3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

In a study undertaken by the National Toxicology Program (2001), groups of 60 male and 60 female $B6C3F_1$ mice, 6 weeks of age, were exposed to particulate aerosols of indium phosphide (purity, > 99%; MMAD, 1.2 μm; GSD, 1.7–1.8 μm) at concentrations of 0, 0.03, 0.1 or 0.3 mg/m^3 for 6 h per day on 5 days per week for 22 weeks (0.1 and 0.3 mg/m^3) or 105 weeks (0 and 0.03 mg/m^3). An interim sacrifice of 10 males and 10 females per group after 3 months showed increased lung weights and lung lesions in animals exposed to 0.1 or 0.3 mg/m^3. The changes were considered sufficiently severe that exposure was discontinued in these groups and the animals were maintained on filtered air from the termination of exposure at week 22 until the end of the study. Survival rates were decreased in exposed males and females compared with chamber controls (survival rates: 37/50 (control), 24/50 (low dose), 29/50 (mid dose) or 27/50 (high dose) in males and 42/50, 13/50, 33/50 or 21/50 in females, respectively; mean survival times: 711, 660, 685 or 679 days in males and 713, 655, 712 or 654 days in females, respectively). Mean body weights were decreased in males exposed to 0.03 and 0.3 mg/m^3 and in all exposed females compared with chamber controls. Incidences of neoplasms and non-neoplastic lesions are reported in Tables 2 and 3.

There was an increased incidence of lung neoplasia in male and female mice exposed to indium phosphide. Alveolar/bronchiolar adenomas and many of the alveolar/bronchiolar carcinomas resembled those which arise spontaneously. However, exposure to indium phosphide did not cause increased incidences of neoplasms in other tissues. The lung carcinomas were distinguished from adenomas by local invasion, metastasis and/or greater anaplasia and/or pleomorphism of component cells. Some of the carcinomas differed somewhat from spontaneous carcinomas. Carcinomas in mice exposed to indium phosphide were very anaplastic with papillary and sclerosing patterns; several appeared to have spread outside the lungs into the mediastinum and some to distant metastases. A few appeared to have extensive intrapulmonary spread which in several instances was diagnosed as multiple carcinoma. Alveolar epithelial hyperplasia in the lung is generally considered to be a precursor to neoplasia in the mouse but was not significantly increased in male or female mice exposed to indium phosphide. There were increased incidences of chronic active inflammation, alveolar proteinosis and foreign bodies (indium phosphide

Table 2. Incidence of neoplasms and non-neoplastic lesions of the lung and associated lymph nodes in mice in a 2-year inhalation study of indium phosphide

Lesions observed	No. of mice exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Males				
Lung				
Total no. examined	50	50	50	50
No. with:				
Alveolar epithelium, hyperplasia	2 (1.5)[b]	5 (2.4)	3 (2.7)	7 (2.1)
Chronic active inflammation	2 (1.0)	50[c] (2.9)	45[c] (1.6)	46[c] (2.1)
Alveolus, proteinosis	0	14[c] (1.0)	0	10[c] (1.0)
Foreign body (indium phosphide particles)	0	49[c] (1.0)	42[c]	49[c]
Serosa, fibrosis	0	50[c] (3.5)	49[c] (2.0)	50[c] (2.4)
Alveolar/bronchiolar adenoma, multiple	1	2	0	3
Alveolar/bronchiolar adenoma (includes multiple)	13	9	7	13
Alveolar/bronchiolar carcinoma, multiple	1	8[d]	3	14
Alveolar/bronchiolar carcinoma (includes multiple)	6	15[c]	22[c]	13[d]
Alveolar/bronchiolar adenoma or carcinoma (includes multiple)	18	23	24	21
Pleural mesothelium, hyperplasia	0	19[c] (2.1)	4 (2.0)	6[d] (1.5)
Lymph node, bronchial				
Total no. examined	35	48	45	48
No. with:				
Hyperplasia	2 (2.5)	36[c] (2.3)	22[c] (2.0)	22[c] (2.0)
Foreign body (indium phosphide particles)	0	43[c] (1.0)	40[c] (1.0)	40[c] (1.0)
Lymph node, mediastinal				
Total no. examined	40	49	45	48
No. with:				
Hyperplasia	0	34[c] (2.5)	17[c] (2.1)	27[c] (2.2)
Foreign body (indium phosphide particles)	0	24[c] (1.0)	14[c] (1.0)	25[c] (1.0)

Table 2 (contd)

Lesions observed	No. of mice exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Females				
Lung				
Total no. examined	50	50	50	50
No. with:				
Alveolar epithelium, hyperplasia	0	1 (2.0)	1 (3.0)	2 (2.0)
Chronic active inflammation	2 (2.5)	49[c] (2.9)	45[c] (1.7)	50[c] (2.1)
Alveolus, proteinosis	0	31[c] (1.1)	0	8[c] (1.4)
Foreign body	0	49[c] (1.0)	36[c]	49[c]
Serosa, fibrosis	0	50[c] (3.8)	47[c] (1.8)	49[c] (2.5)
Alveolar/bronchiolar adenoma, multiple	0	0	1	2
Alveolar/bronchiolar adenoma (includes multiple)	3	6	10[d]	7
Alveolar/bronchiolar carcinoma, multiple	0	1	0	0
Alveolar/bronchiolar carcinoma (includes multiple)	1	6	5	7
Alveolar/bronchiolar adenoma or carcinoma (includes multiple)	4	11[d]	15[d]	14[c]
Pleural mesothelium, hyperplasia	0	16[c] (1.8)	3 (1.7)	13[c] (1.9)
Lymph node, bronchial				
Total no. examined	36	50	48	50
No. with:				
Hyperplasia	5 (1.8)	42[c] (2.8)	31[c] (2.2)	28[c] (2.2)
Foreign body (indium phosphide particles)	0	44[c] (1.0)	33[c] (1.0)	40[c] (1.0)
Lymph node, mediastinal				
Total no. examined	42	48	46	49
No. with:				
Hyperplasia	2 (2.0)	40[c] (3.0)	11[c] (2.2)	29[c] (2.6)
Foreign body	0	20[c] (1.0)	7[c] (1.0)	16[c] (1.0)

From National Toxicology Program (2001)

[a] Exposure stopped after 22 weeks.

[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked

[c] Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test

[d] Significantly different ($p \leq 0.05$) from the chamber control group by the Poly-3 test

Table 3. Incidence of neoplasms and non-neoplastic lesions of the liver in mice in a 2-year inhalation study of indium phosphide

Lesions observed	No. of mice exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Males				
Liver				
No. examined microscopically	50	50	50	50
Eosinophilic focus	10	16[b]	19[b]	18[b]
Hepatocellular adenoma, multiple	8	13	10	14
Hepatocellular adenoma (includes multiple)	17	24	23	32
Hepatocellular carcinoma, multiple	1	7[b]	10[c]	5
Hepatocellular carcinoma (includes multiple)	11	22[b]	23[b]	16
Hepatoblastoma				
Hepatocellular adenoma, hepatocellular	0	1	0	0
carcinomas, or hepatoblastoma (includes multiple)	26	40	37	39
Females				
Liver				
No. examined microscopically	50	50	50	50
Eosinophilic focus	6	9	4	12[b]
Hepatocellular adenoma, multiple	12	14	18	14
Hepatocellular adenoma (includes multiple)	2	4	1	2
Hepatocellular carcinoma, multiple	6	17[c]	8	10
Hepatoblastoma	0	0	0	1
Hepatocellular adenoma, hepatocellular carcinomas, or hepatoblastoma (includes multiple)	18	28[c]	24	23

From National Toxicology Program (2001)

[a] Exposure stopped after 22 weeks.

[b] Significantly different ($p \leq 0.05$) from the chamber control group by the Poly-3 test

[c] Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test

particles) in the lungs of exposed mice. A prominent feature of the inflammatory process was the presence of pleural fibrosis (serosal fibrosis). Usually, these fibrotic areas were associated with areas of inflammation. Pulmonary interstitial fibrosis was an uncommon finding in control animals. The incidence of visceral pleural mesothelial hyperplasia was increased in males and females exposed to 0.03 and 0.3 mg/m^3 indium phosphide. Usually in association with chronic inflammation and fibrosis, the pleural mesothelium from many animals was hypertrophic and/or hyperplastic. Normal visceral mesothelium is a single layer of flattened epithelium, whereas affected mesothelium ranged from a single layer of plump (hypertrophic) cells to several layers of rounded cells (hyperplasia). In the more severe cases, the proliferations formed papillary fronds that projected into the pleural cavity.

There were increased incidences of hepatocellular adenoma and carcinoma in males and females. The incidence of multiple hepatocellular tumours per animal was increased in exposed groups. The incidence of eosinophilic foci was increased in all groups of exposed males and in females exposed to 0.3 mg/m^3. Foci of hepatocellular alteration, hepatocellular adenoma, and hepatocellular carcinoma are thought to represent a spectrum that constitutes the progression of proliferative liver lesions. The increased incidence of liver lesions observed in this study was considered to be related to exposure to indium phosphide. Although there was an increased incidence of rare neoplasms of the small intestine in male mice, this was not statistically significant and it was uncertain whether these neoplasms were a result of exposure to indium phosphide (National Toxicology Program, 2001).

3.1.2 *Rat*

In a study undertaken by the National Toxicology Program (2001), groups of 60 male and 60 female Fischer 344/N rats, 6 weeks of age, were exposed to particulate aerosols of indium phosphide (purity, > 99%; MMAD, 1.2 μm; GSD, 1.7–1.8 μm) at concentrations of 0, 0.03, 0.1, or 0.3 mg/m^3 for 6 h per day on 5 days per week for 22 weeks (0.1 and 0.3 mg/m^3 groups) or 105 weeks (0 and 0.03 mg/m^3 groups). An interim sacrifice of 10 males and 10 females per group after 3 months showed increased lung weights, microcytic erythrocytosis, and lesions in the respiratory tract and lung-associated lymph nodes in animals exposed to 0.1 or 0.3 mg/m^3. These changes were considered sufficiently severe to justify discontinuing exposure after 22 weeks and these animals were maintained on filtered air from termination of exposure at week 22 until the end of the study. No adverse effects on survival were observed in treated males or females compared with chamber controls (survival rates: 27/50 (control), 29/50 (low dose), 29/50 (mid dose) or 26/50 (high dose) in males and 34/50, 31/50, 36/50 or 34/50 in females, respectively; mean survival times: 667, 695, 678 or 688 days in males and 682, 671, 697 or 686 days in females, respectively). No adverse effects on mean body weight were observed in treated males or females compared with chamber controls. Incidences of neoplasms and non-neoplastic lesions are reported in Tables 4 and 5.

Table 4. Incidence of neoplasms and non-neoplastic lesions of the lung in rats in a 2-year inhalation study of indium phosphide

Lesions observed	No. of rats exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Males				
Lung				
Total no. examined	50	50	50	50
Atypical hyperplasia	0	16[c] (3.1)[b]	23[c] (3.3)	39[c] (3.8)
Chronic active inflammation	5 (1.2)	50[c] (3.8)	50[c] (3.4)	50[c] (4.0)
Alveolar epithelium, metaplasia	0	45[c] (3.1)	45[c] (2.8)	48[c] (3.2)
Foreign body	0	50[c] (2.2)	50[c] (1.9)	50[c] (2.1)
Alveolus, proteinosis	0	50[c] (3.7)	48[c] (2.0)	47[c] (3.4)
Interstitium, fibrosis	0	49[c] (3.7)	50[c] (3.5)	50[c] (3.9)
Alveolar epithelium, hyperplasia	11 (1.5)	20 (2.4)	21[d] (2.1)	31[c] (2.6)
Squamous metaplasia	0	1 (2.0)	3 (3.0	4 (2.5)
Squamous cyst	0	1 (4.0)	3 (3.0)	2 (3.0)
Alveolar/bronchiolar adenoma, multiple	1	5	8[d]	12[c]
Alveolar/bronchiolar adenoma (includes multiple)	6	13	27[c]	30[c]
Alveolar/bronchiolar carcinoma, multiple	0	2	1	5[d]
Alveolar/bronchiolar carcinoma (includes multiple)	1	10[c]	8[d]	16[c]
Alveolar/bronchiolar adenoma or carcinoma	7/50	22/50[c]	30/50[c]	35/50[c]
Squamous cell carcinoma	0/50	0/50	0/50	4/50
Females				
Lung				
Total no. examined	50	50	50	50
Atypical hyperplasia	0	8[c] (2.8)	8[c] (2.9)	39[c] (3.8)
Chronic active inflammation	10 (1.0)	49[c] (3.0)	50[c] (2.6)	49[c] (3.9)
Alveolar epithelium, metaplasia	0	46[c] (3.3)	47[c] (2.4)	48[c] (3.8)
Foreign body	0	49[c] (2.1)	50[c] (1.8)	50[c] (2.0)
Alveolus, proteinosis	0	49[c] (3.7)	47[c] (2.0)	50[c] (3.8)
Interstitium, fibrosis	0	48[c] (2.9)	50[c] (2.6)	49[c] (3.9)
Alveolar epithelium, hyperplasia	8 (1.5)	15 (2.1)	22[c] (2.0)	16[d] (1.8)
Squamous metaplasia	0	2 (1.5)	1 (2.0)	4 (2.5)
Squamous cyst	0	1 (4.0)	1 (4.0)	10[c] (3.6)
Alveolar/bronchiolar adenoma, multiple	0	1	1	1
Alveolar/bronchiolar adenoma (includes multiple)	0	7[c]	5[d]	19[c]
Alveolar/bronchiolar carcinoma, multiple	0	1	0	7[c]

Table 4 (contd)

Lesions observed	No. of rats exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Alveolar/bronchiolar carcinoma (includes multiple)	1	3	1	11[c]
Alveolar/bronchiolar adenoma or carcinoma	1/50	10/50[c]	6/50	26/50[c]

From National Toxicology Program (2001)
[a] Exposure stopped after 22 weeks.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked
[c] Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test
[d] Significantly different ($p \leq 0.05$) from the chamber control group by the Poly-3 test

There was an increased incidence of lung neoplasms in male and female rats exposed to indium phosphide but no increased incidence of neoplasms in other tissues was observed. Proliferative lesions of the lung included alveolar/bronchiolar neoplasms and squamous-cell carcinomas as well as alveolar epithelial hyperplasia and atypical hyperplasia of alveolar epithelium. Alveolar/bronchiolar adenomas, typical of those observed spontaneously in Fischer 344/N rats, were generally distinct masses that often compressed surrounding tissue. Alveolar/bronchiolar carcinomas had similar cellular patterns but were generally larger and had one or more of the following histological features: heterogenous growth pattern, cellular pleomorphism and/or atypia, and local invasion or metastasis. A number of exposed males and females had multiple alveolar/bronchiolar neoplasms. It was not usually possible to determine microscopically if these represented intrapulmonary metastases of a malignant neoplasm or were multiple independent neoplasms. Included in the spectrum of lesions was a proliferation of alveolar/bronchiolar epithelium with a very prominent fibrous component not typically seen in alveolar/bronchiolar tumours of rodents. The smallest lesions were usually observed adjacent to areas of chronic inflammation. Small lesions with modest amounts of peripheral epithelial proliferation were diagnosed as atypical hyperplasia, while larger lesions with florid epithelial proliferation, marked cellular pleomorphism, and/or local invasion were diagnosed as alveolar/bronchiolar adenoma or carcinoma. While squamous epithelium is not normally observed within the lung, squamous metaplasia of alveolar/bronchiolar epithelium is a relatively common response to pulmonary injury and occurred in a few rats in each exposed group. Squamous metaplasia consisted of a small cluster of alveoli in which the normal epithelium was replaced by multiple layers of flattened squamous epithelial cells that occasionally formed keratin. Cystic squamous lesions also occurred and were rimmed by a band (varying in thickness from a few to many cell layers) of viable squamous epithelium with a large central core of keratin. Squamous-cell carcinomas were observed in four males exposed to 0.3 mg/m^3 indium phosphide. These

Table 5. Incidence of neoplasms and non-neoplastic lesions of the adrenal medulla in rats in a 2-year inhalation study of indium phosphide

Lesions observed	No. of rats exposed to indium phosphide at concentrations (mg/m^3) of			
	0 (chamber control)	0.03	0.1[a]	0.3[a]
Males				
Adrenal medulla				
Number examined microscopically	50	50	49	50
Hyperplasia	26 (2.2)[b]	26 (2.4)	24 (2.4)	32 (2.3)
Benign pheochromocytoma, bilateral	0	6[c]	4	5[c]
Benign pheochromocytoma (includes bilateral)	10	22	16	23
Complex pheochromocytoma	0	1	0	0
Malignant pheochromocytoma	0	3	3	1
Benign, complex or malignant pheochromocytoma	10	26[d]	18[c]	24[d]
Females				
Adrenal medulla				
Number examined microscopically	50	48	50	40
Hyperplasia	6 (1.8)	13[c] (2.2)	9 (2.3)	15[c] (2.1)
Benign pheochromocytoma, bilateral	0	0	0	2
Benign pheochromocytoma (includes bilateral)	2	6	2	9
Malignant pheochromocytoma	0	0	0	1
Benign or malignant pheochromocytoma	2	6	2	9

From National Toxicology Program (2001)
[a] Exposure stopped after 22 weeks.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked
[c] Significantly different ($p \leq 0.05$) from the chamber control group by the Poly-3 test
[d] Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test

neoplasms ranged from fairly well-differentiated squamous-cell carcinomas to poorly-differentiated and anaplastic ones.

There was an increased incidence of pheochromocytoma in male and female rats and an increased incidence of medullary hyperplasia in females. Focal hyperplasia and pheochromocytoma were considered to constitute a morphologic continuum in the adrenal medulla. There was also a marginal increase in neoplasms typical of those observed spontaneously in male and female Fischer 344/N rats. These included fibromas of the skin in males, mammary gland carcinomas in females, and mononuclear cell leukaemia in males and females. It was uncertain whether these neoplasms were a result of exposure to indium phosphide (National Toxicology Program, 2001).

3.1.3 *Comparison of findings from the rat and mouse inhalation studies*

The alveolar/bronchiolar adenomas found in rats exposed to indium phosphide (National Toxicology Program, 2001) closely resembled those found spontaneously in aged rats. Most alveolar/bronchiolar adenomas and carcinomas in mice exposed to indium phosphide also resembled those occurring spontaneously in $B6C3F_1$ mice (National Toxicology Program, 2001). However, some of the carcinomas were different from those occurring spontaneously in that they were very anaplastic with papillary and sclerosing patterns and often spread outside the lung into the mediastinum and distant metastases. A few appeared extensively throughout the lung and thus were diagnosed as multiple carcinomas. The neoplastic responses in the lungs of mice were even more significant than those in rats, because mice generally do not respond to particulate exposure by developing lung neoplasms, even at higher exposure concentrations.

In mice, exposure to indium phosphide also caused inflammatory and proliferative lesions of the mesothelium of the visceral and parietal pleura, another uncommon response to nonfibrous particulate exposure. Pleural fibrosis was a prominent component of the chronic inflammation and involved both visceral and parietal pleura with adhesions. Significantly, pulmonary interstitial fibrosis was uncommon in mice exposed to indium phosphide.

As a result of discontinuing exposure of the 0.1 and 0.3 mg/m^3 groups to indium phosphide at 21 or 22 weeks, only the groups receiving 0.03 mg/m^3 were exposed for 2 years. Therefore, typical concentration-related responses in neoplasms, based solely on external exposure concentration of particulate indium phosphide, were not expected. The amount of indium retained in the lung and that absorbed systemically must also be considered (see Table 6). The lung deposition and clearance model was used to estimate the total amount of indium deposited in the lungs of mice and rats after termination of exposure, the lung burdens at the end of the 2-year study, and the area under the lung-burden curves (AUC). For both species, the estimates at the end of 2 years indicated that the lung burdens in the groups exposed continuously to 0.03 mg/m^3 were greater than those of the other exposed groups (0.1 or 0.3 mg/m^3), with the lung burdens of the groups exposed to 0.1 mg/m^3 being the lowest. Because of the slow clearance of indium, the lung burdens in the groups exposed to 0.1 and 0.3 mg/m^3 were approximately 25% of the maximum levels in rats and 8% in mice, 83 to 84 weeks after exposure was stopped. The AUCs and the total amount of indium deposited per lung indicated that the groups exposed to 0.3 mg/m^3 received a greater amount of indium phosphide than the other two groups with the group exposed to 0.1 mg/m^3 being the lowest. Regardless of how the total ‘dose’ of indium to the lung was estimated, the group exposed to 0.1 mg/m^3 had less total exposure than the other two groups, implying that this group may be considered the ‘low dose’ in these studies. Therefore, lung-burden data should be considered when evaluating lung neoplasia incidence.

Table 6. Estimates of exposure of rats and mice to indium phosphide for 2 years based on a lung deposition and clearance model

Parameters of exposure	Exposure group		
	Rat/mouse	Rat[a]/mouse[b]	Rat[a]/mouse[b]
	0.03 mg/m^3	0.1 mg/m^3	0.3 mg/m^3
Lung burden at 2 years (μg In/lung)	65.1/6.2	10.2/0.5	31.9/2.3
Total amount deposited per lung (μg In/lung)	72/15	57/11	150/37
First-year AUC (μg In/lung × days of study)	6368/1001	11 502/1764	31 239/6078
Second-year AUC (μg In/lung × days of study)	18 244/2032	6275/486	18 532/1986
Total AUC (μg In/lung × days of study)	24 612/3000	17 777/2200	49 771/8000

AUC, area under the lung burden curve
From National Toxicology Program (2001)
[a] Exposure was discontinued and animals were maintained on filtered air from exposure termination at week 22 until the end of the study.
[b] Exposure was discontinued and animals were maintained on filtered air from exposure termination at week 21 until the end of the study.

3.2 Intratracheal instillation

Hamster

Tanaka and colleagues (1996) studied indium phosphide in hamsters. Groups of 30 male Syrian golden hamsters, 8 weeks of age, received intratracheal instillations of 0 or 0.5 mg phosphorus/animal indium phosphide (purity, ≥ 99.99 %; particle mean count diameter, 3.9 μm [GSD, 2.88 μm]) in phosphate buffer solution once a week for 15 weeks and were observed during their total life span (approximately 105 weeks). Survival after 15 instillations was 29/30 controls and 26/30 treated hamsters. There was no exposure-related mortality (survival time, 433 ± 170 days in exposed hamsters versus 443 ± 169 days in controls) and all exposed animals had died by 689 days (controls, 737 days). Histopathological examination of 23 exposed hamsters showed proteinosis-like lesions in 19/23, alveolar or bronchiolar cell hyperplasia in 9/23, squamous-cell metaplasia in 1/23 and particle deposition in 23/23 animals. There was no treatment-related increase in neoplasms of the lungs or other organs (liver, forestomach, pancreas or lymph nodes). [The Working Group concluded that because of the small number of animals, and because of the extent and duration of exposure by intratracheal instillation, this study may not have provided for adequate assessment of carcinogenic activity.]