Table 1. Relative lung weights and histopathological findings in the lung and lymph nodes of male and female mice exposed to ITO or IO at 4 different concentrations or clean air for 2 wk

		ITO					IO				
Group name (mg/m^3)		Control	0.1	1	10	100	Control	0.1	1	10	100
No. of animals examined		5	5	5	5	5	5	5	5	5	5 a)
<Male>											
Relatve lung weigtht (%)	mean	0.69	0.65	0.79	0.98##	1.10##	0.60	0.63	0.70	0.91##	1.00##
	SD	0.05	0.04	0.06	0.11	0.08	0.07	0.04	0.03	0.14	0.10
Deposition of particles											
Lung		0	0	5	5	5	0	0	5	5	5
MLN		0	0	0	1	5	0	0	0	4	5
NALT		0	0	0	0	0	0	0	0	1	3
Histopathological findings											
Lung											
Alveolar proteinosis		0	0	0	5 <1.0>	5 <1.8>	0	0	0	5 <1.0>	5 <1.0>
Infiltration of alveolar macrophages		0	0	0	1 <1.0>	2 <1.0>	0	0	0	0	1 <1.0>
Infiltration of inflammatory cells		0	0	0	1 <1.0>	5 <1.0>	0	0	0	1 <1.0>	5 <1.0>
Hyperplasia of alveolar epithelium		0	0	0	1 <1.0>	5 <1.0>	0	0	0	0	3 <1.0>
<Female>											
Relative lung weight (%)	mean	0.74	0.76	0.94##	1.06##	1.34##	0.69	0.71	0.76	0.93##	1.12##
	SD	0.12	0.03	0.06	0.15	0.07	0.06	0.04	0.12	0.14	0.08
Deposition of particles											
Lung		0	0	4	5	5	0	0	5	5	4
MLN		0	0	0	3	5	0	0	0	1	4
NALT		0	0	0	0	0	0	0	0	0	1
Histopathological findings											
Lung											
Alveolar proteinosis		0	0	0	5 <1.0>	5 <2.0>	0	0	0	5 <1.0>	4 <1.0>
Infiltration of alveolar macrophages		0	0	0	0	3 <1.0>	0	0	0	0	0
Infiltration of inflammatory cells		0	0	0	0	4 <1.0>	0	0	0	0	3 <1.0>
Hyperplasia of alveolar epithelium		0	0	0	0	4 <1.0>	0	0	0	0	2 <1.0>

Values indicate number of animals bearing lesions. The values in angle bracket 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. MLN: Mediastinal lymph nodes. NALT: Nasal-associated lymphoid tissue. a): Number of female animals was 4, because one female accidentally died before the end of the 2-week exposure period.

Table 2. Relative lung and spleen weights, histopathological findings in the lung, lymph nodes and spleen and indium contents in the lung and blood of male and female mice exposed to ITO or IO at 0.1 or 1 mg/m³ or clean air for 13 wk

		ITO			IO		
Group name (mg/m³)		Control	0.1	1	Control	0.1	1
No. of animals on examined		10	10	10	10	10	10
<Male>							
Relative lung weight (%)	mean	0.51	0.65#	1.01##	0.56	0.57	0.82##
	SD	0.03	0.04	0.08	0.04	0.03	0.06
Relative spleen weight (%)	mean	0.19	0.20	0.33##	0.20	0.20	0.22#
	SD	0.01	0.02	0.07	0.01	0.03	0.02
Deposition of particles							
Lung		0	10	10	0	10	10
MLN		0	4	9	0	0	6
Histopathological findings							
Lung							
Alveolar proteinosis		0	10** <1.0>	10** <1.4>	0	0	10** <1.6>
Infiltration of alveolar macrophages		0	2 <1.0>	10** <1.1>	0	0	10** <1.0>
Infiltration of inflammatory cells		0	0	10** <1.5>	0	0	5* <1.6>
Thickening of pleura		0	0	3 <1.0>	0	0	0
Lymph nodes							
Hyperplasia of MLN		0	0	9** <1.0>	0	0	0
Spleen							
Extramedullary hematopoiesis		0	0	4 <1.0>	0	0	0
Indium contents							
Lung (μg/g as In)		ND	11.5 ± 1.1	77.4 ± 12.2	ND	10.1 ± 1.1	183.3 ± 17.1
Blood (μg/l as In)	a)	ND	ND	0.58	ND	ND	ND
<Female>							
Relative lung weight (%)	mean	0.60	0.68#	1.12##	0.64	0.66	0.97##
	SD	0.04	0.02	0.09	0.05	0.05	0.11
Relative spleen weight (%)	mean	0.29	0.31	0.49##	0.32	0.33	0.38#
	SD	0.02	0.02	0.15	0.04	0.05	0.05
Deposition of particles							
Lung		0	10	10	0	10	10
MLN		0	2	8	0	0	9
Histopathological findings							
Lung							
Alveolar proteinosis		0	6* <1.0>	10** <1.6>	0	0	10** <1.8>
Infiltration of alveolar macrophages		0	0	10** <1.0>	0	0	9** <1.0>
Infiltration of inflammatory cells		0	0	9** <1.6>	0	0	6* <1.3>
Thickening of pleura		0	0	1 <1.0>	0	0	0
Lymph nodes							
Hyperplasia of MLN		0	0	5* <1.0>	0	0	0
Spleen							
Extramedullary hematopoiesis		0	0	6* <1.0>	0	0	0
Indium contents							
Lung (μg/g as In)		ND	7.8 ± 1.3	74.9 ± 10.0	ND	8.6 ± 1.1	166.6 ± 20.8
Blood (μg/l as In)	a)	ND	ND	0.90	ND	ND	ND

Values indicate number of animals bearing lesions. The values in angle bracket 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: #, $p \leq 0.05$; ##, $p \leq 0.01$ by Dunnett's test; *, $p \leq 0.05$; **, $p \leq 0.01$ by Chi-square test. ND: Indium contents were below the quantitative detection limits (lung: 0.006 μg/g tissue, blood: 0.5 μg/l whole-blood). MLN: Mediastinal lymph nodes. a) : The value was obtained from the pooled blood of 10 animals for the indium analysis.

exposed mice.

Lung and blood contents of indium

Lung concentrations of indium expressed as $\mu g/g$ tissue were increased with an increase in the exposure concentrations (Table 2). The contents of indium in the 0.1 mg/m^3 ITO-exposed mice of both sexes were approximately equal to those in the 0.1 mg/m^3 IO-exposed mice of both sexes, but the indium contents in the 1 mg/m^3 ITO-exposed mice was lower by 60% than those in the 1 mg/m^3 IO-exposed mice. Pooled blood contents of indium from ten 1 mg/m^3 ITO-exposed male and female mice were found to be 0.58 and 0.90 $\mu g/l$, respectively.

Discussion

In the present study, incidences and severities of the pulmonary lesions were found to be higher after ITO exposures than after IO exposures. Higher susceptibility of mice to ITO than IO is consistent with the previously reported findings in rats[10]. Species differences in the toxicity are apparent in comparison with the previously reported rat toxicity[10]. First, the severity score of alveolar proteinosis and the incidence of alveolar macrophage infiltration were lower in mice than in rats. Our previous and present results are in sharp contrast to the NTP's findings[11] that mice are more susceptible to the pulmonary toxicity of indium phosphide particles than rats. Second, 4 cases of thickened pleural wall were recognized in the mice exposed to ITO for 13 wk, while only one female case of thickened pleural wall was observed in the ITO-exposed rats at the end of the 26-week post-exposure period[10]. On the other hand, the ITO-exposed rats exhibited a high incidence of alveolar wall fibrosis which occurred only at the end of the 26-week post-exposure period after cessation of the 13-week exposure to ITO[10]. ITO particles were not found in the area of the thickened pleural wall in the ITO-exposed mice, although we did find deposition of the particles in the MLN. Insoluble particles in the deep lung have been reported to translocate through the pleural surface of the pulmonary lymphatic pathway into the MLN[12, 13]. A pathogenic behavior of ITO particles in the pleural surface of mice and the pulmonary interstitium of rats to explain the species difference in the fibrotic response pattern remains to be solved. Third, the significant increase in the erythrocyte parameters and the increased incidence of extramedullary hematopoiesis in the spleen occurred only in the ITO-exposed mice, but not in the ITO-exposed rats[10] , in the 13-week studies. A plausible explanation for this is that ITO-induced lung inflammation and alveolar proteinosis might cause functional impairment of respiration, including possible reduction of blood oxygen saturation, resulting in an adaptive increase in the erythrocyte parameters and extramedullary hematopoiesis in the spleen.

A threshold limit value (TLV) of 0.1 mg/m^3 for indium and its compounds has been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH)[14]. In the present 13-week study, alveolar proteinosis in the ITO-exposed mice was found to occur at the same exposure concentration as the ACGIH's TLV. Therefore, the present mouse study provides novel information about ITO-induced toxicity which leads to the re-consideration of the current occupational exposure limit value for indium.

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