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

4.1 Deposition, retention, clearance and metabolism

4.1.1 *Humans*

Data on excretion of gallium have been collected from cancer patients who had received radioactive gallium for radiotherapy or scintigraphy. There was a wide variation in urinary excretion but most subjects excreted about half of the given dose during the 4 days following administration and the major part during the first 8 h. Brucer *et al.* (1953) reported autopsy data from patients who had received intravenous radioactive gallium (^{72}Ga) which showed accumulation in the lung.

Studies by Krakoff *et al.* (1979) showed that an intravenous injection of gallium nitrate at a concentration of 10 mg/mL to patients with advanced cancer was followed by biphasic clearance with half-lives of 87 min and 24.5 h, respectively. Imaging studies using ^{67}Ga have shown that this element localizes in several major tumour categories (Edwards & Hayes, 1969, 1970; Edwards *et al.*, 1970; Winchell *et al.*, 1970; Ha *et al.*, 2000; Nishiyama *et al.*, 2002).

Following exposure, gallium is known to be transported in blood bound to transferrin and to be capable of up-regulating the transferrin receptor (Chitambar & Zivkovic, 1987; Drobyski *et al*., 1996; Jiang *et al*., 2002). The gallium–transferrin complex hence appears to be the primary mechanism by which the gallium ion is presented to the target cellular system.

4.1.2 *Experimental systems*

(*a*) *In-vitro solubility and dissolution in body fluids*

Although the solubility of gallium arsenide in pure water is very low (see Section 1), its dissolution in body fluids is greatly enhanced by endogenous chelating molecules. When incubated in artificial body fluid (Gamble's solution), gallium arsenide progressively releases both gallium and arsenic. A selective leaching appears to take place, probably by chelating components of the solution, whereby more arsenic than gallium is found in solution. The gallium arsenide particle surface is enriched in arsenic, which migrates from the bulk, and which is ultimately oxidized to arsenic oxide (Pierson *et al.*, 1989). When dissolution of gallium arsenide was tested *in vitro* in phosphate buffer and various acids and bases, the amount of dissolved arsenic was highest in phosphate buffer (Yamauchi *et al.*,

1986). These observations help to explain how arsenic may be released from inhaled gallium arsenide particles.

(*b*) *Respiratory system deposition, retention and clearance of gallium compounds*

(i) *Inhalation studies*

Gallium arsenide

Greenspan *et al*. (1991) studied the clearance of inhaled gallium arsenide in male Fischer 344 rats exposed to 0.1, 1.0, 10, 37 and 75 mg/m^3 gallium arsenide (MMAD, 1.2 μm) for 6 h per day on 5 days per week for 13 weeks. The half-life of clearance from the lung was found to be 17 days for both arsenic and gallium. The findings differ from those obtained using intratracheal instillation which often results in preferential clearance of arsenic over gallium (see below).

The National Toxicology Program (2000) reported results from studies in groups of 10 male and 10 female Fischer 344/N rats exposed to particulate aerosols of gallium arsenide (MMAD, 0.81–1.60 μm) by inhalation of concentrations of 0, 0.1, 1, 10, 37 or 75 mg/m^3 for 6 h per day on 5 days per week for 14 weeks. Tissue burden was evaluated at days 23, 45 and 93. Lung weights increased with increasing exposure concentration in males exposed to 1 mg/m^3 or more when examined on days 23 and 45 and in all exposed groups at week 14. In addition, lung weights of exposed rats continued to increase to a greater extent throughout the study compared with those of chamber controls. The percentages of gallium and arsenic in the lung relative to the total lung burden of gallium arsenide were similar at all exposure concentrations throughout the study. The deposition and clearance rates in the lung for gallium and arsenic were similar within each exposure group. Lung clearance half-lives decreased for gallium, from 56 days in rats exposed to 1 mg/m^3 to 20 days in the highest exposure group (75 mg/m^3). Corresponding values for arsenic were 31 and 19 days.

A 2-year study was subsequently performed in rats exposed to 0.01, 0.1 or 1.0 mg/m^3 gallium arsenide using the same experimental conditions as above. Lung weights measured at months 1, 2, 4, 6, 12 and 18 were increased to a greater extent in all male rats exposed to 0.1 or 1.0 mg/m^3 throughout the study than did lung weights of chamber controls and of the group exposed to 0.01 mg/m^3. The percentages of gallium and arsenic in the lung relative to the total lung burden were similar at all exposure concentrations throughout the study because the deposition and clearance rates in the lung for gallium and arsenic were similar within each exposed group. Deposition rates for gallium and arsenic increased with increasing exposure concentration. Lung clearance half-lives of gallium in the group exposed to 1.0 mg/m^3 were considerably less (37 days) than those for the groups exposed to 0.1 (96 days) or 0.01 mg/m^3 (133 days). Lung clearance half-lives of arsenic were similar to those of gallium. The gallium lung tissue burdens at 18 months were 1.60, 13.86 and 22.87 μg/g for groups exposed to 0.01, 0.1 and 1.0 mg/m^3, respectively. Gallium concentrations in whole blood, serum and testes and arsenic concentrations in serum and

testes were above the limits of detection only at the higher exposure concentrations and at the later time points in the study. The mean gallium concentration in whole blood was 0.05 μg/g at 18 months in the highest exposure group; corresponding values were 0.08 μg/g in serum and 1.5 μg/g in testes (National Toxicology Program, 2000).

Gallium oxide

Wolff *et al*. (1984) studied the deposition and retention of single doses of inhaled aggregate radiolabelled gallium oxide ($^{67}Ga_2O_3$) test particles (MMAD, 0.1 μm) in beagle dogs, Fischer 344 rats and CD-1 mice using a 30-min nose-only exposure. In dogs, total gallium deposition was 39 ± 19% (mean ± SD) of the administered dose, pulmonary deposition was 25%, bronchial deposition was 7% and nasopharyngeal deposition was 7%. Corresponding values in rats were 11, 5 and 9% for pulmonary, bronchial and nasopharyngeal deposition, respectively. Pulmonary deposition in mice was estimated to be 15–20% of the administered dose. Whole-body retention was measured and in dogs the long-term plateau represented more than 70% of the particles, compared with 38% and 28% for rats and mice, respectively. The half-life of the long-term component of clearance was 75 ± 19 days for mice, 65 ± 17 days for rats and 52 ± 25 days for dogs.

Wolff *et al.* (1989) presented results of modelling accumulation of particles in rat lung during chronic nose-only inhalation exposure of Fischer 344 rats to 23 mg/m^3 gallium oxide for 2 h per day on 5 days a week for 4 weeks. Impaired clearance occurred early after accumulation of a low burden of the particles. A half-life in the order of 170 days was observed rather than the 65-day half-life reported earlier (Wolff *et al.*, 1984; see above). This impairment of clearance might influence toxicity and the local dose of particles of low solubility in experimental studies.

Battelle Pacific Northwest Laboratories (1990a) carried out 13-week inhalation studies of gallium(III) oxide in male Fischer 344 rats exposed to 0, 0.12, 0.48, 4.8, 24 or 48 mg/m^3 gallium oxide particles (MMAD, ~0.9 μm). Gallium exposure concentrations were approximately equimolar to those used in the studies of gallium arsenide cited above (Greenspan *et al.*, 1991; National Toxicology Program, 2000). As observed with gallium arsenide, following inhalation of gallium oxide, blood and urinary concentrations of gallium were found to be extremely low and only detectable in animals exposed to 24 and 48 mg/m^3 throughout the study. The results indicated that gallium oxide, like gallium arsenide, is not readily absorbed and that, when absorbed, it is rapidly cleared from the blood and either excreted or sequestered in the tissues. Considerable concentrations of gallium were detected in the faeces. Lung burdens increased with increasing exposure concentration. However, when normalized to exposure concentration, accumulation in the lung during the study increased as exposure concentrations increased. Overload may have occurred at gallium oxide concentrations of 24 mg/m^3 and above; this would be in line with the results of Wolff *et al.* (1989).

(ii) *Instillation studies with gallium arsenide*

Webb *et al.* (1984) investigated absorption, excretion and pulmonary retention of gallium arsenide after intratracheal instillation doses of 10, 30 and 100 mg/kg bw (mean volume particle diameter, 12.7 μm) in male Fischer 344 rats. At day 14, gallium was not detected in the blood and urine at any dosage but was retained in the lungs; arsenic retention (measured by F-AAS) ranged from 17 to 32% of the doses given while gallium retention (measured also by F-AAS) ranged from 23 to 42%. In a later study, Webb *et al*. (1986) exposed male Fischer 344 rats to gallium arsenide (100 mg/kg bw) and gallium trioxide (65 mg/kg bw) (equimolar for gallium) by intratracheal instillation (mean volume particle diameters, 12.7 μm and 16.4 μm, respectively). The mean retention of gallium in the lung at day 14 was fairly similar for the two compounds (44% and 36% for gallium arsenide and gallium trioxide, respectively). Webb *et al*. (1987) showed that smaller gallium arsenide particles (mean volume particle diameter, 5.82 μm) had an increased in-vivo dissolution rate and there was increased severity of pulmonary lesions in male Fischer 344 rats after intratracheal instillation of a suspension containing 100 mg/kg bw. Clearance from lung was faster for arsenic (half-life, 4.8 days) than for gallium (half-life, 13.2 days).

Rosner and Carter (1987) studied metabolism and excretion after intratracheal instillation of 5 mg/kg bw gallium arsenide (mean volume particle diameter, 5.8 μm) in Syrian golden hamsters. Blood arsenic concentrations increased from 0.185 ± 0.041 ppm (2.4 μM) after day 1 to 0.279 ± 0.021 ppm (3.7 μM) on day 2. Blood concentrations of arsenic peaked at day 2 after dosing, indicating continued absorption. Of the arsenic, 5% was excreted in the urine during the first 4 days after gallium arsenide instillation compared with 48% after exposure to soluble arsenic compounds. Arsenic derived from gallium arsenide was converted into arsenate (As^{III}), arsenite (As^{V}) and a major metabolite dimethyl arsinic acid, and rapidly excreted. Twenty-seven per cent of the arsenic derived from gallium arsenide were excreted in the faeces the first day after the instillation; this was probably due to lung clearance into gastrointestinal tract after expectoration.

Omura *et al*. (1996a) exposed hamsters to 7.7 mg/kg bw gallium arsenide, 7.7 mg/kg bw indium arsenide or 1.3 mg/kg bw arsenic trioxide by intratracheal instillation twice a week, 14–16 times. Arsenic concentrations in serum on the day after the last instillation were 0.64 μM after gallium arsenide, 0.34 μM after indium arsenide and 1.31 μM after arsenic trioxide. Serum concentrations of gallium and indium were about 20 μM. The results indicated a high retention of both gallium and indium compared with that of arsenic which might be of importance in toxicity from long-term exposure.

Gallium arsenide might in itself impair lung clearance. Aizawa *et al*. (1993) used magnetometric evaluation to study the effects of gallium arsenide on clearance of iron oxide test particles in rabbits. Instillation of 30 mg or 300 mg gallium arsenide per animal in 2 mL saline significantly impaired clearance at 14, 21 and 28 days after exposure. However, although the effect was clear, the dose was high. Impaired clearance might be caused by gallium arsenide itself or by dissolved arsenic-induced inflammation.

(*c*) *Gastrointestinal exposure to gallium*

(i) *Oral and intraperitoneal studies*

Yamauchi *et al*. (1986) studied metabolism and excretion of gallium arsenide (mean volume particle diameter, 14 μm) in Syrian golden hamsters exposed to single doses of 10, 100 or 1000 mg/kg bw in phosphate buffer administered orally through a stomach tube and 100 mg/kg bw intraperitoneally. Urinary excretion of arsenic during the following 120 h was 0.15, 0.11 and 0.05% of the high, medium and low oral doses, respectively, and 0.29% of the intraperitoneal dose. During the same time period, faecal excretion of arsenic was around 80% of the oral doses and 0.38% of the intraperitoneal dose.

Flora *et al*. (1997) exposed groups of male albino rats to single oral doses of 500, 1000 or 2000 mg/kg bw gallium arsenide. Blood was collected at 24 h, and on days 7 and 15 following exposure. Urinary samples were taken at 24 h. Animals were killed on days 1, 7 and 15 and heart tissue was collected. Blood and heart tissue concentrations of gallium and arsenic were determined using GF-AAS and were found to peak at day 7. In a later study, Flora *et al.* (1998) exposed male Wistar albino rats to single doses of 100, 200 or 500 mg/kg bw gallium arsenide or vehicle (control) by gastric intubation. Concentrations of gallium and arsenic were measured at 24 h, and on days 7 and 21 following administration and peaked at day 7 in the blood, liver and kidney but continued to increase up to day 21 in the spleen.

(ii) *Intravenous injection of gallium-67: tracer studies*

Sasaki *et al.* (1982) studied differences in the liver retention of ^{67}Ga (as gallium citrate) administered intravenously in controls and rats fed with the liver carcinogen 3′-methyl-4-dimethylaminobenzene for 20 weeks. They observed that the accumulation of ^{67}Ga in the carcinogen-fed animals at 20 weeks was about 2.3 times greater (per gram of liver) than in the controls. This increase correlated with increases in γ-glutamyl transpeptidase and glucose-6-phosphatase activities at late stages during hepatocarcinogenesis. The most marked change in ^{67}Ga accumulation occurred in the nuclear/whole cell (800 × g) liver fraction suggesting that ^{67}Ga may bind to components in this fraction, induced by 3′-methyl-4-dimethylaminobenzene.

4.1.3 *Data relevant to an evaluation of gallium arsenide as an arsenic compound*

(*a*) *Metabolism of the arsenic oxides*

Radabaugh and coworkers (2002) recently characterized arsenate reductase enzyme and identified it as a purine nucleoside phosphorylase, an ubiquitous enzyme that required dihydrolipoic acid for maximum reduction of arsenate As^{V} to arsenite As^{III} in mammals. [The valences of different forms of arsenic and their metabolites are indicated by superscript roman numerals such as it is reported in scientific publications.] The As^{III} formed may then be methylated to MMA^{V} and to DMA^{V} by methyl transferases which have been partially characterized (Zakharyan *et al.*, 1995; Wildfang *et al.*, 1998; Styblo *et al.*, 1999).

In mice, the highest methylating activity occurred in testes followed by kidney, liver and lung (Healy *et al.*, 1998). The analogous enzymatic reduction of MMA^{V} to monomethylarsonous acid (MMA^{III}) was also demonstrated in hamster; MMA^{V} reductase-specific activities have been shown in all organs (Sampayo-Reyes *et al.*, 2000).

(*b*) *Variation in arsenic methylation between species*

Most human organs can metabolize arsenic by oxidation/reduction reactions, methylation and protein binding. However, there is a pronounced species difference in this metabolism. Arsenic is strongly retained in rat erythrocytes but not in those of other species. The unique disposition of arsenic in rats may be due to the pronounced biliary excretion of MMA^{III} and erythrocyte of DMA^{III} (Gregus *et al.*, 2000; Shiobara *et al.*, 2001) which may explain the lower toxicity of arsenic in rats. Thus, previous scientific committees have stated that they did not recommend rats for arsenic oxide disposition studies (National Academy of Sciences, 1977; Aposhian, 1997). Most experimental animals excrete very little MMA [valence not specified] in urine compared to humans (Vahter, 1999) and some animal species, in particular guinea-pigs and several non-human primates, are unable to methylate arsenic at all (Healy *et al.*, 1997; Vahter, 1999; Wildfang *et al.*, 2001). The effect of the inability to methylate As^{III} compounds on toxicity following repeated dosing is unknown but methylation has long been considered the primary mechanism of detoxification of arsenic in mammals (Buchet *et al.*, 1981). However, non-methylator animals were not found to be more sensitive to the acute effect of arsenic than methylators in the few tests that have been performed. The toxic response of non-methylators needs to be examined in more detail. At present, the most toxic arsenic species is thought to be the MMA^{III} (Petrick *et al.*, 2000; Styblo *et al.*, 2000; Petrick *et al.*, 2001), leading to the view that this methylation should be considered as bioactivation of the metalloid rather than detoxification.

Arsenic detoxification mechanisms other than methylation have been poorly investigated. The fact that man is more than 10 times more sensitive to the effect of arsenic oxides when compared to all other animal species is remarkable. The explanation of this difference in sensitivity is important in order to understand the mechanism of action of arsenic (see IARC, 2004).

4.2 Toxic effects

4.2.1 *Humans*

There are no published reports specific to the toxicity of gallium arsenide in humans.

4.2.2 *Experimental systems*

(*a*) *Gallium arsenide and gallium oxide*

(i) *Non-neoplastic and pre-neoplastic effects in the respiratory tract*

Results of studies undertaken by the National Toxicology Program (2000) (see also Section 3.1) confirmed that the respiratory tract was the primary site of toxicity, indicated by a spectrum of inflammatory and proliferative lesions of the lung. As described in Sections 3.1.1 and 3.1.2, and in Table 2, groups of 50 male and 50 female $B6C3F_1$ mice and groups of 50 male and 50 female Fischer 344/N rats, 6 weeks of age, were exposed by inhalation to gallium arsenide particulate (purity, > 98%; MMAD, 0.8–1.0 μm; GSD, 1.8–1.9 μm) at concentrations of 0, 0.1, 0.5 or 1 mg/m^3 for mice and 0, 0.01, 0.1 and 1 mg/m^3 for rats, for 6 h per day on 5 days per week for 105 or 106 weeks. In mice, non-neoplastic effects were observed in the lung (which included focal suppurative inflammation, focal chronic inflammation, histiocyte infiltration, hyperplasia of the alveolar epithelium, proteinosis of the alveoli and tracheobronchial lymph nodes). The non-neoplastic effects observed in the lung of exposed rats included atypical hyperplasia, active chronic inflammation, proteinosis and metaplasia of the alveolar epithelium in both sexes. In male rats, hyperplasia of the alveolar epithelium of the lung and chronic active inflammation, squamous metaplasia and hyperplasia of the epiglottis and the larynx were observed (National Toxicology Program, 2000).

The most prominent toxic effect of gallium arsenide after a single intratracheal instillation to rats is pulmonary inflammation (Webb *et al*., 1987; Goering *et al.*, 1988). Histopathological changes and changes in tissue concentrations of protein, lipid, and DNA have been observed (Webb *et al.*, 1986). The effects caused by gallium arsenide (100 mg/kg bw) were compared with those elicited by equimolar gallium oxide (65 mg/kg bw) and maximally-tolerated amounts of (17 mg/kg bw, 0.25 equimolar) arsenious (III) acid (Webb *et al.*, 1986). Two weeks after exposure to gallium arsenide, increases in lipid concentrations, comparable to those observed following exposure to equimolar gallium, and increases in protein concentrations similar to those found after exposure to arsenious acid were observed. DNA concentrations were significantly increased after exposure to gallium arsenide but not to the same magnitude as those seen after arsenious acid exposure (arsenious acid was given at 0.25 times the molar dose of gallium arsenide). Only exposure to arsenious acid resulted in increases in 4-hydroxyproline, an indicator of a fibrotic process. Lung wet weights, lung wet weight/body weight and lung dry weights were all increased after instillation of gallium arsenide but not after instillation of gallium oxide or arsenious acid. Goering *et al.* (1988) reported similar histopathological changes in the lungs of rats treated with gallium arsenide in the same conditions.

In a 16-day inhalation study (National Toxicology Program, 2000) of rats exposed to gallium arsenide at concentrations of 0, 1, 10, 37, 75 or 150 mg/m^3, statistically-significant increases in the weights of lungs and liver relative to body weight were noted in animals exposed to concentrations of 1 mg/m^3 and greater. These effects were noted only for lungs

following exposure to 0.1 mg/m^3 and above in a 14-week study. When the studies were repeated in mice, only the lungs were found to show increases relative to body weights.

(ii) *Haematological effects*

A study (National Toxicology Program, 2000; see Section 4.1.2) of mice and rats exposed to gallium arsenide at chamber concentrations of 0, 0.1, 1, 10, 37 or 75 mg/m^3 for 14 weeks, showed statistically-significant decreases in haematocrit and haemoglobin concentrations, and increased numbers of erythrocytes and reticulocytes at 14 weeks in both species exposed to 37 and 75 mg/m^3. Statistically-significant decreases in leucocyte numbers were noted in rats exposed to the two highest doses, whereas increases in leucocyte numbers were observed in mice exposed to the three highest doses. Zinc protoporphyrin/haeme ratios increased in male and female mice exposed to the two highest doses while methaemoglobin increased only in female rats.

Effects on the haem biosynthetic pathway

In the 14-week exposure study cited above (National Toxicology Program, 2000), concentrations of δ-aminolevulinic acid (ALA) and porphobilinogen were not increased in urine of rats exposed by inhalation to gallium arsenide, suggesting that the effect of the porphyria, as it relates to haeme synthesis, was marginal.

Goering and colleagues (1988) observed systemic effects after intratracheal administration of 50, 100 and 200 mg/kg bw gallium arsenide to rats. Activity of δ-aminolevulinic acid dehydratase (ALAD) in blood and urinary excretion of δ-aminolevulinic acid (ALA) were examined. A dose-dependent inhibition of ALAD activity in blood and an increase in excretion of ALA in urine were observed with a maximum response 3–6 days after exposure. A urinary porphyrin excretion pattern characteristic of arsenic exposure (Woods & Fowler, 1978) was also observed in these animals (Bakewell *et al.*, 1988).

In-vitro studies with gallium nitrate, sodium arsenite and sodium arsenate showed that 75 μM gallium nitrate inhibited the activity of blood ALAD and 2 μM gallium nitrate inhibited liver and kidney ALAD. The inorganic arsenic compounds inhibited ALAD in blood at much higher concentrations (15 mM, 200-fold) (Goering *et al.*, 1988). Subsequent in-vivo and in-vitro studies on ALAD in blood, liver and kidney showed that the mechanism of gallium inhibition involves zinc displacement from the sulfhydryl group of the enzyme active site (Goering & Rehm, 1990).

(iii) *Immunological effects*

A variety of changes have been reported in animals exposed to gallium arsenide including inhibition of T-cell proliferation and suppression of immunological functions at locations distal to a single exposure site (Sikorski *et al*., 1989; Burns *et al.*, 1991; Burns & Munson, 1993; Hartmann & McCoy, 1996). The effects included decreases in both humoral and cellular antibody response. The dissolution of gallium arsenide to form gallium and arsenic oxides may be the origin of the effects; arsenic has been shown to be the primary

immunosuppressive component of gallium arsenide (Burns *et al.*, 1991), but it was unclear whether all the immunological effects reported were caused by dissolved arsenic.

(*b*) *Other gallium compounds*

(i) In vitro

Studies by Chitambar and Seligman (1986), Chitambar and co-workers (1988, 1990, 1991) and Narasimhan *et al.* (1992) have shown that transferrin-gallium exerts its toxic effects at the molecular level by inhibiting ribonucleotide reductase, specifically by displacing iron from the M2 subunit of this enzyme.

(ii) In vivo

Early studies by Dudley and Levine (1949) demonstrated the acute renal toxicity of gallium lactate 3 or 4 days after its intravenous injection in rats. Studies by Hart *et al.* (1971) and Adamson *et al.* (1975) further extended the database on the renal toxicity of gallium nitrate; a limiting factor in its use in the treatment of tumours.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

There have been several studies that have reported that workers in the semiconductor industry experience increased rates of spontaneous abortion, but the evidence is inconclusive (Elliot *et al.*, 1999). No single metal has been denoted as a more possible causative agent than any other because of the complex chemical exposures, and other factors, encountered in these environments (Fowler & Sexton, 2002).

4.3.2 *Animals*

(*a*) *Testicular function changes*

(i) *Gallium arsenide*

Testicular toxicity has been reported in rats and hamsters after intratracheal administration of 7.7 mg/kg bw gallium arsenide twice a week for a total of 8 weeks (Omura *et al.*, 1996a,b). A significant decrease in sperm count and in the proportion of morphologically-abnormal sperm were found in the epididymis in the gallium arsenide-treated rats. In hamsters, gallium arsenide caused testicular spermatid retention and epididymal sperm reduction. Animals treated with arsenic trioxide (1.3 mg/kg) or indium arsenide (7.7 mg/kg bw) did not show any testicular toxicities. The arsenic concentrations in serum of gallium arsenide-treated rats were almost twice those found in arsenic trioxide-treated rats. In addition, the molar concentration of gallium was found to be 10–20-fold higher than that of arsenic in gallium arsenide-treated rats (Omura *et al.*, 1996a). In contrast, the arsenic concentrations in serum of gallium arsenide-treated hamsters were less than half of those

found in arsenic trioxide-treated hamsters. Moreover, the molar concentration of gallium was 32 times higher than that of arsenic in gallium arsenide-treated hamsters. Therefore gallium may play a main role in the testicular toxicity in hamsters (Omura *et al.*, 1996b).

Similar testicular toxicities were observed in 14-week and 2-year gallium arsenide inhalation studies (National Toxicology Program, 2000). The effects included decreases in epididymal weights and sperm motility in both rats and mice exposed to 37 and 75 mg/m^3 in the 14-week study. Decreases in epididymal weights and an epididymal hypospermia were also observed in mice exposed to 10 mg/m^3. Decreased testicular weights, genital atrophy and interstitial hyperplasia were observed in rats exposed to 1 mg/m^3 of gallium arsenide in the 2-year study.

(ii) *Gallium oxide*

In a 13-week study of gallium oxide in male rats and mice, exposure to concentrations of 0, 0.16, 0.64, 6.4, 32 or 64 mg/m^3 were found to have no effect on male rat reproductive parameters. However, exposure to gallium oxide at 32 mg/m^3 or greater caused decreases in cauda epididymis and testis weights. Decreases in epididymal sperm motility and concentration were observed in animals exposed to 64 mg/m^3. Testicular degeneration and increased cellular debris in the epididymis were observed in mice exposed to gallium oxide at 64 mg/m^3 (Battelle Pacific Northwest Laboratories, 1990a,b).

(*b*) *Effects on estrous cycles, gestation and foetal development*

In a 13-week study of gallium oxide in female rats and mice, there was no effect of exposure to concentrations of 0.16–64 mg/m^3 on the estrous cycles of either animal species (Battelle Pacific Northwest Laboratories, 1990a,b).

Studies to assess the developmental toxicity of gallium arsenide were performed with Sprague-Dawley rats and Swiss mice exposed to 0, 10, 37 or 75 mg/m^3 gallium arsenide by inhalation 6 h per day, 7 days per week. Rats were exposed on gestation days 4 through 19. There were no signs of maternal toxicity. Minimal effects on the fetuses were noted, including a marginal reduction in body weight in the group exposed to 75 mg/m^3 and concentration-dependent reduced ossification of the sternebrae. There was a non-significant increase in the incidence of incompletely ossified vertebral centra. Mice were exposed on gestation days 4 through 17. Considerable fetal and maternal toxicity was seen in groups exposed to 37 and 75 mg/m^3 gallium arsenide, with 50% of the female animals found dead or moribund. Most exposed females were hypoactive, had laboured breathing and failed to gain weight. The number of resorptions per litter was significantly increased and occurred earlier, while the number of corpora lutea per dam and the number of live fetuses per litter were significantly decreased. Fetal weights were reduced in all exposed groups. Although not statistically significant, various skeletal malformations were observed including cleft palate, encephalocele, and vertebral defects (Battelle Pacific Northwest Laboratories, 1990c; Mast *et al.*, 1991).

4.4 Genetic and related effects (see Table 3)

Gallium arsenide (10 000 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 or TA1535, with or without induced rat or hamster liver S9 enzymes (Zeiger *et al.*, 1992). No increase in the frequency of micronucleated normochromatic erythrocytes was seen in peripheral blood samples from male or female $B6C3F_1$ mice exposed to gallium arsenide by inhalation in concentrations up to 75 mg/m^3, during a 14-week study (National Toxicology Program, 2000). The majority of these experiments were carried out assuming arsenite (As^{III}) was the toxic species; however, there is evidence that it is not. It appears that dimethyl arsinous acid may be a carcinogen but that the most toxic arsenic species may be MMA^{III} (see Section 4.1.3). It is believed that many studies have assigned a toxic dose to arsenate but the effect was actually the result of the reduction of arsenate (As^{V}) to arsenite (As^{III}) (Carter *et al.*, 1999, 2003). It is also of concern that experiments with arsenate using cells have been done without consideration of the concentration of phosphate, an arsenate uptake inhibitor (Huang & Lee, 1996).

4.5 Mechanistic considerations

The hypothesis used to interpret the carcinogenesis results appears to accept the finding that gallium arsenide causes cancer in female rats and that the non-neoplastic hyperplasia is a precursor to neoplasms. The lung effects appear to be 'point of contact' effects. The mechanism of lung cancer fits with a highly toxic compound which kills many different cells without killing the host organism. This leads to regenerative cell proliferation that magnifies any errors in DNA replication and results in enough errors to make organ neoplastic changes in the lung. Some systemic effects were found to be sex-specific and, therefore, a selectivity of response between males and females is not surprising.

It is clear that there is partial dissolution of gallium arsenide particles *in vivo* and that while the majority of a dose of gallium arsenide remains in the lung, there is redistribution of solubilized gallium and arsenic to other organ systems. This results in a variety of toxic effects including inhibition of haeme biosynthesis in a number of organ systems, testicular damage and impaired immune function. Some of the biochemical effects, such as inhibition of haeme pathway enzymes such as ALAD, appear to be relatively specific. However, more pronounced cellular changes in target organ systems such as the kidney, testes, or immune system may be the result of gallium or arsenic or combined exposure to these elements. Further mechanistic research is needed to elucidate the primary underlying roles played by these elements in organ systems outside the lungs.

There is evidence from in-vitro test systems that ionic gallium, such as the gallium transferrin complex, may influence the carcinogenic process by inducing apoptosis at low doses and producing necrosis at high doses in cancer cell lines (Jiang *et al*., 2002).

Table 3. Genetic and related effects of gallium arsenide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA97, TA98, TA100, TA108, TA1535, reverse mutation	–	–	10 000 µg/plate	Zeiger *et al.* (1992)
Formation of micronuclei in binucleates, cytochalasin-B assay, Syrian hamster embryo cells *in vitro*	–		10 µg/mL	Gibson *et al.* (1997)
Formation of micronuclei, B6C3F$_1$ mice erythrocytes in peripheral blood *in vivo*	–		75 µg/m^3 inhalation (14 wk)	National Toxicology Program (2000)
Cell transformation, Syrian hamster embryo cells *in vitro*	+		0.5 µg/mL	Kerckaert *et al.* (1996)

[a] +, positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose