# The Detrimental Effects of Potassium Bromate and Thioglycolate on Auditory Brainstem Response of Guinea Pigs

**Jiunn-Jye Chuu[1], Chuan-Jen Hsu[2] and Shoei-Yn Lin-Shiau[1,3]**

*Institutes of [1]Toxicology and [3]Pharmacology*
*College of Medicine, National Taiwan University*
*and*
*[2]Department of Otolaryngology*
*National Taiwan University Hospital*
*Taipei 100, Taiwan, ROC*

## Abstract

**Potassium bromate ($KBrO_3$) is known to be an oxidizing agent that is used not only as a food additive, mainly in the bread-making process, but also as a neutralizer in thioglycolate containing hair curling set. Although it has been shown that bromate poisoning could cause severe and irreversible sensorineural hearing loss as well as renal failure, the action mechanism of bromate-induced oto-neurotoxicity especially its combination with thioglycolate remains to be studied.**

**In this study, we attempted to investigate the toxic effects of $KBrO_3$ in combination with or without thioglycolate on the auditory brainstem response (ABR) system in the guinea-pigs which was claimed to be very susceptible to the xenobiotics. In a preliminary test, we have found that after consecutive 2 weeks administration, $KBrO_3$ caused a significant prolongation of wave I-III and the interwave latencies of ABR as well as significantly elevated the threshold of hearing, suggesting that the conduction velocity of the peripheral auditory nerve was delayed. By contrast, the absolute latency of wave IV/V and the interwave latency of wave III-V were not significantly prolonged, suggesting that $KBrO_3$ had no effect on the brainstem. This oto-neurotoxic effect of $KBrO_3$ was markedly enhanced by combining with thioglycolate.**

**Our data also indicated that $KBrO_3$ combined with thioglycolate but not $KBrO_3$ alone prominantly caused a decrease of body weight. However, enzymatic actitivies (including $Na^+/K^+$-ATPase and $Ca^{2+}$-ATPase) and the level of nitric oxide (NO) was significantly affected in the brainstem. Based on these findings, we tentatively conclude that whether $KBrO_3$ alone or $KBrO_3$ combined with thioglycolate induced oto-neurotoxicity majorly through the peripheral auditory nerve rather than via the central brainstem intoxication.**

**Key Word: potassium bromate, thioglycolate, oto-neurotoxicity, brainstem, $Na^+/K^+$-ATPase and $Ca^{2+}$-ATPase, nitric oxide**

## Introduction

Potassium bromate ($KBrO_3$) is an oxidizing agent that has been used as a food additive, mainly in the bread-making process. Potassium bromate is also known to be a complete carcinogen, inducing chromosome aberrations and 8-hydroxydeoxy guanosine generation, and possessing both initiating and promoting activities for rat renal tumorigenesis (1, 19, 20). $KBrO_3$ is now marketed as a neutralizer in home permanent cold wave hair kits (13). Home permanent wave sets consist of the first solution, thioglycolate hair wave lotion, which changes S-S bonds of hair keratins to S-H bonds and makes hair flexible, and the second solution, 2% potassium bromate, which oxidizes the S-H bond to the original

Corresponding author: Dr. Shoei-Yn Lin-Shiau, Institute of Toxicology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei 100, Taiwan, ROC. Fax: 886-2-2341-0217, e-mail:syl@ha.mc.ntu.edu.tw
Received May 19, 2000; Revised June 1, 2000; Accepted June 2, 2000.

S-S bond, thus stabilizing the curling of the hair (8). Several clinical reports indicate that bromate poisoning ($LD_{50}$=200-500mg/Kg of body weight) causes severe and irreversible sensorineural hearing loss as well as renal failure (10).

The pathogenesis of inner ear deafness is thought to be largely due to an osmotic imbalance between endolymph and perilymph (21, 22). $KBrO_3$ has been known to produce maximum fall in potassium ion concentration in the endolymph at present (16). Some reports indicated that cochlear microphonics (CM) and the endocochlear dc potential (EP) were irreversibly suppressed after an injection (200mg/Kg, SC) of $KBrO_3$ into guinea pigs and histological study showed a marked degeneration of the outer hair cells in the stria vascularis (17). Although the neurotoxicity of $KBrO_3$ has been studied, neurotoxicity in guinea pigs determined by auditory brainstem responses (ABRs) was still subjected fewer investigations (14). Audiologic tests can be selected to help determine the dysfunction from the sensory neural and/or central auditory system, which can be monitored by bilateral abnormality in the auditory brainstem response. Recording change in the absolute and interwave latencies from the brainstem auditory evoked potentials can be served as a biomarker of $KBrO_3$ toxicity on the nervous system.

In this study, we attempt to comparatively investigate the ototoxic effects of $KBrO_3$ alone and combined with thioglycolate on hearing by monitoring of ABR. Subjects exposed to the compounds were evaluated with a test battery comprising both peripheral and central auditory tests. In addition, measurement of membrane ATPase activities ($Na^+$/$K^+$-ATPase and $Ca^{2+}$-ATPase) and nitric oxide level of the brainstem approaches in elucidating the sites and action mechanism of $KBrO_3$. We expect that this study will shed light not only on the oto-neurotoxic effects of $KBrO_3$ alone or with thioglycolate but also elucidate the action mechanism of the compound on hearing system. Whether this compound should be banned in the future application, the experimental data are definitely required for this harmful indication.

## Materials and Methods

*Animal Preparation*

Randomly bred guinea pigs were housed at 23 ±2 °C and 55 ±5% humidity and given a solid diet of GB-1 and tap water ad libidum. The study was conducted in accordance with the guideline for the care and use of Laboratory Animals by the Animal Research Committee in National Taiwan University, College of Medicine. In the preliminary experiment animals weighing 230-240g were subcutaneous injection of $KBrO_3$ (50mg/Kg) alone or combined with thioglycolate (15mg/Kg) dissolved in saline or saline alone (control), once a day for consecutive 2 weeks. The neurophysio- logical (ABR testings) parameters were examined before and after consecutive 1-2 weeks administration. In addition, changes of body weights were observed throughout the experimental period. Finally, these animals were sacrificed 24h after all testings under deep anesthesia with pentobarbital (60mg/Kg, ip), then the brainstems were removed. The brainstems were homogenized for enzymatic assays ($Na^+$/$K^+$-ATPase and $Ca^{2+}$-ATPase), the level of nitric oxide analysis.

*Recording of Auditory Brainstem Response (ABR)*

Following head subcutaneous injection of $KBrO_3$ alone or with thioglycolate once daily for two weeks, the guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital (60mg/Kg wt) and all auditory brainstem response (ABR) recordings were obtained at consecutive 1 and 2 weeks administration. An auditory evoked potential system (Nicolet, Spirit, Madison, WI, USA) was used to measure and record the brainstem evoked response in a sound attenuated room (7). Click sounds (57.7/s) and sweep (the number of evoked potentials: 1200) were used to evoke ABR and assess the thresholds of hearing. The extent of auditory dysfunction of experimental guinea pigs was assessed by determining the difference of ABR thresholds while the V wave still appeared (minimal occurrence of evoked responses). The intensity of stimulus was varied in 5-dB stepwise increment and the response signals were recorded by subdermal needle electrodes. The active electrodes were placed in the vertex and the ipsilateral retro-auricular region with a ground electrode on the neck of guinea pigs. We studied the effects on the auditory brainstem response (ABR) by applying a computerized calibration procedure for click stimuli that corrects for individual microphone transducer characteristics and ear canal acoustics (Model Tip-300, Nicolet, WI, USA). Meanwhile, the output from the transducers was channeled through plastic tubes into the animal's ear canals. Two sequences of recordings were made to confirm response reproducibility. Additionally, click sounds (11.1/s) were used to evoke ABR (polarity: postitive consolidation) and measure the absolute latencies of peak waves and interwave latencies at an 85 dB stimulus intensity for all guinea pigs.

*Enzyme Activity Assay*

Membrance ATPase activities were assayed (12, 18). The method allowed quantification of three

distinct $Na^+/K^+$-ATPase, $Ca^{2+}$-ATPase and $Mg^{2+}$-ATPase activities in the same sample. The enzymatic activities were measured in quadruplicate in covered 96 well microtiter plates at 37±0.5°C on a shaker. Ninety microliters of assay buffer (10% sucrose, pH =7.2) containing 2μg of membrance protein was added to each well. The $Na^+/K^+$-ATPase activity was determined by subtracting the ouabain (3.74 mM) sensitive activity from the overall $Na^+/K^+/Mg^{2+}$-ATPase and $Ca^{2+}/Mg^{2+}$-ATPase activities. The $Ca^{2+}$-ATPase activity was determined by subtracting activity measured in the presence of $Ca^{2+}$ and ouabain from that determined in the absence of $Ca^{2+}$ (no added $Ca^{2+}$ plus 0.1mM EGTA) and the presence of ouabain. The plate was preincubated at 37±0.5°C for 20 min, and the assay was started with the addition of 10μl of ATP (final concentration 5mM) making the final reaction volume of 100μl. The reaction was terminated by the addition of 200μl of malachite green (MG) plus ammonium molybdate (AM) (3:1). The plates were read on a microplate ELISA reader (Dynatech MR7000, USA) at 630nm. The absorbance values obtained were converted to activity values by linear regression using a standard curve of sodium monobasic phosphate that included in the assay procedure. Pi μmole represents the concentration of inorganic phosphate and Pi released by hydrolysis of ATP after stopping the enzyme reaction and then was determined colorimetrically. Finally, The ATPase activities were expressed as micromoles inorganic phosphate per milligram protein per 20 minutes. Values reported represent the mean±SEM of at least three separate experiments.

*Nitric Oxide (NO) Detection*

The brainsetm was weighed and homogenized in the homogenate buffer (0.32M sucrose-histidine buffer, pH=7.4 ), and then centrifuged at 4°C for 20 min at 10000xg. To avoid total protein denaturated incompletely, we added 70% ethanol into the pellete overnight. Next day, all samples were centrifuged at 4°C for 20 min at 12000xg; the supernatants of these tissues were collected and assayed by the NO/ozone chemiluminescence (NO Analyzer 280A SIEVERS) for quantitative NO assay as described previously (11). Briefly, we measured the oxidation products ($NO_2^-$ and $NO_3^-$) of NO using a reaction vessel containing a reducing system (0.1M vanadium chloride, Aldrich Co., Germany). Detection of NO is then completed by its reaction with ozone, which leads to the emission of red light ($NO+O_3 \rightarrow O_2^*+O_2$; $NO_2^* \rightarrow O_2+hv$). Standard curves were made right before concentration (1, 5, 10 15 and 20 μM NO), which were prepared using freshly prepared solutions of $NaNO_2$ (10 μl) in distilled water.

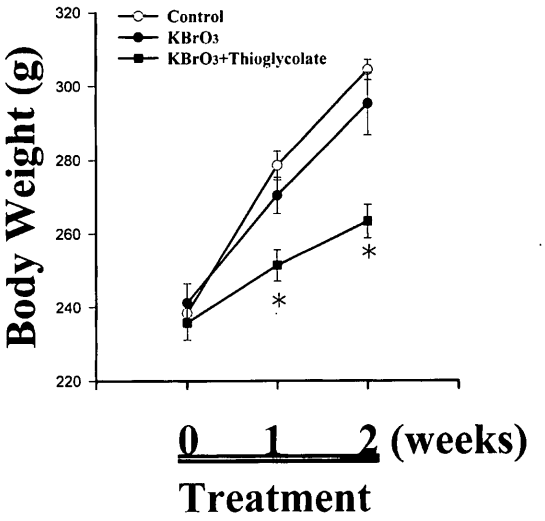


Fig. 1. Effects of $KBrO_3$ on body weight of guinea pigs. The guinea pigs were sub- cutaneously injected with either $KBrO_3$ (50 mg/kg) alone or plus thioglycolate (15 mg /kg) respectively once every day for consecutive 2 weeks. The body weights were recorded during treatment. Data are presented as mean±SEM (n=6 for each group) *P < 0.05 as compared with the respective control.

*Statistical Analysis*

Results are expressed as mean±SEM. When ANOVA was significant, the significance between groups was assessed by means of ANOVA followed by Dunnett's *t*-tests for all testings. It was considered to be significant when P value was less than 0.05.

## Results

*Influences of $KBrO_3$ on Body Weight*

The guinea pigs were subcutaneously injected with either $KBrO_3$ (50 mg/kg, s.c.) alone or combined with thioglycolate once every day for consecutive 2 weeks. We found that $KBrO_3$ (50mg/kg, s.c.) plus thioglycolate (15 mg/kg, s.c.) administration significantly decreased body weight during treatment. However, $KBrO_3$ alone did not produce such detrimental effect (Fig. 1).

*Abnormal ABR Induced by $KBrO_3$*

The purpose of this study aimed at investigating whether $KBrO_3$ alone or combined with thioglycolate can cause the functional abnormalities of auditory brainstem responses (ABR). Following administration of these compounds for consecutive 1 and 2 weeks, the yield in terms of ABR threshold is detailed in Fig. 2. After consecutive 1 week treatment, the results showed that subcutaneous injection of $KBrO_3$ (50 mg/kg, s.c.) together with thioglycolate (15 mg/kg, s.

**Table 1. The Absolute Latencies of ABR Waveforms in Guinea Pigs**

| ABR waveform | Absolute latencies (msec) | | |
|---|---|---|---|
| | Control | $KBrO_3$ | $KBrO_3$+TG |
| I | 1.66±0.19 | 1.90±0.24* | 2.10±0.30* |
| II | 2.60±0.21 | 2.92± 0.31* | 3.16±0.27* |
| III | 3.53±0.19 | 3.92±0.13* | 4.12±0.33* |
| IV | 4.69±0.24 | 5.02±0.33* | 5.25±0.43* |
| V | 5.89±0.29 | 6.27±0.32* | 6.50±0.42* |

Guinea pigs were subcutaneously injected with either $KBrO_3$ (50 mg/kg) alone or plus thioglycolate (TG, 15 mg /kg) respectively once a day for consecutive 2 weeks and then auditory brainstem responses (ABRs) are performed 24h after the last administration. Data are mean±SEM (n=6 for each group), *P < 0.05 as compared with control.

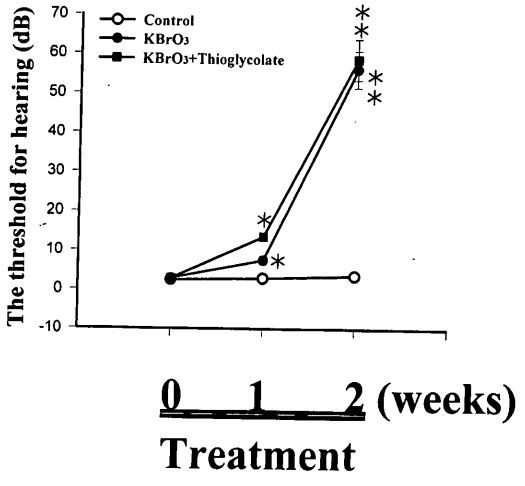


Fig. 2. Increased threshold of hearing for evoked auditory brainstem responses in guinea pigs induced by $KBrO_3$ and thioglycolate. Data are mean±SEM (n=6 for each group) *P < 0.05 as compared with the respective control.

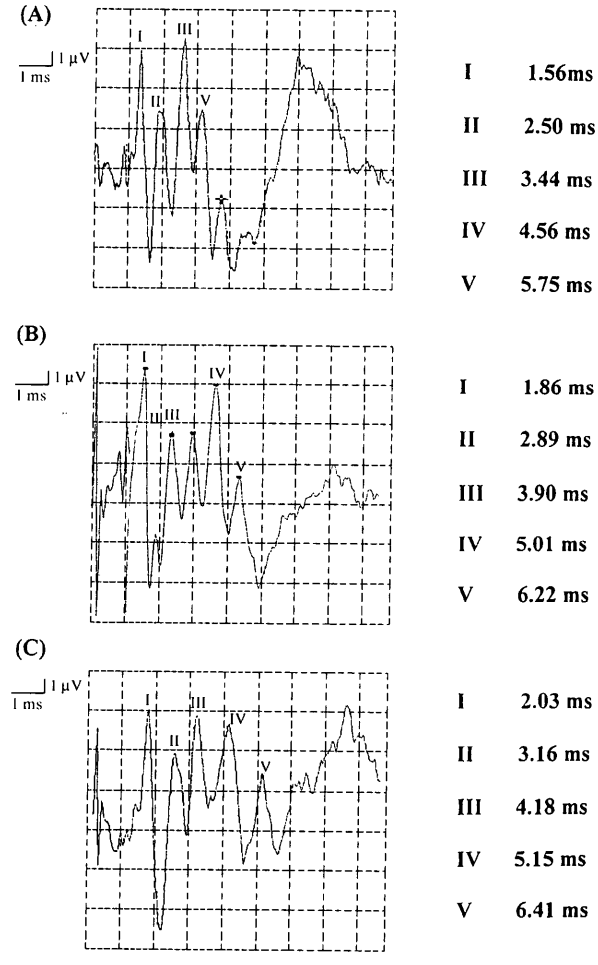


Fig. 3. Representative tracings of altered ABR in guinea pigs treated with $KBrO_3$ and thioglycolate. The waveform of ABR and absolute latencies of peak waves evoked by an 85 dB (click, 11.1/s) stimulus intensity in a representative guinea pig. (A) Normal response in a guinea pig treated with saline for 2 weeks. (B) The waveform is shown in a guinea pig treated with $KBrO_3$ alone (50 mg/kg, s.c.). (C) The waveform is shown in a guinea pig treated with $KBrO_3$ (50 mg/kg, s.c.) plus thioglycolate (15 mg/kg, s.c.). The ABR components are identified with Roman numberals and the absolute latencies of peak waves (msec) were indicated.

c.) caused a marked increase of the threshold for hearing from 3dB to 13dB, while exposure to $KBrO_3$ alone had only a small effect on this threshold (3dB to 7dB). After consecutive 2 weeks treatment, $KBrO_3$ alone or combined with thioglycolate could significantly elevate the threshold of hearing from 3dB to 55dB (Fig. 2).

We then recorded ABR patterns of guinea pigs 24h after consecutive 2 weeks administration and analyzed the ABR tracings including the absolute wave latencies of waves I, II, III, IV and V as well as the interwave intervals of I-II, I-III, I-IV, I-V and III-V. The representative tracings of the abnormal ABR waveform in guinea pigs caused by $KBrO_3$ alone or combined with thioglycolate were shown in Fig. 3.

The mean absolute latencies and interwave latencies of ABR waveforms at the stimulus levels of 85dB for conductive hearing testing were shown in Tables 1 and 2 respectively. As compared with the control group, we found that significant prolongations induced by $KBrO_3$ alone (Fig. 3B) especially in combination with thioglycolate (Fig. 3C) were seen for wave I, II, and III respectively. Meanwhile, significant prolonged interpeak latencies of I-II and I-III waves were seen in the conductively impaired ears as compared to those typical waveforms and latencies in control group (Fig. 3A). In contrast, the latencies of wave IV-V for the two treated groups remained unchanged.

**Table 2. The Interwave Latencies of ABR Waveforms in Guinea Pigs**

| ABR waveform | Interwave latencies (msec) | | |
|---|---|---|---|
| | Control | $KBrO_3$ | $KBrO_3$+TG |
| I-II | 0.94±0.05 | 1.03±0.18* | 1.06±0.06* |
| I-III | 1.87±0.18 | 1.98±0.12* | 2.02±0.22* |
| I-IV | 3.03±0.14 | 3.12±0.20* | 3.15±0.13* |
| I-V | 4.22±0.13 | 4.47±0.30* | 4.30±0.40* |
| III-V | 2.36±0.14 | 2.35±0.22 | 2.38±0.28 |

Guinea pigs were subcutaneously injected with either $KBrO_3$ (50 mg/kg) alone or plus thioglycolate (TG, 15 mg /kg) respectively once a day for consecutive 2 weeks and then auditory brainstem responses (ABRs) are performed 24h after the last administration. Data are mean±SEM (n=6 for each group). *P < 0.05 as compared with control.

**Table 3. Enzyme Activity and Nitric Oxide (NO) were Assayed Respectively in Guinea Pigs**

| Assay | Treatment | | |
|---|---|---|---|
| | Control | $KBrO_3$ | $KBrO_3$+TG |
| $Na^+/K^+$-ATPase (μmole/mg/20min) | 2.63±0.24 | 2.53±0.29 | 2.35±0.1 |
| $Ca^{2+}$-ATPase (μmole/mg/20min) | 1.84±0.18 | 1.8±0.12 | 1.76±0.14 |
| Nitric oxide (μmole/L) | 88.4±7.2 | 75.4±6.5 | 72.8±6.2 |

Guinea pigs were subcutaneously injected with either $KBrO_3$ (50 mg/kg) alone or plus thioglycolate (TG, 15 mg /kg) respectively once a day for 2 consecutive weeks and then biochemical assays were measured 24h after the last administration. Data are mean±SEM (n=6 for each group). *P < 0.05 as compared with control.

*Biochemical Assays*

Following daily subcutaneous injection of $KBrO_3$ alone or plus thioglycolate for consecutive 2 weeks, the data indicated that neither $Na^+/K^+$-ATPase ($KBrO_3$ alone: 2.53±0.29 μmole/mg/20min; $KBrO_3$ plus thioglycolate: 2.35±0.15 μmole/mg/20min respectively, $P$>0.05) nor $Ca^{2+}$-ATPase ($KBrO_3$ alone: 1.8±0.12 μmole/mg/20min; $KBrO_3$ plus thioglycolate: 1.76±0.14 μmole/mg/20min respectively, $P$>0.05) activity of brainstem significantly affected as compared with control vehicle (control: 2.63±0.24 and 1.84±0.18 μmole/mg/20min respectively) (Table 3). Meanwhile, the level of nitric oxide was not detected with the remarkable and discernible changed in brainstem ($KBrO_3$ alone: 75.4±6.5 μmole/L; $KBrO_3$ plus thioglycolate: 72.8±6.2 μmole/L respectively, $P$>0.05 ) as compared with control vehicle (88.4±7.2 μmole/L)

## Discussion

Potassium bromate ($KBrO_3$), marketed as a neutralizer in home permanent cold wave hair kits, has caused several cases of accidental poisoning in children, resulting from the ingestion of this solution (4, 13). The potassium bromate is the flour improving additive for bread making, most widely used. This additive has been reported to have hazardous effects. For this reason it has been forbidden in various countries (2). In order to understand the oto-neurotoxicity induced by the $KBrO_3$, we tried to assess the changes of threshold of hearing and ABR induced by $KBrO_3$ alone or combined with thioglycolate. The obtained results will provide evidence for development or acceleration of impairment of auditory function after consecutive 2 weeks administration either $KBrO_3$ alone or combination with thioglycolate.

It has been reported that there is a linear relationship between wave I latency, the peripheral component of the response, and nerve conduction velocity. In accordance with this notion we have found that $KBrO_3$ significantly prolonged wave I latency, especially in combination with thioglycolate, suggesting that $KBrO_3$ delayed the auditory nerve conduction velocity. On the other hand, the IV-V interval, an index of brainstem transmission, and nerve conduction velocity (6, 15), was not altered by $KBrO_3$ with or without thioglycolate, indicating that no conduction dysfunction in the brainstem was caused by $KBrO_3$. Based on these findings, together with a significant prolongation of wave I-II/I-III and the interwave latencies as well as the elevation of the threshold of hearing, it was suggested that auditory nerve-brainstem conduction velocity singnificantly decreased, leading to peripheral hearing loss. A similar study has been performed to examine a case of a 44 year old man, who had ingested potassium bromate solution for suicide attempt (5). Although the prolongations of interpeak latencies of I-IV/I-V or absolute latencies of IV and V (means the latency from initial to individual wave occurred) were still seen, the changes majorly resulted from the increase in the absolute latencies of I, II and III respectively. Thus, these results demonstrated that either $KBrO_3$ alone or $KBrO_3$ combined with thioglycolate depressesd the peripheral auditory perception but dose not depress the central conduction. Thus, the abnormal findings of ABR from a delay of the absolute latencies and interwave latencies of BAEPs waveforms

can be served as a biomarker of $KBrO_3$ toxicity on peripheral nervous system (PNS). In addition, Deshimaru (3) et al also reported that BAEPs may provide a sensitive tool for detecting subclinical peripheral neurotoxicity caused by $KBrO_3$.

Although our data showed no significant difference in the body weight between the $KBrO_3$ alone (total intake: 0.7 g/Kg body weight) and the control group, some reports indicated that $KBrO_3$ orally in their drinking water at doses of 500.0 ppm for 104 weeks (total intake: 2.5 g/Kg body weight), at the end of which time all the surviving animals had a marked decrease in body weight of male rats (9). Thus, we believed that the possible consequence of the reduced body weight may strengthen the toxicity induced by $KBrO_3$ plus thioglycolate may via rapid bromate accumulation or thioglycolate intoxication itself. In order to understand the machinery underlying the abnormal ABR induced by $KBrO_3$ alone or combined with thioglycolate, we also studied the change in enzymatic activities and nitric oxide (NO) level. As a result, it revealed no significant effect. On the basis of the biochemical findings, we call attention to examine the $KBrO_3$ intoxication in view of its selective oto-neurotoxicity via peripheral auditory nerve pathway. In conclusion, we believe that this study will shed light not only on the hearing loss induced by $KBrO_3$ alone but also the combined effect with thioglycolate; The oto-neurotoxicity is mediated by peripheral auditory nerve dysfunction rather than directly via central brainstem intoxication. However, the mechanism of the enchancing effect of thioglycolate on $KBrO_3$-inducing oto-neurotoxicity requires further studies.

## Acknowledgments

This investigation was supported by a research grant (IBMS-CRC88-T11) Academia Sinica, Taipei, Taiwan, ROC.

## References

1. Cho, D.H., Hong, J.T., Chin, K., Cho, T.S., Lee and B.M. Organotropic formation and disappearance of 8-hydroxydeoxyguanosine in the kidney of Sprague-Dawley rats exposed to adriamycin and $KBrO_3$. *Cancer Lett.* 74: 141-5, 1993.
2. Corrales, X., Guerra, M., Granito, M. and Ferris, J. Substitution of ascorbic acid for potassium bromide in the making of French bread. *Arch Latin Nutr.* 43: 234-40, 1993.
3. Deshimaru, M., Miyagawa, T., Sumiyoshi, S. and Nomura, Y. Peripheral nerve disease associated with acute renal failure due to bromate poisoning. *Brain Nerve* 28: 807-14, 1976.
4. Gradus, D., Rhoads, M., Bergstrom, L.B. and Jordan, S.C. Acute bromate poisoning associated with renal failure and deafness presenting as hemolytic uremic syndrome. *Am. J. Nephrol.* 4: 188-191, 1984.
5. Hamada, F., Ohzono, S., Yamada, S., Baba, Y., Tokuda, Y., Yamashita, W., Nakashima, A., Harada, R, Arima, T. and Morita, T. A case of acute potassium bromate intoxication. *Fukuoka Acta. Med.* 81: 271-276, 1990.
6. Hirata, M., Ogawa, Y., Okayama, A. and Goto S. Changes in auditory brainstem response in rats chronically exposed to carbon disulfide. *Arch. Toxicol.* 66: 334-8, 1992.
7. Hsu, C.J., Liu, T.C. and Lin, K.N. Effect of acoustic trauma on cytochrome oxidase activity in stria vascularis. *J. Otolaryngol.* 60: 314-317, 1998.
8. Hytonen, M., Leino, T., Sala, E., Kanerva, L., Tupasela, O. and Malmberg, H. Nasal provocation test in the diagnostics of hairdressers' occupational rhinitis. *Acta. Oto-laryngol.* 529: 133-136, 1997.
9. Kurokawa, Y., Hayashi, Y., Maekawa, A., Takahashi, M., Kokubo, T. and Odashima, S. Carcinogenicity of potassium bromate administered orally to F344 rats. *J. Nat. Can. Ins.* 71: 965-972, 1983.
10. Kutom, A., Bazilinski, N.G., Magana, L. and Dunea, G. Bromate intoxication: hairdressers' anuria. *Am. J. Kidney Dis.* 15: 84-85, 1990.
11. Lin, C.H., Kang, B.H., Wong, C.H., Mao, S.H. and Wan, F.J. Systemic administration of D-amphetamine induced a delayed production of nitric oxide in the straitum of rats. *Neurosci. Lett.* 276: 141-144, 1999.
12. Liu, S.H., Sheu, Z.J., Lin, R.H. and Lin-Shiau, S.Y. The *in vivo* effect of lipopolysaccharide on neuromuscular transmission in the mouse. *Eur. J. Pharmacol.* 333: 241-247, 1997.
13. Matsumoto, I., Morizono, T. and Paparella, M.M. Hearing loss following potassium bromate: two case reports. *Otolaryng Head Neck Surg.* 88: 625-629, 1980.
14. Matsuyama, Z., Katayama, S. and Nakamura, S. A case of sodium bromate intoxication with cerebral lesion. *Clin. Neurol.* 33: 535-540, 1993.
15. Miller, G., Skouteli, H., Dubowitz, L.M. and Lary, S. The maturation of the auditory brainstem response compared to peripheral nerve conduction velocity in preterm and full-term infants. *Neuropediatrics* 15: 25-27, 1984.
16. Morizono, T. and Ikeda, K. The effects of cetrimide and potassium bromate on the potassium ion concentration in the inner ear fluid of the guinea-pig. *Physiol. Biochem.* 37: 241-247, 1988.
17. Muratsuka, Y., Ueda, H. and Konishi, T. Effects of sodium bromate on ionic concentrations and osmolalities of the cochlear fluids in guinea pigs. *Hearing Res.* 39: 241-249, 1989.
18. Rohn, T.T., Hinds, T..R. and Vincenzi, F.F. Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the $Ca^{2+}$ pump ATPase and $Na^+/K^+$-pump ATPase of human red blood cell membranes. *Biochem. Pharmacol.* 46: 525-534, 1993.
19. Sai, K., Tyson, C.A., Thomas, D.W., Dabbs, J.E., Hasegawa, R. and Kurokawa, Y. Oxidative DNA damage induced by potassium bromate in isolated rat renal proximal tubules and renal nuclei. *Cancer Lett.* 87: 1-7, 1994.
20. Sai, K., Takagi, A., Umemura, T., Hasegawa, R. and Kurokawa, Y. Relation of 8-hydroxydeoxyguanosine formation in rat kidney to lipid peroxidation, glutathione level and relative organ weight after a single administration of potassium bromate. *Jpn. J. Cancer Res.* 82: 165-169, 1991.
21. Shah, D.M., Freeman, D.M. and Weiss, T.F. The osmotic response of the isolated, unfixed mouse tectorial membrane to isosmotic solutions: effect of $Na^+$, $K^+$, and $Ca^{2+}$ concentration. *Hearing Res.* 87: 187-207, 1995.
22. Yeh, T.H., Herman, P. and Tsai, M.C. A cationic nonselective stretch- activated channel in the Reissner's membrane of the guinea pig cochlea. *Am. J. Physiol.* 274: C566-576, 1998.