

This is a provisional English translation of an excerpt from the original full report.

## **Risk Assessment Report Nitrate and Nitrite Nitrogen (beverages)**

Food Safety Commission of Japan (FSCJ)  
October 2012

### **Executive summary**

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on nitrate and nitrite nitrogen, chemical substances, relating to the revision of the standards and criteria for beverages. The data used for the assessment include those from: acute toxicity tests in mice, rats and rabbits, subacute toxicity tests in mice, rats, dogs and pigs, chronic toxicity and carcinogenicity tests in mice and rats, neurovirulence tests in rats, reproductive and developmental toxicity tests in mice, rats, guinea pigs, rabbits and sheep, genotoxicity tests, and epidemiological studies, among others.

In terms of non-carcinogenic toxicity, relation between nitrate exposure through drinking water and methemoglobinemia (MetHb blood disease) in humans, especially in infants, has been reported, and the increase of MetHb level by nitrate exposure has been observed in animals. Epidemiological studies in humans have suggested the effects of nitrate exposure on the cardiovascular system and adrenal gland, and relevance to diabetes. It has been also suggested the effect of exposure to nitrate in utero on the reproduction, and the possibility to result congenital deformities or tumors of the central nervous system in the exposed children. The administration of nitrate and nitrite to test animals reportedly affected the thyroid, adrenal glands and heart.

Nitrate and nitrite are known to generate a carcinogen, N-nitroso compound *in vivo*. In carcinogenicity tests, hepatocarcinogenicity and tumors of the proventriculus were observed in rats exposed to nitrite, through feed and in those exposed through drinking water, respectively. However, FSCJ concluded that it is difficult to conduct a risk assessment on these data at this point, because of the poor reproducibility of the results and limited number of the tests (single dose) in both cases. In addition, the reported effects were considered to be poorly extrapolative to humans. The results from tests with oral administration often fail to reflect the effect of administered nitrite especially when doses are mixed in feed. In addition, many tests were conducted with low doses and nitrite is an unstable substance. Therefore, the FSCJ concluded that further data and information are essential for quantitative assessment of the carcinogenicity of nitrite.

Nitrate is not considered to have any genotoxicity that becomes a particular risk in organisms. High levels of nitrite induce mutation and chromosomal aberration in *in vitro* but not *in vivo*, indicating that the genotoxicity observed in the *in vitro* tests is unlikely to be expressed in organisms.

Hence, FSCJ concluded that it was appropriate to establish a tolerable daily intake (TDI) of nitrate and nitrite nitrogen in terms of non-carcinogenic toxicity.

To establish the TDI of nitrate nitrogen in terms of non-carcinogenic toxicity, the FSCJ established the no

observed adverse effect level (NOAEL) of nitrate nitrogen as 1.5 mg/kg body weight per day, applying the amount of formula taken by infants of the most susceptible age, under three months old, and their body weight on the basis of the fact that methemoglobinemia has not been reported in infants drinking formula prepared with water containing nitrate nitrogen at level less than 10 ppm. . Since the NOAEL was the value evaluated for infants of the most susceptible age, the FSCJ established the TDI of nitrate nitrogen to be 1.5 mg/kg body weight per day without applying the uncertainty factor. .

For evaluation of TDI of nitrite nitrogen in terms of non-carcinogenic toxicity, data of hypertrophic zona glomerulosa of the adrenal cortex observed in a 13-week oral exposure test in rats through drinking water were employed., Consequently, the FSCJ established the NOAEL of nitrite nitrogen to be 1.47 mg/kg body weight per day, and the TDI to be 15  $\mu$  g/kg body weight per day by applying the uncertainty factor of 100 (species difference of 10, individual difference of 10) to the NOAEL.

In conclusion, the FSCJ established the TDI of nitrate nitrogen to be 1.5 mg/kg body weight per day, and the TDI of nitrite nitrogen to be 15  $\mu$  g/kg body weight per day.

### III. Risk assessment

Nitrate and nitrite ions are of natural source, and play a role in the nitrogen cycle. Nitrate ion, which exists in natural water, is partially reduced to nitrite ion in human digestive systems under certain conditions. Nitrite ion reacts with Hb within the blood and generates MetHb, which may cause methemoglobinemia. Nitrate and nitrite are known for their potentials of generating N-nitroso in the stomach by reacting with amine and among others contained in food.

Relation between nitrate exposure through drinking water and methemoglobinemia in humans, especially in infants, has been reported, and the increase of MetHb level by nitrate exposure has been observed in animals. Epidemiological studies in humans have suggested the effects of nitrate exposure on the cardiovascular system and adrenal gland, and relevance to diabetes. It has been also suggested the effect of exposure to nitrate in utero on the reproduction, and the possibility to result congenital deformities or tumors of the central nervous system in the exposed children. The administration of nitrate and nitrite to test animals reportedly affected the thyroid, adrenal glands and heart.

Regarding carcinogenicity, the IARC classified nitrate and nitrite as substances that are probably carcinogenic to humans (Group 2A), and commented that “evidence in humans is insufficient concerning the carcinogenicity of nitrate in drinking water”, and “evidence in humans is limited concerning the carcinogenicity of nitrite in food.”

Carcinogenicity tests demonstrated hepatocarcinogenicity of nitrite in rats exposed through doses mixed in feed, and tumors of the proventriculus in rats exposed through drinking water. However, the FSCJ concluded that it is difficult to conduct a risk assessment at this point, because of the poor reproducibility of the results and limited number of the tests (single dose) in both cases. In addition, the reported effects were considered to be poorly extrapolative to humans. The results from tests with oral administration often fail to reflect the effect of administered nitrite especially when doses are mixed in feed. In addition, many tests were conducted with low doses and nitrite is an unstable substance. Therefore, the FSCJ concluded that further data and information are essential for quantitative assessment of the carcinogenicity of nitrite.

Nitrate is not considered to have any genotoxicity that becomes a particular risk to organisms. High levels of nitrite induce mutation and chromosomal aberration *in vitro* but not *in vivo*, indicating that the genotoxicity observed in the *in vitro* tests is unlikely to be expressed in organisms.

Hence, the FSCJ concluded that it was appropriate to establish a tolerable daily intake (TDI) of nitrate and nitrite nitrogen in terms of non-carcinogenic toxicity.

#### 1. Nitrate nitrogen

Methemoglobinemia has been observed in infants who took formula prepared with water containing a high level of nitrate nitrogen (20 ppm or more), but the case has not been reported with nitrate nitrogen level less than 10 ppm. Based on these findings, the NOAEL of nitrate nitrogen can be evaluated to be 10 ppm (10 mg/L) taking methemoglobinemia as an index.

Additionally, since bacterial can grow in the stomach when gastric pH is five or above, infants (especially aged from 0 to 3 months) that generally have a high pH in the digestive tract are a population with

high-susceptibility to adverse effects of nitrate.

Based on the abovementioned facts, and with the assumption that infants aged two months drink an average of 865 mL per day<sup>2</sup>, and weigh an average of 5.7 kg<sup>3</sup>, the NOAEL is equivalent to 1.5 mg/kg body weight per day. The uncertainty factor does not need to be applied because the value was obtained from infants with the highest susceptibility in terms of this endpoint.

In conclusion, the FSCJ calculated the TDI of nitrate nitrogen to be 1.5 mg/kg body weight per day.

## 2. Nitrite nitrogen

Human epidemiological studies have not been conducted on the carcinogenicity of nitrite itself, but hypertrophy of the adrenal cortex and an increase in MetHb were observed in test animals.

Two cases in Wistar rats (male and female) with oral administration of  $\text{KNO}_2$  through drinking water for 13 weeks showed the lowest NOAEL. With hypertrophic zona glomerulosa of the adrenal cortex as the index, each study established the NOAEL to be 1.64 mg/kg body weight per day and 1.47 mg/kg body weight per day, as nitrite nitrogen respectively. The 59th JECFA commission (2002) considered the slight hypertrophy of the adrenal cortex as reflections of physiological adaptation to small changes in blood pressure, and that there is little toxicological significance. However, although the developmental mechanism of the hypertrophic zona glomerulosa of the adrenal cortex has not been elucidated, the changes observed in administered groups of two different tests were consistent, and therefore, the commission considered the slight hypertrophy of the adrenal cortex as a toxic effect of nitrite nitrogen.

Based on these effects, the NOAEL of nitrite nitrogen was determined to be 1.47 mg/kg body weight per day.

The FSCJ applied an uncertainty factor of 100 to this NOAEL, thus calculated the TDI of nitrite nitrogen in terms of non-carcinogenic toxicity to be 15  $\mu\text{g}$  nitrite nitrogen/kg body weight per day. It is to note that hypertrophic zona glomerulosa of the adrenal cortex has been observed as a naturally occurring change, and no corresponding or related change has been observed in longer term administration tests. Therefore, regarding the uncertainty factor, the FSCJ judged that an additional factor of the subacute toxicity test conducted for the basis was not necessary to apply.

Hence, the FSCJ established the TDI of nitrite nitrogen to be 15  $\mu\text{g}$ /kg body weight per day.

### Nitrate nitrogen

TDI: 1.5 mg/kg body weight per day

(Basis for TDI establishment):	Epidemiological survey
(Observational basis for NOAEL establishment):	Methemoglobinemia in infants
(NOAEL):	1.5 mg/kg body weight per day
(Uncertainty factor):	N/A

<sup>2</sup> Exposure factors handbook of the Research center for chemical risk management, National institution of advanced industrial science and technology  
[http://unit.aist.go.jp/riss/crm/exposurefactors/documents/factor/other\\_intake/intake\\_artificialmilk.pdf](http://unit.aist.go.jp/riss/crm/exposurefactors/documents/factor/other_intake/intake_artificialmilk.pdf)

<sup>3</sup> Average weight of a boy aged 2 - 3 months is 5.88 kg, and that of a girl is 5.53 kg, according to the research report of physical growth of infants in 2000 (Ministry of health, labour and welfare, 2001).

## [Reference]

With non-carcinogenic toxicity as the index, given the abovementioned 1.5 mg/kg body weight per day and the contribution rate of 10%, the concentration of nitrate nitrogen is 3.75 µg/L when a person weighing 50 kg drinks 2 L of drinking water a day.

Nitrite nitrogen

TDI: 1.5 µg/kg body weight per day

(Basis for TDI establishment):	13-weeks oral administration through drinking water
(Species):	Rat
(Period):	13 weeks
(Method of administration):	Oral administration through drinking water
(Observational basis for NOAEL establishment):	Hypertrophic glomerulosa of the adrenal cortex
(NOAEL):	1.47 mg/kg body weight per day
(Uncertainty factor):	100 (species difference of 10, individual difference of 10)

## [Reference]

With non-carcinogenic toxicity as the index, given the abovementioned 15 µg/kg body weight per day, and the contribution rate of 10%, the concentration of nitrate nitrogen is 37.5 µg/L when a person weighing 50 kg drinks 2 L of drinking water a day.