Asian Journal of Chemistry; Vol. 25, No. 10 (2013), 5402-5406

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14400

Investigation of Cd(II) Concentrations in Mother's Milk of Varamin Region of Iran

SIMA NAZARPOUR[1,*], LEILA TEYMOURI[1], SAMANEH TEIMOORI[2], SEYED ALI NOJOUMI[3] and ALI MOGHIMI[4]

[1]Midwifery Department, Midwifery and Nursing Faculty, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[2]Basic Sciences Department, University of Damghan, Semnan, Iran
[3]Microbiology Department, Pasteur Institute of Iran, Tehran, Iran
[4]Department of Chemistry, Varamin (Pishva) Branch, Islamic Azad University, Varamin, Iran

*Corresponding author: E-mail: simanazarpour@yahoo.com

(*Received*: 7 August 2012; *Accepted*: 8 April 2013) AJC-13216

Children's nourishment has the most priority in every plan that follows children's health care; also, milk contamination with various toxic elements can have unfavourable effects on children's health. This study was accomplished to determine the amount of cadmium in women's breast milk in Varamin region of Iran. In this study, the relationship between different factors with the amount of cadmium was assessed. In this research, the amount of cadmium in the breast milk of 100 mothers visiting the selected clinics in Varamin was reviewed 4 to 8 weeks after delivery. Cadmium and chromium concentrations were determined by atomic absorption spectrophotometer. According to our findings, the average cadmium in breast milk was equal to 5± 6.9 mg/mL. The amount of cadmium was more in the breast milk of mothers living nearer to a factory or an industrial center. The present study indicated the relation of some factors with the amounts of elements evaluated. The cadmium levels of breast milk in this study were higher than the reported work.

Key Words: Toxic elements, Heavy metals, Breast milk, Cadmium.

INTRODUCTION

In the recent years, pollution of the environment by heavy metals has received considerable attention. These elements accumulate in living organisms and are of high toxic potential. Their wide technological use (fertilizers, mining, pigments), as well as their production from burning oil and coal and incineration of waste causes an extensive anthropogenic contamination of soil, air and water[1]. Children's nourishment has the most priority in each plan that follows children's health care. Milk is usually the only food source for infants during the first 4 or 5 months of their life and nourishment with breast milk is the best and the most natural food source[1-4]. Breast feeding period is deemed essential in growth and development[5,6]. Therefore, the breast milk is one of the important indicators pertaining to health and it is directly related to the health of infants and next generation. The favourable growth and development of infants is only guaranteed when water and food consumption obtains required concentrations of all essential elements. Normally, breast milk is the only food source in the preliminary stages of life. Consequently, it should provide infants with food requirements[7]. The existence of some metal contaminants, especially cadmium and lead, facilitates their entrance to food chain and finally increases the probability of their toxic effects in human and animal[8]. Most of the chemical materials can be transferred to breast milk through body reserves and blood[2].

Since the breast milk is one of the body ways to transfer toxic elements, breast milk contamination can have harmful effects on the child growth in all conditions. Heavy metals are one of the cases that can first contaminate breast milk and then endanger infant health[9,10]. According to the studies conducted, since the fetus and infants are continually developing and undergoing rapid changes in the structure and function of macro organisms (limbs) they would be more at risk by chemical materials[11]. Infants have some physiological characteristics in the preliminary stages of life such as higher metabolic rate at rest that will increase the vulnerability towards harmful effects of chemical materials. Studies have indicated that there has been the possibility of multiple absorption for the essential trivial elements. Moreover, these materials have toxic effects[7,12]. Nevertheless, favourable daily concentration of elements is still debatable. Recent studies have indicated that the concentration of elements considerably changes during breast feeding and element transmission from mother to infant can be monitored[13,14].

This study was accomplished in order to determine the amount of cadmium in the breast milk of mothers in Varamin on

the basis of above mentioned points. In this study, the relation of different variables with the amount of cadmium was evaluated.

EXPERIMENTAL

This study was conducted by cross sectional method. All the breast feeding mothers who visited the Varamin health Network clinics to provide health care for their children and for themselves during the 4 and 8 weeks after delivery were included in this study. This period was selected since the secretion of colostrums continues 5 days after delivery and then gradually becomes complete milk during next four weeks[15].

Therefore, it was chosen to obtain similar samples 4 weeks after complete conversion of colostrums to milk. The population was 100 breast feeding mothers in Varamin. The selection of 100 samples was on the basis of delivery number in Varamin hospitals, the number of visits paid by breast feeding mothers in 4^{th} or 8^{th} week of delivery to clinics and time limitation of research plan. The sampling was according to random cluster method. In this method, four clinics were randomly selected among the clinics affiliated to Varamin health network in different regions. The breast feeding mothers visiting these clinics between 4^{th} or 8^{th} week after delivery participated in the study considering the merits of probable sample elimination to sample completion in plan. The sample taking was conducted by an official letter by the research deputy of the University to the director of Varamin health network to issue a permission to enter research centers and also by adequate and proper explanation about the subject and its value and importance to obtain their satisfaction.

Collection of samples was carried out by the breast feeding mother after complete hand washing by milking or by sterilized milking system made up polyethylene. The amount of samples was 40 mL that was spilled to polypropylene dishes (sterilized by wet autoclave and γ-ray) and were immediately transferred to a -20 freezer. Then, the samples were transferred to lab in frozen state and the amount of cadmium was measured according to experimental methods.

In this study, the participants provided the data by questionnaire. The questionnaire was accumulated after surveying of the sources and similar studies[2,16-18].

Content validity method was used to determine the scientific validity of questionnaire. The questionnaire was prepared by reviewing books, authentic sources, scientific papers and experiences. Seven academic members and experts were consulted about it and weak points were settled.

The statisticians suggested that we use cronbach α-coefficient for final stage of providing questionnaire, so that a pilot study was conducted by 20 samples. Cronbach α-coefficient was counted 69 according to the results and consequently, the perpetuity of questionnaire was confirmed.

The results were analyzed by SPSS 18 software, descriptive statistics (frequency distribution, average and SD) and illative statistics in order to review correlation between effective factors and the amount of heavy metals). Pearson correlation and linear regression were used to review the relations among variables.

Sample preparation: 20 mL of milk samples was taken and put in the beaker and then in the water bath for 2 h at 100 ºC in order to decrease the amount of sample by evaporation. Then, the resulting samples enter digestion stage (Ursinyova and Masanova, 2005).

Digestion of samples: This operation is done to oxidize milks organic material. To do that, we used wet digestion method accompanying heat and nitric acids solutions, perchloric acid and hydrogen peroxide. Six mL of nitric acid was added to the samples and then put in oven for 1 h in 100 ºC. In this stage, the volume of the samples decreases enormously (to nearly drying stage). Nitric acid digests the milk fats and the resulting solution is relatively a clear yellow solution. The samples are taken out of oven to get the ambient temperature; then, 4 mL of hydrogen peroxide is added and put once again in oven for 0.5 h at 100 ºC.

Final digestion: Finally, 3 mL of dense perchloric acid is added after complete exit of samples from the oven to get the ambient temperature. In this stage, the resulting solution is relatively a clear yellow solution indicating the completion of samples digestion stage. It is noteworthy that some milk samples contain high amount of fat that even after final stage of digestion, forming small spots of oil. These spots were removed by filtration operation using Whatman paper.

Standardization of cadmium: Human milk cadmium absorption is about 0.05-2 mg. This amount will increase 20 times in contaminated regions and in the cases of contaminants consumption.

In this method, some solutions were prepared by adding specified standard of cadmium to tetrazole with 1000 ppm concentration. The digestion of these solutions was read by flame atomic absorption method.

Preparation of 1 ppm standard solution of cadmium: A 1000 ppm concentration of cadmium tetrazole was used to prepare cadmium standard. It is noteworthy that the cadmium concentration assessment is about 0.5-2.6 ppm in flame atomic absorption system. The solution was attained to a certain amount of concentration by standard addition method. Then, the absorption of each sample was read in 3 stages and absorption average was accordingly counted.

Standard addition: The specified cadmium standard with 10 ppm concentration using sampler mL 100, 1 mL was taken and added to samples in 100 digested samples. Then, 9 mL deionized water was added to them in order to prepare the solutions with 1 ppm concentration at the end. The samples were read by JBC AA 932 system compared with prepared standards and also blank (containing, deionized water, nitric acid and perchloric acid) in 222.8 nm wavelength.

RESULTS AND DISCUSSION

The results obtained from questionnaires indicated that most of them belong to the age group of 21-25. The average age of those participated was 26.74 and they were middle aged with 99 % housewives and only 1 % job holders. Most of the participants (35 %) had educated up to guidance level and only 10 % of them had university backgrounds. Most of the participants (73 %) received 5000000 Rials monthly as income ($330 US currency) and the least amount (13.4 %) was related to those whose their monthly income was more than 10 million Rials ($460 US currency). Most of the participants (81.8 %)

TABLE-1
DESCRIPTIVE STATISTICS IN TERMS OF DIFFERENT VARIABLES

	N	Range	Minimum	Maximum	Mean	Std. deviation	Variance
Age	97	29	15	44	26.74	5.403	29.193
The number of children	94	6	0	6	1.85	1.097	1.203
Height	96	25	146	171	158.84	5.459	29.796
BMI	96	27.7	18.7	46.6	27.585	4.5900	21.068
Weight before pregnancy	89	63.0	41	104	64.713	12.7114	161.579
Weight during sampling	99	62	48	110	69.742	11.7248	137.471
Infant's age	99	8	2	10	6.0	1.723	2.968
Weight in the infant's birth time	96	4500	1300	5800	3314.90	597.405	356892.621
The current weight of infants	96	4200	2300	6500	4822.08	793.484	629616.667
Infant's height	97	22	40	62	54.988	3.5012	12.259
Infant's head circumference	97	17.5	32	49.5	37.601	2.071	4.302

lived in a city and only 18.2 % of them lived in village. 42.4 % of those were studied had no pregnancy background and the infant was their first pregnancy while 57.6 % of them had previous pregnancy. The type of delivery in most of the participants (57.6 %) was caesarian and 42.4 of them had natural delivery. The gender relation of infants in participants was equal, so that 50 % was male and 50 % female. Some other variables are indicated in Table-1.

All the participants had no smoking background before the pregnancy or during the pregnancy. 21.2 % of these people had smoking husband. There was at least a person who smoked in the house of 20.2 % of the participants. Only 16.2 % of the participants lived in a congested and heavy traffic area and only 13.1 % of the participants lived near a factory or an industrial estate. There was a river or sewage system near the house of only 8.1 % of the subjects. 77.3 % of the participants used powder milk in addition to breast milk to feed their infants. Only 22.7 % of the participants used pasteurized milk in addition to breast milk. Only 17.2 % of the participants used a kind of medicine (mostly Ferrous sulfate and multi vitamin) during breast feeding. 39.6, 34.1 and 24.2 % of the participants ate fish weekly, monthly and annually, respectively; while, only 2.2 % of the participants didn't eat fish. Most of the participants (49.5 %) ate rice 7 times a week. 51.6 % of the participants used Iranian rice, 42.1 % imported rice and only 6.3 % both rice brands. Most of the participants (99 %) used tap water and only 1 % used mineral water. There was no report of the usage of well water among the subjects. Only 3 % of the participants were on diet and 3 % pointed out about their immune system defects. 31.3 % of the participants declared having stress. The results of cadmium absorption assessment are indicated in Table-2.

Table-2 indicates that cadmium absorption amount ranges from 0.00 to 0.0480 mg/mL and its average is 0.005684 mg/mL.

TABLE-2
CADMIUM AMOUNT IN BREAST MILK OF STUDY SUBJECTS

	N	Min.	Max.	Mean
Cadmium amount in breast milk	100	0.0000	0.0480	0.005684

The correlation between different variables and absorption amount of cadmium indicated that there is no relation among age, occupation, education, income, location, pregnancy background, number of children, height, BMI, weight before pregnancy, mother's weight during sampling, delivery status, infants gender, weight of infant in birth, infant height, smoking husband, smoking at home, living in a congested area, availability of river or a sewage system, infant's nourishment, taking especial medicine, consumption of fish, rice type, type of drinking water, immune system failure, stress and cadmium absorption. Correlation evaluation was not possible because there were no participants who smoked before pregnancy or during pregnancy. There was, however, a correlation among infants age ($p < 0.05$), weight during sampling of the mothers ($p < 0.05$), head circumference ($p < 0.01$), living near a factory or an industrial center ($p < 0.01$), rice consumption ($p < 0.05$) and cadmium absorption.

The cases whose relation is shown were surveyed through linear regression. The predictability of cadmium was assessed by pertaining factors. Linear regression results indicated that cadmium amount was more in breast milk of mothers who lived near a factory or an industrial center (Table-3).

Milk is considered as a complete and main food for the first 6 months of infant's birth and consists of water, fat, protein, vitamin and mineral water. Growth and development of infants depend completely on this complete food. Therefore, breast milk is tremendously vital from the viewpoint of reviewing essential and toxic materials and also effective factors for infants' growth.cadmium concentration has been evaluated in multiple studies in breast milk, dried milk, urine

TABLE-3
LINEAR REGRESSION ASSESSMENT OF FACTORS AFFECTING CADMIUM AMOUNT IN MOTHER'S BREAST MILK

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	β		
(Constant)	-0.018	0.014		-1.320	0.190
Infant's age	0.001	0.000	0.126	1.108	0.271
The current weight of infant	6.093E-7	0.000	0.069	0.475	0.636
Infant's head circumference	0.001	0.000	0.216	1.630	0.107
Living near a factory or an industrial center	-0.006	0.002	-0.315	-3.218	0.002
Consumption of rice	0.000	0.000	0.160	1.634	0.106

and blood sample during these years. In this study, cadmium of breast milk was studied in 100 mothers 4 and 8 weeks after delivery in Varamin.

Various techniques have been utilized to assess and measure heavy metals in different studies. The main methods to study cadmium are as follows[8]. Flame atomic absorption spectrometry[21-23], graphite furnace atomic absorption spectrometry[16], inductively coupled argon plasma emission spectrometry[24], differential pulse anodic stripping voltammetric technique[1], inductively coupled plasma optical emission spectrometry[25]. Flow injection spectrometric methods[26].

Cadmium absorption average in the samples was equal to 0.005 ± 0.0069 µg/mL (5 ± 6.9 µg/mL) according to FAAS method in this study. The cadmium amounts of breast milk in other studies were as follows:

Cadmium amount was equal to 2.54 ± 2.52 µg/L in a study about 29 breast feeding mother in Croatia[20]. A study was accomplished in Germany on 10 rural women and 10 urban women in first 3 month of their breast feeding period. The cadmium amount was found to be 17.3 ± 49 µg/L in rural and 24.6 ± 7.3 µg/L in urban women[27]. In another study accomplished on 68 breast feeding women in Japan, cadmium amount was equal to 0.277 ± 1.82 µg/L[28]. In another study accomplished on 33 breast feeding mothers in Mexico, the cadmium amount was equal to 0.62 ± 0.28 µg/L[29]. In a study carried out on 110 breast feeding mothers in Poland, cadmium amount was equal to 2 µg/L[30]. In a study conducted on 168 breast feeding mothers in Saudi Arabia, the cadmium amount was 1.73 µg/L[18]. In another study conducted in Slovakia on 158 breast feeder mothers, the cadmium amount was equal to 0.43 ± 0.27 µg/L[2]. In a study accomplished in Sudan on 39 breast feeding mothers, the cadmium amount was equal to 0.06 ± 0.27 µg/L[31].

In a study accomplishes in Turkey on 30 breast feeder mothers, the cadmium amount was equal to 2.8 µg/L[32]. In a study about 30 Iranian breast feeding mothers, the cadmium amount was equal to 2.44 ± 1.47 µg/L[16].

Conclusion

In this study, the relation of various factors affecting cadmium amount in breast milk of mothers was surveyed. These factors are as follows:

Mother's age, occupation and education, family income, place of accommodation, background and multiplicity of previous pregnancies, number of children, weight before the pregnancy and the current weight, mother's BMI, type of delivery, infant's gender, age, birth weight, current weight, height, head circumference, smoking mother, husband, other family members, living in a crowded area, existence of a river or a sewage system near living place, type of nourishment of the infant, taking medicine, consumption of the fish, type of rice, diet, immune system failures and stress.

The results indicated that cadmium amount was more in the breast milk of mothers who lived near a factory or an industrial center.

There was no meaningful relation between mother's age and cadmium amount in this study. There have been many studies about cadmium element including studies conducted by Sirkorski *et al.*[30], Yunes *et al.*[33], Drasch *et al.*[34] and Rahimi *et al.*[16] who obtained similar results[16,30,33,34].

There was no meaningful relation between mother's occupation and cadmium amount of breast milk in this study. It is noteworthy that the participants of this study were more housewives and they did not work in factories and industrial centers.

There was no meaningful relation between pregnancy background and cadmium amount of breast milk in this study. Other researchers have obtained the same results such as Sirkorski *et al.*[30], Frkovic *et al.*[20], Ursinyova[2] and Masanova[20,30].

Probable role of smoking on the amount of heavy metals such as cadmium in breast milk of the mothers was reviewed in this study. None of the participants smoked and there was no meaningful relation between smoking by the husband or other family members. Smoking had direct and important effects on the cadmium amount of milk samples according to different studies. Radisch *et al.*[35], Palminger[31], Frkovic *et al*[20], and Eynon *et al.*[36] indicated in their studies that there is a meaningful relation between smoking habit in the family (smoking during pregnancy or/and being affected by others' smoking) and cadmium concentration increase in mother's breast milk.

Leotsinidis *et al.*[19] and Ursinyova and Masanova[2] indicated in their studies that the infant of smoking mothers (20-40 %) are exposed to and affected by cadmium more than the infants of non-smoking mothers and we can see an increase in cadmium concentration of breast milk by smoking mother's before pregnancy (8 %), smoking fathers (17 %) and any other smoking at home (28 %). Rahimi *et al.*[16] indicated that cadmium concentration has meaningful discord in the breast milk of mothers who actively or passively smoked.

This study indicated that cadmium absorption amount was more in breast milk of mothers who lived near a factory or an industrial center. The study of Rahimi *et al.*[16] indicated an increase in cadmium amount of breast milk of the mothers in Zarin Shahr area.

Since rice is one of the families' main foods in our country, surveying rice contamination, especially different types of imported rice, is of great importance. Honda *et al.*[28] and Nishiju *et al.*[37] indicated that cadmium concentration in breast milk of women with high consumption of rice cadmium was accompanied by urine cadmium.

According to our findings, since heavy metals with high amount of cadmium can have unfavourable effects on infant's health, we can have an effective role on infant's health improvement and its proper growth and development by evaluating harmful heavy metals and application of appropriate solutions in order to reduce contamination of mother's breast milk. This should be taken into consideration in health promotion plans.

ACKNOWLEDGEMENTS

The authors acknowledged the financial support of Department of Midwifery and Chemistry, Faculty of Varamin (Pishva) Branch, Islamic Azad University for financial support.

REFERENCES

1. R.M. Tripathi, R. Raghunath, V.N. Sastry and T.M. Krishnamoorthy, *Sci. Total Environ.*, **227**, 229 (1999).
2. M. Ursinyova and V. Masanova Cadmium, *Food Addit. Contam.*, **22**, 579 (2005).

3. O. Wappelhorst, I. Kühn, H. Heidenreich and B. Markert, *Nutrition*, **18**, 316 (2002).
4. N. Koizumi, K. Murata, C. Hayashi, H. Nishio and J. Goji, *Biol. Trace Elem. Res*., **121**, 205 (2008).
5. C. Gundacker, B. Pietschnig, K.J. Wittmann, A. Lischka, H. Salzer, L. Hohenauer and E. Schuster, *Pediatrics,* **110**, 873 (2002).
6. A. Abballe, T.J. Ballard, E. Dellatte, A. Domenico, F. Ferri, A.R. Fulgenzi, G. Grisanti, N. Iacovella, A.M. Ingelido, R. Malisch, R. Miniero, M.G. Porpora, S. Risica, G. Ziemacki and E.D. Felip, *Chemosphere*, **73**, S220 (2008).
7. T. Prohaska, G. KoÈllensperger, M. Krachler, K. De Winne, G. Stingedera and L. Moens, *J. Anal. At. Spectrom.*, **15**, 335 (2000).
8. S. Birghila, S. Dobrinas, G. Stanciu and A. Soceanu, *Environ. Eng. Manage. J.*, **7**, 805 (2008).
9. C.A. Burtis, E.R. Ashwood and D.E. Bruns, Tietz Text Book of Clinical Chemistry and Molecular Diagnostics, edn. 40, Chapter 35 (2006).
10. WHO. Environmental Health Criteria, Inorganic Lead, Organic Lead, Geneva, p. 165 (1995).
11. M. Patriarca, A. Menditto, B. Rossi and T.D.B. Lyon, *Microchem. J.*, **67**, 351 (2000).
12. W. Wertz, Health Evaluation of Heavy Metals in Fnfant Formula and Junior Food. Berlin; E. Schmidt and A. Hildebrand, Springer, 47 (1983).
13. M. Krachler, F. Shi Li, E. Rossipal and K.J. Irgolic, *J. Trace. Elem. Med. Biol.*, **12**, 159 (1998).
14. M. Krachler, E. Rossipal and D. Micetic-Turk, *Eur. J. Clin. Nutr.*, **53**, 486 (1999).
15. F.G. Cunningham, K.J. Leveno, S.L. Bloom, J.C. Hauth, D.J. Rouse and C.Y. Spong, Williams Obstetrics: 23rd Edition. McGraw-Hill (2010).
16. E. Rahimi, M. Hashemi and Z.T. Baghbadorani, *Int. J. Environ. Sci. Technol.*, **6**, 671 (2009).
17. M. Kippler, B. Lönnerdal, W. Goessler, E.C. Ekström, Sh. E. Arifeen, and M. Vahter, *Toxicology*, **257**, 64 (2009).
18. I. Al-saleh, N. Shinwari and A. Mashhour, *Biol. Trace Element Res.*, **96**, 21 (2003).
19. M. Leotsinidis, A. Alexopoulos and E. Kostopoulou-Farri, *Chemosphere*, **61**, 238 (2005).
20. A. Frkovic, M. Kras and A. Alebic-Juretic, *Bull. Environ. Contam. Toxicol.*, **58**, 16 (1997).
21. T. Florea, S. Orban Huszti and M. Costin, Heavy Metal Contaminats in Milk and Cheese. The Annals of the University Dunarea de Jos of Galti; Fascicle VI- Food Tecnology, pp. 26-32 (2006).
22. E. Kondyli, M.C. Katsiari and L.P. Voutsinas, *Food Chem.*, **100**, 226 (2007).
23. P. Phol and B. Prusisz, *Talanta,* **71**, 715 (2007).
24. Y.W. Park, *Small Ruminant Res.*, **37**, 115 (2000).
25. C.S. Kina and V.A. Maihana, *Food Chem*., **100**, 390 (2007).
26. A. Nogueira Rita de Araujo, F. Mockiuti, G. Batista de Souza and O. Primavesi, *Anal. Sci.*, **14**, 559 (1998).
27. H.J. Sternowsky and R. Wessolowski and R. Lead, *Arch. Toxicol*., **57**, 41 (1985).
28. R. Honda, K. Tawara, M. Nishijo, H. Nakagawa, K. Tanebe and S. Saito, *Toxicology*, **186**, 255 (2003).
29. A. Rydzewska and I. Krol, *Ginekol. Pol*., **67**, 125 (1996).
30. R. Sikorski, T. Paszkowski, Jr. T. Radomanski and J. Szkoda, *Gynecol. Obster. Inves*., **27**, 91 (1989).
31. I.P. Hallen, L. Jorhem, B.J. Lagerkvist and A. Oskarson, *Sci. Total Environ.*, **166**, 149 (1995).
32. S. Turan, S. Saygi, Z. Kiliç and A. Orhan, *J. Trop. Pediatrics*, **47**, 81 (2001).
33. B. Younes, A.A. Al-Meshari, A. Al-Hakeem, S. Al-Saleh, F. Al-Zamel, F. Al-Shammari and A. Alwarthan, *Ann. Saudi Med.*, **15**, 249 (1995).
34. G. Drasch, S. Aigner, G. Roider, F. Staiger and G. Lipowsky, *J. Trace Elem. Med. Biol*., **12**, 23 (1998).
35. B. Radisch, W. Luck and H. Nau, *Toxicol. Lett.*, **36**, 147 (1987).
36. G.R. Eynon, J.M. McKenzie-Parnell and M.F. Robinson, *Proc. Univ. Otego Med. School*, **63**, 38 (1985).
37. M. Nishijo, S. Satarug, R. Honda, I. Tsuritani and K. Aoshima, *Mol. Cell Biochem.*, **255**, 87 (2004).