doses of metal chlorides in rats and rabbits, and that indium ions interact most strongly with the simulated biomembrane causing possible impairment of cellular integrity. Blazka *et al.*[11] demonstrated that a single intratracheal administration of indium chloride dissolved in saline (pH 4.1) induces persistent inflammatory responses and development of fibrosis in rats. Lison *et al.*[8] reported that a strong cytotoxic response to ITO particles is induced *in vitro* in macrophages (NR8383 cell line) but not in rat lung epithelial cells. Taken together, it is likely that indium leached from ITO particles in the alveolar macrophages might be involved in the cellular disintegrity of alveolar macrophages, since degenerative alveolar macrophages with swollen cytoplasm engulfing ITO and IO particles were observed microscopically in the present 2- and 13-week studies.

Subacute pulmonary toxicity induced by the 2-week exposure to ITO was characterized by alveolar proteinosis, macrophage infiltration, inflammatory cell infiltration and alveolar epithelial hyperplasia. The former two pulmonary lesions occurred at exposures of 1 mg/m^3 and above, while the latter two appeared primarily at exposures of 10 and 100 mg/m^3. The 13-week inhalation exposure to ITO was found to induce essentially the same pulmonary lesions as those observed in the 2-week exposure, but at lower exposure concentrations. Furthermore, the histopathological examination at the end of the 26-week post-exposure period revealed development of fibrosis of alveolar wall, worsening of alveolar epithelial hyperplasia and persistence of the pulmonary lesions observed at the end of the 13-week exposure to ITO. No recovery from the subchronic effects of indium was indicated. Therefore, the present findings indicate that the pulmonary toxicity of inhaled ITO particles is more severe than that of IO particles and that alveolar macrophages play a critically important role in the induction of indium toxicity as evidenced by alveolar proteinosis, alveolar macrophage infiltration and swollen alveolar macrophages engulfing the particles, all of which occur at the lowest exposure concentration. Lison *et al.*[8] compared *in vivo* and *in vitro* pulmonary toxicity of ITO particles with those of its constituents, tin-oxide, IO and their unsintered mixture. They attributed the reactivity/toxicity of sintered ITO particles to carbon centered radical formation and Fenton-like activity, appearing with a high electron density of the sintering process through which TO molecules were introduced within crystal structure of IO[26]. However, it can be inferred from the present 2-week and 13-week studies that these pulmonary lesions are not causally linked to the dust overload induced by excessive inhalation exposure to ITO or IO aerosol, since the whole-lung contents of indium in the ITO- or IO-exposed rats were far below the levels of dust burden causing overloading, which are reported to be greater than 1–2 mg of persistently retained dust in the lungs of F344 rats[27].

The most remarkable pulmonary lesion found in the present studies was alveolar proteinosis accompanied by alveolar macrophage infiltration. These two lesions were the most sensitive, appearing at the lowest exposure concentration. The alveolar proteinosis observed in the lung of rats exposed to ITO and IO aerosols were characterized by filling of the alveolar space with a granular, pale, eosinophilic material which is positively stained with a PAS reagent. These histological characteristics resemble those of alveolar proteinosis reported in human cases[28,29]. The present result of alveolar proteinosis is consistent with reported findings that a pulmonary administration of ITO to rats and hamsters induces alveolar exudates of proteinaceous materials[8–10]. NTP's study[12] showed that inhalation exposure of rats to indium phosphide aerosol for 14 wk induced accumulation of proteinaceous material within the alveoli, which was diagnosed as alveolar proteinosis. The alveolar proteinosis induced by the inhalation exposures of rats to ITO and IO was similar to that seen in experimental silicosis[30]. Electron-microscopic observation[31] of the lung of rats exposed to pyro-aluminium and quartz showed that pulmonary lesion identical to human alveolar proteinosis is featured by accumulation of PAS-positive alveolar material composed primarily of pulmonary surfactant derived from Type II pneumocytes and large foamy alveolar macrophages with impaired mobility. It is notable that the present finding that the 13-week exposure of rats to 0.1 mg/m^3 ITO induced moderate alveolar proteinosis with a lung content of indium at 24 μg/g lung tissue is comparable with the result of Cummings *et al.*[3], who reported that one of two ITO-exposed workers diagnosed as alveolar proteinosis had 29.3 μg indium per gram of lung tissue. In contrast, occurrence of alveolar proteinosis in workers exposed to ITO has not been definitely demonstrated in any epidemiological studies conducted in Japan[2,4–7]. In particular, Nogami *et al.*[6] reported in their epidemiological study of workers at a Japanese indium plant that neither interstitial nor emphysematous change was recognized in the lung of a worker suffering from bronchioloalveolar carcinoma, while his lung content of indium was 31.2 μg/g lung tissue[6]. These conflicting observations about the occurrence of alveolar proteinosis in indium-exposed workers remain to be resolved, although some etiological factors have been suggested[28–31]. Further experimental toxicology studies will be needed to explore any causative factor of alveolar proteinosis in indium-exposed rodents, including the time- and dose-related changes and species and strain differences.

In the present studies, fibrosis of alveolar wall was found to develop only at the end of 26-week post-exposure period after cessation of the 13-week exposure of rats to ITO at 0.1 mg/m^3. NTP's study[12] also showed that interstitial fibrosis was induced in rats by inhalation exposure to indium phosphide aerosol for 14 wk. The

present finding that 13 wk exposure of rats to ITO induced fibrosis of alveolar wall with the indium burden indicated by 8 μg/g lung and 1 μg/l blood at the end of the 26-week post-exposure period can be contrasted with the case study of Homma *et al.*[32], who showed that an ITO-exposed worker was diagnosed as having pulmonary fibrosis with a serum indium level of 51 μg/l. This apparent difference in the sensitivity to pulmonary fibrosis between rats and humans remains to be resolved and warrants further studies including solubility of ITO in the lung.

ACGIH's recommendation of TLV-TWA[13] for indium and its compounds of 0.1 mg/m^3 was based on pulmonary toxicity of widespread alveolar edema resembling alveolar proteinosis, resulting from 3-month inhalation exposure of rats to IO aerosol[33]. JSOH recommended a BEI of 3 μg/l as a serum level of indium, below which chronic inflammation would not occur[15]. However, it was found in the present studies that both alveolar proteinosis and alveolar macrophage infiltration are induced in rats by 13 wk inhalation exposure to ITO at 0.1 mg/m^3, the same concentration as the ACGIH's TLV-TWA. Moreover, alveolar wall fibrosis and alveolar epithelial hyperplasia develop in all exposed rats at the end of the 26-week post-exposure period, indicating that the persistent fibroproliferative lung lesions develops with a latent period of 26 wk after cessation of the repeated inhalation exposure to 0.1 mg/m^3 ITO. Blood contents of indium are reported to be approximately equal to serum levels of indium[12, 34], while indium levels in blood instead of serum were quantified in the present studies. All the blood levels of indium in the rats exposed to ITO aerosol at 0.1 mg/m^3 measured at the end of the 13-week exposure period and at the end of the 26-week post-exposure period were below the BEI value of 3 μg/l set by JSOH. Therefore, the present findings provide novel information about the animal basis of ITO-induced pulmonary toxicity for reconsideration of the current OEL and BEI for inhaled indium and its compounds.

We consider that an exposure concentration of 0.1 mg/m^3 ITO for 104 wk would be too high for use in a 2-year carcinogenicity study, based on the magnitude of increased lung weights and the increased incidences and severities of pulmonary lesions in the rats exposed to ITO at 0.1 mg/m^3 for 13 wk and for the 26-week post-exposure period after cessation of the 13-week exposure to 0.1 mg/m^3. In the 2-year study, repeated exposure of rats to 0.1 mg/m^3 ITO should be discontinued at the first 26 wk, and then these rats are allowed to continue unexposed in the exposure chamber for the remainder of the study. This exposure discontinuation is based on the 1.7-fold increase in relative lung weight compared with that of the control group, the lack of recovery from alveolar proteinosis, alveolar epithelial hyperplasia, and the development of fibrosis or thickened pleural wall observed at the end of the present study's 26-week post-exposure period, and with reference to NTP's stop-exposure rationale in the 2-year study of indium phosphide carcinogenicity[12]. The middle and lowest exposure concentrations for 104 wk were selected as 0.03 and 0.01 mg/m^3, respectively. The lowest exposure concentration of 0.01 mg/m^3 was set at the lowest concentration that generation of ITO aerosol in the present system and its chamber monitoring of the aerosol can be performed with sufficient reproducibility and accuracy.

Conclusions

Using an aerosol generator and inhalation exposure system with reproducibility and accuracy, rats of both sexes were exposed to ITO and IO at different concentrations for 2 and 13 wk. An exposure concentration-related increase in whole-lung contents of indium tended to be suppressed in the ITO- and IO-exposed rats, and blood contents of indium in the ITO-exposed rats were higher than those in the IO-exposed rats. ITO and IO particles were deposited in the lung, and to a lesser extent in the BALT, MLN and NALT of exposed rats. Two-week exposures to ITO and IO induced alveolar proteinosis, infiltrations of alveolar macrophages and inflammatory cells and alveolar epithelial hyperplasia in addition to increased lung weight. Thirteen-week exposures to ITO and IO induced the similar pulmonary lesions, and some of these lesions were worsened. ITO affected the lung more severely than did IO. Development of fibrosis and worsening of alveolar epithelial hyperplasia were noted at the end of the 26-week post-exposure period following 13 wk exposure. These ITO-induced lesions appeared at the same exposure concentration as ACGIH's TLV and at the blood indium levels below JSOH's BEI.

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Brief Report

Pulmonary Toxicity in Mice by 2- and 13-week Inhalation Exposures to Indium-tin Oxide and Indium Oxide Aerosols

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Abstract: Pulmonary Toxicity in Mice by 2- and 13-week Inhalation Exposures to Indium-tin Oxide and Indium Oxide Aerosols: Kasuke NAGANO, *et al.*, Japan Bioassay Research Center, Japan Industrial Safety and Health Association—Objectives: Inhalation toxicities of indium-tin oxide (ITO) and indium oxide (IO) in mice were characterized in comparison with those previously reported in rats. **Methods:** B6C3F$_1$ mice of both sexes were exposed by inhalation to ITO or IO aerosol for 6 h/day, 5 day/wk for 2 wk at 0, 0.1, 1, 10 or 100 mg/m^3 or 13 wk at 0, 0.1or 1 mg/m^3. **Results:** ITO and IO particles were deposited in the lung, mediastinal lymph node (MLN) and nasal-associated lymphoid tissue. Alveolar proteinosis, infiltrations of alveolar macrophages and inflammatory cells and increased lung weight were induced by 2- and 13-week exposures to ITO and IO, while alveolar epithelial hyperplasia occurred only in the 2-week exposures. Thickened pleural wall, hyperplastic MLN, extramedullary hematopoiesis in the spleen and increased levels of erythrocyte parameters were induced by 13-week exposure to ITO. The ITO- and IO-induced pulmonary lesions were milder in mice than those previously reported in rats, and the fibrotic lesions were different between these two species. Indium levels in the lung and pooled blood were analyzed in the mice exposed to ITO and IO for 13 wk. In the 13-week inhalation exposure of mice to ITO, alveolar proteinosis and significantly increased lung weight were induced at the same exposure concentration as the current threshold limit value for indium and its compounds.
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Key words: Indium oxide, Indium-tin oxide, Inhalation, Lung, Mouse, Toxicity

Serious concerns have been raised over workers' health in the plants where indium-tin oxide (ITO) is manufactured and processed. Fatal case studies[1, 2] and epidemiology studies of workers[3–6] have demonstrated that inhalation of indium is a potential cause of occupational lung disease and increases the risk of interstitial lung damage. Experimental toxicology studies have shown that an intratracheal administration of ITO powder induces persistent inflammation in the lung without any significant fibrotic response in rats[7], and elicits pulmonary inflammatory response with diffuse alveolar or bronchiolar cell hyperplasia and interstitial fibrotic proliferation in hamsters[8, 9]. Our previous study[10] showed that 2- and 13-week inhalation exposures of rats to ITO or indium oxide (IO) aerosol induce pulmonary fibrosis, alveolar proteinosis and macrophage infiltration.

The present studies were intended to characterize inhalation toxicities of ITO and IO aerosols in mice in comparison with those reported in rats[10].

Materials and Methods

The present studies were performed with the approval of the ethics committee of the Japan Bioassay Research Center. The animals were cared for in accordance with a guide for the care and use of laboratory animals. All these related documentations were cited in our previous rat studies[10].

ITO and IO powders were the same as those used in our previous rat studies[10], which were kindly supplied by JX Nippon Mining & Metals, Corp (Tokyo, Japan).

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 before the start of experiment. The mice were individually housed in stainless-steel wire hanging cages

(112 W × 212 D × 120 H mm), and placed in stainless steel inhalation exposure chambers. Environment in the exposure chamber, lighting, and supply of food and drinking water were maintained under the same conditions as described in our previous rat study[10].

In the 2-week study, groups of 5 mice of both sexes were exposed to ITO or IO aerosol at a target concentration of 0.1, 1, 10 or 100 mg/m^3 for 6 h/day, 5 day/wk for 2 wk. In the 13-week study, groups of 10 mice of both sexes were exposed to ITO or IO aerosol at a target concentration of 0.1 or 1 mg/m^3 for 6 h/day, 5 day/wk for 13 wk. Groups of 5 or 10 mice of both sexes were exposed to clean air for 2 or 13 wk, and served as respective controls.

The aerosol generation and exposure system and the methods for measurements of the exposure concentrations and size distributions of ITO and IO aerosols, and for clinical and pathological examinations and analysis of indium in the lung and blood as well as statistical analysis were the same as those described in our previous rat study[10].

Results

Chamber concentrations and size distributions of ITO and IO aerosols

Chamber concentrations of ITO and IO aerosols were controlled precisely within less than 10% in the variation coefficient and accurately within less than 10% deviation from the target concentrations. Mass median aerodynamic diameters (MMADs) of ITO aerosol in the exposure chamber ranged from 2.4 to 3.8 μm in the 2-week study, and from 2.3 to 2.6 μm in the 13-week study, and geometric standard deviations (GSDs) ranged from 1.6 to 2.4. MMADs of IO aerosol ranged from 1.9 to 2.3 μm, and GSDs ranged from 1.5 to 2.1.

Mortality and clinical signs, and hematology

Neither death, abnormal clinical sign nor growth retardation occurred in any group exposed to ITO, IO or clean air for 2 or 13 wk. In the 13-week study, red blood cell counts, hemoglobin concentration and hematocrit value were significantly increased in the 0.1 and 1 mg/m^3 ITO-exposed groups compared with the respective controls (data not shown). However, 13-week exposure to IO did not induce any hematological changes.

Lung and spleen weights

In the 2-week study (Table 1), the relative lung weights were significantly increased in the 1 mg/m^3 ITO-exposed female mice and in the 10 and 100 mg/m^3 ITO- and IO-exposed mice of both sexes compared with the respective controls. In the 13-week study (Table 2), the relative lung weights in the 0.1 and 1 mg/m^3 ITO-exposed mice of both sexes and in the 1 mg/m^3 IO-exposed mice of both sexes were significantly increased compared with the respective controls. The relative spleen weight was significantly increased in the 1 mg/m^3 ITO- and IO-exposed mice of both sexes in the 13-week study compared with the respective control.

Histopathological findings

In the 2-week study, ITO and IO particles were deposited separately as single particles in the lungs of almost all 1, 10 and 100 mg/m^3 ITO- and IO-exposed mice (Table 1). The particles of ITO and IO were pale brown and transparent, looked like amber, and were located primarily within alveolar macrophages. ITO and IO particles were also deposited in the mediastinal lymph node (MLN) of the 10 and 100 mg/m^3 ITO- and IO-exposed mice, and in the nasal-associated lymphoid tissue (NALT) of the nasopharyngeal duct of a few 10 and 100 mg/m^3 IO-exposed male mice. The most remarkable lung lesion found in the 2-week study was alveolar proteinosis, characterized by filling of the alveolar space with a granular, pale eosinophilic material, which was positively stained with a PAS reagent. This lesion was found in all the 10 and 100 mg/m^3 ITO- and IO-exposed mice. The severity score of alveolar proteinosis was higher in the 100 mg/m^3 ITO-exposed mice of both sexes than in the 100 mg/m^3 IO-exposed mice. Hyperplasia of alveolar epithelium and infiltration of inflammatory cells composed of neutrophils and lymphocytes were observed primarily in the 100 mg/m^3 ITO- and IO-exposed mice. Hyperplasia of the alveolar epithelium was characterized by the increased numbers of cuboidal cells which were assumed to be type II pneumocytes. Infiltration of alveolar macrophages was found in a few 100 mg/m^3 ITO-exposed mice and in one 100 mg/m^3 IO-exposed male mouse.

In the 13-week study, ITO and IO particles were deposited separately as single particles in the lungs of all the 0.1 and 1 mg/m^3 ITO- and IO-exposed mice (Table 2). Those particles were also deposited in the MLN of the 0.1 and 1 mg/m^3 ITO-exposed mice and the 1 mg/m^3 IO-exposed mice. Significantly increased incidences of alveolar proteinosis in the 0.1 and 1 mg/m^3 ITO-exposed mice and in the 1 mg/m^3 IO-exposed mice and infiltrations of alveolar macrophages and inflammatory cells in the 1 mg/m^3 ITO- and IO-exposed mice were noted. Swelling of cytoplasm was recognized in the alveolar macrophages engulfing the particles. Alveolar wall fibrosis was not found in any mouse exposed to either ITO or IO. Thickening of pleural wall and hyperplasia of the MLN occurred in the 1 mg/m^3 ITO-exposed mice. The thickened pleural wall was characterized by an increase in collagen-like connective tissue in the interstitium, and was located in focal lung areas. However, ITO particles were not found in the area of the thickened pleural wall. Hyperplasia of the MLN was characterized by increased numbers of lymphocytes resulting in increased MLN size and area of lymphoid follicles. Extramedullary hematopoiesis occurred in the red pulp of the spleen of the 1 mg/m^3 ITO-

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/m³)		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	5	0	0	0	5	5
					<1.0>	<1.8>				<1.0>	<1.0>
Infiltration of alveolar macrophages		0	0	0	1	2	0	0	0	0	1
					<1.0>	<1.0>					<1.0>
Infiltration of inflammatory cells		0	0	0	1	5	0	0	0	1	5
					<1.0>	<1.0>				<1.0>	<1.0>
Hyperplasia of alveolar epithelium		0	0	0	1	5	0	0	0	0	3
					<1.0>	<1.0>					<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	5	0	0	0	5	4
					<1.0>	<2.0>				<1.0>	<1.0>
Infiltration of alveolar macrophages		0	0	0	0	3	0	0	0	0	0
						<1.0>					
Infiltration of inflammatory cells		0	0	0	0	4	0	0	0	0	3
						<1.0>					<1.0>
Hyperplasia of alveolar epithelium		0	0	0	0	4	0	0	0	0	2
						<1.0>					<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 μg/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 μg/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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