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Chronic Toxicity of Indium Arsenide and Indium Phosphide to the Lungs of Hamsters

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Abstract Chronic toxicity of indium arsenide (InAs) and indium phosphide (InP) was studied in male Syrian golden hamsters which received InAs or InP particles containing a total dose of 7.5 mg of arsenic or phosphorus by intratracheal instillations once a week for 15 weeks. As a control, hamsters were treated with the vehicle, phosphate buffer solution. During their total life span, the cumulative body weight gain of hamsters in the InAs group was suppressed significantly compared with that in the control group, but not in the InP group when compared with that in the control group. Concerning the histopathological findings of the lung, the incidence rates of proteinosis-like lesions, alveolar or bronchiolar cell hyperplasia, pneumonia, emphysema and metaplastic ossification observed in the InAs or InP group were significantly higher than those observed in the control group.

From these results, it would seem that InAs and InP produced severe damage to the lungs of hamsters.

Introduction

Indium arsenide (InAs) and indium phosphide (InP) are the III-V compounds of those semiconductor materials of choice such as gallium arsenide (GaAs), for use in high-frequency microwave and millimeterwave telecommunications and for use in ultrafast super computers. To a great extent, it would seen that GaAs is the most extensively used compound in the semiconductor industry[3)13)]. Due to the increasingly frequent industrial use of these materials, the question of whether the exposure of semiconductor employees to these materials is a potential occupational health hazard or not, has been gaining attention.

To date, these materials, in particular InAs or InP, have generated much controversy as new semiconductor materials, especially because of the potential toxicity of arsenic[8)] or indium compounds[4)]. Both toxicological and immunological studies concerning GaAs have already been carried out by some researchers. Webb et al.[21)22)23)] and Goering et al.[5)] reported the acute pulmonary toxicity of GaAs when instilled to the trachea of rats. Regarding chronic toxicity, Ohyama et al.[10)] reported the positive effect of GaAs when instilled to the hamster intratracheally, while Tanaka et al.[19)] showed the increase of spontaneously-occurring tumors in mice which received GaAs, gallium phosphide (GaP) or gallium oxide (Ga_2O_3) intraperitoneally. In addition, Sikorski et al.[15)16)17)] and Burns et al.[1)] demonstrated the

immunotoxicity of GaAs in vitro or in vivo using mice. Although it could be suspected that inorganic arsenic compounds would be tumorigenic when used on laboratory animals, the results of epidemiological studies have also classified these compounds as tumorigenic to the lung and skin[6]. Although Yamauchi et al.[26] recently reported the partial solubility of InAs when given as a single sc injection to hamsters, there have been no available data concerning the chronic toxicity of InAs or InP. It is therefore essential for sufficient toxicity data to be accumulated in order to estimate accurately the risk to semiconductor workers from exposure to these semiconductor materials.

The purpose of this study was to estimate the chronic effects, if any, of InAs and InP. In particular, we focused on the toxicity of these materials to the lungs of hamsters, when instilled intratracheally.

Materials and Methods

Chemicals. InAs and InP, both of which had a purity of more than 99.99 %, were obtained from Sumitomo Electric Industries (Osaka, Japan). The phosphate buffer solution (0.025M, pH 6.9) used was purchased from Katayama Chemicals, Osaka, Japan. The sample of InAs or InP was pulverized in an agate mortar and the mean count diameter for InAs and InP particles was 3.9μm, σg (geometric standard deviation): 2.36 and 3.2μm, σg: 2.88, the particles being measured with an image analyzer (Nikon Co. Ltd, Tokyo, Japan) using scanning electron microscopy (T-220, JEOL Ltd., Tokyo, Japan).

Animals and maintenance. All the hamsters were male and were purchased at six weeks of age from the colony of Kyudo, 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 aluminium cage and fed a commercial diet (CE-2 pellets, Clea Japan, Inc., Tokyo, Japan), with drinking water available *ad libitum*.

Experimental procedure These animals comprised 3 groups: The InAs group, the InP group and a control group as shown in Table 1. Each experimental group was composed of 30 hamsters. Their average body weights (mean±S. D.) at the beginning of the instillations were 125.1±8.3 g in the InAs group, 136.3±10.2 g in the InP group, and 122.5±7.3 g in the control group. The intratracheal instillations were carried out according to the method of Ishinishi et al.[7] at 8 weeks of age. The hamsters were given 0.1 ml 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 semiconductor materials contained 0.5 mg of As or P. The dose was chosen in accordance with that of Ohyama et al.[10], which was half as much in this study. The compounds were suspended in 0.2 ml of phosphate buffer solution and instilled into the tracheas of anesthetized hamsters once a week for 15 weeks by means of a microsyringe with a special metal needle. The control group received 0.2 ml of phosphate buffer solution alone as the weekly dose/animal. The phosphate buffer solution was sterilized in an autoclave and particles of InAs or InP were aseptically suspended in it.

Hamsters were allowed to die spontaneously or were killed when moribund. Except for a few animals lost through post-mortem changes or cannibalism, all were autopsied. Those which died were autopsied, and the principal visceral organs were fixed in 10% formalin solution. For the purpose 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 mortality curve of each group examined was assessed by the Kaplan-Meier method[9] and the cumulative average body-weight gain in each group was evaluated by the 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

Changes in the cumulative average body-weight gain in each group following the initial instillation are shown in Fig. 1. A significant difference in body-weight gain was noted between the InAs and the control groups during the instillation period and the observation period. Although there was a significant difference between the InP and the control group only over the period of from 40 to 42 weeks following the initial instillation, there was a similar trend concerning the change in body-weight during the remainding period.

The survival rates of each group after 15 instillations were 96.7% (29/30) in the InAs group, 86.7% (26/30) in the InP group and 96.7% (29/30) in the control group, as shown in Table 1. During the instillation period, no significant difference in the survival rate was noted between the InAs, the InP and the control groups. The mean number of survival days was 549.3±166.7 days in the InAs group, 432.8±169.6 days in the InP group, 443.0±168.8 days in the control group. All the hamsters had died by the 849th day in the InAs group, by the 689th day in the InP group and by the 737th day in the control group, following the initial instillation.

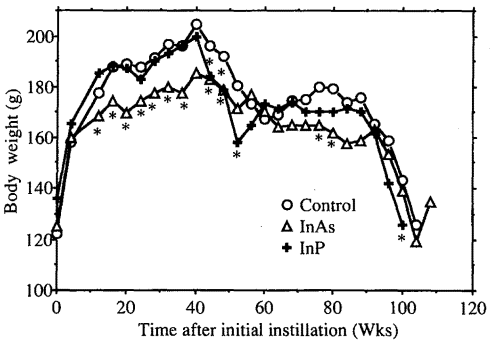

Fig. 1 Changes in the average body weight of the InAs, InP and control groups following the initial instillation (* : Significantly different from the control group, $P<0.05$)

The histopathological findings of the lung in each group, with the exception of tumor formation, are shown in Table 2. The remarkable finding within the lung was a proteinosis-like lesion in both the InAs and the InP groups. This lesion was identified as a greyish-white nodule showing a variation of from 1 to 5 mm in size macroscopically. The alveoli were filled with an eosinophilic, mucinous, amorphous secretion, and were expanded by the secretion which was stained positively by the PAS and alcian blue method, although not by the toluidine blue method (Fig. 2). Alveolar epithelium flattened or disappeared partly, and depositions of InAs or InP particles were observed within or surrounding this lesion. With the secretion, alveolar macrophages or lymphocytes infiltrated the alveolar space slightly. In the region surrounding this lesion, alveolar or bronchiolar cell hyperplasia with or without squamous cell metaplasia could be seen.

In addition to this lesion, hyperplasia of the

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

Group	Dose	Initial no. of hamsters (Sex)	No. of survivors after 15 instillations (%)	No. of hamsters examined
InAs	0.5 mg As×15	30 (M)	29 (96.7)	27 (2)[a]
InP	0.5 mg P×15	30 (M)	26 (86.7)	23 (3)[a]
Control	0.2 ml×15	30 (M)	29 (96.7)	23 (6)[a]

a : No. of cannibalized hamsters
M : Male

Table 2 Histopathological findings of the lung of hamsters in the InAs, InP and control groups

Lung lesions	InAs (27)[a]	InP (23)[a]	Control (23)[a]
Proteinosis-like lesion	20[b]	19[b]	0
Alveolar or bronchiolar cell hyperplasia	16[b]	9[b]	0
Squamous cell metaplasia	2	1	0
Purulent pneumonia	2	4	1
Pneumonia	26[b]	23[b]	7
Emphysema	21[b]	11[b]	0
Metaplastic ossification	18[b]	12[c]	5
Particle deposition	27[b]	23[b]	0

[a] No. of hamsters examined
[b] Significantly different from the control group ($P<0.01$)
[c] Significantly different from the control group ($P<0.05$)

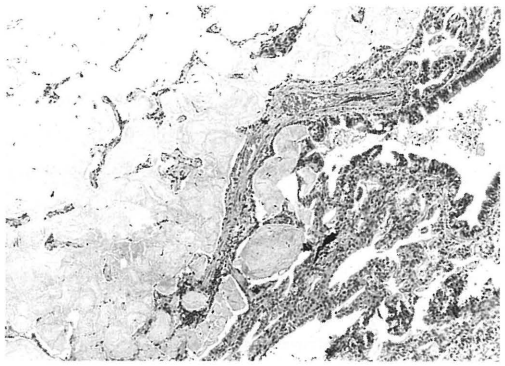

Fig. 2 Proteinosis-like lesion in the caudal lobe in the InAs group. The hamster died on the 663rd day after initial instillation. H. E. stain. ×126.

alveolar or bronchiolar cells, pneumonia, emphysema, or metaplastic ossification of the lung were seen extensively in most of the InAs-or InP-treated hamsters. The difference in the incidence rate of all these lesions, including the proteinosis-like lesion, between the InAs or the InP group, and the control group was significant. Moreover, particles of InAs or InP deposited in the region of the alveolar septum and alveolar space were seen in all of the hamsters in both the InAs group and the InP group. In addition to the lung lesions, deposition of particles was found in the lymph nodes in some of the hamsters.

The tumor incidences including those of the lung in each group are presented in Table 3. Lung tumors were manifested in only 2 hamsters. There was an adenoma in one animal of the InAs group which died on the 686th day following the initial instillation. Microscopically, tumor cells with a multilayered lining were dominant, widely accompanied with squamous cell metaplasia, and the mucous was often abundant in the lumina of the acinar structures (Fig. 3). The other tumor was an adenoma manifested in a hamster of the control group which died on the 737th day following the initial instillation. The morphological pattern of the tumor was that the cells were markedly uniform and cuboidal and formed one or two layers. On the other hand, no tumors of the lung appeared in the InP group. Neoplasms other than lung tumors comprised of 2 cystadenomas of the liver, 1 adenocarcinoma of the pancreas, 1 adenocarcinoma of the adrenal gland and 2 malignant lymphomas of the lymph nodes in the InAs group; 1 phaeochromocytoma of the adrenal gland in the InP group; 1 papilloma of the forestomach in the control group. The total tumor incidence rates when lung tumor were included, were 25.9% in the InAs group, 4.3% in the InP group and 8.7% in the control group. There was no significant difference concerning

Table 3 Tumor incidence in hamsters in the InAs, InP and control groups

Organ in which tumor was manifested	InAs (27)[a]	InP (23)[a]	Control (23)[a]
Lung	1	0	1
Liver	2	0	0
Forestomach	0	0	1
Pancreas	1	0	0
Adrenal gland	1	1	0
Lymph node	2	0	0
No. of tumor-bearing hamsters (%)	7 (25.9)	1 (4.3)	2 (8.7)

[a] No. of hamsters examined

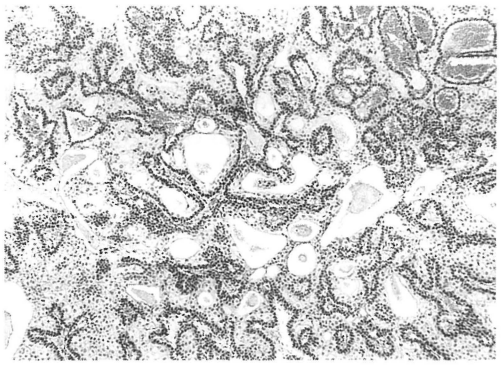

Fig. 3 Adenoma in the caudal lobe in the InAs group. The hamster died on the day 686th day after initial instillation. H. E. stain. ×120.

the tumor incidence rate between each group.

Discussion

The results of the present study indicate the pulmonary or systemic toxicity of InAs or InP particles when instilled intratracheally. Marked histological findings of the lung were noticed in our three groups. The alveolar proteinosis-like lesions which were noted, need to be considered carefully, especially as to their relation to these particles. Corrin and King[2] identified the manifestation of alveolar proteinosis in rats following the inhalation of silica experimentally. Since then, this lesion has also been observed in the case of nickel, carbon dust and some kinds of drugs. However, in this study, accompanying the hyperplasia of the alveolar or bronchiolar cell surrounding this lesion, expansion of the alveolar space plus general disappearance of the alveolar cells were both observed within this lesion. Although, it was not clear whether this histological change was actually alveolar proteinosis or not, it clearly showed that the pathological lesion bearing resemblance to alveolar proteinosis was induced by the exposure of InAs or InP particles and that the secretions which accumulated in the alveolar space may have been secreted from alveolar cell type II.

Concerning other lesions besides alveolar proteinosis like lesion, alveolar or bronchiolar cell hyperplasia and emphysema were observed only in the InAs and InP groups, and the incidence rates of those, together with metaplastic ossification increased significantly in these groups. Such increased incidences of these lesions seem to be due to the chronic physical action of the particles concerned, rather than to their actual chemical properties. The appearance of these lesions may be related to the particle diameter and the given dose of InAs or InP particles. Webb et al.[23] reported that intratracheal instillation of a smaller size of GaAs particles to rats induced more serious acute pulmonary pathogenic lesions and more rapid signs of systemic arsenic toxicity than that seen in their previous study which used larger fractions of GaAs particles. In addition,

Ohyama et al.[10] reported that there were scarcely any particles in the lungs of the hamsters when 0.25 mg of As, as a weekly dose/animal of GaAs particles, were instilled to the trachea for 15 weeks, although no information concerning the particle diameter was given. However, in this study, the mean count diameters of InAs or InP particles were relatively large and the dose (0.5 mg of As or P as a weekly dose/animal) was twice as much compared to that given in their study. A high dose and a large particle diameter are both likely to lead to a decrease in particle clearance from the lung, to produce severe damage to the lung in hamsters, and to influence the significantly suppressed body-weight gain in the InAs group, and probably in the InP group. These findings were inconsistent with the results of Webb. et al.[23]. However, from the fact that arsenic shows a great affinity for red blood cells in rats[20] but not in hamsters, the difference in species (rat or hamster) is a noticeable factor regarding the appearance of toxicity of arsenic, although it is not apparent whether the smaller size of particles caused severe damage to the lung in the chronic toxicity study, or not. On the other hand, there were no marked findings concerning the total tumor incidence or lung tumor incidence in either of the 2 experimental groups compared with the control group. This result supports the finding reported by Ohyama et al.[10] who reported that GaAs had no apparent tumorigenicity when instilled to the trachea of hamsters. Moreover, Tanaka et al.[19] reported the systemic injury and significantly increased spontaneous-occurring tumors of mice when GaAs or gallium phosphide (GaP) particles were injected intraperitoneally, but not subcutaneously. However, many epidemiological studies have shown a causal relationship between exposure to inorganic arsenic compounds and skin or lung cancer[6], and some recent animal experiments have revealed tumorigenicity of inorganic arsenic compounds, especially calcium arsenate, to the respiratory organs[12)24)]. Furthermore, GaAs or InAs was only slightly soluble in vivo, and inorganic arsenic compounds are released partially from GaAs or InAs[14)21)22)25)26)]. In this study, the total tumor incidence rate in the InAs group was increased, but was not significantly different compared with the control group. The larger number of survival days observed in the InAs group may contribute to the increased tumor incidence rate. However, we cannot ignore such an elevated manifestation of tumors in the InAs group, although it was not clarified 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.

There has so far been no study which could explain categorically whether or not there is a relationship between exposure to semiconductor materials and the incidence of adverse health effects. Sorahan et al.[18] reported that there was no increasing mortality from any cancers in a cohort of semiconductor workers, even though threed cases of melanoma were found, since this was not a significant increase. Pastides et al.[11] reported an increased rate of spontaneous abortion and general illness symptoms among workers in semiconductor factories. Based on our findings in this study, taken together with those findings in other studies, we would strongly maintain that InAs and InP particles induce pulmonary or systemic toxicity when instilled intratracheally to hamsters, and we feel that it is thus necessary to pay much greater attention to the exposure to semiconductor materials, such as InAs or InP particles.

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(和文抄録)

インジウムヒ素およびインジウムリンのハムスター肺に対する慢性毒性

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化合物半導体の新素材であるインジウムヒ素(InAs)およびインジウムリン(InP)の生体影響を評価するためにハムスターを用いた慢性毒性実験を行った．8週齢雄性シリアンゴールデンハムスター90匹を用い，InAs群，InP群および対照群(各30匹)の3群を設定した．投与物質としてはInAs(幾何平均粒径；3.7 μm，σg：2.36)およびInP(幾何平均粒径；3.2 μm，σg：2.88)を用い，1回投与量は0.5 mg/animal(AsおよびP当たり)であり，0.2 mlのリン酸緩衝液(pH 6.9)に懸濁し，週1回，15週間にわたってハムスターの気管内に投与した．対照群にはリン酸緩衝液(1回投与量；0.2 ml/animal)を投与した．投与後は無処置で放置し，生涯観察を行った．死亡したハムスターは剖検後，常法により病理標本を作製し，肺を中心とした病理組織学的検索を行った．

各群の観察期間中の体重変化について，InAs群では対照群に比べて有意に体重増加の抑制が観察されたが，InP群では対照群と同様の体重変化の推移を示した．肺の病理学的所見においてInAs群およびInP群の両群で肺胞蛋白症様病変，肺胞および細気管支上皮細胞の増殖，肺炎，肺気腫および骨異形成の発生率が対照群に比べて有意に増加していた．肺腫瘍発生に関しては，InAs群および対照群で各々1例づつ認められたが，InP群では観察されず，InAsおよびInPの肺に対する催腫瘍性は認められなかった．

InAsおよびInPの経気道性曝露により肺に対する障害が発現することが認められ，半導体産業でのInAsやInPの使用に関しては十分留意する必要があると考えられた．