvents	Inhalation	Negative in rats and mice	Not applicable
Stoddard solvent IIC CAS No. 64742-88-7	NTP TR 519, September 2004	Negative	In vivo micronucleus negative	Stoddard Solvent is the most widely used solvent in the paint industry. It is a white spirit/mineral spirit. Component Log $p = 3.16$–7.06	Inhalation	Negative in rats and mice	Not applicable
Decalin CAS No. 91-17-8	NTP TR 513, January 2005	Negative	Equivocal in micronucleus	Log $p = 4.8$. Soluble in water, alcohol, ether, and chloroform	Inhalation	Negative in rats and mice	Not applicable
Isobutene CAS No. 115-11-7	NTP TR 487, December 1998	Negative	Negative in micronucleus	Log $p = 2.34$. Soluble in water, alcohol, ether, and chloroform	Inhalation	Negative in rats and mice	Not applicable
2-Butoxyethanol CAS No. 111-76-2	NTP TR 484, March 2000	Negative	Negative in SCEs, chromosome aberrations	Log $p = 0.83$. Soluble in mineral oil, most organic solvents, and ethanol	Inhalation	Negative in rats and mice	Not applicable
Furfuryl alcohol CAS No. 98-00-0	NTP TR 482, February 1999	Negative	Positive in SCEs, negative in chromosome aberrations, negative in micronucleus	Log $p = 0.28$. Soluble in water, most oils, alcohol, and organic solvents	Inhalation	Negative in rats and mice	Not applicable
Tetrahydrofuran CAS No. 109-99-9	NTP TR 475, June 1998	Negative	Negative in SCE, chromosome aberrations, micronucleus	Log $p = 0.46$. Soluble in water, ethanol, and ketones	Inhalation	Negative in rats and mice	Not applicable
Isobutyraldehyde CAS No. 78-84-2	NTP TR 472, February 1999	Negative	Positive for SCEs and chromosome aberrations	Log $p = 0.77$. Soluble in water, ethanol, and ketones	Inhalation	Negative in rats and mice	Not applicable
Tetrafluoroethylene CAS No. 116-14-3	NTP TR 450, April 1997	Negative	Negative	Log $p = 1.21$ (est.). Soluble in water	Inhalation	Negative in rats and mice	Not applicable

(continued)

Table 8. (continued)

Chemical	Date	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Acetonitrile CAS No. 75-05-8	NTP TR 447, April 1996	Negative	Weakly positive SCE and chromosome aberrations. Positive micronucleus male mice	Log $p = -0.34$. Soluble in water, alcohol, chloroform, and ether	Inhalation	Negative in rats and mice	Not applicable
Hexachlorocyclopentadiene CAS No. 77-47-4	NTP TR 437, February 1994	Negative	Negative in SCE, chromosome aberrations, micronucleus	Log $p = 5.04$. Soluble in water, acetone, CCl_4, and methanol	Inhalation	Negative in rats and mice	Not applicable
l-Epinephrine hydrochloride CAS No. 55-31-2	NTP TR 380, March 1990	Negative	Negative in SCE and chromosome aberrations	Log $p = -2.59$. Soluble in water	Inhalation	Negative in rats and mice	Not applicable
2-Chloroacetophenone CAS No. 532-27-4	NTP TR 379, March 1990	Negative	Weakly positive in chromosomal aberrations	Log $p = 1.93$ (est.). Soluble in ethanol, ether, and benzene	Inhalation	Negative in rats and mice	Not applicable
CS2 (94% o-chlorobenzalmalononitrile) CAS No. 2698-41-1	NTP TR 377, March 1990	Equivocal or negative	Positive in SCE and chromosome aberrations	Log $p = 2.76$. Soluble in acetone, methylene chloride, and benzene	Inhalation	Negative in rats and mice	Not applicable
Toluene CAS No. 108-88-3	NTP TR 371, February 1990	Negative	Negative	Log $p = 2.73$. Soluble in ethanol, benzene, ether, and carbon sulfide	Inhalation	Negative in rats and mice	Not applicable
Methyl methacrylate CAS No. 80-62-6	NTP TR 314, October 1986	Negative	Positive for SCEs and chromosome aberrations	Log $p = 1.38$. Soluble in water and most organic solvents	Inhalation	Negative in rats and mice	Not applicable
Tetrachloroethylene (perchloroethylene) CAS No. 127-18-4	NTP TR 311, August 1986	Negative	Negative	Log $p = 3.40$. Soluble in water, ethanol, chloroform, and benzene	Inhalation	Negative in rats and mice	Not applicable
Propylene oxide CAS No. 75-56-9	NTP TR 267, March 1985	Negative	Induces DNA strand breaks in human diploid fibroblasts	Log $p = 0.03$. Soluble in water, ethanol, and ether	Inhalation	Negative in rats and mice	Not applicable

NTP: National Toxicology Program.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 9. 1/58 Total NTP inhalation studies conducted in rats and mice are positive in the Ames assay test and show lung tumors in neither rats nor mice.

Chemical	Date	Ames assay test	Clastogen	Physicochemical characteristics[a]	Route of exposure	Lung tumors	Nonneoplastic findings
Propylene glycol mono-*t*-butyl ether CAS No. 57018-52-7	NTP TR 515, March 2004	Positive in TA97 without S9	Negative in SCE and chromosome aberrations; small positive micronucleus	Log $p = 0.87$ (est.). Soluble in water.	Inhalation	Negative in rats and mice	

NTP: National Toxicology Program.
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

only cause tumors outside the lung was 25/58 (43.1%) in rats and 10/58 (17.2%) in mice (p-value$_1$ = 0.0024; p-value$_2$ = 0.0016). The fraction of agents that are both positive in the Ames assay test and only cause lung tumors is 1/58 (1.7%) in rats and 6/58 (10.3%) in mice (p-value$_1$= 0.0512; p-value$_2$ = 0.0477). The fraction of agents that are both positive in the Ames assay test and only cause tumors outside the lung is 14/58 (24.1%) in rats and 1/58 (1.7%) in mice (p-value$_1$ = 0.0003; p-value$_2$ <0.0001). The fraction of agents that cause tumors outside the lung in only one rodent species is 16/58 (27.6%) in rats and 0/58 in mice (p-value$_1$ = <0.0001; p-value$_2$ <0.0001).

Eleven out of 58 agents tested in the NTP inhalation studies using rats and mice were negative in the Ames assay test and showed lung tumors in mice only (Table 2). Ten of the 11 chemicals (90.9%) in Table 2 are insoluble or slightly soluble in water, soluble in organic solvents, and have moderately hydrophobic log base 10 octanol–water partition coefficients of 0.17, 1.85, 2.10, 2.13, 2.42, 2.53, 2.61, 3.15, 3.30, 3.66, and 3.80. These moderate log p (log K_{ow}) values are near the optimum values for penetrating the lipid bilayer membranes of cells.[15] These chemicals induce hyperplasia in the airways of mice. Hyperplasia is an increase in the number of cells resulting from cellular proliferation.[16]

Three out of 58 agents tested in the NTP inhalation studies using rats and mice were negative in the Ames assay test and showed lung tumors in rats only (Table 3). These three agents contain metals and are not soluble in water. When laboratory rats are exposed to inorganic particles to the point that lung overload occurs, both benign and malignant tumors may develop. Rats exhibit relatively fast pulmonary clearance of dust and appear to retain pulmonary burdens of dust predominantly in macrophages within alveoli. Mice do not experience similar particle overload effects.[17]

Five out of 58 (8.6%) agents tested in the NTP inhalation studies using rats and mice were negative in the Ames assay test and showed lung tumors in both rats and mice (Table 4). Four out of five agents (80%) in Table 4 are powdered metals. One of the five (20%), Trim VX is a water-soluble oil that forms a chemical emulsion. Each of these five agents caused inflammation and hyperplasia in the lungs. In Table 5, 9/58 (15.5%) agents tested in NTP inhalation studies conducted in rats and mice were positive in the Ames assay test and showed lung tumors in both rats and mice. Three out of nine (33.3%) of these agents were metals.

In Table 6, 6/58 (10.3%) of the total NTP studies conducted using rats and mice were positive in the Ames assay test and showed lung tumors in mice only. All six of these chemicals were direct acting Ames assay mutagens that did not require metabolic activation by rat liver S9 to display mutagenicity. Table 7 shows a stark contrast with the results from Table 6. In Table 7, only 1/58 (1.7%) of the total NTP inhalation studies conducted in rats and mice reported an agent that was positive in the Ames assay test and displayed lung tumors in rats only. In addition, this one positive result might be spurious as the maximum exposure dose in mice was only ¼ the maximum dose in rats due to lethality of 1,2-epoxybutane in mice.

In Table 8, 22/58 (37.9%) total NTP inhalation studies conducted in rats and mice were negative in the Ames assay test and did not show lung tumors in either rats or mice. Since these 22 agents did not show neoplastic changes in the lungs, nonneoplastic changes are not shown for this group of compounds. A number of these agents are either relatively water soluble with log p's of −2.59, −0.38, −0.34, 0.055, 0.28, 0.46, 0.58, 0.77, and 0.83 or extremely hydrophobic with log p's of 4.8, 5.04, and a range of 3.16–7.06 for a multicomponent mixture. Whether these 12 log p values that fall outside the optimum cellular penetration range of about log p of 2[11] reduced their ability to penetrate the lung epithelial cells of the rodents thereby reducing their potential tumorigenicity is unknown.

Table 9 shows that propylene glycol mono-*t*-butyl ether is the only chemical tested via inhalation by NTP in rats and mice reported to be both Ames assay positive and lacking lung tumors in either rats or mice, that is, 1/58 (1.7%). This result is questionable as the only Ames assay data available was a single positive result in Ames assay *Salmonella* strain TA97 without metabolic activation by rat liver S9. Table 10 shows that Ames assay test data were lacking for 2/58 (3.4%) of the total NTP inhalation studies conducted in rats and mice, that is, nickel sulfate hexahydrate which did not cause lung tumors in either rats or mice, and indium phosphide which did cause lung tumors in male and female rats and in male and female mice.

Table 10. 2/58 Total NTP inhalation studies conducted in rats and mice for compounds lacking Ames assay test data.

Chemical	Date	Ames assay test	Clastogen	Physicochemical characteristics	Route of exposure	Lung tumors	Nonneoplastic findings
Nickel sulfate hexahydrate CAS No. 10101-97-0	NTP TR 454, July 1996	No data	Nickel sulfate hexahydrate (500 to 800 g/mL) was tested for induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells. A positive response was observed in the absence of S9. The test was not performed with S9	Soluble in water and ammonium hydroxide	Inhalation	Negative in rats and mice	The incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis were markedly increased in male and female rats exposed to 0.25 or 0.5 mg/m^3. Inflammatory lesions of the lung generally occurred in all exposed groups of male and female mice at the end of the 2-year study. These lesions included macrophage hyperplasia, chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), alveolar proteinosis, and infiltrating cells in the interstitium
Indium phosphide CAS No. 22398-80-7	NTP TR 499, July 2001	No data	Negative in micronucleus	Slightly soluble in acid	Inhalation	Clear evidence in male rats, female rats, male mice, female mice	Chronic active inflammation in mouse lung; atypical hyperplasia and inflammation in rat lung

NTP: National Toxicology Program.

Table 11 shows 7/58 (12.1%) cases where a compound caused tumors in the rat lung, but not outside the lung. Of the seven compounds, four are metals. The seven compounds are as follows: tetranitromethane, isobutyl nitrite, antimony trioxide, vanadium pentoxide, chromium hexavalent compounds, Trim VX, and molybdenum trioxide. Table 11 shows 15/58 (25.9%) cases where a compound only caused tumors in the mouse lung, but not outside the lung. Of the 15 compounds, seven are metals (46.7%). The 15 compounds are as follows: cobalt metal, tetranitromethane, cobalt sulfate heptahydrate, isobutyl nitrite, antimony trioxide, vanadium pentoxide, chromium hexavalent compounds, bis(chloromethyl)ether, Trim VX, nickel oxide, molybdenum trioxide, ozone, vinylidene chloride, naphthalene, and divinylbenzene-HP. All of the seven compounds that caused tumors in the rat lung, but did not cause tumors at other anatomical sites in the rat, also caused tumors in the mouse lung.

Further examination of Table 11 shows that in 34/58 (58.6%) of the compounds tested via inhalation, rats did not show a lung tumor but did show a tumor at another anatomical site outside the lung. In every one of these 34 cases, the chemical was not a metal. The 34 chemicals are as follows: 1,3-butadiene, trichloroethylene, cumene, dichloromethane, isoprene, nitromethane, chloroprene, 1,2-dibromo-3-chloropropane, nitrobenzene, chloroethane, naphthalene, ethylbenzene, CIMSTAR, divinylbenzene-HP, allyl glycidyl ether, propylene glycol mono-*t*-butyl ether, tetralin, propargyl alcohol, α-methylstyrene, methyl isobutyl ketone, Stoddard Solvent IIC, decalin, isobutene, 2-butoxyethanol, furfuryl alcohol, tetrahydrofuran, tetrafluoroethylene, acetonitrile, hexachlorocyclopentadiene, 2-chloroacetophenone, tetrachloroethylene, and propylene oxide.

In contrast with the results for rats, only 10/58 (17.2%) of the compounds tested via inhalation displayed a pattern of no lung tumors in mice, but presentation of tumors at other sites outside the lung. The 10 chemicals are as follows: propylene glycol mono-*t*-butyl ether, propargyl alcohol, methyl isobutyl ketone, Stoddard Solvent IIC, decalin, furfuryl alcohol, tetrahydrofuran, tetrafluoroethylene, tetrachloroethylene, and propylene oxide. All of the 10 compounds that caused tumors at anatomical sites outside the lung, but did not cause lung tumors in mice, also displayed the same tumor presentation pattern in rats.

In Table 11, each of the 58 compounds for which data were available were ranked in descending order of their potential to induce lung tumors in the lungs of rats and mice. Two out of three substances ranked at the highest level of rodent pulmonary tumorigenicity were metals. The compound ranked at the second highest level of pulmonary tumorigenicity to rodent lung was a metal. One of the two compounds ranked at the third highest level of pulmonary tumorigenicity to rodent lung was a metal. In addition, the compound ranked at the fourth highest level of pulmonary tumorigenicity to rodent lung was also a metal. Therefore, 5/7 of the most potent compounds for inducing tumors in the lungs of rats and mice were metals.

Table 11. Relative ranking by lung tumor-producing potency of 58 substances tested via inhalation by NTP in rats and mice (cadmium and cadmium compounds; and diesel exhaust particulates omitted for lack of results regarding a particular compound).

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
Indium phosphide CAS No. 22398-80-7	1	No data	Negative	Not applicable because metallic, not soluble in water	Clear evidence in male rats, female rats, male mice, female mice	Yes—rats; Yes—mice
Cobalt metal CAS No. 7440-48-4	1	Positive	Negative	Not applicable because a metal; only ultrafine cobalt metal is soluble in water	Clear evidence in male rats, female rats, male mice, female mice	Yes—rats; No—mice
Tetranitromethane CAS No. 509-14-8	1	Positive	Positive	Log $p = -2.05$	Clear evidence in male rats, female rats, male mice, female mice	No—rats; No—mice
Cobalt sulfate CAS No. 10124-43-3 cobalt sulfate heptahydrate	2	Negative	Negative	Not applicable, soluble in water	Some evidence in male rats; clear evidence in female rats, male mice, female mice	Yes—rats; No—mice
Isobutyl nitrite CAS No. 542-56-3	3	Positive	Positive in SCE and chromosome aberrations	Log $p = 2.31$	There was clear evidence of carcinogenic activity of isobutyl nitrite in male and female F344/N rats based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was some evidence of carcinogenic activity of isobutyl nitrite in male and female B6C3F$_1$ mice based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males and females	No—rats; No—mice
Antimony trioxide CAS No. 1309-64-4	3	Positive comet assay in mouse lung tissue samples	Positive micronucleus	Not applicable because a metal; not soluble in water	*Some evidence of carcinogenic activity* of antimony trioxide in male and female Wistar Han rats based on increased combined incidences of alveolar/bronchiolar adenoma or carcinoma in the lung. Clear evidence in male and female mice	No—rats; No—mice
Vanadium pentoxide CAS No. 1314-62-1	4	Negative	Negative in micronucleus	Not applicable because a metal; dissolves slightly in water	Under the conditions of this 2-year inhalation study, there was some evidence of carcinogenic activity[a] of vanadium pentoxide in male F344/N rats and equivocal evidence of carcinogenic activity of vanadium pentoxide in female F344/N rats based on the occurrence of alveolar/bronchiolar neoplasms. There was clear evidence of carcinogenic activity of vanadium pentoxide in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms	No—rats; No—mice

(continued)

Table 11. (continued)

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
1,2-Dibromoethane CAS No. 106-93-4	5	Positive—direct acting mutagen	Positive in SCE and DNA binding	Log $p = 1.96$. Soluble in water and most organic solvents	Positive in male and female mice and in female rats. Negative in male rats	Yes—rats; Yes—mice
Chromium hexavalent compounds CAS No. 18540-29-9	6 (no info re rodent sex)	Positive	Positive	Not applicable because a metal	Exposure to chromium(VI) compounds (calcium chromate, chromium trioxide, or sodium dichromate) via inhalation or intratracheal or intrabronchial implantation caused benign and/or malignant lung tumors in rats and/or mice	No—rats; No—mice
Bis(chloromethyl) ether and technical grade chloromethyl methyl ether CAS Nos. 542-88-1 and 107-30-2	6 (no info re rodent sex)	Positive alkylating agent	Positive	Log $p = -0.38$. Reacts with water	Exposure to BCME by inhalation caused lung tumors in rats (benign) and mice (carcinoma)	Ranking would probably be higher if had sex info. Yes—rats; No—mice
Trim VX	7	Negative	Negative	Not applicable—complex mixture	There was *equivocal evidence of carcinogenic activity* of Trim VX in male Wistar Han rats based on the combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *equivocal evidence of carcinogenic activity* of Trim VX in female Wistar Han rats based on the occurrences of alveolar/bronchiolar adenoma of the lung. There was *clear evidence of carcinogenic activity* of Trim VX in male B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was clear evidence of carcinogenic activity of Trim VX in female B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung	No—rats; No—mice
Nickel oxide CAS No. 1313-99-1	8	Negative	Negative in micronucleus; negative in chromosome aberrations	Not applicable because a metal; insoluble in water	Some evidence in male and female rats; equivocal evidence mice; negative in male mice	Yes—rats; No—mice
Molybdenum trioxide CAS No. 1313-27-5	8	Negative	Negative	Not applicable because a metal; in soluble in water	Equivocal evidence in male rats, some evidence of carcinogenic activity in male mice, some evidence in female mice	No—rats; No—mice
Bromoethane (ethyl bromide) CAS No. 74-96-4	9	Positive	Positive for SCEs and negative for chromo some aberrations	Log $p = 1.3$	Some evidence in male rats, equivocal evidence in female rats, equivocal evidence in male mice. Negative in female mice	Yes—rats; Yes—mice

(continued)

Table 11. (continued)

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
Nickel subsulfide CAS No. 12035-72-2	10	Negative	Positive	Not applicable because a metal; insoluble in water	Clear evidence in male and female rats; No evidence in male or female mice	Yes—rats; No—mice
1,3-Butadiene CAS No. 106-99-0	10	Positive	Positive	Log $p = 1.99$	Clear evidence in male and female mice; negative in rats	Yes—rats; Yes—mice
Trichloroethylene CAS No. 79-01-6	10	Negative	Probably negative (caused SCE only)	Log $p = 2.61$	Clear evidence in male and female mice; negative in rats	Yes—rats; Yes—mice
Cumene CAS No. 98-82-8	10	Negative	Positive (weak)	Log $p = 3.66$	Clear evidence in male and female mice; negative in rats	Yes—rats; Yes—mice
Dichloromethane (methylene chloride) CAS No. 75-09-2	10	Positive—direct acting Ames assay mutagen TA98 and TA100	Positive in chromosome aberrations	Log $p = 1.25$	Clear evidence of carcinogenicity in male and female mice; negative in rats	Yes—rats; Yes—mice
Isoprene CAS No. 78-79-5	10	Negative	Negative	Log $p = 2.42$	Positive in male and female mice; negative in rats	Yes—rats; Yes—mice
Nitromethane CAS No. 75-52-5	10	Negative	Negative	Log $p = 0.17$	Positive in male and female mice; negative in rats	Yes—rats; Yes—mice
Chloroprene CAS No. 126-99-8	10	Negative	Negative	Log $p = 2.53$	Positive in male and female mice; negative in rats	Yes—rats; Yes—mice
1,2-dibromo-3-chloropropane CAS No. 96-12-8	10	Positive to TA1535 without S9		Log $p = 2.96$	Positive in male and female mice; negative in rats	Yes—rats; Yes—mice
Ozone	11	Positive—direct mutagen in TA102		Log $p = -0.87$	There was *equivocal evidence* of carcinogenic activity of ozone in male B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was *some evidence* of carcinogenic activity of ozone in female B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. Negative in rats	No—rats; No—mice
Talc containing no asbestos fibers CAS No. 14807-96-6	12	Negative	Negative	Not applicable Insoluble in water	Clear evidence in female rats; no evidence in male rats, or male or female mice	Yes—rats; No—mice
Gallium arsenide CAS No. 1303-00-0	12	Negative	Negative in micronucleus assay	Not applicable because a metal; insoluble in water	No evidence male rats, clear evidence in female rats, no evidence in male and female mice	Yes—rats; No—mice
Nitrobenzene CAS No. 98-95-3	12	Negative	Positive	Log $p = 1.85$	Positive in male mice. Negative in female mice and rats	Yes—rats; Yes—mice

(continued)

Table 11. (continued)

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
Chloroethane (ethyl chloride) CAS No. 75-00-3	12	Positive to TA1535 without S9 alkylating agent		Log $p = 1.43$	Positive in male mice. Negative in female mice and rats	Yes—rats; Yes—mice
Vinylidene chloride CAS No. 75-35-4	12	Negative	Negative	Log $p = 2.13$	Incidence of alveolar/bronchiolar carcinoma significantly increased in 12.5 ppm female mice; negative in male mice and in rats	No—rats; No—mice
Naphthalene CAS No. 91-20-3	12	Negative	Positive	Log $p = 3.3$	Significantly increased incidence of benign lung tumors (adenoma) in female B6C3F1 mice	Yes—rats; No—mice
Ethylbenzene CAS No. 100-41-4	13	Negative	Negative for SCE or chromosome aberrations	Log $p = 3.15$	Some evidence of carcinogenic activity in male mice; negative in female mice and rats	Yes—rats; Yes—mice
CIMSTAR 3800	13	Direct mutagen *E. coli* but negative in TA98 and TA100; considered weakly positive	Negative in micronucleus in vivo	Not applicable—— multicomponent mixture	Some evidence of carcinogenic activity in female mice. Negative in male mice and rats	Yes—rats; Yes—mice
Divinylbenzene-HP CAS No. 1321-74-0	14	Negative	Negative	Log $p = 3.8$	Equivocal evidence of carcinogenic activity in female mice; negative in male mice, male and female rats	Yes—rats; No—mice
Allyl glycidyl ether CAS No. 106-92-3	15	Positive	Positive in SCE and chromosome aberrations	Solubility in water is 14%	No evidence of carcinogenic activity in male Osborne-Mendel rats, no evidence in female rats, no evidence in male mice, no evidence in female mice	Yes—rats; No—mice; nasal passages only
Propylene glycol mono-*t*-butyl ether CAS No. 57018-52-7	15	Positive in TA97 without S9	Negative in SCE and chromosome aberrations; small positive micronucleus	Log $p = 0.83$	Negative in rats and mice	Yes—rats; Yes—mice
Diethylamine CAS No. 109-89-7	15	Negative	Negative in micronucleus	Log $p = 0.58$	Negative in rats and mice	No—rats; No—mice
Tetralin CAS No. 119-64-2	15	Negative	Negative in micronucleus	Log $p = 1.67$	Negative in rats and mice	Yes—rats; No—mice
Propargyl alcohol CAS No. 107-19-7	15	Negative	Negative in vivo micronucleus	Log $p = -0.38$	Negative in rats and mice	Yes—rats; Yes—mice
α-Methylstyrene CAS No. 98-83-9	15	Negative	Negative chromosome aberrations, positive SCEs, positive micronucleus	Log $p = 3.48$	Negative in rats and mice	Yes—rats; Yes—mice
Methyl isobutyl ketone CAS No. 108-10-1	15	Negative		Log $p = 1.31$	Negative in rats and mice	Yes—rats; Yes—mice

(continued)

Table 11. (continued)

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
Stoddard solvent IIC CAS No. 64742-88-7	15	Negative	In vivo micronucleus negative	Not applicable—mixture	Negative in rats and mice	Yes—rats; Yes—mice
Decalin CAS No. 91-17-8	15	Negative	Equivocal in micronucleus	Log $p = 4.8$	Negative in rats and mice	Yes—rats; Yes—mice
Isobutene CAS No. 115-11-7	15	Negative	Negative in micronucleus	Log $p = 2.34$	Negative in rats and mice	Yes—rats; No—mice
2-Butoxyethanol CAS No. 111-76-2	15	Negative	Negative SCEs, chromosome aberrations	Log $p = 0.83$	Negative in rats and mice	Yes—rats; No—mice
Furfuryl alcohol CAS No. 98-00-0	15	Negative	Positive SCEs, negative chromosome aberrations, negative micronucleus	Log $p = 0.28$	Negative in rats and mice	Yes—rats; Yes—mice
Tetrahydrofuran CAS No. 109-99-9	15	Negative	Negative in SCE, chromosome aberrations, micronucleus	Log $p = 0.46$	Negative in rats and mice	Yes—rats; Yes—mice
Isobutyraldehyde CAS No. 78-84-2	15	Negative	Positive for SCEs and chromosome aberrations	Log $p = 0.77$	Negative in rats and mice	No—rats; No—mice
Tetrafluoroethylene CAS No. 116-14-3	15	Negative	Negative	Log $p = 1.21$	Negative in rats and mice	Yes—rats; Yes—mice
Acetonitrile CAS No. 75-05-8	15	Negative	Weakly positive SCE and chromosome aberrations. Positive micronucleus male mice	Log $p = -0.34$	Negative in rats and mice	Yes—rats; No—mice
Hexachlorocyclopentadiene CAS No. 77-47-4	15	Negative	Negative in SCE, chromosome aberrations, micronucleus	Log $p = 5.04$	Negative in rats and mice	Yes—rats; No—mice
l-Epinephrine hydrochloride CAS No. 55-31-2	15	Negative	Negative in SCE and chromosome aberrations	Log $p = -2.59$	Negative in rats and mice	No—rats; No—mice; doses considered too low
2-Chloroacetophenone CAS No. 532-27-4	15	Negative	Weakly positive in chromosome aberrations	Log $p = 1.93$	Negative in rats and mice	Yes—rats; No—mice

(continued)

Table 11. (continued)

Agent	Lung tumor rank	Ames assay +/−	Clastogen +/−	Log Kow	Lung tumor presentation	Tumors at other organ sites
CS2 (94% o-chlorobenzalmalononitrile) CAS No. 2698-41-1	15	Equivocal or neg	Positive in SCE and chromosome aberrations	Log $p = 2.76$	Negative in rats and mice	No—rats; No—mice
Toluene CAS No. 108-88-3	15	Negative	Negative	Log $p = 2.73$	Negative in rats and mice	No—rats; No—mice
Methyl methacrylate CAS No. 80-62-6	15	Negative	Positive for SCEs and chromosome aberrations	Log $p = 1.38$	Negative in rats and mice	No—rats; No—mice
Tetrachloroethylene (perchloroethylene) CAS No. 127-18-4	15	Negative	Negative	Log $p = 3.40$	Negative in rats and mice	Yes—rats; Yes—mice
Propylene oxide CAS No. 75-56-9	15	Negative		Log $p = 0.055$	Negative in rats and mice	Yes—rats (nasal turbinates); Yes—mice (nasal turbinates)
Nickel sulfate hexahydrate CAS No. 10101-97-0	15	No data	Nickel sulfate hexahydrate (500 to 800 g/mL) was tested for induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells. A positive response was observed in the absence of S9. The test was not performed with S9	Not applicable because a metal	Negative in rats and mice	No—rats; No—mice

NTP: National Toxicology Program.
Clear Evidence > Some Evidence > Equivocal Evidence > No Evidence
[a]All physicochemical values are from the Hazardous Substances Database unless otherwise designated.

Table 11 also compares the concordance between rats and mice for each of the 58 compounds tested via the inhalation route to induce tumors at organ sites outside the lung. For 26/58 (44.8%) compounds, both rats and mice showed development of another tumor type outside of the lung. That is, there were 26 cases of positive concordance for development of tumors outside the lung when tested by the inhalation route. Herein follows the unusual result from Table 11. For 16/58 (27.6%) compounds, rats showed tumors outside the lungs while similarly tested mice did not show tumors outside the lungs. In contrast, there is a not a single case where mice showed a tumor outside the lungs while a similarly tested rat did not show a tumor outside the lungs—0/58 compounds.

The entire list of the 10 compounds negative in mouse lung, but positive at other sites, is contained within the list of 34 compounds negative in rat lung but positive at other sites.

In Table 11, there were seven compounds (7/58, 12.1%) which produced neither lung tumors nor tumors at other anatomical sites in either rats or mice. These seven completely negative compounds are as follows: diethylamine, isobutyraldehyde, *l*-epinephrine hydrochloride, CS2 (94% *o*-chlorobenzalmalononitrile), toluene, methyl methacrylate, and nickel sulfate hexahydrate. Ames assay test data were available for 6/7 (85.7%) with the exception of nickel sulfate hexahydrate. All of the six Ames assay tests conducted on these compounds were negative.

In Table 11, indium phosphide was the only compound that produced lung tumors in male and female rats, male and female mice, and at other anatomical sites in both rats and mice. Indium phosphide ranked as the most clearly tumorigenic compound to rodent lung of the 58 tested to date via inhalation by NTP.

Conclusion

For the 58 compounds tested via inhalation by NTP, there is a high degree of discordance between rats and mice in the susceptibility to develop lung tumors. The causation of tumors at anatomical sites outside the lung via the inhalation route is also discordant in rats and mice. This high degree of discordance in the results of two-year inhalation assays suggests that different mechanisms of carcinogenesis might play lesser or greater roles in the development of pulmonary tumors in the two species. In cases where the results from two-year inhalation studies are concordant for lung tumors, the concordant agent might be of special concern to human risk assessment.

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