

Study of the compatibility of oral magnesium oxide preparations sold in Japan with the ICH-Q3D guideline for elemental impurities

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SUMMARY Magnesium oxide has been widely used as an antacid and constipation remedy. Currently in Japan, magnesium oxide preparations manufactured by five medical companies are marketed as prescribed generic drugs. In this study, we focused on metal elemental impurities present in 330 mg magnesium oxide tablets manufactured by each of these companies. The content of such impurities was determined by atomic absorption spectrometry and inductively coupled plasma mass spectrometry. We confirmed whether the content conformed to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guideline for Elemental Impurities (ICH-Q3D) based on the 30% control threshold. The content of these impurities varied among the five products (preparations A-E), but in all cases met the oral permitted daily exposure (PDE) criteria stipulated in ICH-Q3D. In 5 lots of preparation C and all lots of preparation D, the equivalent cadmium (Cd) intake for a daily maximum dosage of 2 g was higher than the 30% control threshold of 1.5 µg/day. By cluster analysis, preparations A-E were classified into preparations A + B and C + D + E and/or preparations A + B, C + D and E. The present study showed that all 5 preparations sold in Japan meet the PDE value standard of ICH-Q3D, and that preparations A and B meet the 30% control threshold. It is important that for preparations failing to meet the criteria, further improvements need to be sought, and impurities in magnesium oxide preparations need to be monitored to ensure their safety.

Keywords Magnesium oxide, metal impurity, ICH-Q3D, control threshold, cluster analysis

1. Introduction

In Japan, magnesium oxide preparations are widely used as low-priced, non-addictive laxatives, and can be used relatively safely by pregnant women and children. With long-term administration, however, it is necessary to pay attention to cumulative effects of any impurities, particularly metal impurities, in the preparation. In addition, for use by pregnant women and children, it is important to comply with the stipulated standards for impurities, as in such cases higher-purity preparations are required.

In recent years, due to globalization of the pharmaceutical market, the need for international risk assessment of metal impurities has been discussed at the International Conference on Harmonization of Technical

Requirements for Registration of Pharmaceuticals for Human Use (ICH). Therefore, the ICH Guideline for Elemental Impurities (ICH-Q3D) for oral preparations has been studied. Table 1 shows the permitted daily exposure (PDE) and control threshold for elemental metal impurities regulated by the ICH-Q3D guidelines in pharmaceutical products. They are grouped into 4 classes based on the toxicity of each element and the likelihood of them appearing as contaminants in pharmaceutical products (1). Elemental metal impurities in preparations can originate from not only the drug substance itself and additives, but also several factors such as the materials and containers used in the manufacturing process and the abundance of the elements themselves in nature (1). Class 1 cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) are highly toxic elements and are subject

Table 1. Oral PDE and 30% control threshold for metals in ICH-Q3D

Class (Toxicity)	Element	Oral PDE (µg/day)	Oral concentration (µg/g) [Option 1]	Maximum daily intake in 2 g/day (µg/g) [Option 3]	30% Control threshold in 2 g/day (µg/g)
1 (Strong)	Cd	5	0.5	2.5	0.75
	Pd	5	0.5	2.5	0.75
	As	15	1.5	7.5	2.25
	Hg	30	3	15	4.5
2A (Moderate)	Co	50	5	25	7.5
	V	100	10	50	15
	Ni	200	20	100	30
2B (Moderate)	Tl	8	0.8	4	1.2
	Au	100	10	50	15
	Pd	100	10	50	15
	Ir	100	10	50	15
	Os	100	10	50	15
	Rh	100	10	50	15
	Ru	100	10	50	15
	Pt	100	10	50	15
	Se	150	15	75	22.5
	Ag	150	15	75	22.5
	3 (Moderate)	Li	550	55	275
Sb		1,200	120	600	180
Ba		1,400	140	700	210
Mo		3,000	300	1,500	450
Cu		3,000	300	1,500	450
Sn		6,000	600	3,000	900
Cr		11,000	1,100	5,500	1,650

PDE: permitted daily exposure.

to the strictest regulatory values. Class 2 elements are those that exhibit toxicity depending on the route of administration. Class 2A cobalt (Co), vanadium (V), and nickel (Ni) are considered highly likely to be present in pharmaceutical products, and therefore risk assessment is essential. It is necessary to prove that Class 2B and Class 3 elements, which are considered unlikely to be present in pharmaceutical products, are below the regulated values when used in catalysts, *etc.* during the manufacture of pharmaceutical products (1). ICH-Q3D stipulates four options - 1, 2a, 2b, and 3 - as risk evaluation methods. Option 1 involves setting an allowable concentration limit for a common element among the components of a drug with a daily intake of 10 grams or less. Option 2a involves setting the permissible concentration limit for an element among the components of a drug for which the daily intake is specified. Option 2b involves setting the permissible concentration limit of elements among the individual components of a product for which the daily intake is specified. Option 3 involves confirming that the elements present in the final product are below the permissible concentration limit (1).

As shown in Table 1, Cd and Pb have a PDE of 5 µg/day for a body weight of 50 kg. In Japan, a maximum of 2 g of magnesium oxide is taken daily for constipation (2). Therefore, when 2.5 µg of Cd and Pb is mixed in a preparation containing 1 g of magnesium oxide, the standard is exceeded. Cd causes Itai-itai disease at high concentrations, but it accumulates in the kidneys and

liver stores even at even low concentrations and is highly toxic (3). Pb has a half-life of about 28 - 36 days in blood and soft tissues, but disappears when taken up by bone and has a long half-life in the order of years (4). In addition, the *in vivo* stagnation rate of Pb is higher in children than in adults. A concentration of 10 µg/dL causes acute poisoning of the blood and nervous system, and long-term exposure also results in chronic poisoning symptoms such as angiopathy, neuropathy and cerebral edema, as well as carcinogenicity and reproductive and developmental toxicity (5).

We have previously reported the results of a pilot survey of the content of heavy metal impurities in magnesium oxide preparations, pointing out differences between the preparations (6). In the present study, the actual condition of metal impurities contained in multiple magnesium oxide preparations sold in Japan was investigated in detail by analyzing the final products. Then, in consideration of the control threshold according to option 3 recommended by ICH-Q3D, the suitability of applying the ICH-Q3D standard to the preparations sold in Japan was examined. Furthermore, we used cluster analysis to examine the risk classification of the drugs.

2. Materials and Methods

2.1. Survey preparation

In April 2020, various dosage forms of magnesium

Table 2. The magnesium oxide formulations available on the Japanese market chosen for the study

Preparation	Formulation						
	200 mg Tablet	250 mg Tablet	300 mg Tablet	330 mg Tablet	400 mg Tablet	500 mg Tablet	Fine Granules 83%
A	○	○		○		○	○
B		○		○		○	
C	○	○	○	○	○	○	○
D		○		○		○	○
E		○		○			

○: Commercially available.

oxide preparations commercially available from 5 pharmaceutical companies (Kenei Pharmaceutical Co. Ltd., Osaka, Japan; Kyowa Chemical Industry Co. Ltd., Kagawa, Japan; Mochida Pharmaceutical Co. Ltd., Tokyo, Japan; Mylan, Tokyo, Japan; Yoshida Pharmaceutical Co. Ltd., Tokyo, Japan) were obtained (Table 2). Among those preparations, 330 mg tablet formulations of magnesium oxide were targeted for the research. Six different lots marketed between 2018 and 2020 were used.

2.2. Tablet weight measurement

Sixty tablets from each lot of each company's preparation were collected and weighed.

2.3. Quantitative analysis of Cd and Pb

Tablets of each preparation were crushed, and after acid treatment of 1 g of the powder, the sample was heated in a muffle furnace at 600°C for 3 hours. The incinerated sample was then dissolved in acid to make a 30 mL solution. The supernatant was separated and the pH adjusted to 5 with 20% aqueous ammonia and 70% nitric acid. The liquid was passed through a chelate resin Novia Skilate column (CHELATE-PA1, Hitachi High-Tech Science Corp., Tokyo, Japan), and Cd and Pb were adsorbed and separated from the matrix metal Mg. Cd and Pb on the chelate resin were then eluted off with 3N nitric acid solution to prepare a measurement solution. Inductively coupled plasma mass spectrometry (ICP-MS) (ELAN DRC II, PerkinElmer Japan Co. Ltd., Kanagawa, Japan) was used to quantify Cd and Pb by the ICP-MS method employing absolute calibration.

2.4. Quantitative analysis of As

Tablets of each preparation were crushed and 0.5 g of the powder was collected, followed by addition of 5 mL of sulfuric acid : nitric acid (1:1) and heat treatment. Then 4 mL of 20% potassium iodide solution was added, the volume was adjusted to 50 mL, and the mixture was left at room temperature for 1 hour. After being allowing to cool, As was quantified using the absolute calibration curve obtained by hydride generation atomic absorption spectrophotometry (AA-

7000, Shimadzu Corp., Kyoto, Japan).

2.5. Quantitative analysis of Hg

Tablets of each preparation were crushed and 1 g of the powder was subjected to acid treatment, followed by addition of a solution of sulfuric acid, nitric acid and potassium permanganate and heat treatment. After being allowing to cool, Hg was quantified using the absolute calibration curve obtained by the reduced vaporization atomic absorption method using a mercury measurement device (HG-400, HIRANUMA Co. Ltd., Ibaraki, Japan).

2.6. Quantitative analysis of Co, Ni, V, Sn and Cr

Tablets of each preparation were crushed and 1 g of the powder was subjected to acid treatment, followed by heating in a muffle furnace at 600°C for 3 hours. The incinerated sample was then dissolved in acid to make 30 mL of solution. The supernatant was separated and ICP-MS (ELAN DRC II, PerkinElmer Japan Co. Ltd.) was used to quantify Co, Ni, V, Sn and Cr by the ICP-MS method using absolute calibration.

2.7. Reagents

ICP-MS analysis standard solution, Cd standard solution, Pb standard solution, As standard solution, Hg standard solution, and Sn standard solution were obtained from FUJIFILM Wako Chemical Corp. (Miyazaki, Japan). For the Co, Ni, V, Sn, and Cr mixed standard solution, a mixed standard solution for ICP-MS analysis was obtained from SPEX (New Jersey, USA). Sulfuric acid, nitric acid, potassium permanganate solution, potassium iodide solution and other reagents (FUJIFILM Wako Chemical Corp.) used were analytical grade. The water used was ultrapure.

2.8. Calculation of acceptable daily intake (ADI)

For Cd, Pb, and As, ADI was calculated using the following formula based on the quantitative results for each formulation.

$$\text{ADI (g/day)} = \text{Oral PDE (}\mu\text{g/day)} / \text{Impurity (}\mu\text{g/g)}$$

2.9. Characterization of heavy metals present in magnesium oxide preparations using cluster analysis

Cluster analysis was performed using JMP Pro13.2.0 (SAS Institute Inc. NC, USA). The heavy metal contents were used to interpret the characteristics of magnesium oxide preparations. Hierarchical cluster analysis was used to classify the magnesium oxide preparations objectively. This analysis used the Ward method based on Euclidean distance from the heavy metal contents. The hierarchical cluster analysis established two clusters.

2.10. Statistics

The experimental data are shown as mean \pm standard error (SE). One-way ANOVA and Tukey's multiple comparison method were used to test the differences in means between each group using JMP Pro13.2.0 (SAS Institute Inc. NC, USA). The significance level was set at $p < 0.05$.

3. Results

3.1. Comparison of weight of each tablet and magnesium oxide content per gram of preparation

The measured weights of the tablets for the 6 lots of magnesium oxide preparations manufactured by drug companies A, B, C, D and E, respectively, are shown in Table 3. There was no significant difference in mass between the preparations, and the differences were all within 10%.

Table 3. Tablet weights of magnesium oxide preparations and their magnesium oxide contents

Preparation	Weight of tablet (g/tablet)	Contents of magnesium oxide (g/1 g)
A	0.378 [0.376-0.378]	0.882 [0.880-0.885]
B	0.395 [0.394-0.397]	0.843 [0.838-0.846]
C	0.396 [0.396-0.397]	0.841 [0.839-0.841]
D	0.408 [0.406-0.410]	0.816 [0.811-0.820]
E	0.402 [0.401-0.403]	0.829 [0.826-0.831]

$n = 6$, mean [range].

3.2. Comparison of metal element impurities per gram of each preparation

Table 4 shows the measured contents of elemental metal impurities contained in preparations A, B, C, D and E. The content of elemental metal impurities varied depending on the preparation, but all of them conformed to the PDE value defined by ICH-Q3D.

The PDE value for Cd in Class 1 is defined as 5 $\mu\text{g}/\text{day}$. The Cd contents of preparations A and B were less than 0.02 $\mu\text{g}/\text{g}$ and 0.01 $\mu\text{g}/\text{g}$, respectively, being significantly lower than those in preparations C, D and E ($p < 0.05$). The PDE value for Pb is 5 $\mu\text{g}/\text{day}$. The Pb contents of preparations C, D and E were 0.18 $\mu\text{g}/\text{g}$, 0.13 $\mu\text{g}/\text{g}$ and 0.12 $\mu\text{g}/\text{g}$, respectively, being significantly higher than those in preparations A and B ($p < 0.05$). The PDE value for As is 15 $\mu\text{g}/\text{day}$. The As contents of preparations C, D and E were 1.57 $\mu\text{g}/\text{g}$, 1.27 $\mu\text{g}/\text{g}$ and 1.70 $\mu\text{g}/\text{g}$, respectively, being significantly higher than those in preparations A and B ($p < 0.05$). The PDE value for Hg is 30 $\mu\text{g}/\text{day}$. The Hg contents of all the preparations were below the measurement limit of 0.05 $\mu\text{g}/\text{g}$.

The PDE value for Class 2A Co is 50 $\mu\text{g}/\text{day}$. The Co content was below the measurement limit of 0.50 $\mu\text{g}/\text{g}$ in all the preparations. The PDE value for V is 100 $\mu\text{g}/\text{day}$. The V content of preparation E was 3.12 $\mu\text{g}/\text{g}$, which was the highest. The V contents of preparations C, D and E were significantly higher than those in preparations A and B, respectively ($p < 0.05$). The PDE value for Ni is 200 $\mu\text{g}/\text{day}$. The Ni content of preparation E was the highest, at 2.55 $\mu\text{g}/\text{g}$. The Ni contents of preparations C, D and E were significantly higher than those of preparations A and B ($p < 0.05$).

The PDE value for Class 3 Sn is 6,000 $\mu\text{g}/\text{day}$. The Sn content of all preparations was less than 0.50 $\mu\text{g}/\text{g}$. The PDE value for Cr is 11,000 $\mu\text{g}/\text{day}$. The Cr content was highest in preparation E, at 6.12 $\mu\text{g}/\text{g}$.

3.3. Comparison of heavy metal element impurity contents of various magnesium oxide formulations at maximum daily dose

ICH-Q3D defines an accurate risk assessment process

Table 4. Pharmaceutical data for elemental impurities in 1 g magnesium oxide preparations

Element	Oral PDE ($\mu\text{g}/\text{day}$)	A	B	C	D	E
Cd	5	0.02 \pm 0.00	0.01 \pm 0.00	0.71 \pm 0.05	0.83 \pm 0.12	0.26 \pm 0.02
Pb	5	0.02 \pm 0.00	0.02 \pm 0.00	0.18 \pm 0.01	0.13 \pm 0.01	0.12 \pm 0.01
As	15	< 0.10	< 0.10	1.57 \pm 0.10	1.27 \pm 0.04	1.70 \pm 0.16
Hg	30	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Co	50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
V	100	< 0.50	< 0.50	2.47 \pm 0.11	2.75 \pm 0.41	3.12 \pm 0.51
Ni	200	0.68 \pm 0.05	1.18 \pm 0.05	1.97 \pm 0.11	2.33 \pm 0.27	2.55 \pm 0.15
Sn	6,000	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
Cr	11,000	< 0.50	2.93 \pm 0.48	4.25 \pm 0.19	5.78 \pm 0.57	6.12 \pm 0.19

$n = 6$, mean \pm SE ($\mu\text{g}/\text{g}$), PDE: permitted daily exposure.

by setting a 30% control threshold to ensure that the elements present in the product are below the permissible concentration PDE. In this study, the content of metal element impurities at the maximum daily dose of 2 g (Option 3) of magnesium oxide described in the attached document was compared for 6 lots of preparations A-E based on the 30% control threshold (Figure 1). In 5 lots of preparation C and all lots of preparation D, Cd present as a metal element impurity in the maximum daily dose of 2 g of magnesium oxide showed a value higher than

the 30% control threshold (Figure 1a).

Table 5 shows the 30% control threshold value at the maximum daily dose of 2 g of magnesium oxide and the contents of the metal element impurities in 6 lots of preparations A to E. The underlined numbers indicate values that exceed the 30% control threshold.

3.4. Risk classification of preparations by cluster analysis

A cluster analysis was performed based on the data in

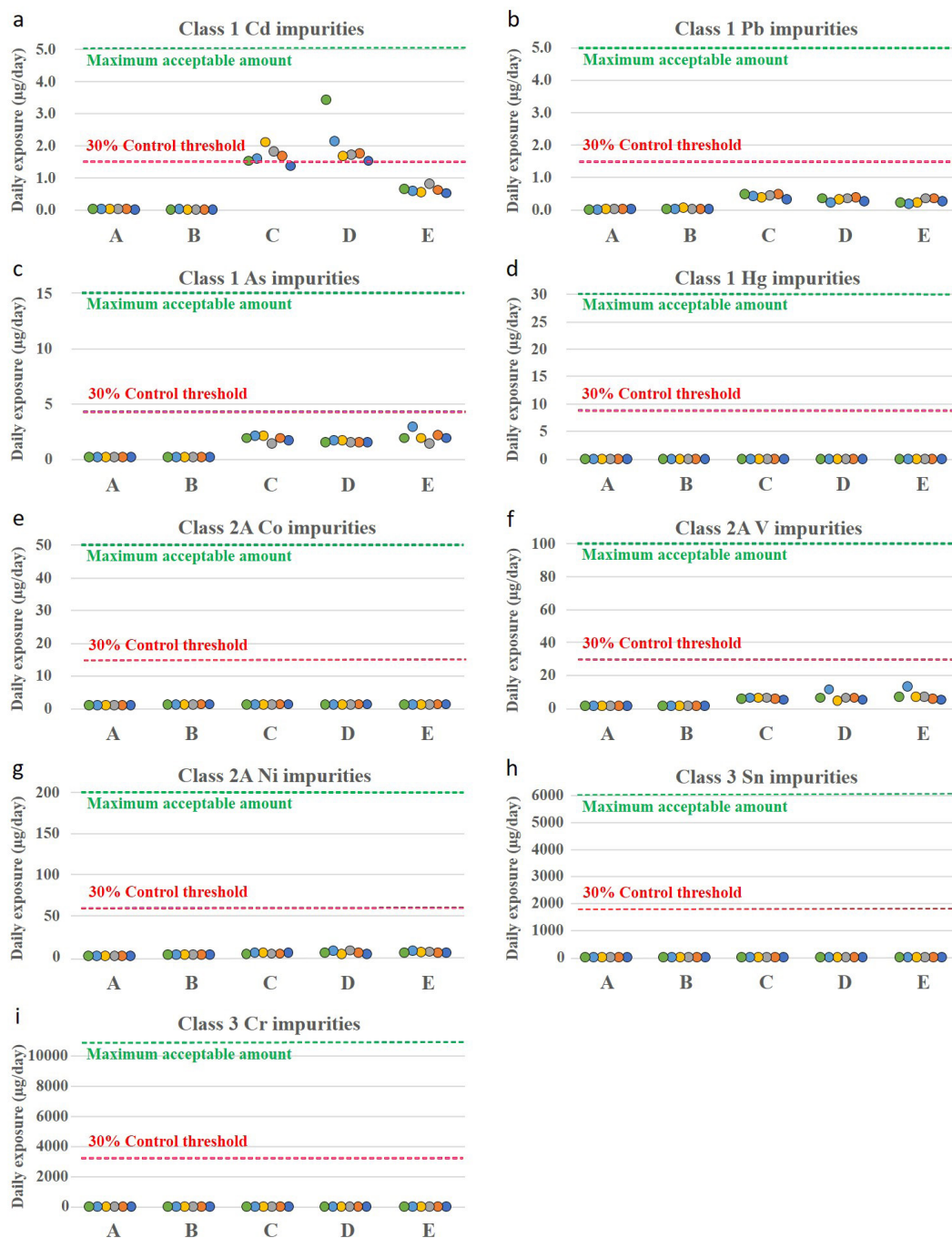


Figure 1. Heavy metal contents of the various magnesium oxide formulations for maximum daily administration. The ordinates represent daily intake exposure for each heavy metal element at the maximum daily dose of 2 g magnesium oxide. Abscissae represent preparations A-E. Each point represents one lot of each preparation. Green line indicates acceptable maximum daily intake, and red line indicates the 30% control threshold based on oral PDE at maximum daily dose of 2 g magnesium oxide.

Table 5. Pharmaceutical data for elemental impurities in magnesium oxide preparation at a maximum daily dose of 2 g

Element	30% Control threshold ($\mu\text{g}/\text{day}$)	A	B	C	D	E
Cd	1.5	0.05 ± 0.01 [0.02 - 0.05]	0.03 ± 0.01 [0.02 - 0.05]	1.68 ± 0.11 [1.35 - 2.12]	2.04 ± 0.39 [1.51 - 3.43]	0.63 ± 0.04 [0.51 - 0.82]
Pb	1.5	0.04 ± 0.01 [0.02 - 0.05]	0.05 ± 0.01 [0.05 - 0.07]	0.42 ± 0.03 [0.31 - 0.50]	0.32 ± 0.03 [0.22 - 0.39]	0.28 ± 0.02 [0.19 - 0.36]
As	4.5	< 0.20	< 0.20	1.85 ± 0.11 [1.40 - 2.10]	1.57 ± 0.05 [1.50 - 1.70]	2.03 ± 0.20 [1.40 - 2.90]
Hg	9	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Co	15	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
V	30	< 1.0	< 1.0	5.88 ± 0.29 [5.00 - 6.70]	6.75 ± 1.04 [4.40 - 11.60]	7.53 ± 1.25 [5.10 - 13.50]
Ni	60	1.57 ± 0.10 [1.40 - 2.00]	2.80 ± 0.15 [2.40 - 3.30]	4.67 ± 0.32 [4.00 - 5.70]	5.72 ± 0.69 [4.10 - 8.10]	6.17 ± 0.39 [5.10 - 7.00]
Sn	1,800	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cr	3,300	< 1.0	6.98 ± 1.22 [3.10 - 10.70]	10.12 ± 0.59 [8.10 - 11.20]	14.20 ± 1.41 [9.10 - 19.50]	14.72 ± 0.45 [14.00 - 16.80]

$n = 6$, mean \pm SE [range] ($\mu\text{g}/\text{day}$), 30% Control thresholds are calculated from PDE, underbars represents value over 30% control threshold.

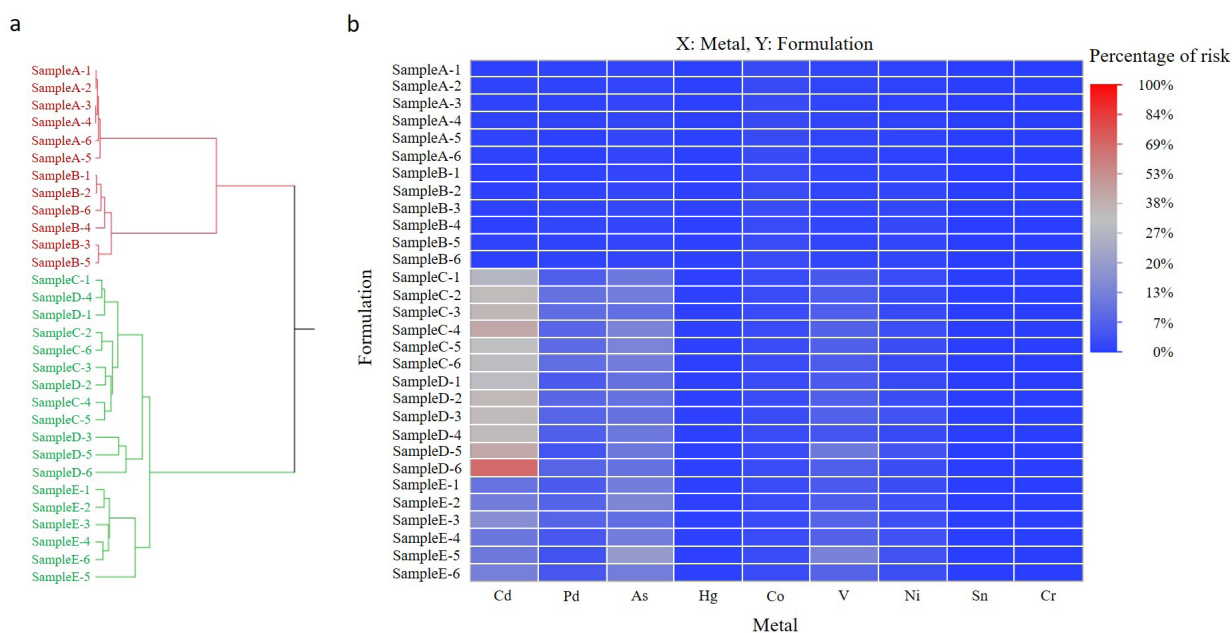


Figure 2. Cluster analysis based on the 30% control threshold for heavy metal contents of each magnesium oxide preparation. The dataset in Figure 1 was used for the cluster analysis. (a): Hierarchical cluster analysis showing the presence of two major groups. (b): Heat map obtained by cluster analysis. Red color shows high impurity and high risk, and blue color shows low impurity and low risk.

Figure 1 and presented as a heat map diagram (Figure 2). The color of the heat map was set to switch from blue to red at the 30% control threshold of PDE, which is the control threshold. Cluster analysis suggested that preparations A-E were classified into two clusters, preparations A + B and preparations C + D + E, or three clusters, preparations A + B, preparations C + D, and preparation E. Preparations A and B were shown to be low in metal impurities and to have a low risk of adverse events due to such impurities.

4. Discussion

Magnesium oxide preparations are pharmaceutical

products containing metal elements. For their manufacture, magnesium is collected from nature as a raw material, then concentrated and purified. Therefore, to produce magnesium oxide pharmaceuticals for humans use, highly industrial technology is required, and it is important to monitor the various metal impurities mixed during both the purification of the raw magnesium and the subsequent commercial processing. In this study, we investigated whether the metal element impurities contained in the magnesium oxide preparations sold in Japan meet the standards specified in ICH-Q3D. We also conducted cluster analysis based on the control thresholds and the adverse effect risk of impurities for Class 1 (Cd, Pb, As, Hg), Class 2A (Co, V, Ni) and Class

3 (Sn, Cr) of each preparation.

It was found that the contents of Cd, Pb, As, and Hg, which have strong toxicity, in the manufactured products, did not contravene the oral PDE standard (Table 4). However, Cd (0.71 µg/g), Pb (0.18 µg/g) and As (1.57 µg/g) in preparation C, Cd (0.83 µg/g), Pb (0.13 µg/g) and As (1.27 µg/g) in preparation D, and Cd (0.26 µg/g), Pb (0.12 µg/g) and As (1.70 µg/g) in preparation E were present as elemental metal impurities. Their contents in preparations C, D and E were significantly higher than those in preparations A and B, respectively ($p < 0.05$). Data for the ADI of magnesium oxide calculated from the contents of Cd and Pb in preparations C and D showed that the ADI for Cd was 7.08 g/day (preparation C) and 6.00 g/day (preparation D) and that for Pb was 28.0 g/day (preparation C) and 38.0 g/day (preparation D). When magnesium oxide preparations are used as laxatives, the maximum daily dose for adults is 2 g/day as magnesium oxide, and therefore it can be said that none of these preparations exceeded the oral PDE value for Cd. As the PDE value for Pb was exceeded when the highest-content preparation C was taken at 28.0 g/day or more, there would be no problem with the use of a normal amount of magnesium oxide, *i.e.* 2 g/day. Furthermore, the Cd and Pb contents of the magnesium oxide preparations A and B are less than one-fifth of those in preparations C, D and E, and thus preparations A and B are considered unlikely to cause health problems.

As an index of the significance of the measured values of elemental impurities, the ICH-Q3D guideline defines the 30% level of the PDE value as a control threshold, and states that this control threshold should be used as a criterion for determining the necessity of further control of the preparation (1). As shown in Figure 1 and Table 5, when magnesium oxide is used clinically at a maximum daily intake of 2 g, the daily intake of Cd is 1.35-2.12 µg for preparation C and 1.51-3.43 µg for preparation D. Five lots of preparation C and all lots of preparation D, tested in this study exceeded the 30% control threshold of 1.5 µg. This suggests that for preparations C and D, it would be necessary to establish further control strategy to ensure that the PDE value is not exceeded.

In this study, based on the ICH-Q3D guidelines, risk classification based on the elemental metal impurity content of each of preparations A-E was attempted by cluster analysis. As shown in Figure 2, this demonstrated two clusters - preparations A + B and preparations C + D + E - or three clusters - preparations A + B, preparations C + D and preparation E. This also suggests that preparations A and B have low metal impurity contents and comply with the ICH-Q3D guidelines, presenting little risk to the living body. On the other hand, as shown in Figure 1 and Table 5, preparations C and D exceeded the 30% control threshold defined in the ICH-Q3D guideline, and preparations C + D were those considered to warrant additional management methods to ensure that

their oral PDE value was not exceeded.

Cd exists in mineral form in nature and is obtained mainly from Cd ore for commercial use (7). Cd is used as a catalyst in organic synthesis, and the problem with oral exposure is nephrotoxicity (8). Therefore, the PDE value is set at 5 µg/day using the evaluation index for nephrotoxicity.

Pb exists in nature as both organic and inorganic forms. Organic Pb compounds are decomposed fairly quickly in the atmosphere and remain as inorganic Pb compounds in water and soil. In humans, exposure to Pb can affect neural, reproductive, developmental, immune, cardiovascular and renal function. In general, susceptibility to Pb toxicity is higher for exposure during the fetal period and childhood than during adulthood. In addition, epidemiological data suggest that blood Pb levels below 5 µg/dL may be associated with neurobehavioral disorders in children (9). Based on these findings, the PDE value of Pb was set at 5 µg/day. Considering the nephrotoxicity of Cd and Pb mentioned above, this point should be taken into consideration when taking magnesium oxide preparations at doses higher than those stated in the package insert or in the case of long-term administration.

As is ubiquitous in the environment and found in food, soil, drinking water and the atmosphere. Inorganic As exists as trivalent (arsenic trioxide, sodium arsenate, *etc.*) or pentavalent (sodium arsenic, arsenic pentoxide, arsenic acid, *etc.*) forms and is highly toxic in the trivalent state. The state of the element is therefore important. Since organic As is also contained in seawater, it is abundant in seaweed. Since the toxicity of organic As is lower than that of inorganic As, and the latter is related to contamination of pharmaceutical products, the standard for safety evaluation is set for inorganic As. It is reported that ingestion of drinking water containing high doses of sodium arsenate by pregnant female rats significantly increased the incidence of liver cancer in next-generation male rats (10). Inorganic As is known to be a carcinogen to humans (11). Oral intake of As is associated with skin, liver, lung, kidney and bladder cancer. The PDE value when taken orally is set at 15 µg/day based on the chronic effect of As on the skin. In this study, the As content was highest for preparation E at 1.70 µg/g, followed by preparation C at 1.57 µg/g, but both met the standard PDE value and there was only a small possibility of health hazards due to chronic toxicity.

Hg is widely distributed in the global environment and exists in three forms: metallic, inorganic and organic Hg. The form of Hg remaining in drug preparations is usually inorganic Hg. Therefore, safety assessment of Hg has been based on toxicological data for metallic Hg and inorganic Hg. The International Agency for Research on Cancer (IARC) has concluded that the carcinogenicity of inorganic Hg to humans does not fit into the previous classification (12). Although inorganic Hg exhibits lower bioavailability *via* oral ingestion than organic Hg,

it has various toxic effects such as neurological effects, corrosiveness, hematopoietic and renal effects, and advanced pain in skin diseases. The underlying toxicity of safety standards for inorganic Hg and its salts is nephrotoxicity. The PDE value for Hg was 30 $\mu\text{g}/\text{day}$, and the Hg content of all the preparations in this survey was less than 0.05 $\mu\text{g}/\text{g}$, which was below the detection limit.

Co, which is classified as Class 2A, is a constituent element of vitamin B12 and an essential element acting as a coenzyme in hemoglobin synthesis. However, cobalt sulfate and other water-soluble Co salts may be carcinogenic to humans. In the case of repeated oral administration, erythrocytosis is the most common problem. Co is classified as Class 2A, because it is associated with heavy metal pneumoconiosis, asthma and contact dermatitis upon inhalation exposure (13,14). The PDE value at the time of oral administration was set at 50 $\mu\text{g}/\text{day}$, but in this study, the content of all of the preparations was less than 0.50 $\mu\text{g}/\text{g}$, which was not problematic.

V exists in the Earth's crust in various oxidized states. V classified into Class 2A as vanadium pentoxide has a carcinogenic risk to humans (7). The gastrointestinal tract, cardiovascular system and blood system are the main targets of its toxicity by oral administration to humans, and the PDE value is set at 100 $\mu\text{g}/\text{day}$. In this study, the V contents of preparations C, D and E were 2.47-3.12 $\mu\text{g}/\text{g}$, being higher than those of preparations A and B at less than 0.50 $\mu\text{g}/\text{g}$. However, all of the preparations were below the PDE value, and thus considered safe.

No report has indicated Ni carcinogenicity as a result of oral administration (15). High oral intake can cause stomach pain, weight loss and adverse effects on the blood and kidneys. It has also been reported that oral intake of Ni in drinking water induces dermatitis in humans (16). The PDE value was set to 200 $\mu\text{g}/\text{day}$, but all the products measured this time were below the PDE value and there was no problem.

Since Sn contained in pharmaceutical preparations contains more inorganic Sn than organic Sn, the safety evaluation is based on inorganic Sn. The problematic adverse effect of repeated oral administration is anemia (17). The PDE value for Sn is 6,000 $\mu\text{g}/\text{day}$, and the Sn content of preparations A-E was less than 0.50 $\mu\text{g}/\text{g}$, which was not considered problematic.

Since Class 3 classified Cr (6+) has strong oxidizing power, Cr-induced skin disorders and carcinogenicity have been confirmed (18). Cr contained in pharmaceutical products is often in the form of Cr (0) or Cr (3+) rather than the highly toxic Cr (6+). Therefore, drug safety assessments are based on Cr (3+) toxicity information, and Cr (6+) is excluded. Since no obvious health effects of oral intake of Cr (3+) have been identified, it is classified as Class 3. According to this measurement, the concentrations of Cr in

preparations B-E were relatively high, with the exception of preparation A. However, since the oral PDE value was set as high as 11,000 $\mu\text{g}/\text{day}$, the results for all the preparations were far below the PDE value, and it was considered that there was no problem.

Various potential sources of elemental impurities are: 1) Residual impurities resulting from elements added intentionally (*e.g.*, catalysts) in the production of the drug substance, excipients, or other drug product components. 2) Elemental impurities that are not added intentionally and are potentially present in the drug substance, water, or excipients used in the preparation of the drug product. 3) Elemental impurities can be potentially introduced into the drug substance and/or drug product from manufacturing equipment. 4) Elemental impurities that have the potential to be leached into the drug substance and drug product from container closure systems. Risk assessment of the drug substance should address the potential for inclusion of elemental impurities in the final drug product. The reason why the content of metal element impurities differed between the various surveyed preparations is considered to be that magnesium oxide is a compound that is affected by the conditions used for sampling of the raw material. Since the standard PDE value was not exceeded in any of the investigated preparations, there would be no problem if the daily dose was maintained within that stated in the package insert. However, for Cd, 5 lots of preparation C and all lots of preparation D exceeded the control threshold. From the viewpoint of risk management, it is necessary to carry out further controls such as changing the steps of the manufacturing process, setting standard values for additives and raw materials, or selecting an appropriate container plugging system for these preparations. In addition, the levels of metal impurities in preparations C, D and E showed were higher except for Hg, Co and Sn in preparations A and B, and the amount exceeded the dose stated in the package insert when the effect of magnesium oxide was insufficient. Therefore, an effect on the human body resulting from long-term administration, or administration to patients with renal impairment, cannot be ruled out. In this context, the present survey clarified that preparation A or B would be desirable in terms of the safety of its heavy metal content.

Currently, there are many generic drugs on the market that can reduce development costs in the pharmaceutical industry. In addition, the Ministry of Health, Labor and Welfare has a policy of promoting the transition from branded drugs to generic drugs with the aim of reducing national medical expenses. As products of the same drug diversify, even equivalence of efficacy and effect is guaranteed, the content of elemental metal impurities will vary depending on the raw materials and additives used by various pharmaceutical companies. Therefore, for long-term administration, it will be necessary to pay attention to accumulation of such impurities in the body.

In order to ensure the globalization of the pharmaceutical industry and the safety of pharmaceutical products, it is considered necessary to conduct preparation tests based on ICH-Q3D in the future. As shown in this study, there may be preparations that meet the current standards but may not meet the ICH standards in the future. For preparations that may not meet these criteria, it will be necessary to seek improvement, and at the same time it is suggested that monitoring of impurities will be required when the ICH standard is introduced.

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