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# Distribution of Various Nickel Compounds in Rat Organs After Oral Administration

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## ABSTRACT

In this study, eight kinds of nickel (Ni) compounds were orally administered to Wistar male rats and the distribution of each compound was investigated 24 h after the administration. The Ni compounds used in this experiment were nickel metal [Ni-M], nickel oxide (green) [NiO(G)], nickel oxide (black) [NiO(B)], nickel subsulfide [$Ni_3S_2$], nickel sulfide [NiS], nickel sulfate [$NiSO_4$], nickel chloride [$NiCl_2$], and nickel nitrate [$Ni(NO_3)_2$]. The solubilities of the nickel compounds in saline solution were in the following order; [$Ni(NO_3)_2$ > $NiCl_2$ > $NiSO_4$] >> [NiS > $Ni_3S_2$] > [NiO(B) > Ni-M > NiO(G)]. The Ni level in the visceral organs was higher in the rats given soluble Ni compounds; $Ni(NO_3)_2$, $NiCl_2$, $NiSO_4$, than that in the rats receiving other compounds. In the rats to which soluble Ni compounds were administered, 80–90% of the recovered Ni amounts in the examined organs was detected in the kidneys. On the other hand, the Ni concentration in organs administered scarcely soluble Ni compounds; NiO(B), NiO(G), and Ni-M were very low. The estimated absorbed fraction of each Ni compounds was increased with the increase of the solubility. These results suggest that the kinetic behavior of Ni compounds administered orally is closely related with the solubility of Ni compounds, and that the solubility of Ni compounds is one of the important factors for determining the health effect of Ni compounds.

**Index Entries:** Ni compounds; oral administration; chemical formula of Ni compounds; solubility; Ni distribution in organs; rat.

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## INTRODUCTION

Although a carcinogenic effect of nickel (Ni) has been suspected, the mechanism underlying the effect has not been clarified as yet.

Ni in the environment is taken into the human body through two routes, orally and through inhalation. The effects of Ni particles inhaled into the body have been reported *(1–3)*.

There are few reports, however, on the toxicity and distribution of Ni compounds taken orally *(4–6)*. The kinetic behaviors of various Ni chemical components taken orally and their effects therefore should be investigated.

In this study, eight Ni compounds, soluble and insoluble, were orally administered to rats, and the in vivo distributions of Ni with different chemical properties were examined.

## MATERIALS AND METHODS

### Test Substances

Eight Ni compounds used in this experiment are shown in Table 1; nickel metal [Ni-M], nickel oxide (green) [NiO(G)], nickel oxide (black) [NiO(B)], nickel subsulfide [$Ni_3S_2$], nickel sulfide [NiS], nickel sulfate [$NiSO_4$], nickel chloride [$NiCl_2$], and nickel nitrate [$Ni(NO_3)_2$].

### Solubility Test

A solubility test was performed for the following five Ni compounds; Ni-M, NiO(G), NiO(B), Ni3S2, and NiS. Three hundred milligrams of each of these compounds was put into 50 mL of distilled water and saline. The suspension was shaken for 1 wk at 37°C and filtered through a filter paper (Toyo filter paper, No. 5C, Ams., Japan) and a membrane filter (pore size 0.22 μm). The Ni concentration of the filtrate was determined by an atomic absorption spectrophotometry (Hitachi Polarized Zeeman Effect Atomic Absorption Spectrophotometer, Model Z-8000, Ams., Japan).

### Animal Experiment

The animals used in this study were Wistar male rats obtained from Kyudo Co., Ltd. (Ams., Japan). Seventy-two rats, 10 wk of age and weighing 200 g, were divided into eight experimental groups and one control group (eight rats per group). They were maintained in a glass metabolic cage for 2 d before the experiment.

Each Ni compound containing 10 mg of Ni was dissolved in a 5% starch saline solution and the solution was administered by gavage to the experimental animals. The control group was also given a 5% starch saline solution.

Table 1
Nickel Compounds Used in the Experiment

| No[a] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Chemical compounds | Ni-M[b] | NiO(G)[c] | NiO(B)[d] | $Ni_3S$ | NiS | $NiSO_4$ -$6H_2O$ | $NiCl_2$ -$6H_2O$ | $Ni(NO_3)$ -$6H_2O$ |
| Ni content, percent | 100 | 78.6 | 78.6 | 73.4 | 64.7 | 22.3 | 24.7 | 20.2 |

[a]Origin of nickel compound samples: No 1, Sumitomo Chem. Machinery Co., Japan; No 2, No 3, No 4, INCO Ltd., Canada; No 5, No 6, No 7, No 8, Wako Pure Chem. Ind., Japan.
[b]Nickel metal.
[c]Green nickel oxide.
[d]Black nickel oxide.

In our preliminary animal experiment, the soluble Ni compounds were excreted for the most part within 72 h after the oral administration and the retained amount of Ni in the organ was small. Therefore, the animals were sacrificed 24 h after the administration. The lungs, liver, kidneys, spleen, pancreas, heart, brain, and blood were taken as samples for the determination of Ni.

### Analysis of Ni Concentration in Organs

After weighing each organ, it was treated with a mixture of nitric acid and sulfuric acid until completely digested, and the solution was brought to a certain volume with 0.1*N* nitric acid. Blood samples were used for analysis after being diluted 10 times with 0.1*N* nitric acid. In addition, 24-h urine was collected by metabolic cage, and each 5 mL of urine was completely digested with nitric acid and brought to a certain volume with 0.1*N* nitric acid in the same way as the organs.

The Ni concentration in the sample solution was determined by a flameless atomic absorption spectrophotometry (Hitachi Polarized Zeeman Effect Atomic Absorption Spectrophotometer, Model Z-9000), and the detection limit was 1 ppb.

### Estimation of Absorbed Fraction

The absorbed fraction of Ni compounds was estimated from the ratio of the total amount in rat organs to the 10 mg administered orally. The total amount of Ni absorbed in the rat was calculated from the sum of the Ni amount of all organs, blood, and urine.

## RESULTS

### Solubility of Ni Compounds

The solubilities of eight Ni compounds are shown in Table 2 and the solubilities of the soluble compounds, $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$, were cited from the values presented in a literature *(7)*. The order of solubility for the eight Ni compounds was as follows: [$Ni(NO_3)_2$ > $NiCl_2$ > $NiSO_4$] >> [NiS > $Ni_3S_2$] > [NiO(B) > Ni-M > NiO(G)]. Five Ni compounds, NiO(G), Ni-M, NiO(B), $Ni_3S_2$, and NiS, known to be insoluble or slightly soluble, were assigned to two classes; one is NiO(G), Ni-M, and NiO(B), all of which showed a solubility of less than 5 μg/mL, and the other, $Ni_3S_2$ and NiS, showed a slight solubility of more than 500 μg/mL. The solubilities in saline were larger than those in distilled water.

### Organ Weight

Wet weights of organs in the rats 24 h after the administration of Ni compounds are shown in Table 3. The organ weight of the lungs and liver in the group that received $Ni(No_3)_2$ was significantly higher ($P <$

Table 2
Solubility of Nickel Compounds

| Ni compounds | Saline solution, $n = 5$, μg/mL | Distilled water, $n = 5$, μg/mL | Solubility (reference value),[7] g/100 g |
|---|---|---|---|
| NiO(G) | 1.34 ± 0.08 | 0.75 ± 0.01 | |
| Ni-M | 3.57 ± 0.22 | 1.13 ± 0.19 | |
| NiO(B) | 4.49 ± 0.20 | 3.90 ± 0.13 | |
| $Ni_3S_2$ | 572 ± 23 | 517 ± 9 | |
| NiS | 2176 ± 91 | 1850 ± 115 | |
| $NiSO_4$ | | | 32.4 |
| $NiCl_2$ | | | 42.3 |
| $Ni(NO_3)_2$ | | | 54.3 |

0.05) than those in the control group, whereas the organ weight in the other experimental groups showed no significant difference from those in the control group.

## *Ni Concentration in Organs*

The Ni concentration in organs 24 h after the administration of Ni compounds is shown in Table 4. In the NiO(G) group, there was no significant difference in Ni concentration between that found in all the organs and controls. Significantly higher concentrations were found in the lungs, kidneys, and pancreas for the Ni-M group, in the lungs and kidneys for the NiO(B) group, and in the lungs, liver, and kidneys for the $Ni_3S_2$ group. Ni concentrations in all organs in the rats given NiS, $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$ were significantly higher than those in the control rats. The Ni concentration in the kidneys was highest in all experimental groups except for the NiO(G) group.

## *Ni Distribution in Organs*

Figure 1 shows the percent distribution of detected Ni in organs classified into four groups: lungs, liver, kidneys, and other organs.

About 84–87% of the Ni amount was found in the kidneys for the $NiCl_2$, $NiSO_4$, and NiS groups. The ratio in the kidneys for the $Ni_3S_2$, $Ni(NO_3)_2$, NiO(B), and Ni-M groups was 76, 73, 62, and 51%, respectively. On the other hand, the Ni amount in the liver was higher than that in the kidneys for the NiO(G) group, showing similar results as the control group. The ratio of Ni in the kidneys depended on the solubility of the administered Ni compound.

## *Ni Amount and Absorbed Fraction*

The Ni amount in all organs are shown in Table 5. In the rats given soluble compounds, such as $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$, the Ni amount

Table 3
Wet Weights of Rat Organs 24 h After Oral Administration of Ni Compounds, mg/g body wt[a]

| Ni compounds | N | Lung | Liver | Kidney | Spleen | Pancreas | Heart | Brain |
|---|---|---|---|---|---|---|---|---|
| NiO(G) | 8 | 4.9 ± 0.4 | 31.5 ± 0.8 | 8.9 ± 0.5 | 2.2 ± 0.4 | 1.4 ± 0.2 | 3.5 ± 0.3 | 5.8 ± 0.4 |
| Ni-M | 8 | 5.0 ± 0.5 | 30.8 ± 1.9 | 8.5 ± 0.6 | 2.6 ± 0.4 | 1.8 ± 0.4 | 3.9 ± 0.3 | 6.2 ± 0.3 |
| NiO(B) | 8 | 4.8 ± 0.3 | 31.3 ± 1.6 | 8.7 ± 0.5 | 2.5 ± 0.4 | 1.8 ± 0.3 | 3.3 ± 0.2 | 5.9 ± 0.3 |
| $Ni_3S_2$ | 8 | 5.1 ± 0.7 | 30.2 ± 1.6 | 8.5 ± 0.5 | 2.4 ± 0.2 | 1.4 ± 0.4 | 3.5 ± 0.4 | 6.1 ± 0.3 |
| NiS | 8 | 4.8 ± 0.4 | 28.4 ± 2.2 | 8.0 ± 0.8 | 2.3 ± 0.3 | 1.2 ± 0.1 | 3.6 ± 0.2 | 6.2 ± 0.5 |
| $NiSO_4$ | 8 | 4.7 ± 0.3 | 30.8 ± 2.8 | 8.2 ± 1.1 | 2.4 ± 0.2 | 1.4 ± 0.3 | 3.6 ± 0.6 | 5.9 ± 0.5 |
| $NiCl_2$ | 8 | 4.6 ± 0.3 | 34.1 ± 7.3 | 8.4 ± 0.7 | 2.0 ± 0.3 | 1.4 ± 0.3 | 3.7 ± 0.2 | 6.2 ± 0.6 |
| $Ni(NO_3)_2$ | 8 | 6.5 ± 1.6* | 36.3 ± 6.0* | 8.7 ± 0.7 | 2.0 ± 0.5 | 1.4 ± 0.3 | 4.0 ± 0.4 | 5.8 ± 0.3 |
| Control | 8 | 4.6 ± 0.3 | 30.7 ± 1.1 | 8.6 ± 0.7 | 2.5 ± 0.4 | 1.5 ± 0.3 | 3.7 ± 0.2 | 5.9 ± 0.5 |

[a]Significantly different from control group by Student's $t$-test, *$p < 0.05$.

Table 4
Nickel Concentration in Rat Organs 24 h After Oral Administration[a]

| Ni compounds | N | Lung, μg/g | Liver, μg/g | Kidney, μg/g | Spleen, μg/g | Pancreas, μg/g | Heart, μg/g | Brain, μg/g | Blood, μg/mL |
|---|---|---|---|---|---|---|---|---|---|
| NiO(G) | 8 | 0.04 ± 0.03 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.04 | 0.03 ± 0.02 | 0.03 ± 0.02 | 0.03 ± 0.02 | 0.03 ± 0.01 |
| Ni-M | 8 | 0.18 ± 0.15[a] | 0.04 ± 0.02 | 0.31 ± 0.24[a] | 0.04 ± 0.01 | 0.06 ± 0.02[a] | 0.04 ± 0.02 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| NiO(B) | 8 | 0.08 ± 0.01[b] | 0.04 ± 0.03 | 0.32 ± 0.23[b] | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| $Ni_3S_2$ | 8 | 0.17 ± 0.14[a] | 0.07 ± 0.03[b] | 1.2 ± 0.9[b] | 0.03 ± 0.03 | 0.05 ± 0.04 | 0.04 ± 0.02 | 0.02 ± 0.01 | 0.05 ± 0.03 |
| NiS | 8 | 0.34 ± 0.27[a] | 0.11 ± 0.07[a] | 6.4 ± 2.7[b] | 0.14 ± | 0.25 ± 0.22[a] | 0.60 ± 0.31[b] | 0.04 ± 0.02[a] | 0.21 ± 0.11[b] |
| $NiSO_4$ | 8 | 2.5 ± 1.4[b] | 0.57 ± 0.35[b] | 25.5 ± 13.8[b] | 0.65 ± | 0.53 ± 0.23[c] | 0.47 ± 0.26[b] | 0.04 ± 0.02[a] | 0.28 ± 0.15[b] |
| $NiCl_2$ | 8 | 3.7 ± 1.9[c] | 0.53 ± 0.36[b] | 28.7 ± 12.2[c] | 0.67 ± | 0.57 ± 0.27[c] | 1.2 ± 0.6[c] | 0.18 ± 0.07[c] | 0.31 ± 0.17[b] |
| $Ni(NO_3)_2$ | 8 | 6.3 ± 3.5[b] | 1.1 ± 0.8[b] | 32.6 ± 12.7[c] | 2.8 ± 2.0[b] | 3.7 ± 1.4[c] | 2.4 ± 1.6[b] | 0.15 ± 0.11[a] | 2.25 ± 1.20[b] |
| Control | 8 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 |

Significantly different from control group by Student's $t$-test, [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

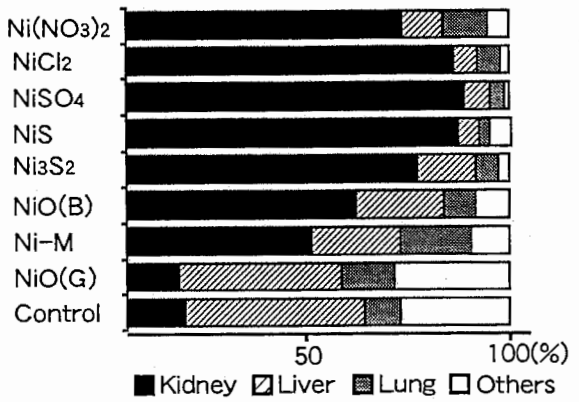



Fig. 1. Distribution of nickel in rat organs.

Table 5
Amounts of Nickel 24 h After Oral Administration

| Ni compounds | Amounts of Nickel (μg) | | | | Absorbed[d] fraction, % |
|---|---|---|---|---|---|
| | Organ | Blood[a] | Urine[b] | Total[c] | |
| NiO(G) | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.9 ± 0.2 | 0.01 |
| Ni-M | 1.0 ± 0.6 | 0.3 ± 0.1 | 7.6 ± 3.3 | 8.9 ± 3.7 | 0.09 |
| NiO(B) | 1.1 ± 0.7 | 0.3 ± 0.1 | 2.6 ± 1.7 | 4.1 ± 2.0 | 0.04 |
| $Ni_3S_2$ | 2.9 ± 2.0 | 0.6 ± 0.4 | 44 ± 16 | 47 ± 15 | 0.47 |
| NiS | 11.9 ± 4.8 | 2.7 ± 2.2 | 197 ± 65 | 212 ± 70 | 2.12 |
| $NiSO_4$ | 61.8 ± 31.5 | 3.7 ± 1.5 | 1047 ± 364 | 1112 ± 388 | 11.12 |
| $NiCl_2$ | 61.2 ± 12.3 | 4.1 ± 2.2 | 914 ± 748 | 976 ± 744 | 9.8 |
| $Ni(NO_3)_2$ | 81.6 ± 30.0 | 42.2 ± 15.6 | 3257 ± 1472 | 3381 ± 1466 | 33.8 |

[a]Estimated values.
[b]Nickel amount excreted in urine for 24 h.
[c]The sum of Nickel in organs, blood, and urine.
[d]Calculated values: [Total amount(c)/administered amount (10 mg)] × 100.

in all organs ranged from 61 to 82 μg. The amounts were 3 μg for the $Ni_3S_2$ group, 12 μg for the NiS group, and 1.0–1.1 μg for the Ni-M and NiO(B) groups. The amount for one of the scarcely soluble compounds, NiO(G), was similar to that of the control.

The absorbed fractions in the rats given Ni compounds are shown in Table 5. The absorbed fraction in the rats given scarcely soluble compounds was estimated to be 0.01% for the NiO(G) group, 0.09% for the Ni-M group, and 0.04% for the NiO(B) group. The observed fraction was 0.5–2.1% for the slightly soluble compounds, $Ni_3S_2$ and NiS groups, and 10–34% for the soluble compounds, $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$ groups.

## DISCUSSION

The solubilities of Ni-M, NiO(G), NiO(B), $Ni_3S_2$, and NiS, known as scarcely soluble or slightly soluble compounds, were determined both in distilled water and saline. The solubilities in saline were found to be higher than those in distilled water (Table 2). In addition, there were marked differences in solubility among Ni compounds of various chemical properties. NiO(B) and NiO(G) are produced at different burning temperatures—550°C for NiO(B) and 1030°C for NiO(G), respectively—although both are considered to be a nickel oxide having the same chemical formula. The difference in the solubility between NiO(B) and NiO(G) found in this study was also reported by M. Yamada et al. *(8)*. The solubilities of Ni-M, NiO(G), and NiO(B) were less than 5 μg/mL, being extremely scarcely soluble. A large difference in solubility between $Ni_3S_2$ and NiS was also found.

The percent distribution in the kidneys was high for soluble compounds; $Ni(NO_3)_2$, $NiCl_2$, and $NiSO_4$. For scarcely soluble compounds, NiO(G) and Ni-M, a higher distribution of Ni was found in the liver or lungs as well as the kidneys (Fig. 1). The Ni distribution in each organ was related to the solubility of the Ni compound. As an increase occurred in the solubility, the Ni distribution in the kidneys became higher. A correlation between the estimated absorbed fraction of each Ni compound and its solubility was observed (Table 5).

In the rats given soluble Ni compounds, a large amount of Ni was found in the organs, especially in the kidneys, suggesting that these compounds are easily absorbed and transferred from the blood to the kidneys and then extracted in urine. Therefore, the excretion from the body seems to occur rapidly in the cases of soluble compounds. On the other hand, in the case of the rat given scarcely soluble Ni compounds, the Ni amount of each organ was comparatively small, but the retention time was longer. Thus, it is suggested that harmful effects of scarcely soluble Ni compounds would be caused by such property.

In the previous inhalation study of Ni, it was confirmed that the rates of deposition and clearance of Ni were dependent on the chemical forms of Ni compounds *(9)*. The present study on oral administration of Ni compounds also revealed that the kinetics behavior of Ni in the rat was considerably varied according to the chemical forms of Ni compounds, especially the solubility had a great effect on the kinetics, suggesting that these properties of Ni compounds might be an important factor for investigating the health effects of nickel.

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