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Pulmonary Toxicity of Indium Arsenide and Arsenic Selenide Following Repeated Intratracheal Instillations to the Lungs of Hamsters

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Chronic toxicity of indium arsenide (InAs) and arsenic selenide (As_2Se_3) was studied in male Syrian golden hamsters which received InAs or As_2Se_3 particles, each containing a total dose of 7.5 mg of arsenic, by intratracheal instillations once a week for 15 weeks. As a control, hamsters were treated with the vehicle, phosphate buffer solution. During their total lifespan, the cumulative body weight gain of the hamsters in the InAs group was suppressed significantly compared with that in the control group, but not in the As_2Se_3 group when compared with that in the control group. However, the survival rate for the InAs group was significantly higher compared with the control group, but not for the As_2Se_3 group when compared with the control group. During the animals' total lifespan, one lung adenoma was seen in the 27 hamsters in the InAs group and one lung adenoma in the 23 hamsters in the control group. No tumors of the lung were observed in the As_2Se_3 group. Malignant tumors outside the lung appeared in four hamsters in the InAs group and in two in the As_2Se_3 group. No non-lung malignant tumours were seen in the control group. Total tumor incidence rates were 25.9% (7/27) in the InAs group, 10.3% (3/29) in the As_2Se_3 group and 8.7% (2/23) in the control group. There were therefore no significant differences in tumor incidence between the InAs or the As_2Se_3 group, and the control group.

Regarding histopathological findings in the lung, incidence rates of proteinosis-like lesions, pneumonia, metaplastic ossification and emphysema were seen only in the InAs group, and alveolar or bronchiolar cell hyperplasia observed in both the InAs and the As_2Se_3 groups were at significantly higher rates than those in the control group.

From these results, it was concluded that InAs and As_2Se_3 particles could induce pulmonary toxicity when instilled intratracheally into hamsters. A great deal of attention should be paid to the toxicity of both InAs and As_2Se_3, even though in this study the adverse health effects of As_2Se_3 appeared to be less than those of InAs.

Keywords: Indium arsenide, arsenic selenide, toxicity, tumorigenicity, histopathology

INTRODUCTION

Indium arsenide (InAs) is a member of the III–V group of semiconductor compound materials of choice, such as gallium arsenide (GaAs). To date, GaAs has been the most widely used III–V compound in the semiconductor industry.[1,2] On the other hand, arsenic selenide (As_2Se_3) is used in electrophotoreceptors which have photoelectric transfer characteristics.[3] With the increasing industrial use of these materials, the question of whether the exposure of employees to them is a potential occupational health hazard has been gaining attention, because InAs and As_2Se_3 both contain arsenic, which is a toxic element suspected of being tumorigenic to humans.[4]

Athough both toxicological[5–10] and immunological[11–14] studies concerning GaAs have already been carried out, there have been no data available on the chronic toxicity of InAs or

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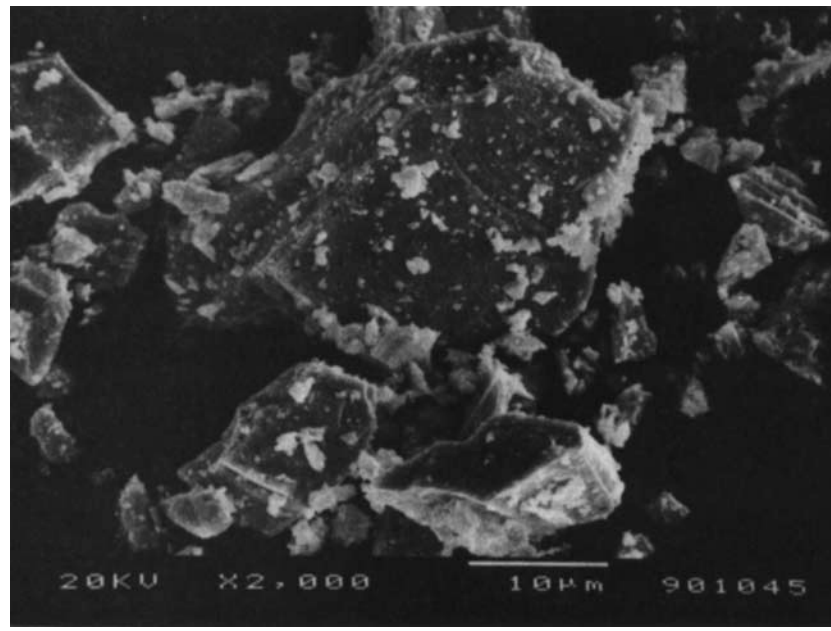

Figure 1 Scanning electron micrograph of InAs particles.

As_2Se_3. It is therefore important for adequate data concerning health effects to be accumulated in order to evaluate accurately the risk to workers from exposure to InAs or As_2Se_3.

The aim of the present study was to evaluate the effect of exposure to InAs and As_2Se_3. In particular, we focused on the chronic toxicity of these materials to the lungs of hamsters when instilled intratracheally.

MATERIALS AND METHODS

InAs (Fig. 1) was obtained from Sumitomo Electric Industries (Osaka, Japan), and had a purity of more than 99.99%. As_2Se_3 (Fig. 2) was obtained from an electronics company in Japan, and also contained few impurities, at the rate of 0.05 ppm manganese, 1.11 ppm iron and 0.42 ppm aluminum, according to fluorescence X-ray analysis. The phosphate buffer solution (0.025 M, pH 6.9) used was purchased from Katayama Chemicals, Osaka. The sample of InAs or As_2Se_3 was pulverized in an agate mortar and the mean count diameter for InAs adn As_2Se_3 particles was 3.9 μm [σ_g (geometric standard deviation) = 2.36] and 1.7 μm ($\sigma_g = 2.65$). The particles were measured with an image analyzer (Nikon Co. Ltd, Tokyo, Japan) using scanning electron microscopy (T-220, JEOL Ltd, Tokyo, Japan).

All the hamsters were male and were purchased at six weeks of age from the Kyudo colony, in Tosu, Japan. The hamsters were raised under conventional conditions at 22–25 °C for two weeks until the beginning of the experiment. Five hamsters were housed in one aluminum cage and fed a commercial diet (CE-2 pellets, Clea Japan, Inc., Tokyo, Japan), with drinking tapwater available *ad libitum*.

The hamsters comprised three groups: the InAs group, the As_2Se_3 group and a control group, as shown in Table 1. Each group was composed of 30 hamsters. The average body weight (mean ± SD) at the beginning of the instillations was 125.1 ± 8.3 g in the InAs group, 111.5 ± 8.6 g in the As_2Se_3 group, and 122.5 ± 7.3 g in the control group. The intratracheal instillations were carried out at eight weeks of age according to the method of Ishinishi *et al.*[15] The hamsters were given 0.1 cm^3 of atropine sulfate subcutaneously and were then anesthetized with a mixture of 5% diethyl ether and 95% oxygen in a desiccator for 5 min. The particles of InAs and As_2Se_3 each contained 0.5 mg of arsenic; the compounds were suspended in 0.2 cm^3 of phosphate buffer solution and instilled into the tracheas of anaesthetized hamsters once a week for 15 weeks by means of a microsyringe with a special metal needle. The

control group received 0.2 cm^3 of phosphate buffer solution alone as the weekly dose per animal. The phosphate buffer solution was sterilized in an autoclave and particles of InAs or As_2Se_3 were aseptically suspended in it.

All the hamsters were observed throughout their entire lifespan. Animals which died were autopsied, and the principal visceral organs were fixed in 10% formalin solution. For the purposes of histopathological examination, sections were prepared by conventional methods and stained with hematoxylin and eosin. Selected sections were stained with periodic acid–Schiff (PAS), Alcian Blue or Toluidine Blue stain. The survival curve of each group examined was assessed by the Kaplan–Meier method[16] and the change in cumulative average body weight was evaluated for significance by Student's *t*-test. The chi-square test was used for statistical comparison of the incidence of tumors or lesions of the lung, in each group.

RESULTS

The survival rate of each of the three groups after 15 instillations was 96.7% (29/30), as shown in Table 1. The mean survival time was 549.3 ± 166.7 days in the InAs group, 467.1 ± 151.1 days in the As_2Se_3 group and 443.0 ± 168.8 days in the control group. All the hamsters had died by the 849th day in the InAs group, by the 826th day in the As_2Se_3 group and by the 737th day in the control group, following the initial instillation. Changes in the survival rate of each group are shown in Fig. 3. During the 11 months following the initial instillation, there was a corresponding tendency regarding the survival rates in the three groups. Howevcer, a high survival rate was observed after 12 months from the intial instillation in the InAs group, in which a significant difference in the survival rate was found when compared with the control group, but not when compared with the As_2Se_3 group.

Changes in the cumulative average body weight gain in each group following the initial instillation are shown in Fig. 4. Significantly suppressed body weight gain was observed in the InAs group compared with the control group during both the instillation period and the observation periods. Although there was a significant difference between the As_2Se_3 and the control group only at 12 and 14 months after the initial instillation, there was a similar trend concerning change in bodyweight during the remaining period.

The tumor incidences, including those of the lung in each group, are shown in Table 2. Lung tumors were developed in only two hamsters among the three groups. One was an adenoma

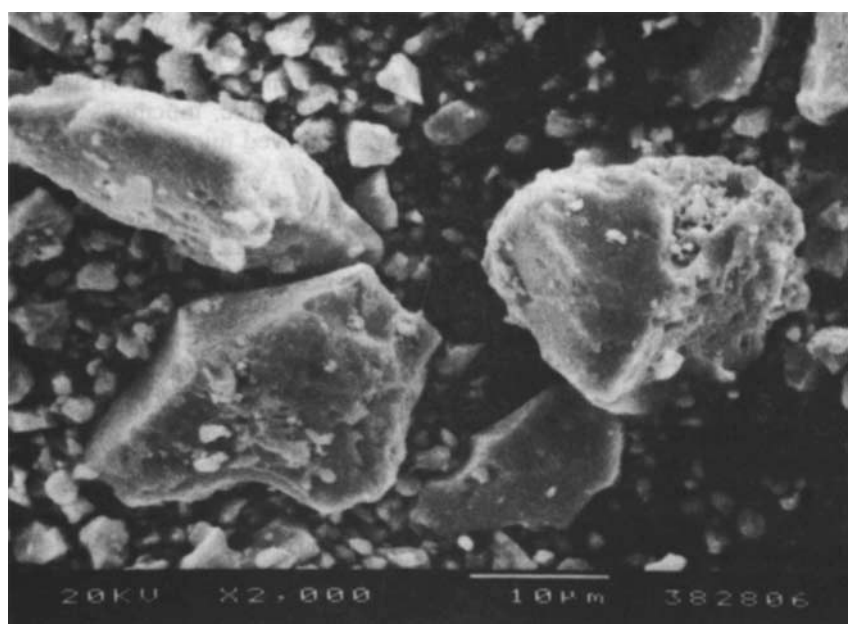

Figure 2 Scanning electron micrograph of As_2Se_3.

Table 1 Dose and number of hamsters in the InAs, As_2Se_3 and control groups

Group	Dose	Initial no. of hamsters	No. of survivors after 15 instillations (%)	No. of hamsters examined
InAs	0.5 mg As × 15 (1.27 mg as InAs × 15)	30	29 (96.7)	27 (2)[a]
As_2Se_3	0.5 mg As × 15 (1.35 mg as As_2Se_3 × 15)	30	29 (96.7)	29
Control	0.2 cm^3 PBS[b] × 15	30	29 (96.7)	23 (6)[a]

[a] No. of cannibalized hamsters is given in parentheses.
[b] PBS, Phosphate buffer solution.

which was observed in a hamster from the InAS group which died on the 686th day following the initial instillation. The other tumor was an adenoma which appeared in a hamster of the control group whch died on the 737th day following the initial instillation. Meanwhile, no tumors of the lung developed in the As_2Se_3 group. With the exception of the tumors of the lung, two cystoadenomas of the liver, one adenocarcinoma of the pancreas, one adenocarcinoma of the adrenal gland and two malignant lymphomas of the lymph nodes in the InAs group, and one adenocarcinoma of the pancreas, one adenocarcinoma and one adenoma of the adrenal gland in the As_2Se_3 group, in addition to one papilloma of the forestomach in the control group, were observed. The total tumour incidence rates were 25.9% in the InAs group, 10.3% in the As_2Se_3 group and 8.7% in the control group. The difference in the rate of tumour manifestation between the InAs or the As_2Se_3 group and the control group was not significant, as determined by the chi-square test.

Histopathological findings in the lung for each group, apart from the appearance of tumors, are given in Table 3. The proteinosis-like leson was a noticeable finding only observed in the InAs group. This lesion was recognized macroscopically as a greyish-white nodule indicating a variation of from 1 to 5 mm in size. Within this lesion, an eosinophilic, mucinous, amorphous secretion was observed microscopically, which was stained

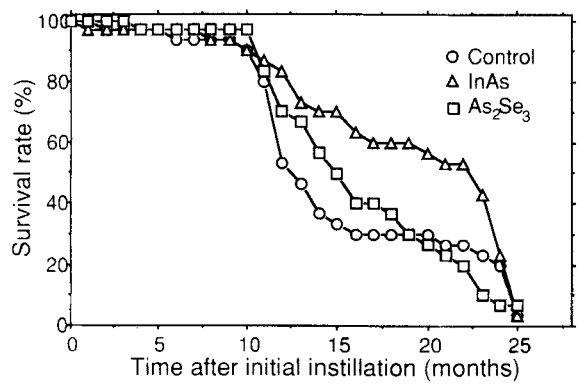

Figure 3 Changes in survival rate (%) of the InAS, As_2Se_3 and control groups following initial instillation.

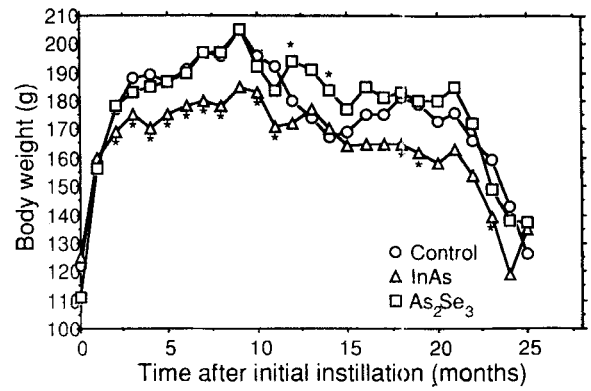

Figure 4 Changes in average body weight of the InAs, As_2Se_3 and control groups following initial instillation.
* Significantly different from the control group, $P<0.05$.

Table 2 Number of tumors which developed in hamsters in the InAs, As_2Se_3 and control groups

Organ in which tumor was manifested	InAs (27)[a]	As_2Se_3 (29)[a]	Control (23)[a]
Lung	1	0	1
Liver	2	0	0
Forestomach	0	0	1
Pancreas	1	1	0
Adrenal gland	1	2	0
Lymph node	2	0	0
No. of tumor-bearing hamsters	7 (25.9)[b]	3 (10.3)[b]	2 (8.7)[b]

[a] No. of hamsters examined is given in parenthesis.
[b] Percentage is in parenthesis.

Table 3 Histopathological findings in the lungs of hamsters in the InAs, As_2Se_3 and control groups

Lung lesion	InAs (28)[a]	As_2Se_3 (29)[a]	Control (23)[a]
Proteinosis-like lesion	20*	0	0
Alveolar or bronchiolar cell hyperplasia	16*	21*	0
Squamous cell metaplasia	2	0	0
Purulent pneumonia	2	3	1
Pneumonia	26*	3	7
Emphysema	21*	0	0
Metaplastic ossification	18*	6	5
Particle deposition	27*	29*	0

[a] No. of hamsters examined is given in parenthesis.
* Significantly different from the control group ($P<0.01$).

positively by PAS and Alcian Blue methods (Fig. 5). There were no such lesions in the As_2Se_3 group. Moreover, emphysema and squamous cell metaplasia were only seen in the InAs group. The difference in the incidence rate of proteinosis-like lesions and emphysema between the InAs and the control group was significant.

Besides these lesions, hyperplasia of the alveolar or bronchiolar cells, purulent pneumonia, pneumonia, metaplastic ossification and particle deposition in the lung were found in both the InAs- and the As_2Se_3-treated hamsters (Fig. 6). The incidence rate of hyperplasia of the alveolar or bronchiolar cells and particle deposition of InAs or As_2Se_3 in both the InAs group and the As_2Se_3 group, in addition to pneumonia and metaplastic ossification in the InAs group, increased significantly when compared with the control group. Particles of the InAs or As_2Se_3 groups were deposited in the region of the alveolar septum and alveolar space, and sometimes alveolar macrophages phagocyted these particles. As well as in the lung, deposition of these particles was observed in the lymph nodes in some of the hamsters.

DISCUSSION

Recent studies using laboratory animals have revealed some positive results concerning the acute and chronic toxicity of semiconductor materials, especially GaAs particles, when instilled intratracheally.[5–9] In the present study, pulmonary toxicity of InAs and As_2Se_3 particles instilled intratracheally was observed. Although the incidence rates of the alveolar or bronchiolar cell hyperplasia were significantly increased in both the InAs and As_2Se_3 groups compared with the control group, from the incidence of other lesions observed in the lung, such as proteinosis-like lesions, emphysema and metaplastic ossification, InAs particles seem to produce more severe injury to the lung of hamsters compared with

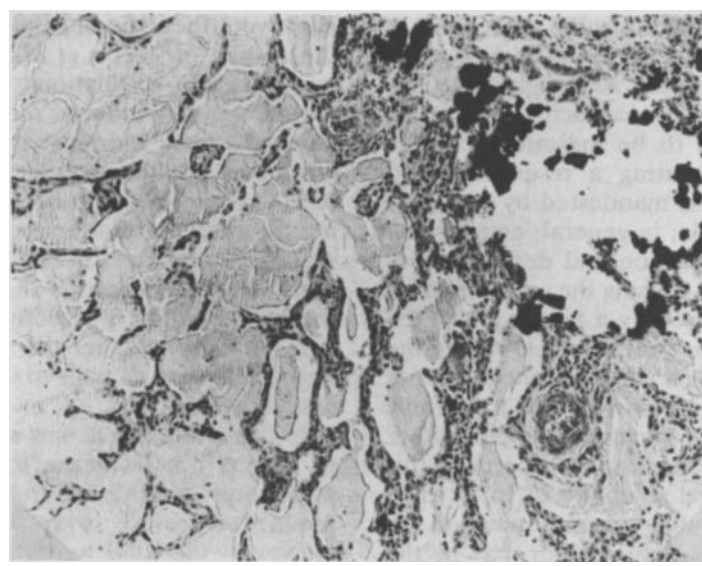

Figure 5 Proteinosis-like lesion and InAs particle deposition in the lung of a hamster which died on the 314th day after the initial instillation of InAs. H.E. stain, ×110.

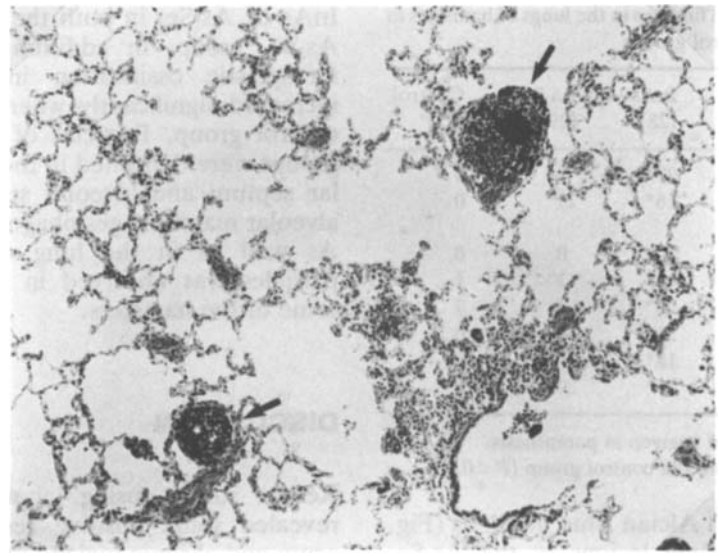

Figure 6 As_2Se_3 particle deposition in the lung of a hamster which died on the 515th day after initial instillation of As_2Se_3. H.E. stain, ×80.

As_2Se_3 particles. Noticeable histological lesions of the lung were the proteinosis-like lesions and emphysema which were only observed in the InAs group. Corrin and King[17] reported the development of alveolar proteinosis in rats following experimental inhalation of silica. Since then, this finding has also been recognized in the cases of nickel, carbon dust and some drugs. However, in our study, accompanying the hyperplasia of the alveolar or bronchiolar cells surrounding this lesion, expansion of the alveolar space plus a general disappearance of the alveolar cells were both seen within this lesion. Although it was not clear whether this lesion was actually alveolar proteinosis, it seemed to be indicated that the pathological change bearing a resemblance to alveolar proteinosis was manifested by exposure to InAs particles. While, in general, emphysema is caused by the obliteration and destruction of respiratory bronchioles causing the entrapment of air, it may also be caused by a narrowing of the terminal and respiratory bronchioles preventing normal expiration.[18] Such increased incidences of these lesions seem to be attributable to chronic physical action by the particles concerned, rather than to their actual chemical properties, this being due to the partial solubility of InAs when given as a single subcutaneous injection to hamsters.[19] Another causative factor may be the particle diameter. The mean count diameter of InAs particles was almost twice as great as that of As_2Se_3 particles, although nearly the same weekly dose per animal was used as in whole particles, these being 1.27 mg as InAs or 1.35 mg as As_2Se_3, respectively. It would appear that a large particle size produces severe damage to the lung in hamsters, especially with regard to the appearance of lesions such as proteinosis-like lesions, emphysema or metaplastic ossification which are observed in the InAs group at a significant increase compared with the control group, although not compared with the As_2Se_3 group. However, these findings were inconsistent with the results of Webb *et al.*,[7] who reported that intratracheal instillations of smaller GaAs particles to rats induced more serious acute pulmonary lesions and more rapid signs of systemic arsenic toxicity than was seen with larger fractions of GaAs particles. On the other hand, considering the evidence that arsenic reveals a great affinity for erythrocytes in rats[20] but not in hamsters, the difference in species is a prominent factor in the manifestation of toxicity of arsenic, although it is not clear whether the smaller particles produced definitive damage to the lung in the chronic toxicity study.

In this study, there was no significant increase of lung tumor incidence following intratracheal instillations in either the InAs group of the As_2Se_3 group compared with the control group. However, the total tumor incidence rate in the InAs group was higher than that in the control group, but not significantly so. It may be that the greater survival time observed in the InAs group

may have contributed to the higher tumor incidence rate. The results of our present study are consistent with those finding reported in our previous study,[10] in which we indicated a significant increase in spontaneously occurring tumors in mice when GaAs or gallium phosphide (GaP) particles were injected intraperitoneally, but not when they were injected subcutaneously. Although there was no definitive conclusion over whether this was due to the effect of arsenic or indium released from the InAs particles or to the direct effect of the InAs particles themselves, it seemed that one causative factor contributing to the increased total tumor incidence rate may have been the longer survival time observed in the InAs group. It remains obscure why such a significantly high survival rate was observed in the InAs group compared with the control group; nevertheless the bodyweight gain was suppressed significantly. To date, some data have veen avaiable on the immunological effects of GaAs exposure,[11–14] but there have been no such data on the effects of InAs. Regarding arsenic compounds, Nunoshiba and Nishioka[21] mentioned that sodium arsenite may have at least two roles to play in the mechanism of its antimutagenesis, these being the inhibition of the *umu*C gene expression and partial increase in the efficiency of error-free repair systems. Since it seems that arsenic may well affect the immune system, further study is needed in order to clarify the long survival period observed in the InAs group.

There have been some epidemiological studies concerning health effects among semiconductor workers.[22–23] Although we cannot ignore the possible adverse health effects of exposure to other semiconductor materials, further study is required to clarify the exact situation regarding toxic effects of InAs and As_2Se_3.

CONCLUSION

Our study indicated that InAs and As_2Se_3 particles produce definite pulmonary lesions when instilled intratracheally into hamsters, even though no tumorigenic effect could be observed. Therefore, a great deal of attention should be paid to both InAs and As_2Se_3, even though As_2Se_3 appeared to be less toxic than InAs.

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