Effects of Intratracheally Administered Indium Phosphide on Male Fischer 344 Rats

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Abstract: Effects of Intratracheally Administered Indium Phosphide on Male Fischer 344 Rats: Takamoto Uemura, et al. Department of Preventive Medicine and Public Health, School of Medicine, Keio University—Objective—To examine the effects of intratracheally administered indium phosphide (InP) and distribution of indium on male Fischer 344 rats. Materials and methods—Rats were intratracheally given 0, 1, 10 or 100 mg/kg of InP with a mean diameter of 0.8 μm and observed for 1 and 7 days. The bronchoalveolar lavage fluid (BALF) was examined biochemically and cytologically. Serum biochemical, hematological and histopathological examinations were done, and the indium concentration in organs and serum was determined. Findings—The number of neutrophils in BALF remarkably increased in a dose-effect manner 1 and 7 days after administration and InP particles were phagocytized in the macrophages. Total protein (TP), lactate dehydrogenase (LDH), total phospholipid (TPL) and total cholesterol (T-Chol) in BALF showed a clear dose-effect relationship 7 days after administration. Indium was detected in the liver and spleen and increased in a dose-related manner on the next day and 7 days after administration. Serum indium was detected in the group given more than 10 mg/kg but did not reveal a dose relationship. Histopathological examination of the lungs showed phagocytized InP particles in the macrophages and the migration of neutrophils in the alveoli. InP particles remained in the bronchioles and alveoli until 7 days after. No histopathological changes were detected in the liver or spleen. A hematological study did not reveal significant findings. Interpretation—Intratracheally administered InP particles cause pulmonary inflammation and those particles remain in the lower airways for at least 7 days. Phagocytosis of macrophages may contribute to their disposal and distribution to the liver and spleen. Further study is required with particles with a lower toxic activity than InP and with the same particle size as the InP used in this study, to clarify their specific toxicity. Simultaneously longer observation is needed to assess toxicity in the other organs after distribution. (J Occup Health 1997; 39: 205-210)

Key words: Indium phosphide, Semiconductor, Intratracheal administration, BALF (Bronchoalveolar lavage fluid), Toxicity, Rats

The use of indium compounds in the semiconductor industry has risen remarkably because III–V semiconductors were discovered to have the better electrical properties than the commonly used siliccon. They are now widely employed in microwave and optoelectronic devices.

Although InP toxicity is thought to be relatively lower, inhalation exposure to its particles is a matter of concern in industry. In vivo toxicity depends on its chemical formula (solubility), particle size, and exposure route. The oral lethal dose 50 (LD50) of metallic indium was 4,200 mg/kg for rats, and that of intraperitoneal and oral LD50 of InP was recently reported to be more than 5,000 mg/kg for mice. This means InP is relatively less toxic than soluble indium compounds. Kabe et al. examined the in vitro solubility of InP and demonstrated that it was hardly soluble in synthetic lung fluid and physiological saline, but was slightly soluble in synthetic gastric fluid with time and temperature dependencies.

Zheng et al. examined tissue distribution and elimination of indium after oral and intratracheal
administration of InP in rats, and reported that indium was relatively evenly distributed among the major organs and almost all administered doses were eliminated by 240 hr after exposure except in the lungs following intratracheal administration\(^4\). Regarding the above investigations, interest in the mechanism of InP toxicity is focused on whether insoluble InP particles remaining in the airway after intratracheal administration will irritate the lungs locally or create a toxic substance that will be distributed to the other organs or the whole body. This study was designed to clarify the biological effects and the basic mechanism of InP toxicity, as well as the route of distribution following intratracheal administration.

**Material and Methods**

Single-crystal InP wafers (99.999% purity, Furukawa Electric) were powdered in a mortar. More than 80% of InP particles were about 0.8 \(\mu\)m or less and the rest were up to 10 \(\mu\)m in diameter. The particles were suspended in aseptic physiological saline and adjusted to the designed concentrations (1, 10, 100 mg/kg) when 0.5 ml of the mixture was administered intratracheally.

Eleven-week-old male Fischer 344 rats (SPF grade, 230 g ± 10 g) were purchased from Charles River Japan and acclimatized for one week prior to administration. Each rat was housed in a stainless-steel cage in the filtered-air ventilated chambers (Shinano Seisakusho) in a clean animal room. The chambers were maintained at 24°C, 60% relative humidity and with a 12 hr light-dark cycle. They were fed pellet food (CE-2, CLEA), and given water ad libitum. The rats' behavior and external appearance were observed and they were weighed every second day.

Rats were fully anesthetized with halothane, 4.0 l/min for 3.5 min, and hooked up to the rubber band on the 45°-angle board. InP particles suspended in aseptic saline were kept at 37°C and instilled into rats through a 22-gauge flexible plastic tube with the aid of a very small electric bulb glued onto a spatula to illuminate the glottis. Seven rats in each group were intratracheally administered 0 (physiological saline), 1, 10, 100 mg/kg of InP for one-day observation, while the other four groups were treated with the same concentrations for a 7-day observation.

Rats were killed by exsanguination from the inferior abdominal vena under sodium pentobarbital anesthesia. After that, the lungs of five rats in each group were lavaged with heparinized (20 units/ml) saline, 40 ml/kg body weight. BALF was collected and then filtered through a strainer (Falcon 2350). Total leukocytes (WBC) were counted with an automated hemocytometer (Celltac MEK 5103, Nihon Kohden) and BALF was centrifuged. Differential leukocytes were counted with the sediment after centrifugation (3,000 rpm for 10 min at 4°C) stained with Mayer's hematoxylin, and a total of 300 cells were counted under a light microscope. Total protein (TP), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total phospholipid (TPL) and total cholesterol (T-Cho) in the supernatant of BALF were determined.

For the blood analysis, the erythrocyte count (RBC), total and differential leukocyte count (WBC), hemoglobin concentration (HB), hematocrit (Ht), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) were determined. Serum \(\delta\)-aminolevulinic acid (\(\delta\)-ALA) was also determined by stopped-flow HPLC\(^5\).

Indium concentrations in the liver, spleen, and serum were determined by graphite-furnace atomic absorption spectrometry (GFAAS), Z-8720, Hitachi\(^6\).

The lungs of two rats in each group were preserved for pathological examination. The lungs, livers, and spleens of all the rats were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin (H-E), periodic acid-Schiff (PAS), and toluidine blue (TB) for light microscopic histopathological examination. Some of the spleen and liver specimens were preserved for the detection of InP particles.

Analysis of variance was employed to assess the significance of the difference in the values of hematological and biochemical parameters, and the BALF cell count. Spearman-rank correlations were adopted in Indium concentrations in the organs from the four groups, and a Student's t-test was conducted for each of the parameters between days 1 and 7.

**Result**

**BALF analysis**

Neutrophils in BALF were noticeably increased in a clear dose-dependent manner both on day 1 and day 7 (Table 1). Even in rats instilled with 1 mg/kg InP, neutrophil counts were 44 times higher on day 1 and 1000 times higher on day 7 than in control rats. The number of macrophages did not change significantly in any of the groups.

In the smear of InP-instilled rats, phagocytized InP particles were seen in the cytoplasm of the macrophages. On day 1, the shape of the...
macrophages seemed to be normal, but on day 7 collapsed or broken macrophages were often observed. As the broken macrophages could not be counted, this led to a decrease in the number of macrophages in the higher concentration group (Table 1). Relatively severe exudation was observed in the background of a microscopic view of the smear in the 100 mg/kg group by day 7. TP and LDH in BALF were dose-dependently increased on day 1. TP, LDH, ALP and T-Cho were noticeably increased in a dose-dependent manner on day 7 (Table 1). Compared with the levels on day 1 in InP-instilled rats, the levels of TP, TPL and LDH on day 7 were significantly increased.

**Hematological and biochemical studies in the blood**

Biochemically, a slight decrease in AST on day 1 in the 10 mg/kg group, and an increase in LDH on day 7 in the 10 mg/kg group were observed, but not in a dose-dependent manner. No systematic changes were identified in the rest of the blood parameters examined. δ-ALA did not reveal any significant changes.

**Indium concentration in serum and organs**

Indium was detected in the liver and spleen of all InP-instilled rats (Table 2). Rank correlation coefficients showed significant positive values, which indicated that indium was accumulated in both organs in a dose-dependent manner, except the liver on day 1. Mean indium concentrations in the spleen were higher than those in the liver in all InP-instilled groups. Comparing day 1 to day 7 for indium concentrations in organs, day 7 showed a notably higher concentration in both organs in the rats instilled with 100 mg/kg InP. But the differences were not statistically significant because of the wide variation in values. Serum indium was detected in some rats instilled with 10 mg/kg InP, and in all rats with 100 mg/kg InP on day 7. Serum concentrations on day 7 were higher than those on day 1.

**Pathological examination**

InP particles remained in the alveoli and bronchioles in all exposed groups both by day 1 and day 7. By day 1, infiltration of macrophages and phagocytosis onto InP particles, and infiltration of neutrophils were observed in the bronchioles. The degree of neutrophil infiltration showed a quantitatively dose-effect relation as well as exudate, and broken macrophages and exfoliated alveolar cells were observed in the higher concentration group. Thickening of the interstitial walls and epithelium of the bronchioles was sometimes seen in the 100 mg/kg group.

By day 7, migration of neutrophils into the lower respiratory lumen was observed more frequently and exudation in the bronchioles was more severe. InP particles still remained even in the 1 mg/kg group and were seen not only in the lumen, but also...
in the interstitial tissues of the lung (Fig. 1). In PAS- and TB-stained specimens, exudation in the alveolus could not be characteristically stained. The spleens in the exposed groups were significantly heavier than in the controls by day 1. The liver and spleen did not show any significant histopathological lesions. Apparent InP particles could not be detected in either of the two organs.

### Table 2. Indium concentration in the liver and spleen and histopathological findings in the lungs

<table>
<thead>
<tr>
<th>Indium concentration</th>
<th>DAY 1</th>
<th>DAY 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=7</td>
<td>Liver (ng/g)</td>
<td>Spleen (ng/g)</td>
</tr>
<tr>
<td>Control</td>
<td>( )</td>
<td>24.71±16.9</td>
</tr>
<tr>
<td>1mg/kg</td>
<td>( )</td>
<td>24.71±16.9</td>
</tr>
<tr>
<td>10mg/kg</td>
<td>( )</td>
<td>24.71±16.9</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>( )</td>
<td>24.71±16.9</td>
</tr>
</tbody>
</table>

Values are the arithmetic means±SD. ND: not detected, ND~value represents range, (-): not performed. Characteristic pulmonary findings for the exposed group were summarized into three categories listed in Table 2 and each degree of severity was evaluated qualitatively. – means the finding was not obtained in any part of the specimen. – means the finding was less sever but could be detected somewhere. – means the finding was often seen anywhere but with some exceptions. – means findings were rather severe and seen frequently everywhere. + – means findings were more sever than – – –. (If the evaluation was more than + , the findings were detected in every specimen in one group, so that severity and frequency were different.)

### Discussion

The degree of acute toxicity of InP particles after intratracheal instillation is relatively low because all rats survived even in the 100 mg/kg group and a histopathological study of the lungs did not reveal severe findings such as bleeding, congestion, or critical decomposition of the lung structure. Kabe et al. reported that InP particles were hardly soluble in the synthetic lung fluid, but slightly soluble in synthetic gastric fluid\(^3\). They also showed that the LDLO of InP following i.p. or p.o. administration in mice was greater than 5,000 mg/kg, which was considered to be less toxic. The severity of toxicity of particulate materials is believed to depend on its solubility, particle size, a route of administration, and a highly soluble particle has a worse affection\(^3\). The low toxicity of InP is therefore apparently due to its low solubility in the body fluids. In contrast, the soluble indium compounds such as indium trichloride and indium nitrate have a higher toxicity; the LD\(50\) was reported to be 5 mg/kg (i.p. mice)\(^4\) for indium trichloride and 5.55 mg/kg (i.p. rats) for indium nitrate\(^7\). On the other hand, LD\(50\) for lower soluble metal indium was reported to be 4,200 mg/kg (p.o. rats)\(^5\). Blazka et al.\(^3\) reported that intratracheally administered 0.00325 mg In/kg indium trichloride was capable of initiating an influx of inflammatory cells, and Downs et al.\(^6\) reported even 0.33 to 3.6 mg of In/kg indium tri-
chloride could cause acute fatality. In the present study, we observed inflammatory changes in the lungs in the 1 mg/kg group but studies with smaller doses of InP will be needed to assess the NOEL (non observed effect level). There have been no studies comparing the toxicity difference according to InP particle size, but the present study employed the smallest particles used in the three preceding studies.\(^1,4,10\)

We employed BALF analysis to detect early signs of pulmonary inflammation. To interpret the increase in neutrophils and the increase in TP, LDH, TPL and T-Chol in the supernatants, InP caused pulmonary inflammation, which developed until at least day 7. In the bronchiolar, and alveolar space, inflammation was more severe on day 7 than on day 1. Exudation was observed to fill alveolar spaces focally, especially in the 100 mg/kg group on day 1. Exudation was observed to fill alveolar spaces focally, especially in the 100 mg/kg group on day 7, and to contain ooze from the alveolar epithelium and broken macrophages after phagocytosis, which were reflected by the increase in TP and TPL in the BALF supernatant. Tanaka et al.\(^10\) reported that alveolar proteinosis-like lesions were manifested on the 663rd day when the animals died.

In the bronchiolar, and alveolar space, inflammation was more severe on day 7 than on day 1. Exudation was observed to fill alveolar spaces focally, especially in the 100 mg/kg group on day 7, and to contain ooze from the alveolar epithelium and broken macrophages after phagocytosis, which were reflected by the increase in TP and TPL in the BALF supernatant. Tanaka et al.\(^10\) reported that alveolar proteinosis-like lesions were manifested on the 663rd day when the animals died spontaneously after multiple dose intratracheal InP instillation. This lesion included a dense accumulation of PAS-positive mucous exudate in the alveolar spaces accompanied by the hyperplasia of the alveolar or bronchiolar epithelial cells. We found the exudation to be similar but less severe. If the difference in the severity of the pulmonary lesion depends on the length of observation and/or frequency of instillation, mucous exudation in our observation may become more severe and develop more characteristic in a longer observation period. We failed to prove the presence of mucopolysaccharides in the exudation by PAS or TB; but if the lesion continues to develop exudation, its contents may change.

Hardly soluble InP particles were considered to remain in the lower airways in their original physical formula, so phagocytosis by the macrophages would play an important role in disposal of the particles. After the phagocytosis, migration of neutrophils may be induced by a chemical substance oozing from broken macrophages. The pathological findings for the neutrophil migration seemed to corroborate the BALF analysis findings. Moreover, when InP particles directly contact with the airway epithelium, physical irritation may also cause local cellular injury. Whereas we observed broken macrophages and exfoliated alveolar cells more frequently in the higher exposed group, TP and TPL might ooze from these cells and exudation from the blood vessels could be the source of TP and TPL. No acute toxicity affecting other organs was detected in the present study, but the questions may remain whether our findings were due to InP-specific toxicity or physical irritation caused by particles. Kevin E et al.\(^11\) reported that intratracheally instilled 100 mg/kg TiO\(_2\), which was about the same particle size as the InP used in our study, caused much less severe changes in the number of cells in BALF.

As for the distribution and disposal of InP particles, we observed indium in the serum, liver and spleen. By day 7, phagocytized macrophages were deposited in the thickening bronchiolar interstitial wall. It seems that the reticuloendothelial system contributes to the disposal of InP and systemic distribution from the lungs, because the route of deposition of cells into interstitial tissue should exist in the thickening bronchiolar interstitial wall, which were considered to be correlate with lymph flow. InP-phagocytized macrophages may also accumulate in the reticuloendothelial system, because the concentration of indium increased dose-dependently and time-dependently by day 7 in the liver, spleen, and serum. The splenic concentration was higher than the serum concentration, which suggested that phagocytized particles in the macrophages were removed mainly by the lymph flow, although some of them were removed by the blood flow.

Zheng et al.\(^4\) reported the tissue distribution of indium over 96 hr after a single intratracheal administration of 10 mg/kg InP particles. They concluded that the high recovery of indium (73% of the dose) in the feces after instillation presumably reflects mucociliary clearance and/or biliary excretion of indium based on the result of a relatively even distribution to the whole blood, brain, liver, skin, stomach, small and large intestines and the content. Smith et al.\(^12\) reported that the kidneys, spleen, and liver contained the highest concentrations after an intratracheal, oral, intramuscular, or subcutaneous injection of indium-114m. In both studies, an increased in the tissue concentration was detected around 4 days after instillation and Smith et al. noted that 30% of the administered indium dose remained after 60 days. Indium was detected in the serum in the present study, which suggests that a little InP can be dissolved or some particles were bound to blood cells and carried to other organs.

In conclusion, intratracheally administered InP particles caused pulmonary inflammation and particles remained in the lower airway for at least seven days. Phagocytosis of the macrophages contributed to their disposal and distribution to other organs. Questions may remain whether this is InP-specific toxicity or physical irritation caused by particles.
Longer observation will be needed to assess toxicity in other organs after InP is removed from the lungs.

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References


