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Analysis of Bromate in Drinking Water Using Liquid Chromatography-Tandem Mass Spectrometry without Sample Pretreatment

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An analytical method for determining bromate in drinking water was developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The ^{18}O-enriched bromate was used as an internal standard. The limit of quantification (LOQ) of bromate was 0.2 μg/L. The peak of bromate was separated from those of coexisting ions (*i.e.*, chloride, nitrate and sulfate). The relative and absolute recoveries of bromate in two drinking water samples and in a synthesized ion solution (100 mg/L chloride, 10 mg N/L nitrate, and 100 mg/L sulfate) were 99 - 105 and 94 - 105%, respectively. Bromate concentrations in 11 drinking water samples determined by LC-MS/MS were <0.2 - 2.3 μg/L. The results of the present study indicated that the proposed method was suitable for determining bromate concentrations in drinking water without sample pretreatment.

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Introduction

Bromate may be carcinogenic in humans and is listed as Group 2B by the International Agency for Research on Cancer (IARC).[1] Bromate is known to be a disinfection by-product during ozonation.[2] It has also been reported that bromate is present as an impurity in sodium hypochlorite solution, a disinfectant.[3,4] The maximum contaminant level (MCL) of bromate in drinking water in the United States of America (USA) is 10 μg/L.[5] The guideline value in the World Health Organization (WHO) Guidelines for drinking water quality and the standard value in drinking water in Japan are 10 μg/L.[6,7]

To determine bromate concentration in drinking water at low concentration, ion chromatography-postcolumn reaction (IC-PCR) has been applied.[8-11] This method is selected as an official method for drinking water in Japan.[12] However, maintenance of the postcolumn module was difficult because high concentrations of sulfate solution and other chemicals were used. Recently, in the IC system, mass spectrometry (MS) has been applied to determine bromate. The methods using both MS (*i.e.*, IC-MS)[13-15] and tandem mass spectrometry (*i.e.*, IC-MS/MS)[16-20] were reported, but the number of studies using IC-MS/MS, a more accurate method, seemed to be larger. Also, more recently, liquid chromatography (LC)-MS/MS has been applied for bromate analysys.[21,22] Although IC-MS/MS and LC-MS/MS are highly sensitive and accurate, these methods have a problem for the application in drinking water. That is, when coexisting ions (*e.g.*, chloride, nitrate and sulfate) contained in drinking water co-eluted with bromate, ion suppression or ion enhancement occurred, which resulted in reduction or increase of the sensitivity. Thus, for the determination of bromate in drinking water using IC-MS/MS or LC-MS/MS, it is necessary to chromatographically separate bromate from coexisting ions, particularly chloride for IC, or to mitigate their effects with sample pretreatment. In the case of IC-MS/MS, chromatographic separation was occasionally achieved and bromate were determined without sample pretreatment.[19,20] On the other hand, in the case of LC-MS/MS, no methods for analysis of bromate without pretreatment have yet been reported. As examples of the sample pretreatment methods, pretreatment cartridges have been used to remove coexisting ions from water.[21] In addition, sample dilution was performed to reduce the effects of coexisting ions and to determine oxyhalide including bromate in sodium hypochlorite solution.[22] In this previous study,[22] ^{18}O-enriched perchlorate and bromate were used as internal standards of perchlorate and bromate, respectively. From these reports, we considered that IC-MS/MS might be more suitable than LC-MS/MS to determine ions including bromate in drinking water. However, compared to LC-MS/MS, more components such as a suppressor and an auxiliary pump are needed for IC-MS/MS. Also, in Japan, application of IC-MS/MS is limited in water utilities and institutions for water quality tests. Therefore, we considered that the development of an analytical method for bromate using LC-MS/MS without pretreatment would be very useful.

In the present study, we developed a method for analysis of bromate in drinking water using LC-MS/MS without the need for sample pretreatment. Bromate concentrations in drinking water were determined using the proposed method.

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Table 1 Compound-dependent parameter of MS/MS

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Declustering potential/ V	Collision energy/ V	Collision cell exit potential/V
Bromate	126.884	110.700	−45	−30	−2
^{18}O-Enriched bromate	132.893	114.800	−45	−30	−2
Chloride	35.000	35.000	−20	−6	−4
Nitrate	62.014	46.100	−30	−30	−4
Sulfate	96.956	79.900	−50	−28	0

Experimental

Reagents and solutions

Ultrapure water purified using a Gradient A10 water purification system (Millipore, Bedford, MA) was used for the experiments (*e.g.*, preparations of the standard and stock solutions and eluents). Standard bromate solution was purchased from Kanto Chemical (Tokyo, Japan), and those of chloride, nitrate, sulfate and chlorite were purchased from Wako Pure Chemicals (Osaka, Japan). Standard haloacetic acid solutions containing nine types of haloaccetic acids were purchased from Kanto Chemical. ^{18}O-Enriched potassium bromate solution was purchased from Cambridge Isotope Laboratories (Andover, MA). All other reagents used in the present study were of analytical grade.

Sample preparation and recovery studies

The ^{18}O-enriched bromate was added to the samples as an internal standard and mixed before analysis (final concentration, 2.0 μg/L). Bromate recovery studies were performed using two types of drinking water (*i.e.*, drinking waters A and B) and synthesized ion water. Drinking water samples A and B were tap water collected in Saitama and Chiba prefectures, respectively. Ammonium chloride (Sigma-Aldrich, St. Louis, MO) was added in drinking water (final concentration, 20 mg/L) to transform residual chlorine into chloramines.[20] The synthesized ion water was prepared by dissolving 100 mg/L of chloride, 10 mg N/L of nitrate and 100 mg/L of sulfate in ultrapure water. For all recovery studies, spiked bromate concentration was 1.0 μg/L. Separation of bromate and haloacetic acids or chlorite was also investigated. Moreover, 11 drinking water samples were collected in June and July 2010 to determine bromate levels in drinking water. The two drinking water samples used for recovery studies were not included in the 11 drinking water samples. As was the case in the recovery studies described above, ammonium chloride was added in drinking water to transform residual chlorine into chloramines. All the sample solutions collected were refrigerated at 4°C.

Analytical methods

Bromate concentrations in water samples were determined by LC-MS/MS. The separation was performed using an Agilent 1200SL binary pump (Agilent Technologies, Palo Alto, CA). The separation column used was an Acclaim Trinity P1 column (3.0 mm × 100 mm, 3 μm; Dionex, Sunnyvale, CA); its temperature was 30°C. This column has reversed-phase, anion-exchange and cation-exchange retention properties.[23] Eluent A was a 40/60 (v/v) mixture of 20 mM ammonium acetate (Wako Pure Chemicals) and 0.05% (v/v) acetic acid (Wako Pure Chemicals) aqueous solution (pH 5)/acetonitrile (high-performance liquid chromatography grade; Wako Pure Chemicals). Eluent B was a 90/10 (v/v) mixture of 200 mM ammonium acetate and 0.5% (v/v) acetic acid aqueous solution (pH 5)/acetonitrile. The gradient conditions of eluent B were as follows: 0% held for 9 min, a linear increase to 95% in 0.5 min and held for 10 min, a linear decrease to 0% in 0.5 min and held for 6 min. After each run, the eluents were flowed for 1 min under the initial conditions. The total run time of each sample was 27 min. The flow rate was 0.7 mL/min, and the injection volume was 50 μL. Detection was performed using a 3200 QTRAP tandem mass spectrometer (Applied Biosystems, Foster City, CA) operated in the negative-ion turbo ion spray mode. In the present study, only MS/MS was investigated and the comparison between MS and MS/MS was not performed. This is because MS/MS is known to be more accurate and the investigation of the analytical conditions without sample pretreatment was considered to be a more interesting topic. The analytical conditions of the tandem mass spectrometer were optimized for bromate analysis. Collision gas flow was 6 psig, curtain gas flow was 30 psig, ion source gas 1 flow was 70 psig, ion source gas 2 flow was 60 psig, ionspray voltage was −4500 V and temperature was 700°C. The multiple reaction monitoring (MRM) transitions were *m/z* 127 ($^{79}BrO_3^-$) to *m/z* 111 ($^{79}BrO_2^-$) for bromate and *m/z* 133 ($^{79}Br^{18}O_3^-$) to *m/z* 115 ($^{79}Br^{18}O_2^-$) for ^{18}O-enriched bromate. The details of the compound-dependent parameters of MS/MS are shown in Table 1. In some cases, the peaks of chloride, nitrate and sulfate were monitored. These MRM transitions were *m/z* 35 ($^{35}Cl^-$) to *m/z* 35 ($^{35}Cl^-$), *m/z* 62 (NO_3^-) to *m/z* 46 (NO_2^-) and *m/z* 97 (HSO_4^-) to *m/z* 80 (SO_3^-), respectively.

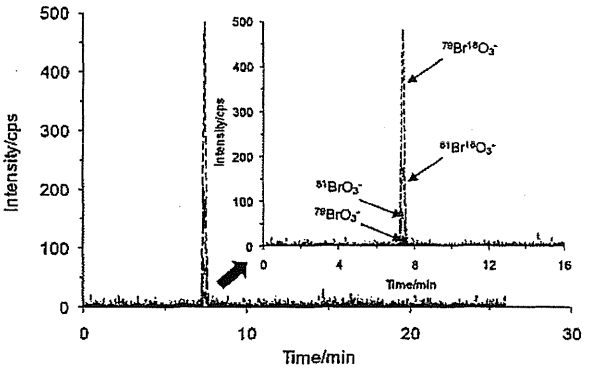

Fig. 1 MRM chromatograms of ^{18}O-enriched bromate at 10 μg/L in ultrapure water.

Results and Discussion

Optimization of analytical conditions of bromate and its limit of quantification

Figure 1 shows the MRM chromatograms of ^{18}O-enriched bromate at 10 μg/L in ultrapure water samples. The ^{18}O-enriched bromate has two isotopes (*i.e.*, $^{79}Br^{18}O_3^-$ and $^{81}Br^{18}O_3^-$). The background of the chromatogram of $^{81}Br^{18}O_3^-$ was higher than that of $^{79}Br^{18}O_3^-$. Thus, the peak area of $^{81}Br^{18}O_3^-$ was lower than that of $^{79}Br^{18}O_3^-$ although the isotope abundance ratios were similar. That is, *m/z* 133 ($^{79}Br^{18}O_3^-$) to *m/z* 115 ($^{79}Br^{18}O_2^-$) was used for the MRM transition of ^{18}O-enriched bromate. Also, 10 μg/L of ^{18}O-enriched bromate solution had bromate peaks ($^{79}BrO_3^-$ and $^{81}BrO_3^-$) although the peak areas were lower

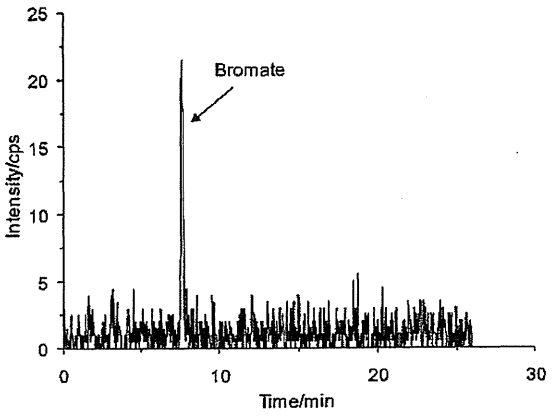

Fig. 2 MRM chromatograms of bromate at 0.2 μg/L in ultrapure water.

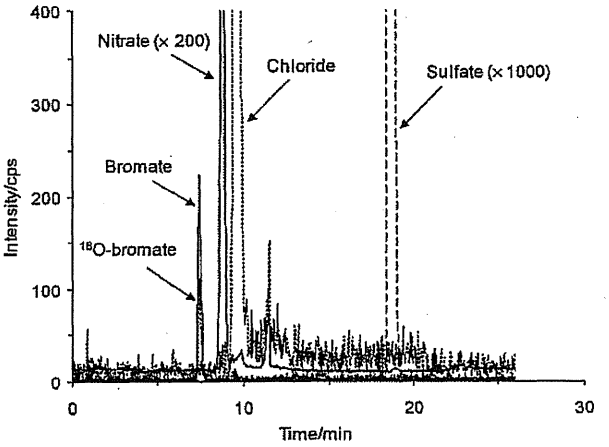

Fig. 3 MRM chromatograms of bromate, chloride, nitrate and sulfate in drinking water.

Table 2 Recovery of bromate in matrix solution ($n = 3$)[a]

Matrix	Native bromate concentration/μg L^{-1}	Relative recovery, %[b,c]	Absolute recovery, %[c]
Drinking water A	<0.2	105 (3.7)	102 (1.0)
Drinking water B	0.4	109 (4.0)	105 (1.0)
Synthesized ion water	—	99 (1.6)	94 (5.5)

a. Spiked bromate concentration was 1.0 μg/L.
b. Recovery corrected by ^{18}O-enriched bromate.
c. Values in parentheses are relative standard deviations (RSDs).

than that of ^{18}O-enriched bromate. The peak area of $^{81}BrO_3^-$ was higher than that of $^{79}BrO_3^-$. In the previous study, the presence of the bromate peak in ^{18}O-enriched bromate solution was not described.[22] Note that the supplier in the previous study was different from that in the present study. When ^{18}O-enriched bromate concentration was reduced to 2.0 μg/L, the peak of $^{79}BrO_3^-$ was not observed. Thus, in the present study, the ^{18}O-enriched bromate concentration added to the sample solution as an internal standard was set to 2.0 μg/L. In the case of bromate, the MRM transition of $^{79}BrO_3^-$ was selected for monitoring.

The bromate calibration points were set at 0.2, 0.5, 1.0, 5.0, and 10 μg/L, and the calibration curve was linear ($R^2 > 0.998$). The method detection limit (MDL) of bromate was determined by repeated analyses ($n = 7$) of 0.2 μg/L of bromate in ultrapure water. Figure 2 shows the MRM chromatograms of bromate at 0.2 μg/L in ultrapure water. The bromate retention time was ~7.5 min. The mean bromate concentration was 0.22 μg/L and its standard deviation (SD) was 0.019 μg/L. The value of 10 × SD was 0.19 μg/L, and therefore the limit of quantification (LOQ) of bromate was set at 0.2 μg/L. It was reported previously that, in the case of IC-MS/MS, the LOQ of bromate was 0.05 μg/L,[21] and the lowest concentration minimum reporting level (LCMRL) was calculated as 0.042 μg/L.[20] It was also reported that the LOQ for bromate using LC-MS/MS was 0.1 μg/L.[18] Thus, the LOQ and LCMRL of bromate using LC-MS/MS in the present study were higher than those in previous studies. On the other hand, the LOQ of the conventional method, IC-PCR, is 0.2 μg/L.[24] The target value for bromate (*e.g.*, MCL in the USA, the guideline value in WHO Guideline for drinking water quality, and a standard value in Japan) is 10 μg/L in many cases.[5-7] Therefore, the LOQ of bromate in the present study was sufficient to determine bromate in drinking water.

Recovery studies of bromate

Next, bromate recovery was investigated ($n = 3$). Table 2 shows the results of bromate in matrix solutions (*i.e.*, two samples of drinking water and synthesized ion water). The mean relative recoveries of bromate were in the range of 99 - 105%. The relative standard deviations (RSDs) were 1.6 - 4.0%. The mean absolute recoveries of bromate were also high (*i.e.*, 94 - 105%), and the RSDs were 1.0 - 5.5%. The addition of ammonium chloride did not affect the recoveries of bromate in drinking water. Thus, we showed that, using the proposed method, sample pretreatment (*e.g.*, using pretreatment cartridges) was not required to determine bromate concentrations.

Separation of bromate with coexisting compounds

Figure 3 shows MRM chromatograms of bromate and coexisting ions (*i.e.*, chloride, nitrate and sulfate) in drinking water. In the case of IC, the chloride retention time is generally shorter than that of nitrate. The bromate peak occasionally overlaps with that of chloride. However, in the present study, the bromate peak was separated from those of chloride, nitrate and sulfate. In addition, the nitrate retention time was shorter than that of chloride. The column used has the multiple retention properties[23] and we considered that the separation of the ions was obtained by the combined effects of these properties. Thus, we presumed that the separation mechanism of the ions by the column used was different from that by IC although the main mechanism was unclear. This chromatographic separation was considered to be one reason for the high recoveries of bromate observed in the present study (Table 2). In the present study, the gradient condition of LC was changed back to the initial state (*i.e.*, 0% of eluent B) after the elution of sulfate (Fig. 3). We considered that the retention times of some compounds in drinking water are longer than that of sulfate. For example, the retention times of some haloacetic acids were longer than that of sulfate in IC-MS/MS system.[18-20] Elution of nine haloacetic acids were investigated under the condition that the final gradient conditions in the present study (*i.e.*, 95% of

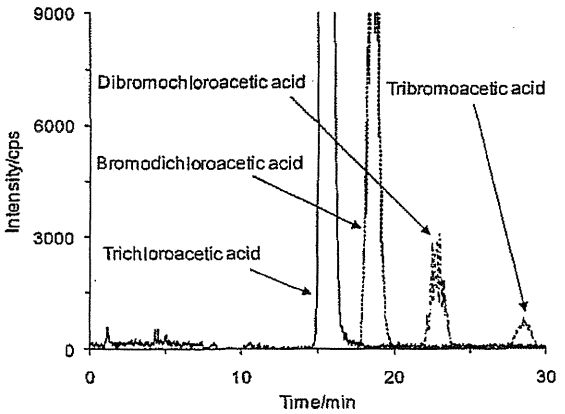

Fig. 4 MRM chromatograms of some haloacetic acids at 5 mg/L in ultrapure water.

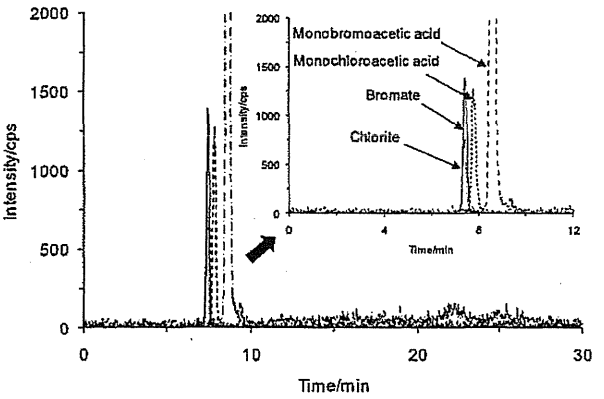

Fig. 5 MRM chromatograms of bromate (10 μg/L), chlorite (100 μg/L), monochloroacetic acid (100 μg/L) and monobromoacetic acid (100 μg/L) in ultrapure water.

eluent B) were continued. As in the case of IC-MS/MS system,[18-20] the retention times of trihaloacetic acids were longer than those of mono- and dihaloacetic acids (data not shown). The retention time of tribromoacetic acid was around 30 min and was the slowest among the four trihaloacetic acids (Fig. 4). In the figure, the MRM transitions of trichloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid and tribromoacetic acid were *m/z* 161 ($C^{35}Cl_3COO^-$) to *m/z* 117 ($C^{35}Cl_3^-$), *m/z* 161 ($C^{79}Br^{35}Cl_2^-$) to *m/z* 79 ($^{79}Br^-$), *m/z* 209 ($C^{81}Br_2{}^{35}Cl^-$) to *m/z* 81 ($^{81}Br^-$) and *m/z* 251 ($C^{79}Br_2{}^{81}Br^-$) to *m/z* 79 ($^{79}Br^-$), respectively. The temperature of the MS/MS system was set at 300°C in Fig. 4. This value was lower than that of the analytical conditions of bromate (*i.e.*, 700°C) (see section of analytical methods). This is because the sensitivities of the trihaloacetic acids in the MS/MS system were low at 700°C, and their peaks were not observed. It was considered that trihaloacetic acids were easy to degrade by thermal decomposition. The result in Fig. 4 indicated that the longer gradient condition of 95% of eluent B seemed to be better from the point of the accumulation of compounds in the column. However, as described above, the recovery of bromate in the gradient conditions used in the present study was high (Table 2). Thus, this gradient condition in the present study was employed. Moreover, it was reported that the peak of bromate was close to that of monobromoacetic acid and was relatively close to those of monochloroacetic acid and chlorite in IC-MS/MS system.[13] In LC-MS/MS system, the peak of bromate was close to that of monochloroacetic acid and was relatively close to that of monobromoacetic acid (Fig. 5). In the figure, the MRM transitions of monochloroacetic acid, monobromoacetic acid and chlorite were *m/z* 93 ($C^{35}ClH_2COO^-$) to *m/z* 35 ($^{35}Cl^-$), *m/z* 139 ($C^{81}BrH_2COO^-$) to *m/z* 81 ($^{81}Br^-$) and *m/z* 67 ($^{35}ClO_2^-$) to *m/z* 51 ($^{35}ClO^-$), respectively. On the other hand, the peak of bromate was overlapped with that of chlorite. In drinking water regulation in Japan, chlorite is selected as the management item and its target value is 600 μg/L.[12] Our results suggested that it was difficult to determine bromate using the proposed method without sample pretreatment in the samples containing chlorite at high concentration, although chlorite concentration in drinking water is generally low.

Application in drinking water samples

Moreover, the bromate concentrations in 11 drinking water samples were determined using LC-MS/MS. Three drinking water samples had conventional purification systems (*i.e.*, coagulation, flocculation and sand filtration). Bromate was detected in two of the three drinking water samples, and its concentrations were <0.2 - 0.4 μg/L. The purification systems of the remaining eight drinking water samples involved conventional purification systems with ozone/biological activated carbon (BAC) treatment. Bromate was detected in all eight drinking water samples at concentrations in the range 0.5 - 2.3 μg/L. These results were in agreement with those of previous studies indicating that bromate is produced during ozonation.[2] The absolute recoveries of ^{18}O-enriched bromate in the 11 drinking water samples were 94 - 101%. The results of the present study indicated that the proposed LC-MS/MS method is applicable for determination of bromate concentrations in drinking water without sample pretreatment.

Conclusions

(1) The LOQ of bromate using LC-MS/MS with ^{18}O-enriched bromate as an internal standard was 0.2 μg/L.
(2) The relative and absolute recoveries of bromate in two drinking water samples and one synthesized ion solution were 99 - 105 and 94 - 105%, respectively.
(3) Bromate concentrations in 11 drinking water samples determined using LC-MS/MS were <0.2 - 2.3 μg/L. The proposed method using LC-MS/MS was applicable to determination of bromate in drinking water without sample pretreatment.

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（2） 水道におけるN-ニトロソアミン類とその前駆物質の実態調査

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全国19浄水場を対象に，夏季，冬季の原水と浄水中の3種のN-ニトロソアミン類（N-ニトロソジメチルアミン（NDMA），N-ニトロソモルホリン（NMor），N-ニトロソピロリジン（NPyr））の実態調査を行った．原水，浄水中のNDMAは，それぞれ延べ36試料中11試料，36試料中9試料から検出された．NMorはそれぞれ延べ36試料中4試料，36試料中4試料から検出された．NPyrはいずれの試料からも検出されなかった．下水処理場放流水と事業所排水口直下の水路の水の場合，NDMAとNMorは，10試料全てから検出され，NPyrは検出されなかった．原水や排水をクロラミン処理，オゾン処理したところ，NDMAの場合，クロラミン処理後の方が濃度が増加した試料数は多かった．NMorの場合，いずれの処理後でも濃度は増加せず，NPyrは，一部の排水試料において，クロラミン処理後に濃度が増加した．3試料を対象に，オゾン処理時間とNDMAの生成との関係について検討した結果，試料によって時間は異なったが，いずれも溶存オゾンが検出され始めるまでの処理時間に設定すると，NDMAの生成量は最大になることがわかった．

Key Words : *N-nitrosamines, N-nitrosodimethylamine (NDMA), N-nitrosamine precursors, chloramination, ozonation, water supply*

1. はじめに

N-ニトロソジメチルアミン（NDMA）は，国際がん研究機関（IARC）においてグループ2A（ヒトに対しておそらく発がん性がある）[1]に，統合的リスク情報システム（IRIS）においてクラスB2（人に対して発がんの可能性がある）[2]に分類されている．また，IRISは10^{-5}の生涯発がんリスクに相当する飲料水中濃度として7 ng/L[2]を，世界保健機関（WHO）は飲料水中のガイドライン値として100 ng/L[3]を示している．国内の飲料水に係わる規制では，要検討項目に指定され，目標値として100 ng/L[4]が定められている．

NDMA以外の複数のN-ニトロソアミン類も，IARCでグループ2Aやグループ2B（人に対する発がん性が疑われる）[1]に，IRISでクラスB2[5]に分類されている．また，米国環境保護庁（USEPA）の第3次未規制物質候補リスト（CCL3）[6]にNDMAを含む5種のN-ニトロソアミン類が，第2次未規制物質監視規則（UCMR2）[7]にNDMAを含む6種のN-ニトロソアミン類が指定されている．

海外では，NDMAに関する研究は，カナダ[8]やカリフォルニア州[9]の飲料水中から検出されて以降，実態調査や新規の消毒副生成物としてクロラミン処理による生成機構等が，複数の研究者によって進められてきた[8),10)-16)]．また，NDMAは，オゾン処理による副生成物でもあることも報告された[17),18)]．また，当初は，N-ニトロソジメチルアミンのうち，NDMAに対する研究が主であったが，それ以外のN-ニトロソジメチルアミン類についての調査研究も行われている[19)-21)]．

国内では，NDMAに関する研究は，数年前から幾つか報告が行われてきた．例えば，全国の浄水場や浄水プロセスにおけるNDMAの実態調査[22)]，淀川流域や利根川流域を対象とした下水処理場放流水や河川水中の実態調査[23)-25)]，東京の地下水中のNDMAの実態調査[26)]等が挙げられる．また，NDMAの前駆物質に係わる研究では，淀川流域では，下水処理場放流水中にオゾン処理によるNDMA前駆物質が含まれていることも明らかとなってい

る[22,23,27]．一方，NDMA以外のN-ニトロソアミン類に関する調査研究は，淀川流域の浄水場，上流の下水処理場放流水，河川水等について行われているが[26]，報告は少ない．このため，N-ニトロソアミン類やクロラミン処理，オゾン処理による前駆物質について，全国的な存在状況の把握することは，重要であると考えられる．

また，現状，国内水道では，クロラミン処理を採用している浄水場はごく一部に限られ，浄水中でNDMA濃度が相対的に高い濃度で検出された浄水場は，特に冬季の淀川流域のオゾン処理を導入している浄水場であった[22]．これらのことから，国内水道では，オゾン処理によるNDMAの生成が課題となっていると考えられるが，その生成能を評価する際，どのような条件で実施したら適切であるか明らかになっていない．試験室ごとで，反応槽や対象水が異なることから，オゾン処理条件に対する何らかの共通の目安を示すことができれば，非常に有用であると考えられる．

本研究は，全国の浄水場を対象に，N-ニトロソアミン類について調査を行った．N-ニトロソアミン類として，NDMA およびこれまでの国内外の調査を参考にして，N-ニトロソモルホリン（NMor），N-ニトロソピロリジン（NPyr）を対象とした．また，浄水場の上流域にある下水処理場等の排水についても同様に調査した．クロラミン処理やオゾン処理による影響についても検討し，前駆物質の調査を行った．さらに，オゾン処理によるN-ニトロソアミン類の生成を評価する際の処理条件についても検討した．

2. 実験方法

(1) 試薬および保存溶液

NDMA，NMor，NPyrの標準液は，Supelcoから購入した．NDMA-d_6はC/D/N Isotopesから購入し，NMor-d_8およびNPyr-d_8はCambridge Isotope Laboratoriesから購入した．クロラミン溶液は，次亜塩素酸ナトリウム溶液とpH 8.5に調製した塩化アンモニウム溶液を1：1.2 mol/molで混合させ，冷蔵庫で1～2時間静置させた後，実験に使用した[20]．クロラミン溶液は，実験日ごとに作成した．各溶液の調製には，Gradient A10（Millipore）で精製した超純水を使用した．ただし，超高速液体クロマトグラフタンデム質量分析計（UPLC-MS/MS）の溶離液の場合のみ蒸留水（LC-MS用，関東化学）を使用した．

(2) 試料の採取

2010年7～9月（夏季）と2011年1，2月（冬季）に，全国の19浄水場（A～S浄水場）の原水と浄水を採取し，N-ニトロソアミン類濃度の測定を行った．ただし，F，H，N浄水場は冬季の調査は行わず，O浄水場は夏季については2回調査を行った．5浄水場（F，G，H，N，O浄水場）については，プロセス水の調査も実施した（O浄水場の夏季調査の場合，プロセス水の調査は2回のうち1回のみ）．これら浄水場は，高度浄水プロセス（オゾン/活性炭処理）を採用している．原水の場合，F浄水場を除き，クロラミン処理による影響についての調査も行った．

2010年11，12月，淀川流域の5下水処理場放流水（a～e下水処理場，b下水処理場については2放流口があったためb-1，b-2と表記）と淀川河川水（YR）を採取した．淀川流域の一部の下水処理場にはオゾンによる前駆物質が流入し，放流水中にも存在していることが報告されている[22,23,27]．2011年3月，利根川流域の3下水処理場放流水（f～h下水処理場），事業所排水口直下の水路の水（j），利根川河川水（TR）を採取した．YR，TRのいずれも調査対象とした下水処理場，事業所排水口直下の水路の下流に，また，浄水場（D～H浄水場：利根川流域；N，O浄水場：淀川流域）の上流に位置している．これら淀川流域，利根川流域で採取した排水や河川水について，N-ニトロソアミン類濃度，クロラミン処理，オゾン処理による前駆物質について調査した．なお，淀川流域，利根川流域の下水処理場や事業所は，浄水場の上流に位置している．全ての試料の採取はスポット採取であり，また，プロセス水の採取では到達時間の考慮は行っていない．

(3) クロラミン処理，オゾン処理によるNDMAの生成

クロラミン処理，オゾン処理のいずれも反応容器には容量1 Lのガラス製のねじ口瓶を用いた。クロラミン処理によるN-ニトロソアミン類の生成は，試料量は600 mL，反応時間は24時間，pH 7（5 mMりん酸緩衝液），水温は20℃，24時間後のクロラミン濃度が3.0±0.5 mg/Lの条件で評価した[24,25]．本研究でのクロラミン処理条件では，処理前後でpHは特に変化しなかった．オゾン 処理によるN-ニトロソアミン類の生成は，半回分式で行った．高圧ガスによる純酸素を原料とし，オゾンガス発生器はPOX-20（富士電機）を用いた．試料量は800 mL，pH 7（5 mMりん酸緩衝液），水温は20℃，オゾンガス濃度は5 mg/L，オゾンガス流量は250 mL/分の条件とした．オゾン処理時間は，2～10分であった．オゾン処理条件の検討では，対象水のオゾンとの反応の進行の程度とNDMA生成との関連性の観点から，オゾン処理時間の影響を評価した．一方，他の運転パラメータであるオゾンガス濃度やオゾンガス流量は一定とした．これは，これら運転パラメータは，オゾンの液相から液相への移動速度に影響するが，対象水のオゾンとの反応の進行の程度

については，これらを一定にしても，基本的にオゾン処理時間を変えれば評価できると考えたためである．ただし，ばっ気による気散に対しては，オゾンガス濃度やオゾンガス流量は影響すると考えられるが，本研究では，その点の考慮はできていない．

(4) 測定方法

N-ニトロソアミン類濃度は，これまで著者らが報告してきたNDMAの場合[22),24),25)]と同様，固相抽出で試料の濃縮を行った後，UPLC-MS/MSを用いて測定した．まず，内部標準として用いた各*N*-ニトロソアミン類の同位体を試料に添加し，その後に濃縮を行った．表流水，下水処理水の場合，濃縮前にガラス繊維ろ紙（GF-F；Whatman）でろ過を行った．濃縮用カートリッジとしてAC-2（400 mg×2，Waters）を，精製用カートリッジとしてFlorisil（1 g，Waters）を用いた．濃縮手順は，既報のとおりである[22),24),25)]．UPLC（Acquity UPLC；Waters）は，分離カラムにAcquity UPLC BEH C18（1.7 mm×150 mm；Waters）を使用した。移動相は，0.1%ギ酸水溶液とアセトニトリルとし，アセトニトリルの割合が 20%（0 min）→ 20%（1 min）→ 90%（3 min）→ 90%（4.5 min）→ 95%（4.6 min）→ 95%（6 min）→ 20%（6.35 min）→ 20%（8.35 min）のグラディエント条件で，流量0.2 mL/minで送液した．試料注入量は30 μLとした．MS/MSは，Acquity TQDタンデム質量分析計（Waters）を用い，イオン化方法は電気化学イオン化法の正イオンモード（ESCI+）とした．多反応モニタリング（MRM）は，NDMAではm/z 74.9/43.1（定量用）と74.9/57.9（確認用）を，NDMA-d_6ではm/z 81.0/46.0を，NMorではm/z 117.0/87.0を，NMor-d_8ではm/z 125.0/95.0を，NPyrではm/z 100.97/55.0を，NPyr-d_8ではm/z 109.0/62.0を選定した．対象物質の定量下限値（LOQ）は，精製水を用いた添加回収試験で，相対回収率（内部標準の回収率で補正した値）の変動係数（CV）が10%以下であった最低の添加濃度とした．すなわち，NDMAとNMorのLOQは1.0 ng/L（n=6，NDMAの相対回収率の平均値は120%，NMorでは100%），NPyrのLOQは3.0 ng/L（n=4，相対回収率の平均値は120%）であった．このとき，それぞれの絶対回収率の平均値は，50%，62%，46%であった．また，河川水と水道水については，添加回収試験（添加濃度：10 ng/L）を行ったところ，NDMAの相対回収率は100%と110%，NMorでは99%と93%，NPyrでは110%と130%であった（CVは6%以下（n=2））．下水処理場放流水を用いた添加回収試験は行わなかったが，本研究の実態調査において，下水処理場放流水を対象とした場合，NDMA，NMor，NPyrの内部標準の絶対回収率の平均値は，それぞれ50%，64%，32%であった．

クロラミン濃度は，*N,N*-ジエチル-*p*-フェニレンジアミンと硫酸第一鉄アンモニウムを用いた滴定法により測定した[30)]．オゾンガス濃度はPG-620HA（荏原実業）によって，溶存オゾン濃度はインジゴを用いた吸光光度法によって測定した[30)]．全有機炭素（TOC）濃度はTOC計（TOC-V CPH；島津製作所）により，全窒素（TN）濃度はTOC計に接続したTN計（TNM-1；島津製作所）により測定した．硝酸態窒素および亜硝酸態窒素濃度はイオンクロマトグラフ（DX-500；ダイオネクス）を用いて測定した．アンモニア態窒素濃度はインドフェノール法[31)]により測定した．有機態窒素（TON）濃度は，TN濃度と硝酸態窒素，亜硝酸態窒素，アンモニア態窒素濃度との差とした．

3. 結果および考察

(1) 全国の浄水場におけるNDMA濃度とクロラミン処理の影響

表-1に，夏季，冬季における19浄水場の原水，浄水中の*N*-ニトロソアミン類の実態調査結果を示す．夏季において同一浄水場で2回調査を実施したり，冬季では実施していない浄水場があるため，対象浄水場数と試料数は一致していない．

原水の場合，NDMAは，夏季では20試料水中5試料水から検出され，その濃度範囲は1.1～2.0 ng/Lであった．冬季では16試料水中6試料水から検出され，その濃度範囲は1.2～3.8 ng/Lであった．冬季の方が，検出濃度の最大値が高い結果が得られたが，夏季に検出された地点で冬季には不検出となった場合もあり，今回の調査では冬季の方が濃度が高いとは判断できなかった．

表-1 浄水場での*N*-ニトロソアミン類濃度の調査結果

対象物質	原水		浄水	
	検出率*	濃度 (ng/L) **	検出率*	濃度 (ng/L) **
NDMA				
夏季	5/20	1.1～2.0	5/20	1.0～2.2
冬季	6/16	1.2～3.8	4/16	1.3～8.3
NMor				
夏季	0/20		2/20	1.1～1.3
冬季	4/16	1.2～4.2	2/16	1.1～3.3
NPyr				
夏季	0/20		0/20	
冬季	0/16		0/16	

*検出試料数/測定試料数，** 検出試料の濃度範囲

また，NDMAが検出された原水の多くは，淀川流域か利根川流域の浄水場で，最も濃度が高い試料も淀川流域の浄水場であった．すなわち，両流域の浄水場の原水中からは，NDMAが検出されやすく，これは過去の調査結果と一致していた[24]．また，原水中のNDMA濃度と一般水質項目との関連性は，認められなかった．

浄水中のNDMAについて見ると，夏季では20試料水中5試料水から検出され，その濃度範囲は1.0～2.2 ng/Lであった．冬季では16試料水中4試料水から検出され，その濃度範囲は1.3～8.3 ng/Lであった．原水の場合と同様に，冬季の方が濃度が高かった．特に，浄水中の濃度が8.3 ng/Lであった浄水場は，オゾン処理を導入している淀川流域のO浄水場であった．これは，同浄水場の原水には，オゾン処理によるNDMAの前駆物質が存在しているため，オゾン処理の結果，NDMA濃度が上昇したことによると考えられた（次節参照）[22)23)27)]．

原水中のNMor濃度は，夏季では全ての試料で<1.0 ng/Lであったが，冬季では16試料水中4試料水から検出され，その濃度範囲は1.2～4.2 ng/Lであった．浄水中のNMor濃度は，夏季では20試料水中2試料水から検出され，その濃度範囲は1.1～1.3 ng/Lであった．冬季では16試料水中2試料水から検出され，その濃度範囲は1.1～3.3 ng/Lであった．これらの結果から，NMorは，NDMAよりは頻度は高くはないが，原水，浄水中に存在していること，NDMAの場合と同様に冬季に検出率が高くなる傾向があることがわかった．また，原水中と浄水中でNMor濃度を比較すると，増加している場合や減少している場合もあったが，ほとんど変わらない場合が多かったことから，浄水プロセスでNMorは除去されにくいことがわかった．NPyrについては，原水と浄水のいずれの試料からも検出されなかった．

次に，クロラミン処理による影響について検討した．図-1に，原水のクロラミン処理後のNDMA濃度を示す．クロラミン処理の影響について検討しなかったF浄水場は除いている．H，N浄水場は冬季の調査は行っていない．O浄水場では夏季の調査を2回実施し，それぞれ4.7，17 ng/Lであったためその平均11 ng/Lを記載した．

夏季，冬季で，それぞれ19試料中18試料，15試料中14試料からNDMAは検出され，1.0～17 ng/L，1.6～25 ng/Lの濃度範囲であった．また，今回の調査では，夏季よりも冬季において，クロラミン処理後のNDMA濃度が高い，すなわち，NDMA前駆物質が存在している傾向にあった．クロラミン処理後のNDMA濃度が，夏季，冬季のいずれかで5.0 ng/L以上であったのは9浄水場あり，その中に淀川流域あるいは利根川流域の6浄水場は全て含まれていた（D～H浄水場：利根川流域；N，O浄水場：淀川流域）．この結果は，著者ら[24)]が2008年に実施した調査と

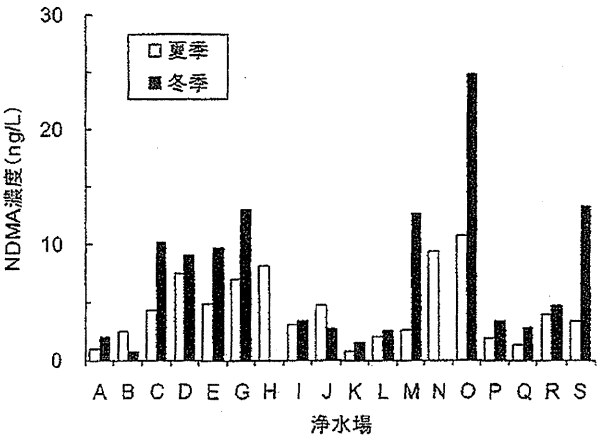

図-1　原水のクロラミン処理後のNDMA濃度（H，N浄水場は冬季の調査は未実施，夏季に2回調査したO浄水場は平均値を表示，図中<1.0 ng/Lの場合も値を表示）

同様の傾向であった．一方，淀川流域，利根川流域以外の3浄水場でもクロラミン処理後のNDMA濃度が比較的高かったが，この結果は，2008年に行った調査とは異なる傾向にあった[24)]．この理由として，過去の報告の調査時期は夏季であり，本研究で，クロラミン処理後のNDMA濃度が比較的高かったのは冬季の結果であったためと考えられた．また，クロラミン処理後のNDMA濃度と一般水質項目との関連性は，認められなかった．NMorとNPyrの場合，いずれの試料でも，クロラミン処理後に濃度が増加する傾向はほとんど認められなかった．

(2) 高度浄水プロセスでのN-ニトロソアミン類の挙動

図-2に，オゾン処理を導入している5浄水場における，高度浄水プロセスでのNDMAの挙動について示す．G，O浄水場のみ夏季と冬季の両方で調査した．砂ろ過の設置地点が，凝集沈殿後，活性炭処理後と浄水場によって異なっていたため，図では省略した．また，活性炭処理後に，砂ろ過と塩素処理，あるいは塩素素処理を行っているため，活性炭処理水と浄水とは異なる水である．

淀川流域の浄水場（O，N浄水場）の場合，夏季の調査では，浄水プロセスでそれほど濃度は変わらなかった．冬季の調査では，N浄水場において，オゾン処理でNDMA濃度が増加し，後段の活性炭処理で濃度が低下するが，除去しきれずに浄水中でもNDMAが検出された．この挙動は，これまで報告されていた，冬季における淀川流域の高度浄水プロセスでの挙動と一致した[23)]．一方，利根川流域の浄水場（F～H浄水場）の場合，夏季の調査では，浄水プロセスでそれほど濃度は変わらなかったが，そのうちのG浄水場の冬季の調査では，N浄水場ほどではないが，オゾン処理でNDMA濃度が増加した．ただし，その後，活性炭処理で濃度が低下した．これまで

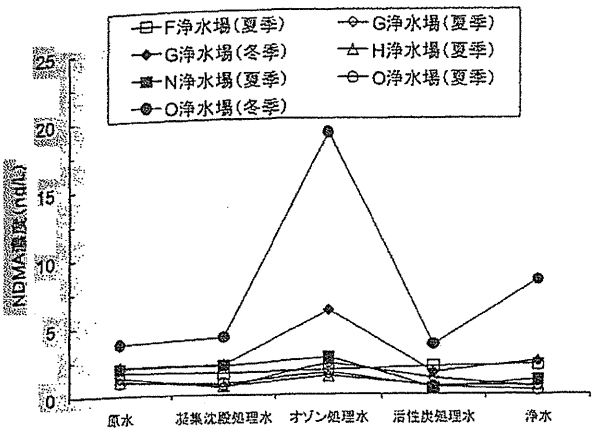

図-2 高度浄水プロセスにおけるNDMAの挙動（図中 <1.0 ng/L の場合も値を表示）

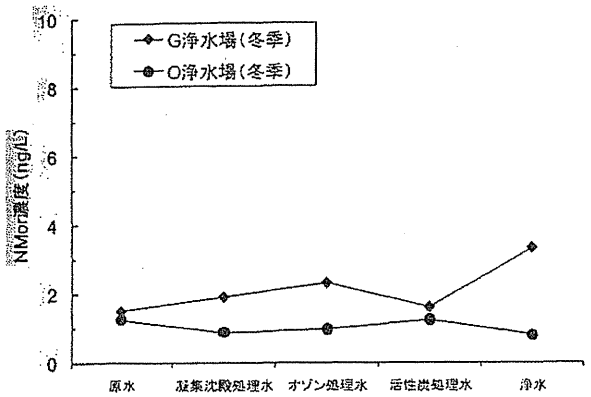

図-3 高度浄水プロセスにおけるNMorの挙動（図中 <1.0 ng/Lの場合も値を表示）

オゾン処理によるNDMA濃度の増加は，淀川流域のみであった．クロラミン処理によるNDMA前駆物質は，排水等の人為的発生源の寄与の方が多いと考えられている[13),32)]．オゾン処理による前駆物質の場合，知見が少ないため明確なことは言えないが，クロラミン処理の場合と同様に，人為的発生源の方が大きいとすれば，一般的に河川流量が低下し，それに占める排水の割合が高くなる傾向にある冬季では，利根川流域の浄水場でもオゾン処理でNDMA濃度が若干増加する場合があると推測された．

また，NMorの場合，夏季の調査では，ほとんど検出されず，高度浄水プロセスでの挙動は把握できなかった．一方，冬季のG，O浄水場の場合，オゾン処理を含め，浄水プロセスで濃度の変動は認められたが，大きくは変わらなかった（図-3）．NPyrについては，いずれの浄水場においても，原水～浄水にいたるまで，全ての試料で濃度が<3.0 ng/Lであった．したがって，対象とした浄水場では，NMor，NPyrのオゾン処理による前駆物質は存在していないか，存在していたとしてもLOQ未満であり，今回の調査では評価できない濃度範囲であった．

(3) 排水中のN-ニトロソアミン類濃度とクロラミン処理，オゾン処理の影響

前節まででは，浄水場におけるN-ニトロソアミン類の調査を行った．本節では，NDMAとNMorが比較的検出頻度が高かった淀川流域と利根川流域を対象に，上流域の下水処理場等の排水について調査した．これら排水は，NDMAやその前駆物質の発生源（の一つ）であることが報告されている[23)-25),27)]．表-2に，淀川流域の6下水処理場放流水と淀川河川水，および利根川流域の3下水処理場放流水，事業所排水口直下の水路の水，利根川河川水中のN-ニトロソアミン類の調査結果を示す．

NDMAは，全ての試料から検出され，河川水では2.0, 5.0 ng/L，水路の水は15 ng/L，下水処理場放流水では5.0～200 ng/Lの範囲であった．これら濃度は，概してこれまでの報告値と同等の値であった[23)-25),27)]．しかし，水路の水については，過去の報告では9300 ng/L[25)]であったが，本研究での結果では15 ng/Lと非常に小さい値であった．この報告では，採取地点より下流の河川水を数回採取，測定し，NDMA濃度は，370～2100 ng/Lとばらつきは大きいが常に高かったことを示している．にもかかわらず，本研究では濃度が低かった理由として，過去の報告の調査時期は6～7月，本研究の調査時期は3月と時期が異なっていたこと，事業所の生産形態や排水処理システム等が変わった可能性があること等が推測された．

NMorは，利根川河川水からは検出されなかったが，それ以外からは検出され，河川水では1.0 ng/L，水路の

表-2 河川水，水路の水，下水処理場放流水中のN-ニトロソアミン類濃度の調査結果

対象	NDMA (ng/L)	NMor (ng/L)	NPyr (ng/L)
淀川流域			
a下水処理場放流水	31	47	<3.0
b下水処理場放流水-1	9.0	3.0	<3.0
b下水処理場放流水-2	19	22	<3.0
c下水処理場放流水	5.0	2.0	<3.0
d下水処理場放流水	11	25	<3.0
e下水処理場放流水	200	4.0	<3.0
淀川河川水（YR）	5.0	1.0	<3.0
利根川流域			
f下水処理場放流水	21	16	<3.0
g下水処理場放流水	20	10	<3.0
h下水処理場放流水	170	30	<3.0
水路の水（i）*	15	18	<3.0
利根川河川水（TR）	2.0	<1.0	<3.0

*事業所排水口直下の水路

水は18 ng/L，下水処理場放流水では2.0～47 ng/Lの濃度範囲であった．したがって，NMorは，NDMAより濃度は低いが，地域によらず，下水処理場放流水や事業所排水中に広く存在していることが示された．これは，海外での報告[21),33)]や淀川流域の下水処理場や事業所排水を対象とした調査[28)]と同様の傾向であった．また，NPyrの場合，いずれの試料からも検出されなかった．

図-4に，下水処理場放流水中，水路の水，河川水中のNDMA濃度とクロラミン処理後，オゾン処理後のNDMA濃度を示す．オゾン処理時間は，河川水と下水処理プロセスでオゾン処理が導入されているe下水処理場放流水は2分間，それ以外は8分間に設定した．処理後の溶存オゾン濃度は，b下水処理場放流水では検出されなかったが，それ以外の試料では検出され，0.09～0.35 mg/Lの範囲にあった．

下水処理場放流水や水路の水の場合，b下水処理場放流水-2，e下水処理場放流水，水路の水中については，NDMA濃度はクロラミン処理後でほとんど変化しなかった．しかし，それ以外の7試料では，NDMA濃度は24～320 ng/Lになり，処理前に比べて数十～数百ng/L増加した．一方，オゾン処理後では，NDMA濃度が増加したのは，a下水処理場放流水，b下水処理場放流水-1，b下水処理場放流水-2，h下水処理場放流水の4試料で，それぞれ3800，95，49，1200 ng/Lであった．これらの結果から，クロラミン処理によるNDMA前駆物質は，比較的排水中に広く存在し，オゾン処理によるNDMA前駆物質は限られた排水中にのみ存在していることが示された．なお，e下水処理場については，流入水やプロセス水中にオゾン処理によるNDMA前駆物質は存在しているが[27)]，オゾン処理を導入しているため，放流水をクロラミン処理やオゾン処理してもNDMA濃度がほとんど変化しなかったと考えられた．また，これまで，排水中のオゾン処理によるNDMA前駆物質の存在は，淀川流域の排水中からのみ報告されていたが，利根川流域のh下水処理場放流水中にも存在していることがわかった．

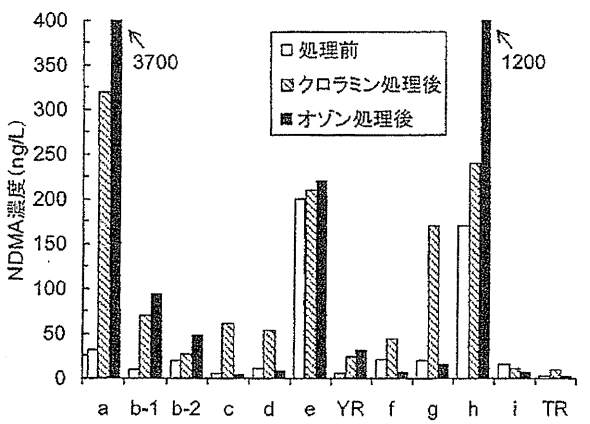

図-4 河川水，水路の水，下水処理場放流水中のNDMA濃度とクロラミン処理，オゾン処理後のNDMA濃度

ところで，a下水処理場放流水やh下水処理場放流水では，オゾン処理後のNDMA濃度の方が，クロラミン処理後のNDMA濃度よりも5～10倍程度高かった．この理由として，オゾン処理とクロラミン処理でNDMA前駆物質が異なることが挙げられる．また，本研究のクロラミン処理の条件は浄水プロセスを想定し，NDMA前駆物質のうち比較的速やかにNDMAに変換しやすい成分を対象としているが，オゾン処理の条件は全NDMA前駆物質を対象とした条件となっていることも理由に考えられた（次節参照，ただし，h下水処理場放流水の場合，処理後の溶存オゾンが検出されなかったため，全NDMA前駆物質を評価できなかった可能性がある）．

NMorの場合，クロラミン処理後，オゾン処理後のいずれも，処理前に比べ濃度が増加する傾向は認められなかった．NPyrの場合，オゾン処理後に濃度が増加する傾向は認められなかったが，クロラミン処理では，a，g下水処理場放流水，水路の水中のNPyr濃度が増加した．これらの値は，それぞれ51，22，48 ng/Lであった．したがって，一部の排水中にはNPyrのクロラミン処理による前駆物質が存在していることがわかった．

図-5に，排水，水路の水中のNDMA濃度とNMor濃度の関係を示す．多くのプロットについては，ある程度の関連性があるように認められたが，淀川流域と利根川流域のそれぞれ1下水処理場放流水は，大きく外れていた．これは，NDMAの方が濃度が非常に高くなる場合があること，上述したように，下水処理プロセスでオゾン処理が導入されたり，また，塩素処理を行うと処理水中のアンモニアと反応してクロラミンへと変換されることにより，NDMA濃度が増加したためと考えられた．さらに，排水中あるいはクロラミン処理後のNDMA濃度，NMor濃度と一般水質項目との関連性について検討したところ，関連性は認められなかった．

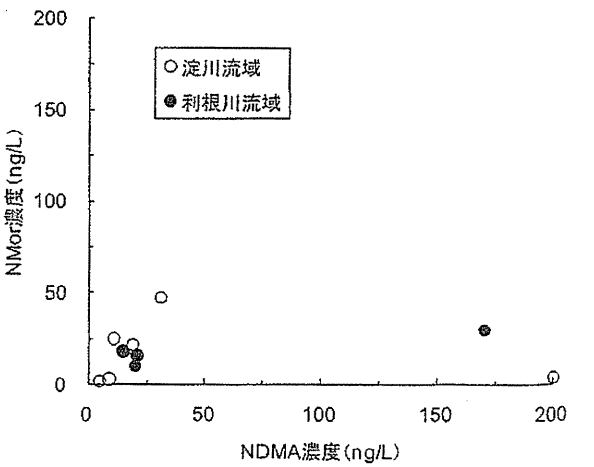

図-5 下水処理場放流水，水路の水中のNDMA濃度とNMor濃度の関係

(4) オゾン処理による*N*-ニトロソアミン類の生成と処理時間との関係

前節では，オゾン処理による*N*-ニトロソアミン類の生成について検討したが，このとき，処理時間は，試料によって異なるものの，ある処理時間における評価であった．本節では，オゾン処理における処理時間とNDMAの生成との関係について検討した．NMor, NPyrについては，いずれの対象水でも生成しなかったため対象としなかった．

図-6に淀川河川水を，図-7にe下水処理場の二次処理水（オゾン処理前）を，図-8にa下水処理場放流水を対象とした場合の，オゾン処理時間とNDMA濃度の関係について示す．e下水処理場二次処理水は，図-4の結果と別の日に採取した試料である．

淀川河川水の場合，NDMA濃度はオゾン処理時間の増加にともなって増加したが，2分以降は約30 ng/Lでほぼ一定の値となった．e下水処理場二次処理水の場合も，NDMA濃度はオゾン処理時間の増加にともなって増加し，その値は5分以降90～100 ng/Lでほぼ一定の値となった．a下水処理場放流水の場合，処理時間7分のNDMA濃度が8，10分よりも高く，淀川河川水やe下水処理場二次処理水の場合よりもばらつきは大きかったが，NDMA濃度は，同様にほぼ一定の範囲に達した（3700～4500 ng/L程度）．これらNDMA濃度がほぼ一定となった処理時間は，対象水によって異なったが，いずれの場合も溶存オゾンが検出され始めた時間であった（淀川河川水:0.11 mg/L，e下水処理場二次処理水：0.09 mg/L，a下水処理場放流水：0.12 mg/L）．ただし，本研究ではNDMA濃度はほぼ一定の範囲であったが，処理時間がより長くなるとNDMAが分解し，その濃度が低下していくと考えられる．

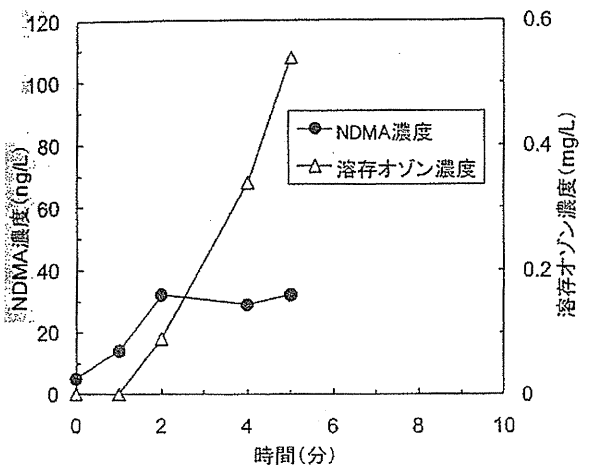

図-6　淀川河川水のオゾン処理における処理時間とNDMA濃度との関係

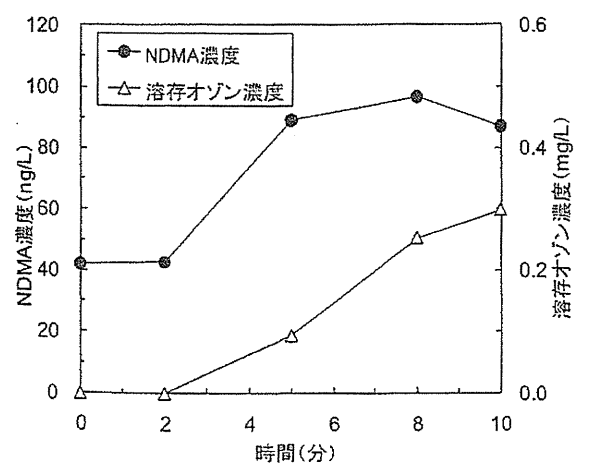

図-7　e下水処理場二次処理水のオゾン処理における処理時間とNDMA濃度との関係

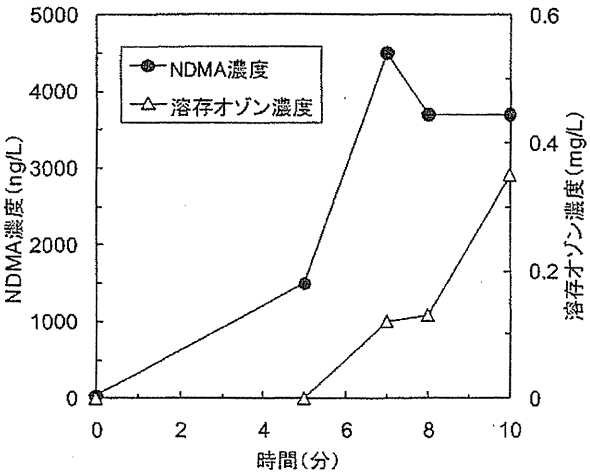

図-8　a下水処理場放流水のオゾン処理における処理時間とNDMA濃度との関係

したがって，少なくとも淀川流域の試料を対象とする場合，オゾン処理によるNDMA前駆物質は，オゾンとの反応が高いため，溶存オゾンが検出されるまでの反応初期の段階で，NDMAへと変換されていることが明らかとなった．言い換えると，全NDMA前駆物質の量を評価するには，対象水によって処理時間は異なるが，処理時間を溶存オゾンが検出され始める時間に設定するのが適切であると考えられた．このことから，図-4の結果は，h下水処理場放流水を除き，ほぼ全NDMA前駆物質を評価していたと推測された．また，通常，浄水場でのオゾン処理は，反応槽やその後の滞留槽出口の溶存オゾン濃度によるフィードバック制御をしており，この条件では，NDMA前駆物質はほぼ全てNDMAに変換していると考えられた．

4. まとめ

(1) 全国19浄水場の原水，浄水中のNDMAは，それぞれ

夏季，冬季で延べ36試料中11試料，36試料中9試料から検出され，その濃度は1.1～3.8，1.0～8.3 ng/Lの範囲にあった．原水，浄水中のNMorは，それぞれ延べ36試料中4試料，36試料中4試料から検出され，その濃度は1.2～4.2，1.1～3.3 ng/Lの範囲にあった．NPyrはいずれの試料からも検出されなかった．

(2) 原水をクロラミン処理した後のNDMAは，夏季，冬季の延べ34試料中32試料から検出され，その濃度は1.6～25 ng/Lの範囲にあった．淀川流域，利根川流域の浄水場の高度浄水プロセスにおいて，オゾン処理後にNDMA濃度が増加する場合が認められた．NMor，NPyrは，クロラミン処理後，オゾン処理後のいずれにおいても濃度の増加は認められなかった．

(3) 淀川流域，利根川流域の下水処理場放流水，事業場排水口直下の水路の水では，NDMA，NMorのいずれも10試料全てから検出され，それぞれ5.0～200，2.0～47 ng/Lの範囲にあった．NPyrは，いずれの試料からも検出されなかった．

(4) 下水処理場放流水，事業場排水口直下の水路の水のクロラミン処理後のNDMA濃度は，10試料中7試料で増加した．このとき，NDMA濃度は24～320 ng/Lの範囲にあり，処理前に比較して数十～数百ng/L増加した．オゾン処理の場合，10試料中4試料でNDMA濃度は増加し，49～3800 ng/Lの範囲にあった．オゾン処理によるNDMA濃度の増加は，淀川流域，利根川流域のいずれの試料からも認められた．NMorは，クロラミン処理，オゾン処理のいずれでも処理後に濃度は増加しなかった．Npyrは，オゾン処理後は濃度の増加は認められなかったが，クロラミン処理後では，10試料中3試料で濃度が増加し，22～48 ng/Lの範囲であった．

(5) オゾン処理によるNDMAの前駆物質の評価を行う場合，少なくとも淀川流域の試料では，溶存オゾンが検出し始めた段階までオゾン処理を行うと，全NDMA前駆物質量の評価が可能であることがわかった．

謝辞：浄水場，下水処理場の試料の採取にあたり，関係上下水道局の方々，京都大学越後信哉先生にご協力いただいた．試料の測定において，中嶋淳氏（茨城県企業局），平野雄大氏（愛知県企業庁）にご協力いただいた．本研究の一部は，CREST「都市地下帯水層を利用した高度リスク管理型水再利用システムの構築」（研究代表者：伊藤禎彦教授），厚生労働科学研究費補助金「水道における水質リスク評価および管理に関する総合研究」（研究代表者：松井佳彦教授）によって行われた．記して謝意を表する．

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Occurrence of *N*-nitrosamines and their precursors on chloramination and ozonation in water supply in Japan

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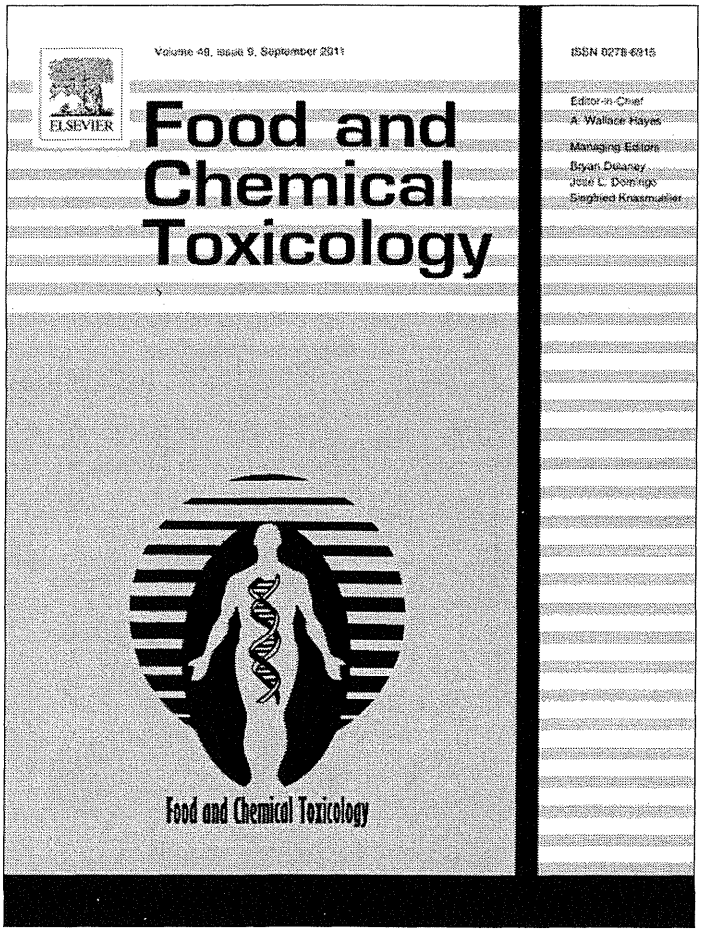
Occurrence of *N*-nitrosamines (*N*-nitrosodimethylamine (NDMA), *N*-nitrosomorpholine (NMor) and *N*-nitrosopyrrolidine (NPyr)) in raw and finished waters at 19 water purification plants were investigated. NDMA was detected in 11 of total 36 raw waters and 9 of total 36 finished water. NMor was detected in 4 of total 36 raw waters and 4 of total 36 finished water. NDMA and NMor were detected in all ten sewage effluents and the waterway which is located just downstream of effluent from a factory. NPyr was not detected in all samples of purification plants and effluents. The number of samples whose NDMA concentrations increased after chloramination was larger than those after ozonation. In case of NMor, NMor concentrations in the samples did not increase after chloramination or ozonation. NPyr concentrations in the samples did not increase after ozonation, but increased after chloramination in some cases. The effects of ozonation time on NDMA formation were examined using three different samples. For all samples, NDMA formation became stable when dissolved ozone in the samples was detected.



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Evaluation of the reproductive and developmental toxicity of aluminium ammonium sulfate in a two-generation study in rats

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ABSTRACT

Aluminium ammonium sulfate (AAS) was tested for reproductive/developmental toxicity in a two-generation study. Male and female rats were continuously given AAS in drinking water at 0, 50, 500 or 5000 ppm. Water consumption was decreased in all AAS-treated groups, and the body weight of parental animals transiently decreased in the 5000 ppm group. In either generation, no compound-related changes were found in estrous cyclicity, sperm parameters, copulation, fertility and gestation index, number of implantations and live birth pups, sex ratios of pups or viability during the preweaning period. Male and female F1 pups in the 5000 ppm group showed a lower body weight on postnatal day 21, while there were no differences in the birth weight of F1 and F2 pups between the control and AAS-treated groups. Preweaning body weight gain in F2 males and females indicated a similar decreasing tendency at 5000 ppm. In F1 and F2 weanlings, the weight of the liver, spleen and thymus decreased at 5000 ppm, but no histopathological changes were found in these organs. In F1 females in the 5000 ppm group, vaginal opening was delayed slightly. There were no compound-related changes in male preputial separation or in other developmental landmarks. In behavioral tests conducted for F1 animals at 4–6 weeks of age, no compound-related changes were found in spontaneous locomotor activity and performance in a water-filled multiple T-maze. In conclusion, the NOAEL of AAS for two-generation reproductive/developmental toxicity was considered to be 500 ppm in rats. Considering the aluminium content in the basal diet, the total ingested dose of aluminium from drinking water and food in this 500 ppm group was calculated to be 5.35 mg Al/kg bw/day.

1. Introduction

Aluminium compounds are widely used as food additives in various food products; for example, aluminium ammonium sulfate [AAS; CAS No.: 7784-25-0 (anhydrous), 7784-26-1 (dodecahydrate)] is added as a firming agent or stabilizer in egg products, processed fish and vegetables, candied fruit, etc. (Codex Alimentarius Commission, 2010). Other aluminium compounds used as food additives include acidic and basic sodium aluminium phosphate (SALP), sodium and calcium aluminium silicate, aluminium sodium sulfate, and aluminium lakes of various food dyes and colors (IPCS, 2007). While aluminium is ubiquitous in the environment (IPCS, 1997), the major route of aluminium exposure by the general public is considered to be dietary exposure, particularly through foods containing such aluminium compounds as food additives (WHO, 2008). Total dietary exposure to aluminium has been calculated to range from 14 to 280 mg Al/week in the adult population (IPCS, 2007).

Concerning the effects of aluminium on human health, many international and national organizations have conducted toxicological evaluations (ATSDR, 2008; EFSA, 2008; IPCS, 1997; WHO, 2007). Recently, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated aluminium from all sources, including food additives. While it established a provisional tolerable weekly intake (PTWI) of 1 mg Al/kg bw for all aluminium compounds in food, a review of the toxicology database disclosed that there is a need for an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioral endpoints to be conducted on relevant aluminium compound(s) (WHO, 2007).

Aluminium has been reported to affect the male reproductive system, causing necrosis of spermatocytes/spermatids in the testes, decreases in testicular spermatid counts and epididymal spermatozoa counts, and reduction of fertility etc., in various laboratory animals (Guo et al., 2005; Kamboj and Kar, 1964; Krasovskii et al., 1979; Llobet et al., 1995; Roy et al., 1991; Yousef et al., 2005). Although the oral bioavailability of aluminium is extremely low

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(less than 1%) (Jouhanneau et al., 1997; Zafar et al., 1997), such effects were observed in oral gavage studies (Krasovskii et al., 1979; Roy et al., 1991; Yousef et al., 2005), the lowest effective dose of which was reported to be 2.5 mg Al/kg bw/day in a 6-month exposure study in rats. However, because the toxicokinetics after a bolus dose by gavage must differ significantly from those after actual continuous exposure via the diet in humans, the relevance of these oral gavage studies for human risk assessment is unclear. Further, the dietary intake of aluminium was not considered in oral gavage studies even though laboratory animal feed contains a significant portion of aluminium (ranging from 60 to 8300 ppm) (ATSDR, 2008). There is therefore a possibility that the toxic effects of aluminium on the male reproductive system might have been overestimated in these studies. In fact, Hicks et al. (1987) demonstrated that 28-day continuous exposure to diets containing basic SALP or aluminium hydroxide did not affect testicular histopathology up to 302 mg Al/kg bw/day in rats. In the 26-week feeding study of SALP basic in dogs, germinal epithelial cell degeneration and atrophy in the seminiferous tubules were observed at 75 mg Al/kg bw/day (Pettersen et al., 1990), but no such effects on male reproductive organs were detected up to 88 mg Al/kg bw/day in a similar subchronic dietary study of SALP acidic in dogs (Katz et al., 1984). These dietary exposure studies took into account the aluminium content in the basal diet, but they all used water-insoluble or sparingly-soluble forms of aluminium (ATSDR, 2008; IPCS, 2007), which are widely assumed to be less bioavailable than soluble compounds, such as AAS, aluminium chloride and aluminium lactate (IPCS, 2007; Taimei Chemicals Co. Ltd., year not specified a). Thus, it is necessary to investigate the effects on the male reproductive system after continuous exposure to water-soluble aluminium compounds.

As for developmental toxicity, most of the reported continuous exposure studies focused on specific neurobehavioral endpoints (Bernuzzi et al., 1986, 1989; Muller et al., 1990). In particular, a group of researchers from the University of California have provided many reliable results in this field (Donald et al., 1989; Golub and Germann, 2001; Golub et al., 1992, 1995, 2000). In these studies, Swiss Webster mice were given a diet containing aluminium lactate throughout the gestation and/or lactation period, and neurobehavioral effects, such as greater or lower grip strength, decreased sensitivity to heat and impaired learning in a maze, were found in the offspring from dams given $\geqslant$500 mg Al/kg diet (100–210 mg Al/kg bw/day). In contrast, insufficient information is available regarding the effects of continuous aluminium exposure on the physical and sexual development of offspring.

In order to fill these data gaps, we previously conducted a two-generation reproductive toxicity study of aluminium sulfate with continuous administration in drinking water, according to OECD test guidelines under GLP (Hirata-Koizumi et al., 2011). Aluminium sulfate is a water-soluble salt of aluminium, and is primarily used as a flocculant for water purification, paper-sizing agent, fire extinguisher material, etc. (Donaldson, year not specified; Taimei Chemicals Co. Ltd., year not specified b). The results of a two-generation study showed possible effects on postnatal growth, but no definitive effects were found in reproductive or developmental toxicity, including neurobehavioral parameters up to 3000 ppm (31.2–87.0 mg Al/kg bw/day). In the present study, a two-generation reproductive toxicity study was conducted of another water-soluble aluminium compound, AAS, which is used as a food additive.

2. Materials and methods

This study was conducted in accordance with OECD guideline 416 "Two-generation Reproduction Toxicity Study" (OECD, 2001) and the Japanese guidelines for the designation of food additives and revision of the standards for the use of food additives (MHW, 1996). All procedures involving the use and care of animals complied with the principles for Good Laboratory Practice (ME, METI and MHLW, 2003, 2008) and applicable animal welfare regulations ["Act on Welfare and Management of Animals"(Japanese Animal Welfare Law, 2005), "Standards Relating to the Care, Management of Laboratory Animals and Relief of Pain"(ME, 2006) and "Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in the Testing Facility under the Jurisdiction of the Ministry of Health, Labour and Welfare"(MHLW, 2006)].

2.1. Animals and housing conditions

Crl:CD(SD) rats (4 weeks old) were purchased from Atsugi Breeding Center, Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were acclimated to the laboratory for 8 days, and rats found to be in good health were divided into 4 groups of 24 males and 24 females each by stratified random sampling based on body weight. Ear tattoos were used for parent animal identification, and limb tattoos for live pup identification (after postnatal day (PND) 4).

Throughout the study, animals were maintained in an air-conditioned room with controlled temperature (22 ± 3 °C) and humidity (50 ± 20%). Light was provided on a 12-h light/dark cycle (light: 8:00–20:00). The animals were housed individually, except for the acclimation, mating and nursing periods, in metal bracket-type cages with wire-mesh floors. From day 17 of gestation to day 21 after delivery, individual dams and litters were reared using wood chips as bedding (White Flake; Charles River Laboratories Japan, Inc.). All animals were fed *ad libitum* with a standard rat diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan), and were supplied with drinking water containing different concentration of AAS, as mentioned below, through two generations. Aluminium concentration in the standard diet, analyzed by flame atomic absorption spectrometry for each lot of diet, ranged from 22 to 29 ppm.

2.2. Chemical and dosing

AAS (99.5% pure anhydrous; Lot No. A81009) was obtained from Taimei Chemicals Co., Ltd. (Nagano, Japan). The bulk of the sample was stored with a desiccating agent at room temperature (20–25 °C). The test article was dissolved in ion-exchanged water, and served as drinking water for the animals, which were 5 weeks old when the treatment started. Control rats were given ion-exchanged water alone as drinking water, in which AAS was contained at less than the quantitation limit (5 μg/mL). Before the start of the study, the stability of AAS in ion-exchanged water at concentrations of 0.05 and 10 mg/mL was confirmed after 5-day storage at room temperature following 7-day refrigerated storage; therefore, dosing solutions were prepared at least once every 7 days and kept in a cool place until serving, and the drinking water was replaced at least once every 5 days. During the study, the concentrations of AAS in drinking water were analyzed at the first and last preparation and once every 3 months, and were confirmed to be 99.4–104.4% of the target by high performance liquid chromatography.

Prior to the present two-generation reproductive toxicity study, a dose-finding study was performed in male and female rats (6/sex/dose) given drinking water containing AAS at 0, 300, 1000, 3000 or 10,000 ppm. In that study, males were dosed for 7 weeks, beginning 14 days before mating, and females were dosed from 14 days before mating to day 4 of lactation throughout the mating and gestation periods. AAS reduced water consumption in all treatment groups, and there were decreases in body weight at 3000 ppm and above. At necropsy, thickening of the limiting ridge in the stomach was detected at 10,000 ppm, although no animals died at any doses. There were no changes in any other reproductive/developmental parameters. Taking into account the results of this dose-finding study, the dose levels of AAS in the present study were set as 50, 500 or 5000 ppm.

2.3. Experimental design

A graphic representation of the experimental design is presented in Fig. 1. The study began with 24 rats/sex/group (F0 generation), and they were exposed to AAS in drinking water at 0, 50, 500 or 5000 ppm. After 10-week administration of AAS, each female was mated with a male from the same dosage group, and pregnant females were allowed to deliver and nurse their pups. F0 parental male rats were necropsied after the parturition of paired females, and F0 females were necropsied after weaning of their pups. Administration of AAS was continued throughout the mating, gestation and lactation periods until necropsy.

For the second generation, 24 male and 24 female weanlings (1 or 2 weanlings/sex in each litter) in each group were selected as F1 parents on PNDs 21–25 to equalize the mean body weights among groups as much as possible. The day on which F1 parental animals were selected was designated as day 0 of dosing for the F1 generation. F1-selected rats were given drinking water with the respective formulation, and mated after 10-week administration. They were allowed to deliver and nurse their F2 pups, and necropsied in the same manner as described for F0 rats. Unselected F1 weanlings and all F2 weanlings were necropsied on PND 26.

2.4. Parental data (F0 and F1)

Throughout the study, all F0 and F1 parental rats were observed twice daily for general appearance and behavior, as well as for any signs of AAS intake. Food and water consumption was determined once and twice a week, respectively, throughout the exposure period, except during cohabitation. The body weights of males

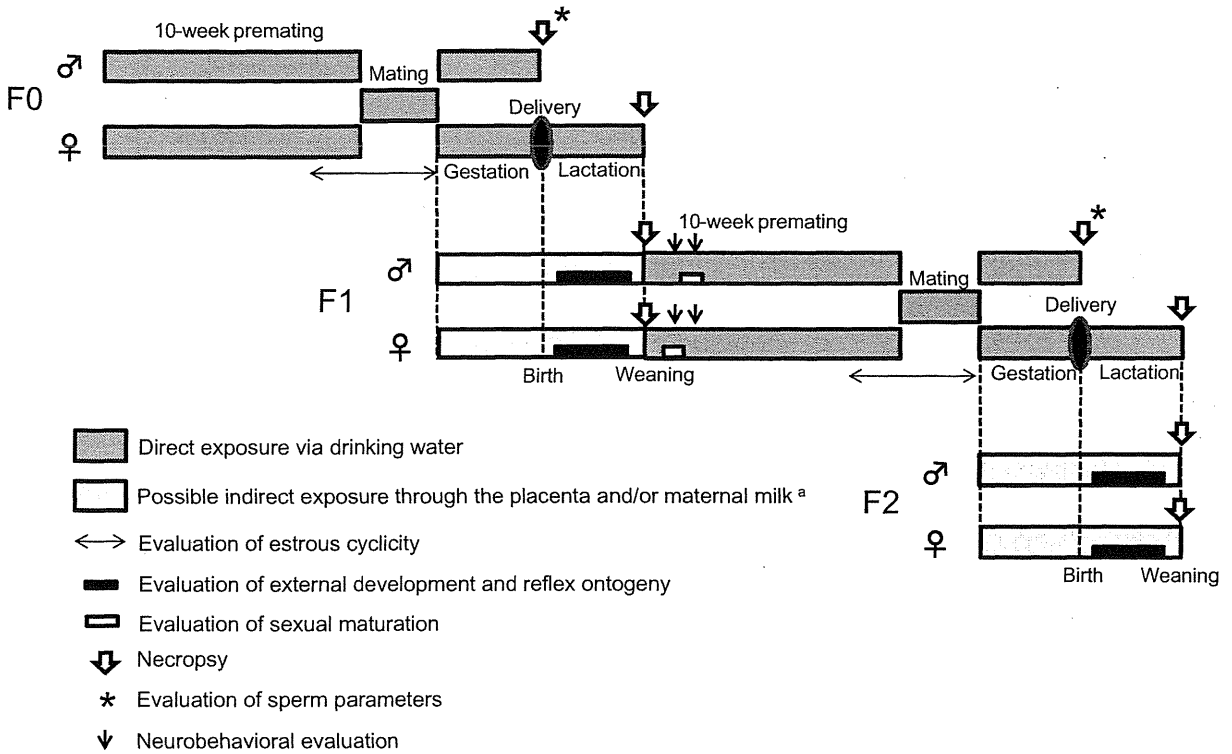

Fig. 1. Presentation of study design. [a]Pups could be also exposed to AAS directly via drinking water at the end of weaning period.

were measured weekly throughout the study. Body weight measurement for females was also performed weekly until evidence of copulation was detected, and thereafter on days 0, 7, 14 and 20 of gestation and on days 0, 4, 7, 14 and 21 of lactation.

2.4.1. Assessment of reproductive performance

After the 10-week premating period, each female was transferred to the home cage of a randomly chosen male from the same exposure group, and cohabited on a 1:1 basis until successful copulation occurred or the mating period of 2 weeks had elapsed. Beginning 2 weeks before the cohabitation period, vaginal smears were prepared daily to determine the stage of estrus for each F0 and F1 female until evidence of copulation was detected. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as evidence of successful mating, and the day of successful mating was designated as day 0 of gestation. Females that did not mate successfully during the 2-week mating period were cohabited with another male from the same group who had been proven to copulate within not less than 7 days. Following confirmation of mating, females were returned to their home cages, and allowed to deliver spontaneously and nurse their pups until PND 21 (day of weaning). They were checked at least three times daily on days 21–25 of gestation, and the day on which dams held their pups under the abdomen in the nest by 13:00 was designated as day 0 of lactation or PND 0.

2.4.2. Necropsy and histopathology

F0 and F1 parental males were euthanized by exsanguination under ether anesthesia after the parturition of paired females. The females were evaluated for estrous cycle stage by examination of the vaginal smear after weaning of pups, and euthanized in the proestrus stage by exsanguination under ether anesthesia. After external examination of these parental animals, the abdomen and thoracic cavity were opened and gross internal examination was performed. The number of uterine implantation sites was recorded for each female. The brain, pituitary, thyroid, thymus, liver, kidney, spleen, adrenal, testis, epididymis, seminal vesicle, ventral prostate, uterus and ovary were weighed (weights of the thyroid and seminal vesicle were measured after fixation). The testis and epididymis were fixed with Bouin's solution and preserved in 70% ethanol, and the other organs were stored in 10% neutral-buffered formalin.

Histopathological evaluations were performed for the testes, epididymides, seminal vesicles, ventral prostate, coagulating gland, ovaries, uterus and vagina of all F0 and F1 animals in the control and highest dose groups. These organs were embedded in paraffin by a routine procedure. They were then sectioned, stained with hematoxylin–eosin and examined histopathologically under a light microscope.

The number of primordial follicles was counted for 10 F1 females, each randomly selected from the control and highest dose groups. The right ovary was dehydrated and embedded in paraffin in longitudinal orientation by routine procedures, and sectioned serially at 5 μm. Every 20th section was mounted on a slide and stained with hematoxylin–eosin. About 40 sections per ovary were used to determine the primordial follicles.

2.4.3. Sperm parameters

At the time of F0 and F1 parental male sacrifice, the right testis was immediately removed, weighed and homogenized in a physiological solution, and the number of homogenization-resistant spermatid heads was counted in a hemacytometer. Right epididymal cauda was also extracted and weighed, and an epididymal sperm suspension was prepared to assess the sperm number, motility, and morphology. Caudal sperm numbers were enumerated with a hemacytometer under a light microscope. Sperm motility (percentage of motile sperm and progressively motile sperm, swimming speed and pattern) was determined using a computer-assisted cell motion analyzer (TOX IVOS; Hamilton Thorne Bioscience, Beverly, MA, USA). Sperm morphology was evaluated for 200 sperm per male under a light microscope, using sperm slides stained with eosin.

2.5. Offspring evaluation (F1 and F2)

All pups derived from F0 and F1 parents (F1 and F2 litters, respectively) were examined as soon as possible on the day of birth to determine the number and sex of pups, the number of liveborn and stillborn members of each litter, and gross abnormalities. To reduce variability among litter size, each litter was randomly adjusted to eight pups of four males and four females on PND 4. The pups were observed daily for clinical signs of toxicity, and the body weight of live pups was recorded on PNDs 0, 4, 7, 14 and 21.

2.5.1. Developmental landmarks

All F1 and F2 live pups were evaluated for pinna unfolding daily for 4 days after birth. The anogenital distance (AGD) of these animals was measured using calipers on PND 4, and the AGD/cube root of the body weight ratio was calculated to correct the influence of body size on the AGD value (Gallavan et al., 1999). One male and one female F1 and F2 pup selected from each dam were observed for incisor eruption beginning on PND 8 and eye opening beginning on PND 12 until each pup achieved the criterion. For the same F1 and F2 pups, the surface righting reflex, negative geotaxis and mid-air righting reflex were assessed on PND 5, 8 and 18, respectively. All F1 offspring selected as F1 parents were observed daily for male preputial

separation beginning on PND 35 or female vaginal opening beginning on PND 25 until completion. The body weight of the respective F1 rats was recorded on the day the criteria were fulfilled.

2.5.2. Necropsy and histopathology

F1 weanlings not selected to become parents and all F2 weanlings were euthanized under ether anesthesia on PND 26. For one male and one female F1 and F2 weanling selected from each dam, major organs were removed, fixed and preserved, as described for the adults. Weights of the brain, thymus, liver, kidney, spleen, adrenal, testis, epididymis, ventral prostate, uterus and ovary were recorded before fixation.

Since test substance-related change was found in the thymus, liver and spleen weight of males and in the liver and spleen weight of females in the highest dose group, histopathological evaluations of these organs were performed for 10 male and 10 female F1 and F2 weanlings in the control and highest groups. The examined animals were selected randomly from the animals whose organs were stored. Paraffin sections were routinely prepared, stained with hematoxylin–eosin and examined histopathologically under a light microscope.

2.6. Neurobehavioral evaluation

Spontaneous locomotor activity was evaluated at 4 weeks of age for 10 male and 10 female F1 rats randomly selected from each group. The animals were placed individually in transparent polycarbonate cages [285(W) × 450(D) × 210(H) mm, CL-0108-1; CLEA Japan Inc., Tokyo, Japan], and spontaneous motor activity was measured with SUPERMEX (Muromachi Kikai Co., Ltd., Tokyo, Japan), which was mounted above the cage to detect changes in heat across multiple zones of the cage with an infra-red sensor. Spontaneous motor activity was determined for 10 min periods and for a total of 60 min.

Spatial learning ability was assessed using a water-filled multiple T-maze (Biel's type) for 10 male and 10 female F1 rats selected from each group at 6 weeks of age. The water temperature of the maze was maintained at 21.0–22.0 °C. On the first day, the animals were given triplicate swimming trials in a straight channel, and the time required to reach the goal was recorded. On the following three days, they were subjected to three maze trials per day, and the time required to reach the goal and the number of errors were recorded. None of the rats were allowed to remain in the water for more than 3 min in any trial to prevent exhaustion.

2.7. Statistical analysis

The body weight of parental animals, food and water consumption, length of estrous cycle, gestational length, precoital interval, number of implantations and pups born, delivery index, reflex response time, age at sexual maturation, parameters of behavioral tests, organ weight and sperm parameters were evaluated by Bartlett's test for homogeneity of variances ($P < 0.05$). The body weight of preweaning pups, AGD, viability and age at the completion of developmental landmarks were similarly analyzed using the litter as the experimental unit. When homogeneity was recognized, one-way analysis of variance was applied ($P < 0.10$). If a significant difference was found, Dunnett's test was used for pairwise comparisons between control and individual treatment groups ($P < 0.01$ or 0.05). Data without homogeneity were subjected to the Kruskal–Wallis rank sum test ($P < 0.10$), and if significant differences were detected, the Mann–Whitney U test was used to compare AAS-treated groups with the control group ($P < 0.01$ or 0.05). The incidence of parental animals with clinical signs and necropsy and histopathological findings, incidence of females with normal estrous cycles, incidence of weanlings with histopathological findings, copulation, fertility and gestation index, neonatal sex ratio and completion rate of negative geotaxis were compared between the control and each dosage group using Fisher's exact test ($P < 0.01$ or 0.05). Wilcoxon's rank sum test was performed for the incidence of pups with clinical signs or necropsy findings per litter, completion rate of pinna unfolding in each litter, and the success rate of surface and mid-air righting reflex ($P < 0.01$ or 0.05). The number of primordial follicles was compared between the control and highest dose groups using Student's t-test ($P < 0.01$ or 0.05).

3. Results

3.1. Clinical observations, water and food consumption and body weight of parental animals (F0 and F1)

Throughout the study, there were no treatment-related parental deaths or clinical signs of toxicity at any of the three exposure levels evaluated. Water consumption was dose-dependently reduced in males and females of both generations, as shown in Fig. 2. Significant changes were observed throughout or almost throughout the dosing period in F0 males of all AAS-treated groups, and in F0 females and F1 males and females in the 500 and 5000 ppm groups. Significant decreases in water consumption were also found during weeks 1, 9 and 10 of dosing, week 1 of gestation and week 1 of lactation in 50 ppm-treated F0 females and during weeks 4 and 8–10 of dosing in 50 ppm-treated F1 females. Food consumption was significantly lower during week 1 of dosing in F0 males of the 5000 ppm group and in F0 females of the 500 and 5000 ppm groups (data not shown). In 5000 ppm-treated F0 and F1 females, there were also significant decreases in food consumption in the 2nd and 3rd weeks of lactation. Body weight was significantly lower in the 2nd week of dosing in both sexes of F0 rats and on day 21 of lactation in F0 females at 5000 ppm (Fig. 3). In the 5000 ppm group, the body weight of F1 males and females was significantly lower in the first 2 and 3 weeks of dosing, respectively (Fig. 3).

3.2. Daily intake of AAS and aluminium in parental animals (F0 and F1)

For each of the AAS-treated groups, daily AAS intake was estimated based on water consumption and body weight during the premating and postmating periods in males and during the premating, gestation and lactation periods in females. Calculated average intake of AAS during the whole period was 3.78, 33.5 and 305 mg/kg bw in F0 males, 6.52, 58.6 and 500 mg/kg bw in F0 females, 4.59, 41.8 and 372 mg/kg bw in F1 males, and 6.65, 61.9 and 517 mg/kg bw in F1 females for the 50, 500 and 5000 ppm groups, respectively. Considering aluminium content in the basal diet, dietary aluminium exposure of F0 and F1 animals was estimated from the food consumption and body weight in the control and AAS-treated groups. Average aluminium intake from drinking water and food combined was calculated to be 1.56, 1.98, 5.35 and 36.3 mg Al/kg bw/day in F0 males, 2.20, 2.89, 8.81 and 59.0 mg Al/kg bw/day in F0 females, 1.83, 2.35, 6.57 and 44.2 mg Al/kg bw/day in F1 males, and 2.39, 3.10, 9.36 and 61.1 mg Al/kg bw/day in F1 females for control through high-dose groups.

3.3. Reproductive effects (F0 parents/F1 offspring and F1 parents/F2 offspring)

During the premating period, a few AAS-treated F0 and F1 female rats had persistent diestrus; however, the incidence of females with normal estrous cycles (4–5 days) was not changed significantly compared with the control. There were no significant differences in the estrous cycle between control and AAS-treated groups (data not shown).

Reproductive performance of F0 and F1 parental animals is summarized in Table 1. Although some animals failed to copulate, impregnate or deliver live pups, no significant changes were found in the copulation, fertility or gestation index between the control and AAS-treated groups in F0 and F1 generations. There were also no significant differences in the precoital interval and gestation length in either generation.

Sperm analysis of schedule-sacrificed F0 and F1 adults revealed no significant differences in the number of testis sperm and cauda epididymal sperm, the percentage of motile sperm and progressively motile sperm, the swimming speed and pattern, and the percentage of morphologically abnormal sperm between control and AAS-treated groups (data not shown).

3.4. Developmental effects (F1 and F2)

No significant changes were found in the number of implantations or pups delivered, delivery index, sex ratio of pups and the viability index during the preweaning period in either generation (Table 2). During the preweaning period, external gross examination revealed microphthalmia, a rudimentary tail, trauma and scab

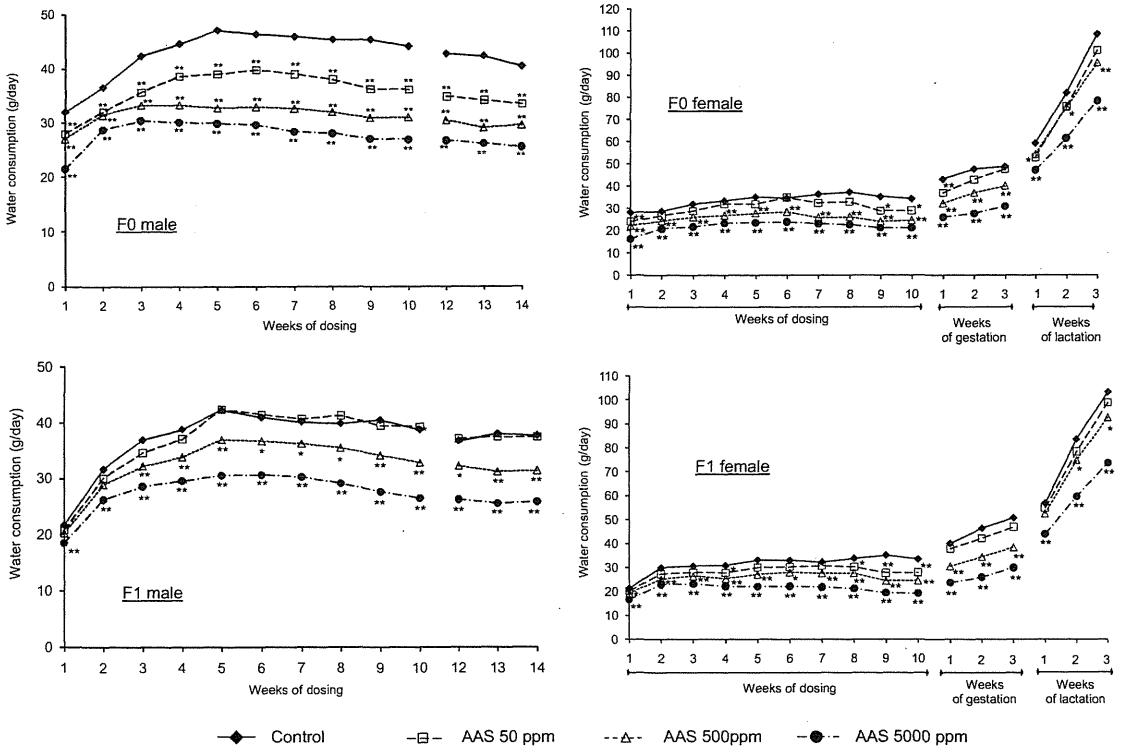

Fig. 2. Water consumption of F0 and F1 parental animals. *Significantly different from the control. $P < 0.05$, **Significantly different from the control, $P < 0.01$.

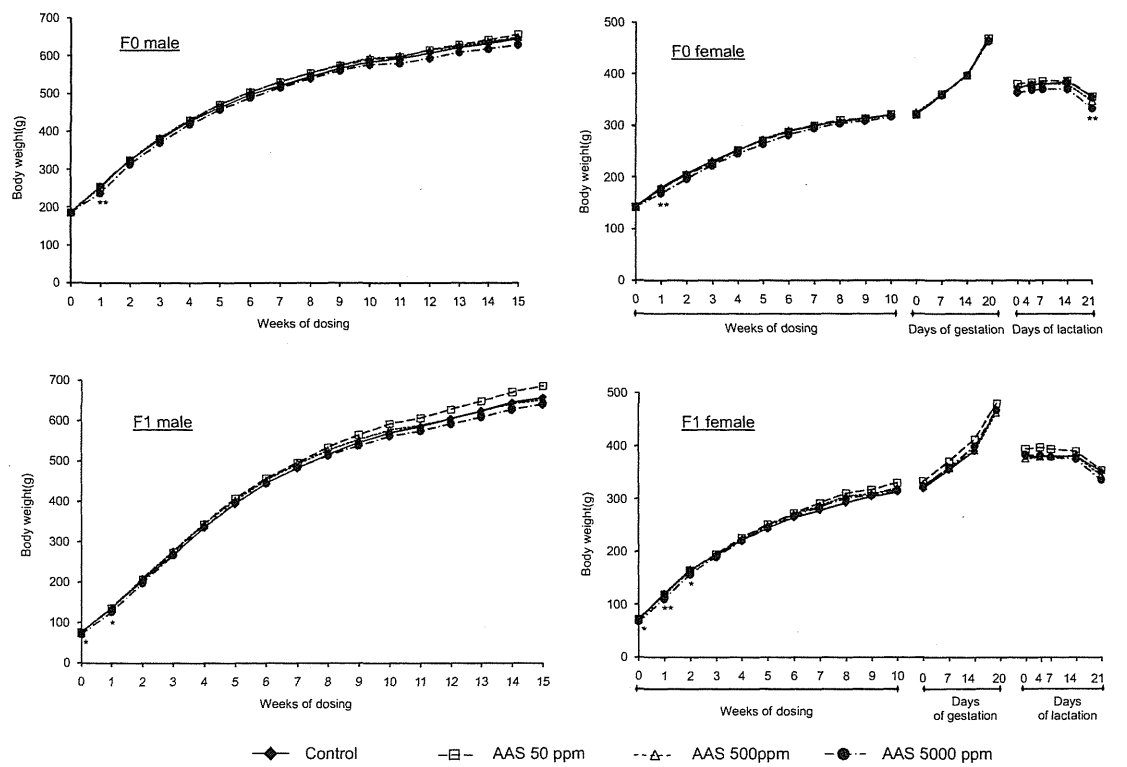

Fig. 3. Body weight change of F0 and F1 parental animals. *Significantly different from the control, $P < 0.05$. **Significantly different from the control, $P < 0.01$.

on right hindlimb and crushing of incisor/malocclusion in a few F1 pups in control and AAS-treated groups; however, there were no significant differences in incidence between the control and AAS-treated groups (data not shown). No gross abnormalities were found in any F2 pups. While there were no significant differences in birth weight between the control and AAS-treated groups, the