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# Inhalation Carcinogenicity and Chronic Toxicity of Indium-tin Oxide in Rats and Mice

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**Abstract: Inhalation Carcinogenicity and Chronic Toxicity of Indium-tin Oxide in Rats and Mice: Kasuke NAGANO, *et al*. Japan Bioassay Research Center, Japan Industrial Safety and Health Association—Objectives:** Carcinogenicity and chronic toxicity of indium-tin oxide (ITO) were examined by inhalation exposure of rats and mice to ITO aerosol. **Methods:** Fifty mice of both sexes were exposed to ITO at 0 (control), 0.01, 0.03 or 0.1 mg/m$^3$ for 6 h/day, 5 day/wk for 104 wk, and 50 rats of both sexes were exposed to 0, 0.01 or 0.03 mg/m$^3$ ITO for the same time period. The repeated exposure of 50 rats of both sexes to 0.1 mg/m$^3$ ITO was discontinued at the 26th wk, followed by clean air exposure for the remaining 78 wk. **Results:** In rats, incidences of bronchiolo-alveolar adenomas and carcinomas, bronchiolo-alveolar hyperplasia, alveolar wall fibrosis and thickened pleural wall, alveolar proteinosis and infiltrations of alveolar macrophages and inflammatory cells were significantly increased. Combined incidences of malignant lung tumors in male rats and total lung tumors in male and female rats were significantly increased at exposure to 0.01 mg/m$^3$ ITO. In mice, no carcinogenic response occurred, but thickened pleural wall, alveolar proteinosis and alveolar macrophage infiltration were induced. Mice were less susceptible to ITO than rats. The lung content of indium was the greatest, followed by the spleen, kidney and liver. Blood indium levels increased dose-dependently. **Conclusions:** There was clear evidence of carcinogenicity of inhaled ITO in male and female rats but not clear evidence in mice, together with occurrence of the chronic pulmonary lesions in both rats and mice.
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**Key words:** Carcinogenicity, Indium-tin oxide, Inhalation, Lung, Mouse, Rat

Indium-tin oxide (ITO), a sintered material containing 90% indium oxide (IO) and 10% tin oxide, has been extensively used in liquid-crystal displays and other display devices. In recent years, touch-screen applications are a developing market. The average annual growth of indium in the form of ITO has increased by an average 18 percent per year[1, 2]. However, this promising material poses a serious threat to the health of workers engaged in manufacturing, processing and handling of ITO. A worker engaged in finishing compacted ITO by wet grinding in a Japanese ITO plant was diagnosed as interstitial pneumonia and died of bilateral pneumothorax[3]. An operator of a hydrogen furnace with a rarely functioned ventilation system in an American ITO plant died of respiratory failure due to alveolar proteinosis[4]. Recent epidemiology studies on the health of workers in Japanese ITO plants have demonstrated that inhalation exposure of workers to hardly soluble indium could be a potential cause of a risk for interstitial lung disease[5–8]. Experimental toxicology studies have shown that pharyngeal aspiration or intratracheal instillation of ITO induces persistent inflammation in the lung of rats[9] and pulmonary inflammatory response with diffuse alveolar hyperplasia and interstitial fibrotic proliferation in hamsters[10, 11]. Thirteen-week inhalation exposure to ITO aerosol was reported to induce alveolar proteinosis, hyperplasia of alveolar epithelium and fibrotic response in rats and mice[12, 13]. A bioassay carcinogenicity study by the National Toxicology Program (NTP)[14] demonstrated that repeated inhalation exposure to indium phosphide aerosol for 2 yr increased incidences of alveolar/bronchiolar tumors in rats and mice. Gottschling *et al*.[15] reported that 2-year inhalation exposure of rats to indium phosphide aerosol induced pulmonary inflammation associated with

oxidative stress, resulting in progression to pre-neoplastic lesions and lung tumors. An increased frequency of micronucleated cells in Type II pneumocytes of rats treated with pharyngeal aspiration of ITO particles has also been reported[9].

In order to assess health risks of workers engaged in manufacturing, processing and handling of ITO, the present studies were intended to characterize the carcinogenicity and chronic toxicity induced by 2-year inhalation exposure of rats and mice of both sexes to ITO aerosol, and to provide dose-response relationships between concentrations of inhalation exposure to ITO aerosol, target tissue contents of indium, and carcinogenic and pre- and non-carcinogenic responses. The current occupational exposure limit (OEL) and biological exposure index (BEI) for indium and its compounds are discussed on the basis of the present findings.

## Materials and Methods

We conducted inhalation exposure of rats and mice of both sexes to ITO aerosol at different concentrations for 2 yr, in accordance with the Organization for Economic Cooperation and Development's (OECD's) Good Laboratory Practice[16], and with reference to the OECD's Guidelines for Testing of Chemicals 451; "Carcinogenicity Studies"[17]. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals[18] and the present studies were approved by the ethics committee of the Japan Bioassay Research Center (JBRC).

*Test material*

ITO powder was kindly supplied by JX Nippon Mining & Metals, Corp. (former Nippon Mining & Metals Co., Ltd.) (Tokyo, Japan). The supplier prepared the ITO powder by grinding sintered ITO plate. According to the supplier's data, the mean powder diameter was 3.5 $\mu$m with a 90% cumulative diameter of 8.9 $\mu$m; the powder composition was 90.06% IO and 9.74% $SnO_2$ with an ITO purity of 99.8% and trace amounts of aluminium, chromium, copper, iron, nickel, lead, silica, zirconium and zinc as impurities. The ITO powder was colored black.

*Animals*

F344/DuCrlCrlj rats and B6C3F$_1$/Crlj mice of both sexes were obtained at the age of 4 wk from Charles River Japan, Inc (Kanagawa, Japan). The animals were quarantined and acclimated for 2 wk, and then divided by stratified randomization into 4 body weight-matched groups, each comprising 50 rats and 50 mice of both sexes. The animals were individually housed in stainless-steel wire hanging cages (170 W × 294 D × 176 H mm for a rat and 112 W × 212 D × 120 H mm for a mouse) which were placed in stainless steel inhalation exposure chambers. Four exposure chambers of 7,600 liters in volume for rats and 4 exposure chambers of 3,700 liters for mice were used in the present studies. The environment in the exposure chamber was maintained at a temperature of 20–24°C and a relative humidity of 30–70% with 12 air changes/h. The exposure chambers were installed in a barrier system animal room. Fluorescent lighting was controlled automatically to give a 12-hour light/dark cycle. All rats and mice were given sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*. The body weight measured immediately before the first exposure to ITO or clean air was 123 ± 5 (mean ± SD) g for male rats, 96 ± 4 g for female rats, 24.6 ± 0.9 g for male mice and 20.1 ± 0.8 g for female mice.

*Experimental design*

Groups of 50 rats of both sexes were exposed to airflow containing ITO aerosol at a target concentration of 0.01 or 0.03 mg/m$^3$ as ITO for 6 h/day, 5 day/wk for 104 wk, while 50 rats of both sexes were exposed to 0.1 mg/m$^3$ ITO aerosol for 6 h/day, 5 day/wk for 26 wk, and then exposed to clean air for the remainder of the 2-year study. The discontinuation of ITO exposure in the 0.1 mg/m$^3$-exposed group was based on the results of our previous 13-week inhalation study[12] and with reference to the NTP's stop-exposure rationale in the 2-year study of indium phosphide carcinogenicity[14]. Fifty rats of both sexes were exposed to clean air for 104 wk under the same conditions and served as respective controls. For the mouse study, groups of 50 mice of both sexes were exposed to airflow containing ITO aerosol at a target concentration of 0.01, 0.03 or 0.1 mg/m$^3$ for 6 h/day, 5 day/wk for 104 wk, and 50 mice of both sexes were exposed to clean air for 104 wk under the same conditions and served as respective controls.

A 26-week interim evaluation study was conducted to assure the validity of our protocol for rats of both sexes exposed to ITO at 0.1 mg/m$^3$ for 26 wk and then to clean air for the remaining 78 wk. Groups of 10 rats of both sexes were exposed to 0.1 mg/m$^3$ ITO for 6 h/day, 5 day/wk for 26 wk or clean air as control for the same time period, and were sacrificed for analysis of tissue indium and histopathology at the end of the 26-week exposure period.

*Aerosol generation and exposure to ITO*

ITO aerosol at an exposure concentration of 0.1 mg/m$^3$ was prepared, using the aerosol generation and inhalation exposure system with the multiple feedback control systems described in our previous study[12]. The exposure concentrations of 0.03 and 0.01 mg/m$^3$ ITO were generated with the slightly modified aerosol generation and exposure system, using an optical particle counter (OPC) (OPC-AP-600, Sibata Scientific Technology, Ltd., Tokyo, Japan) for monitoring of particle number concentrations in the

exposure chambers and a digital dust indicator (DDI) (Type AP-632T, Sibata Scientific Technology, Ltd.) for monitoring of mass-equivalent concentrations in the reservoir chambers. The multiple feedback loops were set up between OPC or DDI and an ejector for preparation of a stabilized aerosol concentration in the exposure chamber or the reservoir chamber. Airflow containing ITO aerosol from the first reservoir chamber (about 50 mg/m$^3$) was passed through an ejector for further dilution with clean air and delivered into the second reservoir chamber where the aerosol concentration was kept constant at about 20 mg/m$^3$. Then, the aerosol was sent to an exposure chamber where the filtered clean air was kept flowing downward at 12 air changes/h and the aerosol concentration was maintained at 0.03 mg/m$^3$. The aerosol-containing airflow from the second reservoir chamber was diluted with clean air in the ejector and delivered to the third reservoir chamber where the aerosol concentration was kept constant at about 7 mg/m$^3$. Then, the aerosol from the third reservoir chamber was further diluted with clean air in the ejector and introduced to a final exposure chamber where the aerosol concentration was maintained at 0.01 mg/m$^3$ under 12 air changes/h. The exposure chambers were kept at a negative pressure (–100 Pa), in order to prevent ITO aerosol from leaking into the outside air. Exhaust air from the exposure chamber was passed through a HEPA filter to remove ITO particles before release into the atmosphere. The concentrations of indium in the exhaust air were below a detection limit of 0.0001 mg/m$^3$ after the treatment with the HEPA filter.

*Chamber concentrations and size distribution of ITO aerosol*

The methods for the measurement of the exposure concentrations and size distributions of ITO aerosol in the exposure chamber were described in our previous study[12]. The exposure concentrations of ITO aerosol in the exposure chamber were derived from the observed levels of elemental indium (Table 1).

*Clinical observations and pathological examinations*

The animals were observed daily for their clinical signs and mortality. Body weight and food consumption were measured once a wk for the first 14 wk, and every 4 wk thereafter. All rats and mice received complete necropsy. For indium analysis, hematology and blood biochemistry, blood was collected from the abdominal aorta under etherization. Urinary parameters were measured in the last wk of the 2-year study period with Ames Reagent Strips (Siemens Healthcare Diagnostics, IL, USA). The organs and tissues designated in the OECD test guidelines[17] were examined macroscopically and microscopically. The tissues were fixed in 10% neutral buffered formalin, and embedded in paraffin. Tissue sections of 5 $\mu$m in thickness were prepared, and stained with hematoxylin and eosin (H & E). The sections of lung tissue were also stained with a periodic acid Schiff (PAS) reagent. Lesions of the lung and lymph nodes were evaluated for their severities, and scored on a scale of "slight" to "severe" with reference to the criteria by Shackelford *et al.*[19].

*Determination of indium concentrations in blood and tissues*

Indium concentration in whole-blood was quantified in the 26-week interim evaluation study using 10 rats/group/sex, and in the 104-week studies using 10 rats and 10 mice/group/sex. Blood of 10 mice in each group was pooled for indium analysis, because sufficient volume of blood could not be collected from individual mice for quantitative analysis of indium, and because the pooled blood contents of indium from 10 mice exposed to 1 mg/m$^3$ ITO for 13 wk were found to be close to the quantitative detection limit of 0.5 $\mu$g/*l*[13]. Contents of indium in various organs or tissues were quantified in the 26-week interim evaluation study. Cranial, caudal and accessory lobes of the right lung were used for indium analysis with 10 rats/group/sex. Tissue samples of 0.5 g or more were obtained from the liver, kidney, brain, muscle (femur), testis and epididymis of 10 rats/group/sex, and from the spleen and pancreas of 5 rats/group/sex. The bone marrow of femur and ovary of 10 rats/group/sex were pooled for analysis of indium. Individual or pooled tissues or 1.0 m*l* of individual or pooled whole-blood were added with ultra-pure nitric acid and digested with a microwave digestion apparatus (Microwave Digestion System, Model 7295, O·I·Analytical, TX, USA). The digested sample was added with ultra-pure water and injected into an inductively coupled plasma mass spectrometer (ICP-MS) (Type 7500i, Agilent Technologies, Ltd., CA, USA). Cesium was used as an internal standard for the indium measurement. The quantitative detection limit of indium was 0.006 $\mu$g/g for all tissues and 0.5 $\mu$g/*l* for whole-blood.

*Statistical analysis*

Incidences of neoplastic lesions were analyzed for a dose-response relationship indicated by a significant positive trend by Peto's test[20] and for a significant difference from the clean air-exposed group by Fisher's exact test. However, Peto's test was not applied to the 0.1 mg/m$^3$-exposed rat groups, because the exposure to ITO was discontinued at the 26th wk. The log-rank test[21] and Fisher's exact test were used to test a statistically significant difference in the survival rate between any ITO-exposed rat or mouse group of either sex and the clean air-exposed group. Incidences of pre- and non-neoplastic lesions were analyzed by chi-square test. Body weight, food consumption, and hematological, blood biochemical parameters and organ weight were analyzed by Dunnett's test. A two-tailed test was used for all statistics except for Peto's test. In all cases, a $p$ value of 0.05 was used as the

**Table 1.** Mass concentrations and mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSDs) of ITO aerosol in the exposure chamber

| Dose | Rats | | | | Mice | | | |
|---|---|---|---|---|---|---|---|---|
| | Mass concentration ($mg/m^3$) (Mean ± SD) | | Aerodynamic diameter Median ($\mu$m) | GSD | Mass concentration ($mg/m^3$) (Mean ± SD) | | Aerodynamic diameter Median ($\mu$m) | GSD |
| | ITO | In a) | | | ITO | In a) | | |
| 0.01 $mg/m^3$ | 0.010 ± 0.001 | 0.007 ± 0.001 | 1.8 | 2.2 | 0.010 ± 0.000 | 0.007 ± 0.000 | 1.8 | 2.1 |
| 0.03 $mg/m^3$ | 0.030 ± 0.001 | 0.022 ± 0.001 | 1.9 | 2.1 | 0.030 ± 0.001 | 0.022 ± 0.001 | 2.1 | 2.0 |
| 0.1 $mg/m^3$ | 0.100 ± 0.004 | 0.075 ± 0.003 | 2.4 | 2.1 | 0.100 ± 0.004 | 0.075 ± 0.003 | 2.4 | 2.0 |

a): Mass concentration as indium.

level of significance.

## Results

1) Exposure chamber concentrations and size distributions of ITO aerosol

Table 1 shows mass concentrations (mean ± SD) of ITO derived from the observed levels of elemental indium, and mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) of ITO aerosol in the exposure chamber. The exposure concentrations were controlled precisely within 10% of the variation coefficient and accurately within 10% deviation of the target concentration. MMADs of ITO aerosol tended to slightly increase with an increase in the exposure chamber concentrations, although their GSDs were maintained at about 2.

2) 26-week rat interim evaluation study

Table 2 shows relative lung weights and pulmonary lesions in the 0.1 $mg/m^3$-exposed rats of both sexes sacrificed at the end of the 26-week exposure period. The exposed rats of both sexes exhibited a 2-fold increase in lung weight as compared with the respective controls. Increases in absolute lung weights were evident as well (data not shown). Histopathological examination revealed that the deposition of particles in the lung, bronchus-associated lymphoid tissue (BALT), mediastinal lymph node (MLN) and nasal-associated lymphoid tissue (NALT), and fibrosis of the alveolar wall, alveolar proteinosis, infiltration of alveolar macrophages and inflammatory cells and hyperplasia of the alveolar epithelium in the lung occurred in the exposed rats. The particles were pale brown and transparent, looked like amber, and were located primarily within the alveolar macrophages in the lung and within the macrophages in the lymph tissue and nodes. Notably, severity of the alveolar proteinosis was scored as moderate to marked grade. A significantly increased incidence of granulomas was noted in the MLN of the exposed females as compared with the control. No tumor or pre-neoplastic lesion was observed in any organ or tissue in the present 26-week interim study.

In the ICP-MS analysis of indium in the 0.1 $mg/m^3$-exposed rats at the end of the 26-week exposure period, indium was detected in the lung, spleen, kidney, liver, bone marrow, ovary, pancreas, testis, epididymis and blood (Fig. 1), but the indium contents of the brain and muscle were below the quantitative detection limit of 0.006 $\mu$g/g tissue. Accumulation of indium in the lung was the greatest, followed by the spleen, kidney and liver. The lung concentrations of 20.2 ± 0. 8 and 21.1 ± 1.3 $\mu$g indium/g tissue (mean ± SD) in the exposed male and female rats were equivalent to whole-lung contents of 43.0 ± 3.0 and 29.8 ± 2.3 $\mu$g indium/whole-lung, respectively. The contents of indium in the extrapulmonary tissues were less than one percent of the lung contents. The blood contents of indium of male and female rats exposed to ITO at 0.1 $mg/m^3$ were less than 0.01 percent of the lung concentrations. The blood content of indium in the exposed females was 2-fold higher than that in the exposed males, although the lung content of indium in the exposed females was equal to that in the exposed males.

3) 104-week rat study

*Survival, body weight and clinical sign*

There was no significant decrease in the survival rate at any time point between any ITO-exposed group of either sex and the respective control (data not shown). The survival rates at the end of the 2-year study period were 78, 76, 82 and 80% for males and 82, 84, 82 and 86% for females exposed to 0, 0.01, 0.03 and 0.1 $mg/m^3$, respectively. Slight but significant retardation of body weight gain was observed in the ITO-exposed male groups during the first half of the 104-week exposure period (data not shown). There was no significant difference in body weight gain between any ITO-exposed female group and the respective control. Body weights of 0.01, 0.03 and 0.1 $mg/m^3$-exposed male groups at the end of the 104-week exposure period were 95, 92 and 97% of the control group, respectively. In the observation of clinical signs, irregular respiration was noted in the 0.03 $mg/m^3$-exposed group of both sexes (5 and 7 cases out of 50 males and 50 females,

**Table 2.** Relative lung weights and histopathological findings in the lung and lymph nodes of male and female rats exposed to ITO at 0.1 mg/m$^3$ or clean air for 26 wk and sacrificed at the end of the 26-week exposure period

| | | Male | | Female | |
|---|---|---|---|---|---|
| Group name (mg/m$^3$) | | Control | 0.1 | Control | 0.1 |
| No. of animals examined | | 10 | 10 | 10 | 10 |
| Relative lung weight (%) | mean | 0.288 | 0.586 ## | 0.400 | 0.764 ## |
| | SD | 0.013 | 0.023 | 0.022 | 0.043 |
| Deposition of particles | | | | | |
| Lung | | 0 | 10 | 0 | 10 |
| BALT | | 0 | 10 | 0 | 10 |
| MLN | | 0 | 7 | 0 | 10 |
| NALT | | 0 | 2 | 0 | 0 |
| Histopathological findings | | | | | |
| Lung | | | | | |
| Fibrosis of alveolar wall | | 0 | 6 * | 0 | 9 ** |
| | | | <1.0> | | <1.0> |
| Alveolar proteinosis | | 0 | 10 ** | 0 | 10 ** |
| | | | <2.6> | | <2.5> |
| Infiltration of alveolar macrophages | | 0 | 10 ** | 0 | 10 ** |
| | | | <1.0> | | <1.0> |
| Infiltration of inflammatory cells | | 0 | 10 ** | 0 | 9 ** |
| | | | <1.0> | | <1.0> |
| Hyperplasia of alveolar epithelium | | 0 | 10 ** | 0 | 10 ** |
| | | | <1.0> | | <1.0> |
| Lymph nodes | | | | | |
| Granuloma of MLN | | 0 | 1 | 0 | 10 ** |
| | | | <1.0> | | <1.0> |

Values indicate number of animals bearing lesions. The values in angle brackets indicate the average of severity grade index of the lesion. The average of severity grade is calculated with a following equation. Σ (grade × number of animals with grade) / number of affected animals. Grade: 1, slight; 2, moderate; 3, marked; 4, severe. Significant difference: ##, $p \leq 0.01$ by Dunnett's test; *, $p \leq 0.05$; **, $p \leq 0.01$ by Chi-square test. BALT: Bronchus-associated lymphoid tissue. MLN: Mediastinal lymph nodes. NALT: Nasal-associated lymphoid tissue.

respectively).

*Hematological and blood biochemical changes*

No significant change in hematological or blood biochemical parameters was observed in any ITO-exposed group, except for increases in white blood cell counts of the 0.03 and 0.1 mg/m$^3$-exposed female rats, and except for increases in hemoglobin and hematocrit values and a decrease in serum albumin of the 0.03 mg/m$^3$-exposed female group compared with the respective controls (data not shown).

*Lung weight and pathology*

Relative lung weights of all ITO-exposed rat groups of both sexes were significantly increased compared with the respective controls (Table 3). Increases in absolute lung weights were evident as well (data not shown).

Bronchiolo-alveolar carcinomas in male and female rats and bronchiolo-alveolar adenomas in male rats occurred in an exposure concentration-dependent manner, as indicated by the significantly positive trend by Peto's test (Table 3). The incidences of bronchiolo-alveolar carcinomas in the 0.03 and 0.1 mg/m$^3$-exposed male and female rats and bronchiolo-alveolar adenomas in the 0.03 and 0.1 mg/m$^3$-exposed male rats and in the 0.1 mg/m$^3$-exposed female rats were significantly increased as compared with those of respective control groups by Fisher's exact test. One adenosquamous carcinoma was found in the 0.01 mg/m$^3$-exposed rats of both sexes, and one squamous cell carcinoma was found in a female rat in each of the 0.01 and 0.1 mg/m$^3$-exposed groups. Both the adenosquamous carcinoma, composed of both glandular and squamous components, and the squamous cell carcinoma, with only a squamous component, are rarely occurring malignant tumors (adenosquamous carcinoma: null case/2,399 males and 1 case/2,197

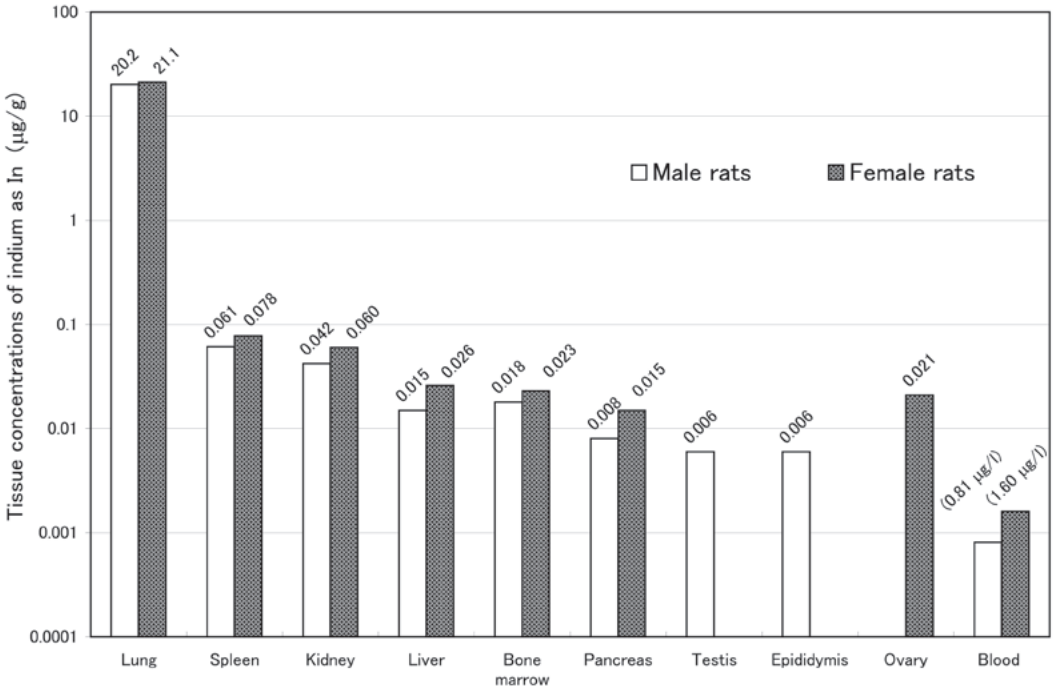



**Fig. 1.** Average concentrations of indium in various tissues ($\mu$g/g tissue) and blood ($\mu$g/*l* whole-blood) of male and female rats exposed to ITO at 0.1 mg/m$^3$ for 26 wk and sacrificed at the end of the 26-week exposure period. The average concentrations of indium are given at the top of vertical bars. Brain and muscle concentrations of indium were below the quantitative detection limit of 0.006 $\mu$g/g tissue.

females, squamous cell carcinoma; null case/2,197 females in the JBRC historical control data); thus, we regarded them as being ITO-related. Notably, the combined incidence of malignant lung tumors including the bronchiolo-alveolar carcinoma and adenosquamous carcinoma in the 0.01 mg/m$^3$-exposed male rats was significantly higher than that of the control group by Fisher's exact test. The combined incidences of total lung tumors exhibited a significantly positive trend by Peto's test, and were significantly increased in all the exposed rats of both sexes. The first lung tumor was observed in a female rat which died in the 79th wk, but the majority of lung tumors were found in the ITO-exposed rats surviving to the end of the 2-year study period. It is noteworthy that these ITO-induced lung tumors were often accompanied by proliferative, fibrous connective tissue (Fig. 2A), which is uncommonly seen in control F344 rats.

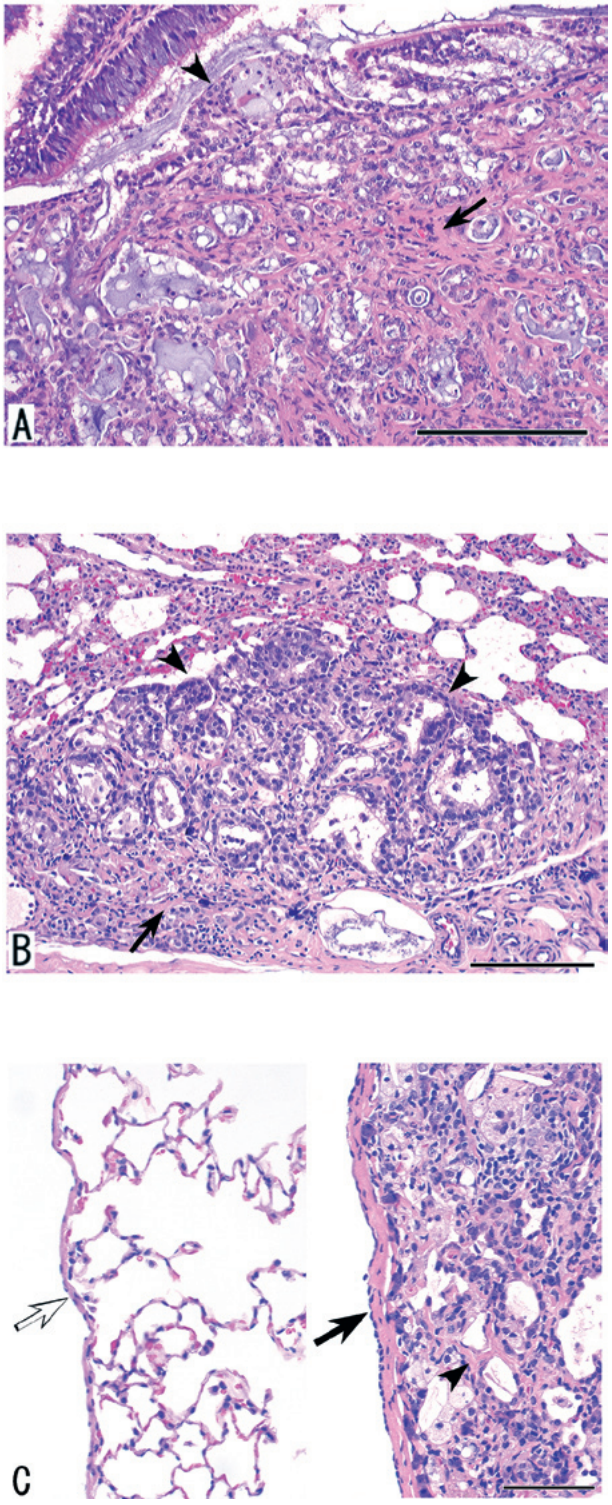
As for pre-neoplastic lung lesions, incidences of bronchiolo-alveolar hyperplasia were significantly increased in the 0.03 and 0.1 mg/m$^3$-exposed male rats and in all the exposed females as compared with the respective controls. Most of the bronchiolo-alveolar hyperplasias were adjacent to the alveolar wall fibrosis (Fig. 2B). Atypical hyperplasia and squamous cell metaplasia occurred in the lung of the exposed rats of both sexes, but the incidences of these two pre-neoplastic lesions were not significantly increased. The squamous cell metaplasia was presumed to be a pre-neoplastic lesion of squamous cell carcinoma.

As for non-neoplastic lung lesions, significant and high incidences of fibrosis of alveolar wall and thickening of the pleural wall were noted in all the exposed rat groups of both sexes. The incidences of the alveolar wall fibrosis in the 0.1 mg/m$^3$-exposed rats sacrificed at the end of the 2-year study were higher than those in the 0.1 mg/m$^3$-exposed rats sacrificed at the end of the 26-week exposure. The fibrosis of alveolar wall was composed of the proliferative and fibrous connective tissue and was adjacent to the lung tumors and pre-neoplastic hyperplasia. Thickening of pleural wall, composed of the collagenous and fibrous connective tissue, was located adjacent to the alveolar wall fibrosis (Fig. 2C). Incidences of alveolar proteinosis, infiltrations of alveolar macrophages and inflammatory cells and hyperplasia of the alveolar epithelium were significantly increased in all the groups of both sexes exposed to ITO at 0.01 mg/m$^3$ and above. It is noteworthy that the severities of alveolar proteinosis in the 0.1 mg/m$^3$-exposed groups of both sexes were milder than those in the 0.01 and 0.03 mg/m$^3$-exposed groups or in the 0.1 mg/m$^3$-exposed groups sacrificed at the end of the 26-week exposure period, indicating that alveolar

**Table 3.** Relative lung weights and histopathological findings in the lung and lymph nodes of male and female rats exposed to ITO at 0.01 or 0.03 mg/m$^3$ or clean air for 104 wk and at 0.1 mg/m$^3$ for 26 wk and then to clean air for the remainder of the 2-year study

| | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group name (mg/m$^3$) | | Control | 0.01 | 0.03 | 0.1 | Control | 0.01 | 0.03 | 0.1 |
| No. of animals examined | | 49 | 50 | 50 | 50 | 50 | 49 | 50 | 49 |
| Relative lung weight (%) | mean | 0.374 | 0.628 ## | 0.773 ## | 0.521 ## | 0.390 | 0.580 ## | 0.814 ## | 0.530 ## |
| | SD | 0.048 | 0.289 | 0.121 | 0.066 | 0.085 | 0.066 | 0.272 | 0.124 |
| Deposition of particles | | | | | | | | | |
| Lung | | 0 | 50 | 50 | 50 | 0 | 49 | 50 | 49 |
| BALT | | 0 | 43 | 48 | 45 | 0 | 41 | 44 | 44 |
| MLN | | 0 | 37 | 38 | 38 | 0 | 32 | 38 | 41 |
| NALT | | 0 | 1 | 5 | 2 | 0 | 0 | 2 | 1 |
| Neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Bronchiolo-alveolar adenoma | | 3 | 5 | 10 F | 12 F ↑ | 1 | 5 | 6 | 7 F |
| Bronchiolo-alveolar carcinoma | | 0 | 4 | 5 F | 5 F ↑ | 0 | 1 | 9 FF | 5 F ↑↑ |
| Adenosquamous carcinoma | | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Squamous cell carcinoma | | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Combined malignant lung tumors [1] | | 0 | 5 F | 5 F | 5 F ↑ | 0 | 3 | 9 FF | 6 F ↑↑ |
| Combined all lung tumors [2] | | 3 | 10 F | 15 FF | 16 FF ↑↑ | 1 | 8 F | 14 FF | 13 FF ↑↑ |
| Pre-neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Bronchiolo-alveolar hyperplasia | | 2 | 6 | 24 ** | 21 ** | 1 | 12 ** | 22 ** | 10 ** |
| | | <1.0> | <1.0> | <1.1> | <1.1> | <1.0> | <1.0> | <1.0> | <1.0> |
| Atypical hyperplasia | | 0 | 1 | 3 | 2 | 0 | 0 | 2 | 1 |
| | | | <1.0> | <1.0> | <1.0> | | | <1.0> | <1.0> |
| Squamous cell metaplasia | | 0 | 1 | 2 | 1 | 0 | 2 | 0 | 0 |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | | |
| Non-neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Fibrosis of alveolar wall | | 0 | 47 ** | 50 ** | 49 ** | 0 | 48 ** | 50 ** | 49 ** |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | <1.0> | <1.0> |
| Thickening of pleural wall | | 0 | 50 ** | 50 ** | 49 ** | 0 | 48 ** | 50 ** | 49 ** |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | <1.0> | <1.0> |
| Alveolar proteinosis | | 0 | 50 ** | 50 ** | 50 ** | 0 | 49 ** | 50 ** | 49 ** |
| | | | <1.6> | <2.1> | <1.0> | | <1.9> | <2.3> | <1.0> |
| Infiltration of alveolar macrophages | | 0 | 50 ** | 50 ** | 50 ** | 0 | 48 ** | 50 ** | 49 ** |
| | | | <1.0> | <1.2> | <1.0> | | <1.0> | <1.2> | <1.0> |
| Infiltration of inflammatory cells | | 0 | 34 ** | 36 ** | 20 ** | 0 | 33 ** | 36 ** | 12 ** |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | <1.0> | <1.0> |
| Hyperplasia of alveolar epithelium | | 0 | 48 ** | 49 ** | 48 ** | 0 | 48 ** | 50 ** | 48 ** |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | <1.0> | <1.0> |
| Granuloma of BALT | | 0 | 11 ** | 12 ** | 15 ** | 0 | 6 * | 9 ** | 21 ** |
| | | | <1.0> | <1.0> | <1.0> | | <1.0> | <1.0> | <1.0> |
| Lymph nodes | | | | | | | | | |
| Granuloma of MLN | | 6 | 33 ** | 34 ** | 34 ** | 12 | 36 ** | 44 ** | 47 ** |
| | | <1.0> | <1.0> | <1.0> | <1.1> | <1.0> | <1.0> | <1.1> | <1.1> |

Values indicate number of animals bearing lesions. 1) Number of animals bearing malignant lung tumors including bronchiolo-alveolar carcinomas, adenosquamous carcinomas and squamous cell carcinomas (combined). 2) Number of animals bearing all lung tumors including bronchiolo-alveolar adenomas and malignant lung tumors (combined). The values in angle brackets indicate the average of severity grade index of the lesion. The average of severity grade is calculated with a following equation. Σ (grade × number of animals with grade) / number of affected animals. Grade: 1, slight; 2, moderate; 3, marked; 4, severe. Significant difference: ##, $p \leq 0.01$ by Dunnett's test; F, $p \leq 0.05$; FF, $p \leq 0.01$ by Fisher's exact test; ↑, $p \leq 0.05$; ↑↑, $p \leq 0.01$ by Peto's test; *, $p < 0.05$; **, $p < 0.01$ by Chi-square test. BALT: Bronchus-associated lymphoid tissue. MLN: Mediastinal lymph nodes. NALT: Nasal-associated lymphoid tissue.




proteinosis tended to recover after cessation of the repeated exposure to ITO. The incidences of inflammatory cell infiltration in the 0.1 mg/m$^3$-exposed rats of both sexes were lower than those observed at the end of the 26-week exposure period, and were lower than those in the 0.01 or 0.03 mg/m$^3$-exposed groups. Increased incidences of granulomas composed of particle-laden macrophages were noted in both BALT and MLN of all exposed groups of both sexes. ITO particles were found in the lungs and in the BALT, MLN and NALT of the exposed rats of both sexes. The particles were located primarily within the alveolar macrophages in the lung and within the macrophages in the lymph tissue and nodes, and were deposited separately as single particles. The extent of deposition was less in the lymph tissue and nodes than in the lung.

No exposure-related histopathological changes were observed in any other organ or tissue of the ITO-exposed rats of either sex.

4)104-week mouse study

*Survival, body weight and mortality and clinical sign*

There was no significant difference in the survival rate at any time point between any ITO-exposed group of either sex and the respective control (data not shown). The survival rates at the end of the 2-year study period were 62, 66, 56 and 60% for males and 76, 64, 68 and 68% for females exposed to 0, 0.01, 0.03 and 0.1 mg/m$^3$, respectively. Body weights were slightly but significantly decreased in the 0.1 mg/m$^3$-exposed group of both sexes during the latter half of the 2-year exposure period as compared with the controls (data not shown). The body weights of 0.01, 0.03 and 0.1 mg/m$^3$-exposed groups at the end of 104-week exposure period were 98, 99 and 93% of the control group for males and 102, 102 and 97% of the control group for females. No ITO exposure-related clinical signs were

**Fig. 2.** A) A microphotograph showing bronchiolo-alveolar carcinoma in a female rat exposed to ITO at 0.03 mg/m$^3$. Note that the tumor is accompanied by the proliferative, fibrous connective tissue (arrow) and invading the bronchus (arrowhead). H & E stain. Bar indicates 200 $\mu$m. B) A microphotograph showing a pre-neoplastic hyperplasia in a male rat exposed to ITO at 0.1 mg/m$^3$. Note that the bronchiolo-alveolar hyperplasia (arrowheads) was adjacent to the alveolar wall fibrosis (arrow). H & E stain. Bar indicates 200 $\mu$m. C) Microphotographs showing the normal lung (left side) of a female rat exposed to clean air for 104 wk and the affected lung (right side) of another female rat exposed to ITO at 0.1 mg/m$^3$. Note that the pleural wall is thickened (filled arrow) as compared with the thickness of the normal pleural wall (open arrow) and that the alveolar wall fibrosis (arrowhead) is located beneath the thickened pleural wall. H & E stain. Bar indicates 200 $\mu$m.

**Table 4.** Relative lung weights and histopathological findings in the lung and lymph nodes of male and female mice exposed to ITO at 0.01, 0.03 or 0.1 mg/m$^3$ or clean air for 104 wk

| | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group name (mg/m$^3$) | | Control | 0.01 | 0.03 | 0.1 | Control | 0.01 | 0.03 | 0.1 |
| No. of animals examined | | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 47 |
| Relative lung weight (%) | mean | 0.634 | 0.654 | 0.862 ## | 1.004 ## | 0.867 | 0.758 | 1.046 ## | 1.271 ## |
| | SD | 0.303 | 0.120 | 0.143 | 0.149 | 0.996 | 0.099 | 0.274 | 0.310 |
| Deposition of particles | | | | | | | | | |
| Lung | | 0 | 50 | 50 | 50 | 0 | 47 | 50 | 47 |
| BALT | | 0 | 9 | 29 | 41 | 0 | 11 | 40 | 45 |
| MLN | | 0 | 9 | 27 | 33 | 0 | 12 | 25 | 29 |
| Neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Bronchiolo-alveolar adenoma | | 5 | 4 | 5 | 5 | 1 | 0 | 2 | 4 ↑ |
| Bronchiolo-alveolar carcinoma | | 7 | 1 | 4 | 5 | 2 | 0 | 1 | 3 |
| Combined all lung tumors 1) | | 12 | 5 | 9 | 10 | 3 | 0 | 3 | 7 ↑↑ |
| Pre-neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Bronchiolo-alveolar hyperplasia | | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 1 |
| | | <1.0> | | <1.0> | <1.0> | <1.0> | | | <1.0> |
| Non-neoplastic lesions | | | | | | | | | |
| Lung | | | | | | | | | |
| Thickening of pleural wall | | 0 | 0 | 18** | 23** | 0 | 0 | 17** | 32** |
| | | | | <1.0> | <1.0> | | | <1.1> | <1.0> |
| Alveolar proteinosis | | 0 | 26** | 50** | 49** | 0 | 18** | 40** | 44** |
| | | | <1.0> | <1.0> | <1.1> | | <1.0> | <1.0> | <1.0> |
| Infiltration of alveolar macrophages | | 0 | 8* | 30** | 48** | 2 | 11** | 37** | 43** |
| | | | <1.1> | <1.0> | <1.0> | <1.5> | <1.0> | <1.0> | <1.0> |
| Infiltration of inflammatory cells | | 0 | 0 | 8** | 15** | 0 | 0 | 12** | 14** |
| | | | | <1.0> | <1.1> | | | <1.2> | <1.0> |
| Hyperplasia of BALT | | 2 | 0 | 7 | 16** | 11 | 7 | 20 | 24** |
| | | <1.0> | | <1.0> | <1.0> | <1.0> | <1.0> | <1.1> | <1.2> |
| Lymph nodes | | | | | | | | | |
| Hyperplasia of MLN | | 2 | 2 | 7 | 10* | 2 | 1 | 11* | 16** |
| | | <1.0> | <1.0> | <1.0> | <1.0> | <1.0> | <1.0> | <1.0> | <1.0> |

Values indicate number of animals bearing lesions. 1) Number of animals bearing all lung tumors including bronchiolo-alveolar adenomas and carcinomas (combined). The values in angle brackets indicate the average of severity grade index of the lesion. The average of severity grade is calculated with a following equation. Σ (grade × number of animals with grade) / number of affected animals. Grade: 1, slight; 2, moderate; 3, marked; 4, severe. Significant difference: ##, $p \leq 0.01$ by Dunnett's test; ↑, $p \leq 0.05$; ↑↑, $p \leq 0.01$ by Peto's test; *, $p \leq 0.05$; **, $p \leq 0.01$ by Chi-square test. BALT: Bronchus-associated lymphoid tissue. MLN: Mediastinal lymph nodes.

observed in any exposed group of either sex.

*Hematological and blood biochemical changes*

No remarkable change was observed in the hematological parameters of any ITO-exposed group of either sex. In the blood biochemical examination, potassium was decreased in all the exposed groups of both sexes as compared with the control. An increase in AST and LDH and a decrease in the A/G ratio of the 0.1 mg/m$^3$-exposed male group and an increase in LDH of the 0.1 mg/m$^3$-exposed female group and a decrease in A/G ratio of the 0.03 and 0.1 mg/m$^3$-exposed female groups were found in comparison with the respective controls (data not shown).

*Lung weight and pathology*

Relative lung weights of the 0.03 and 0.1 mg/m$^3$-exposed mice of both sexes were significantly increased in a dose-dependent manner as compared with the respective control groups (Table 4). Increases in absolute

lung weights were evident as well (data not shown).

Histopathological findings in the lung and lymph nodes of ITO-exposed mice are shown in Table 4. Although incidences of bronchiolo-alveolar adenomas and combined incidences of the bronchiolo-alveolar adenomas and carcinomas in the exposed females showed a significantly positive trend by Peto's test, those tumor incidences did not attain any statistically significant level in any ITO-exposed mouse group as compared with those of the control group by Fisher's exact test. No increase in the incidence of pre-neoplastic lesions was noted in any organ.

Regarding the non-neoplastic lesions, the incidences of thickening of the pleural wall were significantly increased in the 0.03 and 0.1 mg/m$^3$-exposed mice of both sexes as compared with the respective controls, but these incidences were lower than those in the ITO-exposed rats which appeared at the lowest exposure concentration. Significant and dose-dependent increases in alveolar proteinosis and alveolar macrophage infiltration were evident in all the exposed mice of both sexes as compared with the respective controls. The incidences of inflammatory cell infiltration were significantly increased in the 0.03 and 0.1 mg/m$^3$-exposed mice of both sexes as compared with the controls. In the lymph nodes, increased incidences of the hyperplasias were noted in the BALT of the 0.1 mg/m$^3$-exposed mice and in the MLN of 0.03 and 0.1 mg/m$^3$-exposed mice as compared with the respective controls. ITO particles were found in the lungs and in the BALT and MLN of the exposed mice of both sexes. Those particles were located primarily within the alveolar macrophages in the lung and within the macrophages in the lymph tissue and nodes, and were deposited separately as single particles. The extent of deposition was less in the lymph tissue and nodes than in the lung.

No exposure-related histopathological changes were observed in any other organ or tissue in the ITO-exposed mice of either sex.

5) Blood contents of indium in rats and mice of 104-week studies

In the exposed rats, blood contents of indium above the quantitative detection limit were found in 7, 10 and 1 males and in 6, 10 and 7 females of the 0.01, 0.03 and 0.1 mg/m$^3$-exposed groups, respectively. The mean contents (minimum–maximum) in the 0.01, 0.03 and 0.1 mg/m$^3$-exposed groups were 0.72 (0.60–0.80), 1.96 (1.12–3.04) and 0.68 $\mu$g/*l* for males and 0.97 (0.80–1.32), 2.10 (0.92–2.80) and 0.67 (0.60–0.76) $\mu$g/*l* for females, respectively. Inhalation exposure of rats to ITO at 0.01 and 0.03 mg/m$^3$ for 104 wk increased the blood contents of indium in an exposure concentration-related manner. In the exposed mice, the pooled blood contents of indium were 0.64 and 1.96 $\mu$g/*l* in the 0.1 mg/m$^3$-exposed males and females, respectively, and 0.72 $\mu$g/*l* in the 0.03 mg/m$^3$-exposed females. On the other hand, the pooled blood contents of indium in the 0.01 mg/m$^3$-exposed mice of both sexes and in the 0.03 mg/m$^3$-exposed male mice were below the detection limit. These blood data indicate that the blood content of indium was higher in female mice than in males.

## Discussion

In the present studies, repeated inhalation exposure to ITO aerosol was found to significantly increase incidences of bronchiolo-alveolar carcinomas, brochiolo-alveolar adenomas, combined incidences of malignant lung tumors and combined incidences of total lung tumors in male and female rats in an exposure concentration-dependent manner. Rarely occurring malignant lung tumors, including adenosquamous carcinoma and squamous cell carcinoma, were also found to occur only in the ITO-exposed rats. It is noteworthy that these combined incidences of malignant lung tumors in male rats and total lung tumors in rats of both sexes were significantly increased at the lowest exposure concentration of 0.01 mg/m$^3$. In contrast, neither malignant or benign lung tumors nor pre-neoplastic lesions were induced in any mice of either sex exposed to ITO at the same range of the exposure concentrations for 104 wk. Therefore, it can be concluded that there is clear evidence of carcinogenic activity of ITO in male and female rats exposed by inhalation to ITO aerosol for 2 yr, while no clear evidence of ITO carcinogenicity in male or female mice could be obtained under the present experimental conditions.

The present findings of ITO-induced lung tumors in rats are consistent with the findings of NTP's rat study on indium phosphide[14] that 2-year inhalation exposure to indium phosphide aerosol induces malignant and benign neoplasms of the lung in the rats of both sexes. Gottschling *et al*.[15] reported that the 2-year inhalation exposure of rats to indium phosphide particles induced pulmonary inflammation associated with oxidative stress by infiltrating alveolar macrophages, resulting in progression to pre-neoplastic lesions and lung tumors. Lison *et al*.[9] attributed the reactivity/toxicity of sintered ITO particles to carbon centered radical formation and Fenton-like activity, suggesting the preponderance of secondary genotoxic mechanisms due to reactive oxygen species in the lung carcinogenesis of ITO. Since infiltration of alveolar macrophages occurred persistently in the lungs of rats exposed to ITO aerosol for 13 wk in our previous study[12] and for 2 yr in the present study, persistent pulmonary inflammation associated with oxidative stress by infiltrating alveolar macrophages is considered to play a critically important role in the lung carcinogenesis of inhaled ITO.

Non-neoplastic lesions found in the present 2-year studies were characterized by fibrosis of alveolar wall in rats but not in mice and thickening of the pleural wall, alveolar proteinosis and infiltrations of alveolar

macrophages and inflammatory cells in both rats and mice. The alveolar proteinosis and alveolar macrophage infiltration occurred significantly in the rats and mice of both sexes exposed to ITO at 0.01 mg/m$^3$. It is noteworthy that the pre-neoplastic lesions including the bronchiolo-alveolar hyperplasia and atypical hyperplasia occurred adjacent to the alveolar wall fibrosis and were accompanied by infiltrating alveolar macrophages, and that the ITO-induced lung tumors were accompanied by the proliferation of fibrous connective tissue. Consistent with our histological observation, the NTP's 2-year study pointed out that the fibroproliferative lesions were adjacent to areas of inflammation and appeared to be part of a morphologic continuum that might progress to neoplasia[14]. Thus, it can be inferred that the alveolar wall fibrosis is morphologically linked to the pre-neoplastic and neoplastic lung lesions induced by the exposure to ITO aerosol, and that pulmonary fibrosis might play an important part in ITO-induced lung carcinogenicity.

ITO particles were microscopically found in the lungs of rats and mice exposed to ITO aerosol at the lowest exposure concentration of 0.01 mg/m$^3$, and were deposited separately as single particles. The ICP-MS analysis revealed that the mean lung contents of indium in the male and female rats exposed to ITO at 0.1 mg/m$^3$ were 20 and 21 $\mu$g/g tissue, respectively, at the end of the 26-week exposure period. Cummings *et al.*[4] reported that the lung concentration of indium was 29.3 $\mu$g/g in an ITO-exposed worker who was diagnosed as alveolar proteinosis. This finding corresponds well to our present animal data that alveolar proteinosis was induced by the 26-week exposure of rats to ITO at 0.1 mg/m$^3$, resulting in lung deposition of indium as 20 $\mu$g/g. It is also interesting to note that the alveolar proteinosis tended to recover in the rats which were allowed to continue unexposed for 78 wk after the repeated exposure to 0.1 mg/m$^3$ ITO had been discontinued in the 26th wk. In contrast, Nogami *et al.*[7] reported that no interstitial change was observed in the lung of an ITO plant worker who was diagnosed as bronchioloalveolar carcinoma, while his lung content of indium was 31.2 $\mu$g/g tissue. These conflicting observations on the occurrence of alveolar proteinosis in indium-exposed workers between the epidemiological studies of indium-exposed workers in Japan[3, 5-8] and in the USA[4] remain to be resolved.

In the present ICP-MS analysis, indium was detected in the extrapulmonary tissues including the spleen, kidney, liver, bone marrow, ovary, pancreas, testis, epididymis and blood in the 0.1 mg/m$^3$-exposed rats at the end of the 26-week exposure period. There seems to be three plausible explanations for the deposition of indium in the extrapulmonary tissues. First, hardly soluble ITO particles might be dissolved in the acidic pH environment of the phagolysosomes of the ITO particle-laden alveolar macrophages, as suggested by Brain *et al.*[22] , and then move through the lung capillary vessels to general circulation. Second, orally ingested ITO particles might also be dissolved by the acidic gastric juice in the stomach[23] and then be absorbed in the bloodstream, since the ITO-exposed animals lick ITO particles off the fur, and since the ITO particles depositing on the upper and lower respiratory tracts are conveyed by the muco-ciliary escalator back into the esophagus. Third, inhaled ITO particles might migrate through the MLN into the reticuloendothelial system, since the pulmonary lymphatic pathway is known to drain through the MLN into the blood stream at the subclavian vein[24, 25], and since inhaled particles have been reported to migrate and deposit in the macrophages of the spleen and the Kupffer cells of the liver[26]. Kabe *et al.*[23] reported that intraperitoneally administered indium phosphide particles were translocated to the spleen and liver by way of lymphokinetics, and caused reticuloendothelial responses in mice. However, we could not detect ITO particles in the spleen or liver, even though relatively high concentrations of indium in the reticuloendothelial system, such as the spleen and liver, were found in the ITO-exposed rats as compared with the indium concentrations in the other extrapulmonary tissues. The pathogenic behavior and effect of ITO particles migrating from the deep lung through the MLN to the reticuloendothelial system remain to be clarified.

The three different aerosol concentrations of 0.01, 0.03 and 0.1 mg/m$^3$ were selected for the present 2-year studies on the basis of the toxicity found in our previous 13-week rat and mouse studies[12, 13]. The current OEL value of 0.1 mg/m$^3$ for indium and its compounds which has been recommended by both the American Conference of Governmental Industrial Hygienists (ACGIH)[27] and the National Institute for Occupational Safety and Health (NIOSH)[28] and the technical feasibility for preparation of ITO aerosol at the lowest exposure concentration of 0.01 mg/m$^3$ were also considered for the selection. According to the criteria of maximum tolerated dose (MTD) set up by the guidelines of the National Cancer Institute (NCI)[29] and the International Agency for Research on Cancer (IARC)[30], the highest dose of the test agent given during the carcinogenicity study should not exceed the MTD that can be predicted not to alter the animals' normal longevity from toxic effects other than carcinogenicity, or no more than a 10% weight decrement, as compared to the concurrent control groups. The survival rates and body weight changes throughout the 2-year exposure period in the present study were found to fulfill these MTD criteria. Cessation of inhalation exposure of rats to ITO at 0.1 mg/m$^3$ at the 26th wk followed by exposure to clean air for the remainder of the 2-year study was based on development of the fibrosis and alveolar epithelial hyperplasia at the end of the 26-week post-exposure period after the 13-week exposure of rats to ITO at 0.1 mg/m$^3$ found in our previous 13-week rat study[12]. Since the present 26-week rat interim evaluation study showed

essential similarities in the incidences and severities of the fibrosis and proliferative lesions to those found at the end of the 26-week post-exposure period[12], the effects on the lung of rats exposed to 0.1 mg/m$^3$ for 26 wk were considered sufficiently severe that the exposure to ITO was discontinued at the 26th wk, and this group was allowed to continue unexposed in the chamber for the remainder of the 2-year study. In the present 2-year studies, the 0.1 mg/m$^3$ ITO-exposed rats of both sexes exhibited the ITO-induced carcinogenicity and chronic toxicity without any significant decrease in survival rates or body weight gain at the end of the 2-year study. Therefore, the exposure to ITO at the concentration of 0.1 mg/m$^3$ for 26-week followed by clean air exposure is considered to fulfill the MTD criteria.

The IARC evaluated carcinogenicity of indium phosphide as being probably carcinogenic to humans (Group 2A), upgrading it from 2B to 2A even in the absence of data on cancer in humans[31]. This evaluation was based on the NTP's 2-year carcinogenicity study[14]. The ACGIH recommended an OEL as a TLV-TWA value of 0.1 mg/m$^3$ and the same value was assigned as a Recommended Exposure Limit (REL) by NIOSH. The Japan Society for Occupational Health (JSOH) recommended a serum indium concentration of 3 $\mu g/l$ for BEI[32], based primarily on the findings of the epidemiological studies[5, 6]. Notably, 2-year inhalation exposure to ITO aerosol at 0.01 mg/m$^3$ was found to significantly increase the combined incidences of malignant lung tumors in male rats and total lung tumors in male and female rats in the present studies. These findings indicate that the 2-year exposure to ITO at one tenth of the ACGIH's TLV-TWA induces malignant lung tumors in rats. Moreover, alveolar wall fibrosis and thickening of the pleural wall in rats of both sexes as well as the alveolar proteinosis and alveolar macrophage infiltration in rats and mice of both sexes were induced at an ITO aerosol concentration of 0.01 mg/m$^3$. The lung tumors, fibrosis, thickened pleural wall, alveolar proteinosis and macrophage infiltration were induced in the rats which were exposed for 26 wk to ITO at 0.1 mg/m$^3$, the same level as ACGIH's TLV. In addition, our present blood indium analysis revealed that mean blood levels of indium in the male and female rats exposed to ITO at 0.01 mg/m$^3$ were 0.72 and 0.97 $\mu g/l$, respectively, indicating that these blood levels of indium are below the JSOH's BEI of 3 $\mu g/l$ for indium. Indeed, it has been reported that serum levels of indium are approximately equal to indium levels in whole-blood[14, 33]. Therefore, the present findings of the 2-year rodent studies of the carcinogenicity and chronic toxicity of ITO provide novel information about the lung carcinogenicity and chronic toxicity in animal models which can be used for re-consideration of the current OEL and BEI for indium and its compounds.

## Conclusions

Repeated inhalation exposure of rats to ITO aerosol at concentrations of 0.01 and 0.03 mg/m$^3$ for 104 wk and at 0.1 mg/m$^3$ for the first 26 wk followed by the exposure to clean air for the remaining 78 wk significantly increased the incidences of benign and malignant lung tumors and a pre-neoplastic lesion of bronchiolo-alveolar hyperplasia, in addition to the fibrosis of alveolar wall and thickening of pleural wall. Therefore, it can be concluded that there is clear evidence of the carcinogenic activity of ITO in male and female rats exposed by inhalation to ITO aerosol for 2 yr. The combined incidences of malignant lung tumors in male rats and total lung tumors in the rats of both sexes were significantly increased at 0.01 mg/m$^3$. On the other hand, the 2-year exposure of mice to ITO at the same concentrations did not induce any carcinogenic response but thickening of the pleural wall. Other principal non-neoplastic lesions observed were alveolar proteinosis, and infiltrations of alveolar macrophages and other inflammatory cells in rats and mice. Mice were less susceptible to inhaled ITO than rats. The ITO-induced lung tumors, fibrosis and alveolar proteinosis appeared at the exposure concentrations below the ACGIH's TLV and at the whole-blood levels of indium below the JSOH's BEI for indium and its compounds.

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