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Oral Toxicity of Indium in Rats: Single and 28-Day Repeated Administration Studies

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Abstract: Oral Toxicity of Indium in Rats: Single and 28-Day Repeated Administration Studies: Keiko ASAKURA, *et al*. Department of Preventive Medicine and Public Health, Keio University School of Medicine—Indium is widely used in the electronics industry to make semiconductors, liquid-crystal panels, and plasma display panels, and its production is increasing. However, it is necessary to handle it more cautiously than before, because the pulmonary toxicity of inhaled indium has been identified. The present study aimed to characterize the potential toxic effects of indium through oral administration and observation for fourteen days following a single dose of 0 or 2,000 mg/kg (acute oral toxicity study), and repeated oral administration for 28 days at dose levels of 0, 40, 200, or 1,000 mg/kg daily (28-day repeated oral dose toxicity study) to male and female Crj:CD (SD) IGS rats (SPF). No deaths and no abnormalities in clinical signs, body weights, and necropsy findings were observed for any of the animals in the acute oral toxicity study. Furthermore, no changes related to indium were also observed in the dose groups up to 1,000 mg/kg of the 28-day repeated oral dose toxicity study. From the results described above, the lethal dose 50% (LD_{50}) of indium is greater than 2,000 mg/kg under these study conditions, and the no-observed-adverse-effect-level (NOAEL) is considered to be 1,000 mg/kg for males and females under these conditions.
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Indium is an essential metal for the electronics industry and is used to make semiconductors, liquid-crystal panels, and plasma display panels. Initially, it was assumed to be a stable, harmless material, so its production and consumption have increased rapidly. However, the first case of fatal interstitial pulmonary disease induced by inhalation of indium occurred in Japan in 2001, and this case was reported in 2003[1)]. A milder case was also reported in 2005[2)]. Nowadays, it is realized that adequate protection against inhalation of indium is essential for indium-processing workers.

The toxicity of inhaled indium has been confirmed in several animal experiments using mice, rats, and hamsters[3–7)]. These experiments have clearly shown that indium inhalation causes pulmonary inflammation, fibrosis and carcinogenesis. In addition, it has been reported that elimination of indium from the lung is slow, and that the effects of indium might continue for a long time[6)].

On the other hand, indium might also enter the human body orally because it is used as a component of dental crowns or inlays[8)]. It also used as a material in some medications for diagnostic and therapeutic purposes[9, 10)]. At the same time, the use of indium as a substitute metal for lead is increasing in lead-free solder production. This trend will continue because the toxicity of lead is widely known. However, the toxicity of orally administered indium has not been clarified. Therefore, in the present study, we attempted to define the oral toxicity of indium in animal experiments using rats.

Materials and Methods

Chemicals

Indium (abbreviation: In; purity, 99%; CAS No: 7440-74-6; lot number, 67246G; particle diameter: M45 μm pass) was provided by Kojundo Chemical Laboratory Co.,

Ltd. and was kept at room temperature. A stability analysis of the lot used in this study was not conducted, since it was considered stable based on the characteristics of indium.

Animals

We used 12 male and 12 female Crj: CD(SD) IGS rats (SPF) which were purchased from Charles River Japan, Inc for the acute oral toxicity study. The animals were observed for general condition for 6 days during the quarantine and acclimatization period to confirm that there were no abnormalities. For the 28-day repeated oral dose toxicity study, 36 male and 36 female rats were used after a 7-day quarantine and acclimatization period. On the day before the start of dosing, animals were assigned to groups by the stratified-by-weight randomization method so that they were evenly assigned with respect to the mean body weight. On the dosing day, the male and female rats were 5 wk old. The body weights just before the administration ranged from 125 to 145 g in male and 116 to 130 g in female rats in the acute oral toxicity study, and from 172 to 201 g in males and 135 to 161 g in females in the 28-day repeated oral dose toxicity study. The body weights of rats in the acute oral toxicity study were lighter than those for the 28-day repeated oral dose toxicity study due to an 18-h fast before indium administration.

The animals were fed a pellet diet (MF, Oriental Yeast Co., Ltd.) and given tap water irradiated by UV rays after passing through a 5-μm filter *ad libitum*. The rats were housed under the following conditions: a temperature of 22.2 ± 2°C, relative humidity of 55 ± 15%, ventilation frequency of approximately 12 changes per hour, and light for 12 h per day (7:00 to 19:00). This experiment was approved by the Committee for Ethics in Animal Studies of the Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd. (Approval number (date); acute oral toxicity study: 2000-0281 (October 23, 2000), 28-day repeated oral dose toxicity study: 2000-0235 (September 25, 2000))

Administration

We performed the acute oral toxicity study in accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline 401[11]. Indium was administered once, orally via a stomach tube, after the animal had fasted for approximately 18 h prior to dosing; diet was withheld for 3 h after dosing. Since there were no deaths during a preliminary study, doses of 2,000 mg/kg (2 rats of each sex), a dosage of 2,000 mg/kg was set for the study. The test substance was suspended in corn oil (Junsei Chemical Co., Ltd.). The dosing volume was set at 10 m*l*/kg, and the dose volume for individual animals was calculated based on the body weight measured just before dosing. The preparation of the dosing suspension was done on the administration day. The control group was treated with the vehicle (corn oil) alone.

Prior to the 28-day repeated oral dose toxicity study, we conducted a preliminary dose-finding study entitled "A Fourteen-Day Repeated Oral Toxicity Preliminary Study in Rats" (dose levels: 0, 100, 500, and 1,000 mg/kg; number of animals: three males and three females per group). No changes attributable to the test substance were observed in clinical signs, body weights, hematology, or organ weights (brain, thymus, liver, kidney, adrenal glands, spleen, testis, and ovary) on necropsy. As such, the highest dose level was set at 1,000 mg/kg, and the middle and lower dose levels were set at 200 and 40 mg/kg, respectively. The test substance was suspended in corn oil (Junsei Chemical Co., Ltd.), and the preparation frequency was once a week. The dosing preparations were stored under dark, refrigerated conditions until administration. The homogeneity of the dosing suspension was confirmed at concentrations of 4.0 and 100 mg/m*l* before administration. Thereafter, oral administration (gavage) was performed once daily in the morning throughout the 28-day dosing period, according to the Chemical Substances Control Law in Japan (1986), to 6 rats of each sex in the 200 and 40 mg/kg groups, and to 12 rats of each sex in the control and 1,000 mg/kg groups. The dosing volume (10 m*l*/kg) for individual animals was calculated based upon the most recent body weight. Moreover, 6 rats in each of the control and 1,000 mg/kg groups were given a 14-day recovery period after completion of the dosing period. The control group was treated with the vehicle (corn oil) alone.

For the acute oral toxicity study, clinical signs, body weight, and pathological examination were performed. In addition to these examinations, food consumption, hematology, blood chemistry, and urinalysis were performed in the 28-day repeated oral dose toxicity study. The parameters examined are described in detail below.

Clinical signs, body weight, and food consumption

In the acute oral toxicity study, clinical signs of the animals were observed five times (shortly before dosing, and 0.5, 1, 3, and 5 h after dosing) on the dose day and thereafter once a day for 14 days. In the 28-day repeated oral dose toxicity study, observation for the same parameters was done twice a day (before and after administration) throughout the dosing period, and once a day in the morning during the recovery period. The body weights of all animals were measured before dosing and on days 4, 8, and 15 (the day of administration was designated as day 1) with an electronic balance (EB-3200S, Shimadzu Corp.) in the acute oral toxicity study. In the 28-day repeated oral dose toxicity study, the body weights and the gross weights of the feeders were

measured once a week, and the mean daily food consumption per animal for each weighing period was calculated.

Hematology and blood chemistry

On the days of the scheduled necropsy after the completion of dosing and recovery periods (days 29 and 43), the animals were anesthetized by intraperitoneal injection of thiopental sodium (Ravonal, Tanabe Seiyaku Co., Ltd.). Thereafter, blood samples were collected via the posterior vena cava. For hematology, we measured the erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count (Reti), platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), leukocyte count (WBC), and differential leukocyte count. For blood chemistry, we used the obtained serum samples to measure the aspartate aminotransferase (AST), alanine animotransferase (ALT), γ-glutamyltransferase (γGT), alkaline phosphatase (ALP), total bilirubin (T-bil), blood urea nitrogen (BUN), creatinine (Cr), glucose, total cholesterol (T-cho), triglycerides (TG), total protein (TP), albumin, albumin/ globulin ratio (A/G), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K), and chlorine (Cl).

Urinalysis

Fresh urine samples from six males and six females in each group were collected on day 27, and the following test items were measured: pH, protein, glucose, ketone bodies, bilirubin, occult blood, and urobilinogen. No changes attributed to indium were found in the examinations during the dosing period. Therefore, examinations were not conducted during the recovery period.

Pathological and histopathological examination

In the acute oral toxicity study, all animals were euthanized after the final observation (day 15) by exsanguination from the abdominal aorta under intraperitoneal thiopental sodium anesthesia. In the 28-day repeated oral dose toxicity study, all animals were sacrificed by the same method after blood sampling, and then subjected to necropsy. The following organs of all animals were weighed: brain, liver, kidney, adrenal grands, thymus, spleen, testis, and ovary. Relative organ weights ((each organ weight/ body weight)*100) were calculated from body weights on each necropsy day.

In the 28-day repeated oral dose toxicity study, histopathological examinations were performed on the heart, liver, spleen, kidneys, and adrenals obtained from animals of the control and 1,000 mg/kg groups, and on gross lesions of any group. Specimens were stained with hematoxylin and eosin according to a standard procedure and then microscopically examined. No changes attributed to indium were observed. Therefore, histopathological examinations of organs and tissues except for gross lesions in the animals of the recovery group were not conducted.

Statistical analysis

We used multiple comparison tests to analyze statistical significances in the numerical data (body weight, food consumption, hematology, blood chemistry, and organ weights). First, the homogeneity of the variances of the data was tested by Bartlett's test. Second, if the variances of the treatment group and the control group were homogenous, one-way analysis of variance was performed, while if the variances of the both groups were heterogeneous, the Kruskal-Wallis test was performed. Third, if there were statistical significances in the data between groups, Dunnett's test or a Dunnett-type rank-sum test was conducted. Furthermore, statistical significances in graded categorical data (urinalysis) were analyzed by the a×b chi-square test. If statistical significances were found, we compared data obtained from the control group with those obtained from each dose group using Armitage's chi-square test. Significance levels of 5% and 1% were set for all statistical analyses. Statistical analyses were not conducted on clinical signs, necropsy findings, and histopathological findings.

Results

Acute oral toxicity study

We found no abnormal clinical signs in any animal. The body weights of all animals increased from the day of dosing to the end of the observation period, day15. The body weight gain of all animals was not significantly different between the control group and the treatment group. No abnormalities attributed to the administration of indium were observed during necropsy. With regard to spontaneous changes, pelvic dilatation of the kidneys was observed in one male of the 2,000 mg/kg group.

28-day repeated oral dose toxicity study

Clinical signs, body weights, and food consumption: No abnormal clinical signs and no significant body weight or food consumption differences between the control group and any treatment group were observed in any animal during the dosing and recovery periods.

Hematology and blood chemistry: The results of blood analysis in males and females are shown in Table 1. We found no significant hematological change attributable to indium after the dosing period. After the recovery period, a significantly higher value of the ratio of lymphocyte and a significantly lower value of the ratio of monocyte were observed in females of the 1,000 mg/

Table 1. Results of hematology and blood chemistry in the 28 day repeated oral dose toxicity study

a) Just after dosing period (5 wk)

		Male				Female			
Indium dose		0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg	0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg
Animal number		6	6	6	6	6	6	6	6
RBC	$\times 10^4/\mu l$	733.0 ± 38.5	746.5 ± 17.4	750.0 ± 13.9	732.7 ± 15.6	717.3 ± 30.0	703.3 ± 35.2	720.2 ± 34.2	722.5 ± 26.7
Hb	g/d*l*	14.73 ± 0.73	14.95 ± 0.74	14.75 ± 0.33	14.18 ± 0.60	14.55 ± 0.59	14.10 ± 0.87	14.33 ± 0.63	14.07 ± 0.38
Ht	%	43.22 ± 2.07	43.67 ± 2.73	43.32 ± 0.77	41.72 ± 1.72	41.73 ± 1.86	40.30 ± 2.24	41.07 ± 2.19	40.75 ± 1.06
MCV	fl	59.00 ± 1.56	58.50 ± 3.58	57.75 ± 0.72	56.95 ± 2.44	58.20 ± 2.17	57.30 ± 1.56	57.02 ± 2.07	56.42 ± 1.50
MCH	pg	20.10 ± 0.51	20.03 ± 0.82	19.67 ± 0.45	19.33 ± 0.89	20.32 ± 0.88	20.05 ± 0.41	19.92 ± 0.52	19.48 ± 0.41
MCHC	%	34.10 ± 0.36	34.27 ± 1.09	34.05 ± 0.39	34.02 ± 0.81	34.87 ± 0.69	34.98 ± 0.69	34.92 ± 0.47	34.53 ± 0.27
Reti	‰	31.38 ± 3.25	27.97 ± 5.18	30.12 ± 4.14	27.07 ± 2.23	23.82 ± 5.47	21.13 ± 4.34	25.28 ± 5.10	21.87 ± 3.75
PLT	$\times 10^4/\mu l$	92.00 ± 10.00	88.77 ± 29.61	89.90 ± 6.12	92.62 ± 5.98	90.18 ± 9.11	100.68 ± 15.10	92.85 ± 8.42	87.72 ± 9.64
WBC	$\times 10^2/\mu l$	109.38 ± 17.10	96.52 ± 23.26	109.03 ± 13.25	100.57 ± 17.41	84.98 ± 36.35	100.57 ± 24.42	75.87 ± 12.50	88.17 ± 20.12
Lymphocyte	%	92.2 ± 3.0	93.5 ± 3.8	93.5 ± 2.2	92.5 ± 2.7	93.8 ± 2.6	94.0 ± 3.0	93.7 ± 4.1	94.5 ± 2.0
Monocyte	%	1.3 ± 0.8	0.5 ± 0.8	0.3 ± 0.5	0.8 ± 0.8	1.0 ± 1.5	1.0 ± 0.6	1.5 ± 1.5	0.7 ± 0.8
ASAT	U/*l*	88.3 ± 8.5	78.7 ± 11.9	78.8 ± 11.0	86.7 ± 8.8	84.8 ± 18.3	82.5 ± 20.5	75.8 ± 8.4	85.8 ± 11.4
ALAT	U/*l*	44.0 ± 7.2	42.0 ± 9.9	41.5 ± 7.9	45.7 ± 3.9	30.2 ± 6.0	28.0 ± 5.2	29.8 ± 5.1	34.7 ± 3.8
γGT	U/*l*	ND	ND	ND	ND	ND	ND	ND	ND
ALP	U/*l*	798.7 ± 226.0	757.8 ± 152.8	746.5 ± 277.8	832.0 ± 150.8	448.8 ± 73.4	419.2 ± 108.3	446.2 ± 40.6	544.5 ± 134.8
T-bil	mg/d*l*	ND	ND	ND	ND	ND	ND	ND	ND
BUN	mg/d*l*	13.38 ± 3.04	14.82 ± 2.26	13.43 ± 1.00	13.48 ± 1.71	15.05 ± 2.14	16.58 ± 1.59	12.92 ± 1.79	16.15 ± 2.72
Cr	mg/d*l*	0.42 ± 0.04	0.45 ± 0.08	0.40 ± 0.00	0.40 ± 0.00	0.45 ± 0.05	0.52 ± 0.08	0.43 ± 0.05	0.47 ± 0.05
Glucose	mg/d*l*	158.9 ± 7.1	154.2 ± 11.1	160.0 ± 5.1	159.0 ± 9.1	146.5 ± 14.3	164.5 ± 23.3	143.7 ± 10.8	143.8 ± 4.5
T-cho	mg/d*l*	59.2 ± 10.4	63.2 ± 8.7	60.0 ± 7.1	65.2 ± 7.0	63.3 ± 7.5	55.2 ± 9.3	59.7 ± 3.9	58.5 ± 10.4
TG	mg/d*l*	58.0 ± 39.2	63.3 ± 16.7	51.8 ± 18.1	48.8 ± 15.8	17.0 ± 4.8	13.7 ± 4.5	16.8 ± 3.2	15.5 ± 4.6
TP	g/d*l*	6.58 ± 0.15	6.43 ± 0.16	6.60 ± 0.28	6.40 ± 0.20	6.42 ± 0.31	6.22 ± 0.21	6.18 ± 0.15	6.37 ± 0.22
Albumin	g/d*l*	3.47 ± 0.05	3.42 ± 0.08	3.53 ± 0.14	3.45 ± 0.10	3.50 ± 0.15	3.48 ± 0.15	3.38 ± 0.08	3.55 ± 0.10
A/G		1.115 ± 0.052	1.137 ± 0.080	1.155 ± 0.052	1.173 ± 0.064	1.202 ± 0.046	1.278 ± 0.101	1.212 ± 0.075	1.262 ± 0.028
Ca	mg/d*l*	9.77 ± 0.28	9.58 ± 0.21	9.80 ± 0.31	9.63 ± 0.31	9.33 ± 0.27	9.23 ± 0.20	9.18 ± 0.10	9.40 ± 0.34
P	mg/d*l*	8.73 ± 0.19	8.82 ± 0.50	8.58 ± 0.38	8.55 ± 0.41	8.00 ± 0.50	8.18 ± 1.11	7.92 ± 0.50	7.98 ± 0.25
Na	mmol/*l*	143.0 ± 0.0	142.8 ± 1.0	143.0 ± 1.3	142.3 ± 0.5	142.8 ± 1.2	142.3 ± 1.6	142.5 ± 0.5	142.3 ± 0.5
K	mmol/*l*	4.43 ± 0.12	4.60 ± 0.23	4.40 ± 0.23	4.52 ± 0.18	4.10 ± 0.21	4.08 ± 0.18	4.03 ± 0.26	4.02 ± 0.16
Cl	mmol/*l*	99.0 ± 0.9	100.0 ± 1.5	98.8 ± 1.0	99.7 ± 1.0	100.7 ± 1.0	101.5 ± 1.8	101.0 ± 1.1	100.3 ± 1.0

Table 1. continued

b) Just after recovery period (7 wk)

		Male				Female			
Indium dose		0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg	0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg
Animal number		6	0	0	6	6	0	0	6
RBC	$\times 10^4/\mu l$	788.7 ± 24.6	–	–	792.8 ± 47.3	743.3 ± 29.2	–	–	766.5 ± 22.2
Hb	g/d*l*	15.08 ± 0.73	–	–	14.87 ± 0.43	14.50 ± 0.66	–	–	14.45 ± 0.36
Ht	%	43.12 ± 1.24	–	–	41.92 ± 1.39	41.07 ± 2.01	–	–	41.20 ± 0.99
MCV	fl	54.70 ± 1.96	–	–	52.97 ± 2.73	55.27 ± 2.28	–	–	53.77 ± 1.06
MCH	pg	19.13 ± 0.62	–	–	18.80 ± 1.13	19.50 ± 0.76	–	–	18.87 ± 0.44
MCHC	%	35.00 ± 0.33	–	–	35.47 ± 0.60	35.32 ± 0.77	–	–	35.10 ± 0.43
Reti	‰	24.72 ± 3.25	–	–	26.02 ± 2.75	21.27 ± 2.35	–	–	24.55 ± 5.05
PLT	$\times 10^4/\mu l$	97.88 ± 8.61	–	–	101.85 ± 7.46	87.72 ± 8.16	–	–	92.92 ± 7.36
WBC	$\times 10^2/\mu l$	99.87 ± 18.26	–	–	94.80 ± 16.52	93.77 ± 17.63	–	–	89.87 ± 23.30
Lymphocyte	%	88.8 ± 4.1	–	–	90.2 ± 3.0	89.5 ± 1.8	–	–	93.5* ± 2.7
Monocyte	%	3.2 ± 2.1	–	–	2.0 ± 1.4	4.5 ± 2.2	–	–	2.0* ± 0.6
ASAT	U/*l*	80.5 ± 4.7	–	–	84.3 ± 4.5	69.0 ± 3.3	–	–	76.7 ± 10.6
ALAT	U/*l*	27.8 ± 4.4	–	–	28.5 ± 2.4	26.0 ± 2.0	–	–	25.3 ± 4.4
γGT	U/*l*	0.2 ± 0.4	–	–	ND	0.2 ± 0.4	–	–	ND
ALP	U/*l*	665.5 ± 139.2	–	–	551.7 ± 85.6	349.7 ± 26.3	–	–	419.2 ± 126.7
T–bil	mg/d*l*	ND	–	–	ND	0.02 ± 0.04	–	–	ND
BUN	mg/d*l*	17.88 ± 1.37	–	–	17.40 ± 2.31	20.02 ± 1.69	–	–	18.02 ± 2.33
Cr	mg/d*l*	0.43 ± 0.05	–	–	0.40 ± 0.00	0.42 ± 0.04	–	–	0.43 ± 0.05
Glucose	mg/d*l*	159.5 ± 15.7	–	–	156.8 ± 5.1	148.2 ± 10.4	–	–	148.7 ± 7.6
T–cho	mg/d*l*	69.5 ± 5.0	–	–	74.0 ± 11.3	81.0 ± 7.4	–	–	71.2* ± 5.1
TG	mg/d*l*	106.2 ± 71.3	–	–	100.3 ± 45.5	34.0 ± 19.2	–	–	29.2 ± 14.3
TP	g/d*l*	6.87 ± 0.30	–	–	6.98 ± 0.18	6.67 ± 0.25	–	–	6.68 ± 0.37
Albumin	g/d*l*	3.50 ± 0.09	–	–	3.52 ± 0.10	3.52 ± 0.15	–	–	3.60 ± 0.14
A/G		1.042 ± 0.070	–	–	1.015 ± 0.053	1.115 ± 0.035	–	–	1.173 ± 0.079
Ca	mg/d*l*	10.07 ± 0.26	–	–	9.95 ± 0.19	9.65 ± 0.26	–	–	9.68 ± 0.30
P	mg/d*l*	8.13 ± 0.25	–	–	8.32 ± 0.39	7.08 ± 0.33	–	–	7.07 ± 0.30
Na	mmol/*l*	144.0 ± 1.1	–	–	142.8 ± 1.3	143.0 ± 1.3	–	–	143.8 ± 0.8
K	mmol/*l*	4.38 ± 0.20	–	–	4.47 ± 0.12	4.07 ± 0.22	–	–	4.02 ± 0.12
Cl	mmol/*l*	99.8 ± 1.0	–	–	99.5 ± 0.5	102.3 ± 1.8	–	–	101.5 ± 1.2

ND: not detected. Each value is the Mean ± SD. Significantly different from control: *, $p<0.05$.

kg group, as compared with the control group.

At the same time, no significant differences in blood chemistry were observed between the control group and any treatment group after the indium-dosing period. After the recovery period, a significantly lower value of total cholesterol was observed in females of the 1,000 mg/kg group.

Urinalysis: In the examination during the dosing period, a significantly higher value of urobilinogen was observed in females of the 1,000 mg/kg group, as compared with the control group (data not shown).

Pathological and histopathological examination: The necropsy and histopathological findings are shown in Tables 2 and 3, respectively. There were no abnormalities attributed to the administration of indium in any male or female in these examinations. However, in the necropsy, we found significantly lower values of absolute kidney weights in female rats of the 200 mg/kg group after the dosing period (Table 2). In addition, a brown patch in the lungs (one male, 200 mg/kg group), an hepatodiaphragmatic nodule in the liver (one male, 200 mg/kg group), pelvic dilatation in the kidneys (one male, 40 mg/kg group), unilateral ureteral dilatation (one male, 40 mg/kg group), and thickening of the cranial bone (one female, 200 mg/kg group) were observed.

Furthermore, the following spontaneous changes in histopathological findings were observed in animals of the 1,000 mg/kg groups after the dosing period: focal myocardial degeneration/fibrosis in the heart, periportal fatty change of hepatocytes, focal infiltration of inflammatory cells and microgranuloma in the liver, basophilic renal tubule, renal cysts, hyaline droplets of proximal tubular epithelium and focal inflammatory cell infiltration in the kidneys, and an increase in lipid droplets of the fascicular zone in the adrenals (Table 3). Most of the changes were also observed in the control group as well as in the 1,000 mg/kg group. In addition, a brown patch and inflammatory cell infiltration with slight hemorrhage of the lungs was observed in a male rat of the 200 mg/kg group, and thickening of the cranial bone was observed in a female rat of the 200 mg/kg group. Pelvic dilatation and dilatation of the distal tube of the kidneys, and unilateral ureter dilatation with inflammatory cell infiltration was also found in a male rat of the 40 mg/kg group.

Discussion

The aim of the present study was to characterize the potential toxic effects of indium and the reversibility of these effects. Although occupational indium exposure primarily occurs through inhalation, indium compounds are used in medical treatments such as in materials for dental crowns or inlays. Therefore, our indium ingestion experiments will provide important information regarding the toxic effects of indium as a basic toxicological study.

We found no changes attributable to indium administered to rats in an acute oral toxicity study. Although pelvic dilatation of the kidneys was observed in one male of the 2,000 mg/kg group, this change was considered to be a spontaneous change because no abnormalities were found in urinalysis and blood chemistry. Based on our results, we conclude that the lethal dose 50% (LD_{50}) dosage is greater than 2,000 mg/kg for both sexes.

In the 28-day repeated oral dose toxicity study, a significantly higher value of the ratio of lymphocytes and a significantly lower value of the ratio of monocytes were observed in females of the 1,000 mg/kg after the recovery period. Concurrently, a significantly lower value of total cholesterol was observed in the same group after the recovery period. These changes may not be related to indium because they were not observed at the end of the dosing period, and both total white blood cell count and overall nutritional condition were stable. Also, a significantly higher value of urobilinogen was observed in females of the 1,000 mg/kg group during the dosing period. We considered that this change was not necessarily related to indium because neither liver dysfunction nor anemia coincided with it. Experiments using more animals might demonstrate the irrelevancy of indium to changes of parameters more clearly.

In the pathological examination, we found significantly lower values of absolute kidney weights in female rats of the 200 mg/kg group after the dosing period. However, this difference was considered as a spontaneous change because it occurred without dose-dependency, and relative kidney weights were not significantly different between treated groups and the control group. Some histopathological changes were observed in several rats. These findings were also considered as changes that were unrelated to indium because they were slight, nonspecific, and not dose-dependent.

Thus, we conclude that there were no changes related to indium with regard to clinical signs, body weights, food consumption, hematology, blood chemistry, urinalysis, organ weights, necropsy, or histopathological findings in the 28-day repeated oral dose toxicity study. As a result of these findings, we determined the no-observed-adverse-effect level (NOAEL) of indium to be 1,000 mg/kg for males and females.

Today, indium is widely used in the electronics industry. Several kinds of indium-containing materials such as indium arsenide, indium phosphide, and indium-tin oxide are used to make semiconductors, injection lasers, solar cells, photodiodes, and light-emitting diodes. Concerns about occupational exposure to indium are rising with the increasing use of these materials.

The toxicity of inhaled indium has been shown in

Table 2. Organ weights in the 28–day repeated oral dose toxicity study

a) Absolute organ weights

		Male				Female			
Indium dose		0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg	0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg
Animal number		6	6	6	6	6	6	6	6
Final body weight	g	427.0 ± 33.7	401.0 ± 24.5	419.5 ± 33.2	398.7 ± 30.5	248.5 ± 19.5	234.8 ± 19.0	239.0 ± 19.8	241.8 ± 14.4
Brain	g	2.703 ± 0.135	2.005 ± 0.140	2.052 ± 0.061	2.058 ± 0.040	1.853 ± 0.078	1.855 ± 0.071	1.935 ± 0.063	1.890 ± 0.090
Thymus	mg	663.8 ± 124.3	621.0 ± 95.5	586.7 ± 96.8	576.0 ± 54.6	542.7 ± 135.6	518.5 ± 74.9	483.7 ± 58.4	542.8 ± 99.8
Liver	g	17.11 ± 1.92	15.32 ± 1.46	16.73 ± 1.83	15.29 ± 1.52	9.518 ± 1.375	8.717 ± 0.655	8.823 ± 1.074	9.433 ± 0.875
Spleen	g	0.883 ± 0.117	0.773 ± 0.092	0.808 ± 0.099	0.763 ± 0.103	0.548 ± 0.044	0.535 ± 0.066	0.562 ± 0.098	0.587 ± 0.096
Kidneys	g	2.935 ± 0.217	2.882 ± 0.429	2.940 ± 0.193	2.715 ± 0.309	1.818 ± 0.091	1.705 ± 0.136	1.655* ± 0.084	1.802 ± 0.076
Adrenals	mg	56.10 ± 7.51	55.25 ± 4.56	55.93 ± 5.40	54.75 ± 1.88	64.18 ± 9.93	62.82 ± 8.31	63.40 ± 4.03	70.55 ± 8.16
Testis	g	3.230 ± 0.265	3.270 ± 0.125	3.272 ± 0.161	3.310 ± 0.218	–	–	–	–
Ovaries	mg	–	–	–	–	99.42 ± 17.59	85.27 ± 11.85	98.10 ± 14.60	96.77 ± 13.79

Each value is the Mean ± SD. Significantly different from control: *, $p<0.05$.

b) Relative organ weights

		Male				Female			
Indium dose		0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg	0 mg/kg	40 mg/kg	200 mg/kg	1,000 mg/kg
Animal number		6	6	6	6	6	6	6	6
Final body weight	%	100	100	100	100	100	100	100	100
Brain	%	0.488 ± 0.056	0.500 ± 0.032	0.490 ± 0.028	0.520 ± 0.034	0.748 ± 0.076	0.793 ± 0.052	0.813 ± 0.066	0.783 ± 0.045
Thymus	%	0.156 ± 0.033	0.155 ± 0.025	0.139 ± 0.017	0.145 ± 0.020	0.217 ± 0.044	0.221 ± 0.031	0.184 ± 0.021	0.225 ± 0.042
Liver	%	4.000 ± 0.170	3.818 ± 0.207	3.985 ± 0.208	3.832 ± 0.148	3.813 ± 0.261	3.717 ± 0.144	3.683 ± 0.177	3.898 ± 0.222
Spleen	%	0.207 ± 0.019	0.192 ± 0.027	0.193 ± 0.014	0.193 ± 0.028	0.222 ± 0.023	0.227 ± 0.019	0.235 ± 0.032	0.243 ± 0.029
Kidneys	%	0.688 ± 0.053	0.717 ± 0.090	0.702 ± 0.023	0.680 ± 0.055	0.735 ± 0.028	0.727 ± 0.063	0.697 ± 0.039	0.747 ± 0.047
Adrenals	$*10^{-3}$%	13.20 ± 2.17	13.78 ± 0.97	13.42 ± 2.02	13.80 ± 1.07	25.82 ± 3.26	26.75 ± 2.93	26.67 ± 2.57	29.22 ± 3.40
Testis	%	0.757 ± 0.069	0.818 ± 0.074	0.783 ± 0.079	0.833 ± 0.055	–	–	–	–
Ovaries	$*10^{-3}$%	–	–	–	–	39.83 ± 4.63	36.50 ± 5.61	41.05 ± 5.06	39.97 ± 4.59

Relative organ weights: (Organ weight/Final body weight)*100, Each value is the Mean ± SD.

Table 3. Histological findings in the 28-day repeated oral dose toxicity study

	Male		Female	
Indium dose	0 mg/kg	1,000 mg/kg	0 mg/kg	1,000 mg/kg
Animal number	6	6	6	6
Heart				
Myocardial degeneration/fibrosis, focal	2	1	1	2
Liver				
Fatty change, hepatocyte, periportal	0	0	2	2
Inflammatory cell infiltration, focal	0	1	1	2
Microgranuloma	1	0	2	2
Kidney				
Basophilic tubule	3	3	1	1
Cyst	0	0	1	2
Hyaline droplet, tubular epithelium, proximal	6	6	0	0
Inflammatory cell infiltration, lymphocyte, interstitium, focal	2	1	0	0
Mineralization, corticomedullary junction	0	0	1	0
Adrenal				
Increase in lipid droplet, fascicular zone	0	1	0	0

Each value is the number of animals with histological findings.

several reports[3–7]. Pulmonary inflammation (especially, interstitial pneumonia) and pulmonary carcinogenesis is known to occur as a result of indium inhalation in animal experiments. For example, Blazka *et al*. have reported that indium trichloride induces an inflammatory response and fibrosis of the lung in rats[3, 4]. In addition, the National Toxicology Program has shown that incidents of alveolar and bronchiolar adenomas and carcinomas are increased by continuous inhalation of indium for 2 yr both in rats and in mice. Increased incidences of benign and malignant pheochromocytomas of the adrenal gland in rats and hepatocellular neoplasms in mice have also been observed[6]. Furthermore, Gottschling *et al*. have suggested that oxidative stress is associated with pulmonary inflammation induced by indium phosphide inhalation, which also results in progression to atypical hyperplasia and neoplasia[7].

In Japan, several case reports have been published in recent years, and these reports clearly demonstrate that occupational indium inhalation affects human health. Homma *et al*. reported the first case of interstitial pneumonia induced by inhaled indium in 2003[1]. The affected person had worked for 3 yr in a metal-processing factory, where indium-tin oxide sputtering targets were produced for transparent conductive films used in flat panel displays, and died in 2001 due to bilateral pneumothorax. On histopathological examination, numerous fine particles were observed in this person's alveolar spaces, alveolar septum, and bronchiolar lumens. These particles were considered to be indium as a result of examination using a scanning electron microscope; therefore, the authors concluded that the interstitial pneumonia of this case was induced by indium. Homma *et al*. also reported another, non-fatal case in 2005[2].

Thereafter, several cross-sectional studies have been performed which have confirmed the association of indium inhalation with interstitial pulmonary disease[12–14]. For instance, Chonan *et al*. have indicated that higher serum levels of KL-6 (a sensitive marker of interstitial lung disease) and high-resolution computed tomography abnormalities are prevalent among indium workers, and that serum indium concentrations, which reflect the amount of inhaled indium, are positively correlated with these abnormalities[12].

On the other hand, no previous research has been done to examine the oral toxicity of indium, except Zheng *et al*. who have reported that indium is poorly absorbed from the gastrointestinal tract in both single and multiple oral dose animal experiments[15]. In addition, since most serum indium is thought to be derived from inhaled indium and it seems to take a long time to decrease serum indium levels[16], oral administration of indium is unlikely to affect health conditions. Actually, in the animal experiments of the present study, no adverse health effects were observed. Although the toxic effects of indium administered orally to humans should be confirmed in further studies, inhaled indium might be more toxic than orally administered indium.

In conclusion, the toxicity of indium administered orally was not observed under the conditions tested in our study. We should pay closer attention to the adverse effects of inhaled indium.

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