4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Deposition, retention, clearance and metabolism

The absorption and distribution of indium is highly dependent on its chemical form. Indium phosphide has low solubility in synthetic simulated body fluids (Gamble solution) (Kabe *et al*., 1996).

4.1.1 *Humans*

A study (Miyaki *et al.*, 2003) of concentrations of indium in blood, serum and urine of workers exposed (n = 107) or not exposed (n = 24) to water-insoluble indium-containing particulates in workplace air is described in detail in Section 1.3.2. In each of the three biological fluids, concentrations of indium were clearly higher in exposed workers than in unexposed workers.

4.1.2 *Experimental systems*

(*a*) *Indium phosphide*

(i) *Inhalation studies in rats and mice*

The deposition and clearance of indium phosphide have been studied by the National Toxicology Program (2001). Groups of 15 male Fischer 344 rats designated for tissue burden analyses and five male rats designated for post-exposure tissue burden analyses were exposed to particulate aerosols of indium phosphide at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6 h (plus 12 min build-up time) per day on 5 days per week for 14 weeks. Indium continued to accumulate in lung tissue, blood, serum and testes throughout the exposure period. At day 5, the concentrations of indium ranged from 13 to 500 μg/g lung and concentrations of up to 1 mg/g lung were measured after exposure to 100 mg/m^3 indium phosphide for 14 weeks.

Lung clearance half-lives during exposure were in the order of 47–104 days. At 14 days after exposure, the half-life increased to about 200 days. Blood and serum indium concentrations in all exposed animals were found to be similar at the end of exposure and at 112 days after exposure. Concentrations of indium in testis tissue continued to increase more than twofold after exposure ended in rats exposed to 10- and 30-mg/m^3 concentrations of indium phosphide. Indium concentrations reached 7.20 ± 2.4 μg/g testis 14 days after the end of exposure to 100 mg/m^3.

In a further study (National Toxicology Program, 2001), groups of 60 male and 60 female rats and mice were exposed to particulate aerosols of indium phosphide at concen-

trations of 0, 0.03, 0.1, or 0.3 mg/m^3 (MMAD ~1.2 μm), for 6 h (plus 12 min build-up time) per day on 5 days per week for 22 weeks (rats) and 21 weeks (mice) (0.1 and 0.3 mg/m^3 groups) or 105 weeks (0 and 0.03 mg/m^3 groups, rats and mice). Animals in the 0.1- and 0.3-mg/m^3 groups were maintained on filtered air from exposure termination at week 22 until the end of the study. In rats, the lung indium burden at 5 months was proportional to exposure. At 12 months, 34.3 ± 1.87 μg indium per lung was measured in the male rats of the 0.03-mg/m^3 exposure group. The estimated lung clearance was long (half-life, 2422 days) and the mean indium concentration in serum at 12 months was high (3.4 ± 0.2 ng/g) in the 0.03-mg/m^3 exposure group. Results for B6C3F$_1$ mice exposed to 0.03, 0.1 or 0.3 mg/m^3 were similar although there were quantitative differences in lung burden and kinetic parameters. The mean indium concentration in the lungs at 12 months was 4.87 ± 0.65 μg per lung for male mice in the low-exposure group (0.03 mg/m^3). Lung clearance half-lives of 144 and 163 days were estimated for mice in the 0.1- and 0.3-mg/m^3 exposure groups, respectively, compared with 262 and 291 days for rats exposed to the same concentrations.

Exposure of male rats for 5 days per week for 2 years to 0.03 mg/m^3 indium phosphide resulted in a mean indium concentration of 7.65 ± 0.36 μg/g lung tissue at 5 months, i.e. a fourfold lower concentration compared with that found at 14 weeks exposure to 1 mg/m^3 indium phosphide. Lung clearance half-lives for indium phosphide in male rats in the 2-year studies were estimated to be 2422, 262 and 291 days for 0.03-, 0.1- and 0.3-mg/m^3 exposure concentrations of indium phosphide, respectively. In male B6C3F$_1$ mice exposed to 0.03 mg/m^3 for 2 years, the mean indium concentration in the lung at 5 months was 8.52 ± 1.44 ng/g lung. Indium phosphide lung clearance half-lives were 230, 144 and 163 days for male mice exposed to 0.03, 0.1 and 0.3 mg/m^3 indium phosphide, respectively (National Toxicology Program, 2001).

Deposition and clearance during long-term exposure of rats and mice to indium phosphide appeared to follow zero-order (constant rate) kinetics. The burden of indium retained in the lung throughout the experiments was proportional to exposure concentration and duration. The studies indicated that elimination of indium was quite slow. For both species, estimates at the end of 2 years indicated that the lung burdens in the groups continuously exposed to 0.03 mg/m^3 were greater than those in the groups exposed to 0.1 or 0.3 mg/m^3 where exposure was terminated at 22 weeks. Because of the slow clearance of indium, the lung burdens in the groups exposed to 0.1 and 0.3 mg/m^3, 83 weeks after exposure was stopped, were approximately 35–50% and 16–28% of the maximum concentrations in rats and mice, respectively. These findings were also compatible with the results from the 14-week study in which concentrations in testes of rats exposed to 10 and 30 mg/m^3 indium phosphide continued to increase more than twofold after exposure ended (National Toxicology Program, 2001).

(ii) *Intratracheal administration in rats*

After an intratracheal instillation into male Fischer rats of 10 mg/kg bw particulate indium phosphide (1.73 ± 0.85-μm particles), Zheng *et al.* (1994) found minimal absorp-

tion, i.e. < 0.23% urinary excretion over a 10-day period. Retention at 96 h in the body (except in lung) was 0.36%; 73% of the administered dose was recovered in faeces, probably reflecting mucociliary transport followed by ingestion.

Uemura *et al*. (1997) exposed Fischer 344 rats to 0, 1, 10 and 100 mg/kg bw particulate indium phosphide (80% of the particles were < 0.8 μm in diameter) by intratracheal instillation. Indium, determined by use of AAS was detected at concentrations of 25 ng/g and 58 ng/g in liver and spleen, respectively, 1 day after instillation of 1 mg/kg bw indium phosphide. On day 7, the concentrations were 14 and 19 ng/g in these organs. Indium concentrations in serum increased significantly from day 1 to day 7 in animals that had received the highest dose. Toxic effects were obvious in the lungs but all rats survived. In this experiment, toxicity of indium phosphide was found to be much lower than that of more soluble compounds, such as indium chloride and indium nitrate (see e.g. Zheng *et al.*, 1994).

(iii) *Intraperitoneal administration and gavage*

Kabe *et al*. (1996) studied male ICR mice after gavage and intraperitoneal injection of 0, 1000, 3000 and 5000 mg/kg bw indium phosphide suspended in 0.3 mL physiological saline and found minimal absorption after gavage with 2.4-μm particles but a dose-dependent increase in indium concentrations in serum after intraperitoneal administration. Mean indium concentrations were 1 and 4 μg/g in the liver and kidney, respectively, in mice given a single oral dose of 5000 mg/kg bw. Intraperitoneal administration resulted in accumulation of indium mainly in the lung (> 200 μg/g) and liver (about 300 μg/g) as measured by GF-AAS.

(*b*) *Other indium compounds*

(i) *Mice*

After intravenous injection of ^{113}In in mice, Stern *et al.* (1967) found that 50–60% of the injected radioactivity remained in the blood after 3 h. Castronovo and Wagner (1973) studied ^{114}In administered to mice as ionic indium chloride or as colloidal hydrated indium oxide and reported biphasic excretion patterns for both compounds, with half-life values of 1.9 and 69 days for indium chloride and 2 and 74 days for indium oxide. Ionic indium chloride concentrated primarily in the kidney while colloidal indium oxide was concentrated in the liver and reticuloendothelial system 4 days after a dose sufficient to cause the death of all animals.

(ii) *Rats*

Smith *et al*. (1960) studied the metabolism of $^{114}InCl_3$ in rats and found that more than half of the administered dose had been absorbed or excreted 4 days after intratracheal instillation, and intramuscular and subcutaneous injections. At 30 days after administration, 33–40% of the indium dose had been eliminated via faeces and urine independent of the route of administration.

Blazka *et al*. (1994) studied the distribution of indium trichloride after intratracheal instillation of 1.3 mg/kg bw in Fischer 344 rats. The rats were killed at different time-points up to 56 days after exposure and indium content of the lungs was determined. During the first 8 days after treatment, 87% of the indium was removed from the lung. Over the following 48 days less than 10% of the indium retained at 8 days was eliminated. It was concluded that indium chloride was capable of causing severe lung damage. [The Working Group noted the significant pulmonary retention for this soluble indium compound.]

4.2 Toxic effects

4.2.1 *Humans*

There are no published reports specific to the toxicity of indium phosphide in humans. A study by Raiciulescu *et al*. (1972) reported vascular shock in three of 770 patients injected with colloidal ^{113}In during liver scans.

4.2.2 *Experimental systems*

There is little information about the toxic effects in animals of indium phosphide either *in vivo* or *in vitro*. In general, the toxicity of indium compounds is dependent upon the form (solubility), the dose and the route of administration. When compared with the acute toxicity of other indium compounds, indium phosphide is less toxic (Venugopal & Luckey, 1978; National Toxicology Program, 2001).

(*a*) *Indium phosphide*

Oda (1997) investigated the toxicity of indium phosphide particles (78% < 1 μm diameter) administered by intratracheal instillation of 0, 0.2, 6.0 and 62.0 μg/kg bw in male Fischer 344 rats that were subsequently observed for 8 days. Indium was not detected in the serum, liver, kidney, spleen, thymus or brain. A dose-related increase in SOD activity was observed in BALF on day 1 in all exposed groups, with no increase in inflammatory cells or total protein. LDH activity was increased on day 1 in the group that received the highest dose. On day 8, an increase in neutrophil and lymphocyte counts, LDH activity, and total protein, phospholipid and cholesterol concentrations was observed in BALF, together with desquamation of alveolar epithelial cells and the presence of amorphous exudate in the alveolar lumen as determined by histopathological examination, but only in rats that received the highest dose (62.0 μg/kg bw).

In another experiment from the same laboratory (Uemura *et al*., 1997), male Fischer 344 rats (SPF grade) were exposed to intratracheal instillations of 0, 1, 10 or 100 mg/kg indium phosphide (mean diameter, 0.8 μm). The number of neutrophils in BALF increased considerably, in a dose-dependent manner, 1 and 7 days after indium phosphide administration. Indium phosphide particles were phagocytosed by macrophages and there was a large number of collapsed or broken macrophages at 7 days. LDH activity and the concen-

trations of total protein, total phospholipid and total cholesterol in BALF had increased in a dose-dependent manner 7 days after administration of indium phosphide. Histopathological examination of the lungs showed infiltration of macrophages and neutrophils, accompanied by broken macrophages, exfoliated alveolar cells and eosinophilic exudate. Indium phosphide particles were observed in the interstitium as well as in the lumen of the lung.

In a study conducted by the National Toxicology Program (2001) (described in Section 4.1.2), rats and mice were exposed to 0, 1, 3, 10, 30 or 100 mg/m^3 of indium phosphide by inhalation 5 days per week for 14 weeks. Examination of the lungs at the end of the exposure period revealed pulmonary inflammation characterized by alveolar proteinosis, chronic inflammation, interstitial fibrosis and alveolar epithelial hyperplasia. In addition, microcytic erythrocytosis, consistent with bone-marrow hyperplasia and haematopoietic cell proliferation of the spleen, were observed in both rats and mice. Hepatocellular necrosis was indicated by the increased activities in serum of alanine aminotransferase and sorbitol dehydrogenase in all groups of male and female rats exposed to concentrations of 10 mg/m^3 or greater. These findings were confirmed by histopathological examination of the liver in both sexes exposed to 100 mg/m^3.

In further studies (National Toxicology Program, 2001; see also Section 4.1.2), groups of 60 male and 60 female B6C3F$_1$ mice and 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 (rats) and 21 weeks (mice) (0.1 and 0.3 mg/m^3) or 105 weeks (0 and 0.03 mg/m^3). Exposure to indium phosphide caused dose-related increases in the incidence of proliferative and inflammatory lesions, especially in the lung, in both rats and mice (see Tables 2 and 3 in Section 3). In a subsequent evaluation of lung tissues collected during the 2-year National Toxicology Program study, Gottschling *et al.* (2001) used immunohistochemical techniques to show that concentrations of inducible nitric oxide synthase and cyclooxygenase-2 were elevated in inflammatory foci after 3 months of exposure to indium phosphide. In lungs of animals exposed for 2 years, inducible nitric oxide synthase, cyclooxygenase-2 and glutathione-*S*-transferase Pi were expressed and 8-OHdG was increased in non-neoplastic and neoplastic lesions. Glutathione-*S*-transferase Pi and 8-OHdG enhancement was observed in cells of carcinoma epithelium, atypical hyperplasia and squamous cysts. The results suggested that oxidative stress in pulmonary lesions may contribute to the carcinogenic process (Upham & Wagner, 2001).

(*b*) *Other indium compounds*

In a study by Tanaka *et al.* (1996), male Syrian golden hamsters received indium arsenide or indium phosphide particles by intratracheal instillation of a dose containing 0.5 mg arsenic or phosphorus once a week for 15 weeks and were observed until the animals died [for about 105 weeks]. The cumulative gain in body weight was suppressed significantly in the indium arsenide-treated hamsters and not in the indium phosphide-treated group, compared with the control animals. Histopathological examination of the lungs showed that, in the animals treated with indium phosphide or indium arsenide, the inci-

dence of proteinosis-like lesions, alveolar or bronchiolar cell hyperplasia, pneumonia, emphysema and metaplastic ossification, including infiltration of macrophages and lymphocytes into the alveolar space was significantly higher than that observed in controls. Particles of each compound were observed in the region of the alveolar septum and space as well as in the lymph nodes (Tanaka *et al*., 1996).

A number of studies (Woods *et al*., 1979; Fowler, 1986; Conner *et al*., 1995) have shown that soluble indium administered as indium chloride, or indium arsenide particles, is a potent inducer of haeme oxygenase which is the rate-limiting enzyme in the haeme degradation pathway. Induction of this enzyme is used as a molecular marker of oxidative stress and, following acute administration of indium, is associated with marked decreases in cytochrome P450 and attendant mixed function oxidase activities in the liver of rats. Alterations in the activities of these mixed function oxidases may change cellular responsiveness to a number of known organic carcinogens found in semiconductor production facilities (Woods *et al*., 1979; Fowler *et al.*, 1993).

Exposure to indium, indium arsenide and indium chloride has been shown to produce a number of effects on gene-expression patterns, including inhibition of expression of a number of stress proteins induced by arsenic (Fowler, 1986, 1988; Conner *et al*., 1993). The marked inhibitory effects of indium on protein synthesis may play a role in altering the activities of DNA repair enzymes and the expression of proteins involved in regulating apoptosis: low doses of indium chloride induced apoptosis in rat thymocytes, whereas higher doses caused necrotic cell death (Bustamante *et al*., 1997). These results provide another possible mechanism by which this element may contribute to the carcinogenic process, depending upon dose.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Six studies in experimental animals have been published; indium nitrate ($In(NO_3)_3 \cdot 4.5H_2O$) (Ferm & Carpenter, 1970) or indium trichloride ($InCl_3$) (Chapin *et al*.,1995; Nakajima *et al*., 1998, 1999, 2000) were used in five of these studies and given by oral gavage or intravenous injection. The overall results show that fetal development in rats is more affected than female or male reproductive capacity. Gross congenital malformations were observed in rat embryos. Mice were less susceptible to the teratogenicity of indium.

In the National Toxicology Program (2001) study, developmental toxicity was examined in Swiss (CD-1) mice and Sprague-Dawley rats exposed to 0, 1, 10 or 100 mg/m^3 indium phosphide by inhalation. Rats were exposed on gestation days 4–19 and mice were exposed on days 4 –17. In rats, exposure to indium phosphide by inhalation did not

induce maternal or fetal toxicity, malformations or effects on any developmental parameters. Exposure of mice to the highest dose resulted in early deaths and slightly reduced body weight gain (not statistically significant); lung weights were significantly increased in all mice exposed to indium phosphide. Renal haemorrhage was observed in some fetuses in the group exposed to 100 mg/m^3, but no significant teratogenicity or developmental effects could be attributed to exposure.

4.4 Genetic and related effects

No reports of genetic effects of indium phosphide in humans were found in the literature.

In a study carried out by the National Toxicology Program (2001) (described in detail in Section 3.1.1), no significant increases in the frequencies of micronucleated normochromatic erythrocytes were noted in the peripheral blood samples of male or female B6C3F$_1$ mice exposed by inhalation to indium phosphide in concentrations up to 30 mg/m^3 in a 14-week study. There was a significant increase in micronucleated polychromatic erythrocytes in male, but not in female mice exposed to 30 mg/m^3. The percentage of polychromatic erythrocytes was not altered in males or females (National Toxicology Program, 2001).

In the 2-year inhalation study of indium phosphide (0.03 and 0.3 mg/m^3) in male and female B6C3F$_1$ mice (National Toxicology Program, 2001), β-catenin and *H-ras* mutations were assessed in hepatocellular adenomas and carcinomas. The frequency of *H-ras* codon 61 mutations in the indium phosphide-induced hepatocellular neoplasms was similar to that observed in controls. The frequency of β-catenin mutations was concentration-dependent: in the group exposed to 0.3 mg/m^3 indium phosphide, 40% of the hepatocellular neoplasms showed β-catenin mutations compared with 10% in controls.

4.5 Mechanistic considerations

Inhalation of indium phosphide causes pulmonary inflammation associated with oxidative stress. The data of Gottschling *et al.* (2001) suggest that this inflammation may progress to atypical hyperplasia and neoplasia in the lungs in rats.

It has been suggested that induction of apoptosis *in vitro* in rat thymocytes by indium chloride at low concentrations occurs through alterations of the intracellular redox status, or of intracellular homeostasis (Bustamante *et al.*, 1997). This apoptotic effect has been shown to trigger repair-associated cell proliferation and may contribute to the risk for development of neoplasia.

Analysis of genetic alterations in indium phosphide-induced hepatocellular adenomas and carcinomas revealed mutations in H-*ras* and β-catenin that were identical to those found in human hepatocellular neoplasms (De la Coste *et al.*, 1998). This suggests a similar pathway of carcinogenesis in both species.