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

Carr J Smith1,2 and Steven P Anderson1

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

Date received: 29 April 2017; accepted: 22 May 2017

Introduction
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.

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. Two of the 60 inhalation
studies were conducted on “cadmium and cadmium compounds” and on “diesel exhaust particulates.” 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 B6C3F1 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.

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

| Species | Fraction of 58 agents causing lung tumors only | Fraction of 58 agents causing non-lung tumors only | Fraction of Ames assay test negative agents causing lung tumors only | Fraction of Ames assay test negative agents causing non-lung tumors only | Fraction of Ames assay test positive agents causing lung tumors only | Fraction of Ames assay test positive agents causing non-lung tumors only | Fraction of agents causing non-lung tumors in one species only |
|---------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| 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                                           |

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

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

NTP: National Toxicology Program.

Borderline at 95% significant difference between rats and mice at $p$-value$_1 = 0.0588; p$-value$_2 = 0.0549$.

Statistically significant difference between rats and mice at $p$-value$_1 <0.0001; p$-value$_2 <0.0001$.

Statistically significant difference between rats and mice at $p$-value$_1 = 0.0226; p$-value$_2 = 0.0198$.

Statistically significant difference between rats and mice at $p$-value$_1 = 0.0024; p$-value$_2 = 0.0016$.

Statistically significant difference between rats and mice at $p$-value$_1 = 0.0512; p$-value$_2 = 0.0477$.

Statistically significant difference between rats and mice at $p$-value$_1 = 0.0003; p$-value$_2 = 0.0001$.

Statistically significant difference between rats and mice at $p$-value$_1 <0.0001; p$-value$_2 <0.0001$.

No significant difference in Ames assay positive and Ames assay negative agents at $p$-value$_1 = 0.2543; p$-value$_2 = 0.2501$.

Statistically significant difference in Ames assay positive and Ames assay negative agents at $p$-value$_1 <0.0001; p$-value$_2 <0.0001$.

No significant difference between rats and mice at $p$-value$_1 = 0.0751; p$-value$_2 = 0.0703$.

No significant difference between rats and mice at $p$-value$_1 = 0.0751; p$-value$_2 = 0.0703$.

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, 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.
### 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 | Route of exposure | Lung tumors                                      | Nonneoplastic findings                                                                 |
|---------------------------|--------------------------|-----------------|-----------------------------------------------|---------------------------------|-------------------|-------------------------------------------------|------------------------------------------------------------------------------------------|
| Nitrobenzene CAS No. 98-95-3 | RoC 13th edition          | Negative        | Positive in chromosome aberrations in humans. | 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 |
| I-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 3. 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                        | Reference      | Ames assay test | Clastogen                          | Physicochemical characteristics | Route of exposure | Lung tumors                                      | Nonneoplastic findings                                      |
|---------------------------------|----------------|----------------|------------------------------------|---------------------------------|-------------------|-------------------------------------------------|-------------------------------------------------------------|
| Gallium arsenide CAS No. 1303-00-0 | NTP TR 492, September 2000 | Negative | Negative micrornucleus 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.

*All physicochemical values are from the Hazardous Substances Database unless otherwise designated.*
Table 4. 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. | 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) |
|                 |                             |                 |           |                                 |                   | 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. | 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

| Chemical        | References                  | Ames assay test | Clastogen | Physicochemical characteristics | Route of exposure | Lung tumors                                                                 | Nonneoplastic findings                                                                                                                                 |
|-----------------|-----------------------------|-----------------|-----------|---------------------------------|-------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
|                 | 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, alkales (forming vanadates), and acetone. | Inhalation | Under the conditions of this 2-year inhalation study, there was some evidence of carcinogenic activity 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)
| Chemical                        | References                     | Ames assay test | Clastogen | Physicochemical characteristics                                                                 | Route of exposure | Lung tumors                                                                 | Nonneoplastic findings                                                                                                                                                                                                 |
|--------------------------------|--------------------------------|----------------|-----------|-------------------------------------------------------------------------------------------------|------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cobalt sulfate heptahydrate    | NTP TR 471, August 1998        | Negative        | Negative  | Cobalt sulfate is a reddish, crystalline, water-soluble powder (Smith and Carson, 1981).<sup>7</sup> | 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, histiocytic 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 |
Table 4. (continued)

| Chemical                  | References            | Ames assay test | Clastogen                          | Physicochemical characteristics                                                                 | Route of exposure | Lung tumors                              | Nonneoplastic findings                                                                 |
|---------------------------|-----------------------|-----------------|-------------------------------------|-------------------------------------------------------------------------------------------------|-------------------|-------------------------------------------|---------------------------------------------------------------------------------------|
| Nickel oxide              | 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) is an olive gray powder with a melting point of 2090°C and a density of 7.45 g/cm³. It is insoluble in water and soluble in acids (Merck Index, 1989). 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 pigmentations 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 pigmentations 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.

*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         | NTP TR 590        | Positive Comet Assay in mouse | 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 |
| CAS No. 1309-64-4          | (February, 2016)   | lung tissue samples           |                                        |                                                   |                   |                                                                               |                                                                                        |
| 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 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). | Bronchiolar and alveolar hyperplasia                                                     |
| Tetranitromethane CAS No. 509-14-8 | NTP TR 386, March 1990 | Positive chromosome aberrations and SCEs | Log $K_{ow}$=2.05. Soluble in ethanol, ether, and CCl₄. Soluble in water. Freely soluble in alcoholic KOH |                                                   | Inhalation        | Clear evidence of carcinogenic activity of tetranitromethane for male and female F344/N rats and male and female B6C3F1 mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats | Alveolar hyperplasia. Hyperplastic and squamous metaplasia or respiratory epithelium |

(continued)
| Chemical                                      | References                  | Ames assay test | Clastogen                                      | Physicochemical characteristics | Route of exposure | Lung tumors                      | Nonneoplastic findings                                      |
|----------------------------------------------|----------------------------|----------------|-----------------------------------------------|---------------------------------|-------------------|---------------------------------|------------------------------------------------------------|
| Allyl glycidyl ether                         | 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 |
| CAS No. 106-92-3                             | 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                    |
| Bromoethane (ethyl bromide) CAS No. 74-96-4 |                            |                |                                               |                                 |                   |                                 |                                                            |
| 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 supplicative 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.

*All physicochemical values are from the Hazardous Substances Database unless otherwise designated.*
| 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 bronchiolar 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 B6C3F1 mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was some evidence of carcinogenic activity of ozone in female B6C3F1 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 centricinar region |
| 1,3-Butadiene | 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) | 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 | 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 | 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.

*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:

\[ H_0 : p_1 - p_2 = 0 \]

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

Unpooled test:

\[ H_0 : p_1 - p_2 = 0 \]

\[ z = \frac{(\hat{p}_1 - \hat{p}_2)}{\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}} \]

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 \( p\)-value1 = 0.0588 (pooled test) and \( p\)-value2 = 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 (\( p\)-value1 < 0.0001; \( p\)-value2 < 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 (\( p\)-value1 = 0.0226; \( p\)-value2 = 0.0198). The fraction of agents that are both positive in the Ames assay test and show lung tumors in rats only.
### 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<sup>a</sup> | 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<sub>4</sub>, 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         |
| \( \alpha \)-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         |
| Isobutyaldehyde 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)
| Chemical                                      | Date             | Ames assay test | Clastogen                                              | Physicochemical characteristics | 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 |
| 1-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 |
| CS$_2$ (94% o-chlorobenzylnonitrile) 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.

*All physicochemical values are from the Hazardous Substances Database unless otherwise designated.*
only cause tumors outside the lung was 14/58 (24.1%) in rats and 0/58 (1.7%) in mice (p-value1 = 0.0003; p-value2 < 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-value1 = <0.0001; p-value2 < 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 values are near the optimum values for penetrating the lipid bilayer membranes of cells. These chemicals induce hyperplasia in the airways of mice. Hyperplasia is an increase in the number of cells resulting from cellular proliferation.

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.

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 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-tert-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 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, C11-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. One of the two compounds 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 a metal. Therefore, 5/7 of the most potent compounds for inducing tumors in the lungs of rats and mice were metals.

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                  | Route of exposure | Lung tumors | Nonneoplastic findings                                                                 |
|-----------------------------------|-----------------------|-----------------|-----------------------------|-------------------|-------------|---------------------------------------------------------------------------------------|
| Nickel sulfate hexahydrate        | NTP TR 454, July 1996 | No data         | Nickel sulfate hexahydrate  | 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/m³. 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                  | NTP TR 499, July 2001 | No data         | Slightly soluble in acid    | Inhalation        | Clear evidence in male rats, female rats, male mice                                  | Chronic active inflammation in mouse lung; atypical hyperplasia and inflammation in rat lung |

NTP: National Toxicology Program.
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 B6C3F1 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 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)
| Agent | Lung tumor rank | Lung tumor presentation | Log Kow | Tumors at other organ sites |
|-------|-----------------|-------------------------|---------|---------------------------|
| 1,2-Dibromoethane CAS No. 106-93-4 | 5 | Positive in male and female mice and in female rats. Negative in male rats | Log $p = 1.96$. Soluble in water and most organic solvents | Yes—rats; Yes—mice |
| Chromium hexavalent compounds CAS No. 18540-29-9 | 6 (no info re rodent sex) | 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 | Not applicable because a metal | 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) | Exposure to BCME by inhalation caused lung tumors in rats (benign) and mice (carcinoma) | Log $p = -0.38$. Reacts with water | Ranking would probably be higher if had sex info. Yes—rats; No—mice |
| Trim VX | 7 | 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. | Not applicable—complex mixture | Yes—rats; No—mice |
| Nickel oxide CAS No. 1313-99-1 | 8 | Some evidence in male and female rats; equivocal evidence in male mice; negative in male mice | Not applicable because a metal; insoluble in water | Yes—rats; No—mice |
| Molybdenum trioxide CAS No. 1313-27-5 | 8 | Equivocal evidence in male rats, some evidence of carcinogenic activity in male mice, some evidence in female mice | Not applicable because a metal; in soluble in water | No—rats; No—mice |
| Bromoethane (ethyl bromide) CAS No. 74-96-4 | 9 | Some evidence in male rats, equivocal evidence in female rats, equivocal evidence in male mice. Negative in female mice | Log $p = 1.3$ | Yes—rats; Yes—mice |
| Agent                                                                 | Lung tumor rank | Lung tumor presentation | Tumors at other organ sites |
|----------------------------------------------------------------------|-----------------|-------------------------|----------------------------|
| Nickel subsulfide CAS No. 12035-72-2                                  | 10              | Positive, Not applicable because a metal; insoluble in water | Yes—rats; No—mice          |
| 1,3-Butadiene CAS No. 106-99-0                                       | 10              | Positive, Log p = 1.99  | Yes—rats; Yes—mice         |
| Trichloroethylene CAS No. 79-01-6                                    | 10              | Probably negative (caused SCE only), Log p = 2.61 | Yes—rats; Yes—mice         |
| Cumene CAS No. 98-82-8                                               | 10              | Positive, weak, Log p = 3.66 | 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 | Yes—rats; Yes—mice         |
| Isoprene CAS No. 78-79-5                                             | 10              | Negative, Log p = 2.42  | Yes—rats; Yes—mice         |
| Nitromethane CAS No. 75-52-5                                          | 10              | Negative, Log p = 0.17  | Yes—rats; Yes—mice         |
| Chloroprene CAS No. 126-99-8                                         | 10              | Negative, Log p = 2.53  | Yes—rats; Yes—mice         |
| 1,2-dibromo-3-chloropropane CAS No. 96-12-8                          | 10              | Positive to TA1535 without S9, Log p = 2.96 | Yes—rats; Yes—mice         |
| Ozone                                                                | 11              | Positive—direct mutagen in TA102, Log p = –0.87 | No—rats; No—mice          |
| Talc containing no asbestos fibers CAS No. 14807-96-6                | 12              | Negative, Not applicable Insoluble in water | Yes—rats; No—mice          |
| Gallium arsenide CAS No. 1303-00-0                                   | 12              | Negative in micronucleus assay, Not applicable because a metal; insoluble in water | Yes—rats; No—mice          |
| Nitrobenzene CAS No. 98-95-3                                         | 12              | Positive, Log p = 1.85  | Yes—rats; Yes—mice         |
| Agent                                      | Lung tumor rank | Lung tumor presentation                                                                 | Tumors at other organ sites |
|--------------------------------------------|-----------------|-----------------------------------------------------------------------------------------|----------------------------|
| Chloroethane (ethyl chloride) CAS No. 75-00-3 | 12              | Positive in male mice. Negative in female mice and rats                                  | Yes—rats; Yes—mice         |
| Vinylidene chloride CAS No. 75-35-4        | 12              | 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              | Significantly increased incidence of benign lung tumors (adenoma) in female B6C3F1 mice    | Yes—rats; No—mice          |
| Ethylbenzene CAS No. 100-41-4              | 13              | Some evidence of carcinogenic activity in male mice; negative in female mice and rats     | Yes—rats; Yes—mice         |
| CIMSTAR 3800                               | 13              | Some evidence of carcinogenic activity in female mice. Negative in vivo micronucleus in vivo | Yes—rats; Yes—mice         |
| Divinylbenzene-HP CAS No. 1321-74-0        | 14              | Equivocal evidence of carcinogenic activity in female mice; negative in male mice, male and female rats | Yes—rats; No—mice          |
| Allyl glycyl ether CAS No. 106-92-3        | 15              | 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              | Negative in rats and mice                                                                | Yes—rats; Yes—mice         |
| Diethylamine CAS No. 109-89-7              | 15              | Negative in rats and mice                                                                | No—rats; No—mice           |
| Tetralin CAS No. 119-64-2                  | 15              | Negative in rats and mice                                                                | Yes—rats; No—mice          |
| Propargyl alcohol CAS No. 107-19-7         | 15              | Negative in rats and mice                                                                | Yes—rats; Yes—mice         |
| α-Methylstyrene CAS No. 98-83-9            | 15              | Negative in rats and mice                                                                | Yes—rats; Yes—mice         |
| Methyl isobutyl ketone CAS No. 108-10-1    | 15              | Negative in rats and mice                                                                | Yes—rats; Yes—mice         |
| 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 | 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 | 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               |
| 1-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               |
| Agent | Lung tumor rank | Ames assay | Clastogen | Log Kow | Lung tumor presentation | Tumors at other organ sites |
|-------|----------------|------------|-----------|---------|------------------------|---------------------------|
| CS2 (94% o-chlorobenzalmononitrile) 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
*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, isobutylaldehyde, l-epinephrine hydrochloride, CS2 (94% o-chlorobenzalmononitrile), 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.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. National Toxicology Program. NTP Vision & Roadmap Future Directions, 2016, https://ntp.niehs.nih.gov/about/ vision/index.html (accessed 15 December 2016).
2. National Toxicology Program (NTP) Technical Reports, 2016, http://ntp.niehs.nih.gov/results/longterm/reports/ longterm/index.html (accessed 15 December 2016).
3. National Toxicology Program. Scientific review of cadmium and cadmium compounds, 2015, https://ntp.niehs.nih.gov/ pubhealth/roc/listings/c/cadmium/summary/index.html (accessed 01 November 2016).
4. National Toxicology Program. Scientific review of diesel exhaust particulates, https://ntp.niehs.nih.gov/ntp/roc/content/profiles/dieselexhaustparticulates.pdf (2015, accessed 01 November 2016).
5. Hanna JM and Onaitis MW. Cell of origin of lung cancer. J Carcinog 2013; 12: 6. doi:10.4103/1477-3163.109033
6. Harkema JR, Carey SA and Wagner JG. The nose revisited: A brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol 2012; 40: 887–898.
7. Smith IC and Carson BL. Trace Metals in the Environment. Ann Arbor, MI: Ann Arbor Science Publishers, 1981.
8. Boldt JR. The Winning of Nickel: Its Geology, Mining, and Extractive Metallurgy. Van Nostrand Company, 1967, p. 487.
9. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals, Budavari S, ed. 11th ed. 1989, p. 1606.
10. National Toxicology Program. Scientific review of diesel exhaust particulates, http://ntp.niehs.nih.gov/pubhealth/roc/listings/b/bromopropane/summary/index.html (2016, accessed 01 November 2016).
11. Ashby J and Tennant RW. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the US NTP. Mutat Res 1991; 257: 229–306.
12. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile of antimony and related compounds. September, 1992. www.atsdr.cdc.gov/toxprofiles/tp23.pdf (accessed 15 December 2016).
13. Tennant RW, Margolin BH, Shelby MD, et al. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science 1987; 236: 933–941.
14. Zeiger E, Haseman JK, Shelby MD, et al. Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals. Environ Mol Mutagen 1990; 16(S18): 1–14.
15. Newcombe RG. Statistics in Medicine, vol. 17. New York: John Wiley & Sons, Ltd, 1998, pp. 857–872.
16. Cambridge MedChem Consulting. Brain penetration, a work in progress, 2012. www.cambridgemedchemconsulting.com/resources/ADME/brian_penetration.html (accessed 15 December 2016).
17. Kumar V, Abbas AK, Fausto N, et al. Robbins Basic Pathology. Philadelphia: Saunders Elsevier, 2007, p. 4.