

4. 消毒副生成物に関する基準値設定上の課題

本稿では、消毒副生成物を例として取り上げ、飲用奇与率の設定問題について論じてきた。ここでは基準値設定上、残された他の課題について紹介することとしたい。それらは、手元のテクニカルな問題から、背後に広がる水質基準の本質に関わる問題に及ぶ。

4.1 毒性評価の方法について～DCAAを中心に～

4.1.1 DCAAの基準値設定上の扱いの変遷

HAAsのうち、現行の水質基準項目にリストアップされているのは、クロロ酢酸であるMCAA, DCAA, TCAAの3種類であり、基準値はそれぞれ、0.02 mg/L, 0.04 mg/L, 0.2 mg/Lである。毒性の強さは、この3種類を比較するとMCAAがもっとも強く、TCAAがもっとも弱いので、毒性の順序と基準値の大小関係との間には整合性がある。これに対して、現在、DCAA²¹⁾, TCAA²²⁾ともに0.03 mg/Lを基準値とする原案が示されており、平成27年度から施行される可能性がある。特に、TCAAは0.2 mg/Lが0.03 mg/Lとなるので、施行されれば大変な基準値強化となる。なお、MCAA²³⁾については現行の0.02 mg/Lを変更する予定はない。

上記3種類のうち、特にDCAAの指針値および基準値設定の経緯を図12に示す。この物質は、基準値設定上の扱いがその時々によって変化してきた物質である。

まず、1992年(平成4年)時の水質基準体系の中では、DCAAは監視項目に位置付けられ、指針値0.04 mg/L

(暫定値)が設定されていた。

これが1998年(平成10年)に見直された²⁴⁾。DCAAは、従来から発がん性は認められるが、遺伝子障害性の有無は不明瞭であった。このため、遺伝子障害性があると仮定した計算と、ないと仮定した計算の2通りが行われた。そして遺伝子障害性を仮定しVSD法にしたがって計算すると、評価値は0.05 mg/Lとなり、非遺伝子障害性であるとしてTDI法にしたがうと0.02 mg/Lが得られた。これより安全側として0.02 mg/Lが採用されることとなる。しかし同時に、遺伝子障害性の有無について不明瞭なままであり毒性評価法を確定できないため、この値は暫定値とされた。

これに対し、2003年(平成15年)における評価²⁵⁾では異なる方法がとられている。依然として遺伝子障害性の有無については不明瞭であるので、同様に評価するのであれば、2通りの計算方法によって評価値を算出し、低い値を採用すべきである。しかしこの時は、遺伝子障害性を前提とするVSD法のみで計算が行われた。その考え方は「安全側に立った評価を行う観点から、遺伝子障害性があると仮定するのが適切である」というものである。この結果、評価値として0.04 mg/Lという1種類の値のみが得られ、これを確定値として水質基準値とした。もしこの時、1998年と同様に2通りの計算を行ってより小さい値を採用したとすると、それは確定値とはならず暫定値となっていた。

1998年における考え方と2003年の考え方との間には一貫性がない。遺伝子障害性の有無に関する知見に変化がないにもかかわらず、評価時の見方ひとつで(表

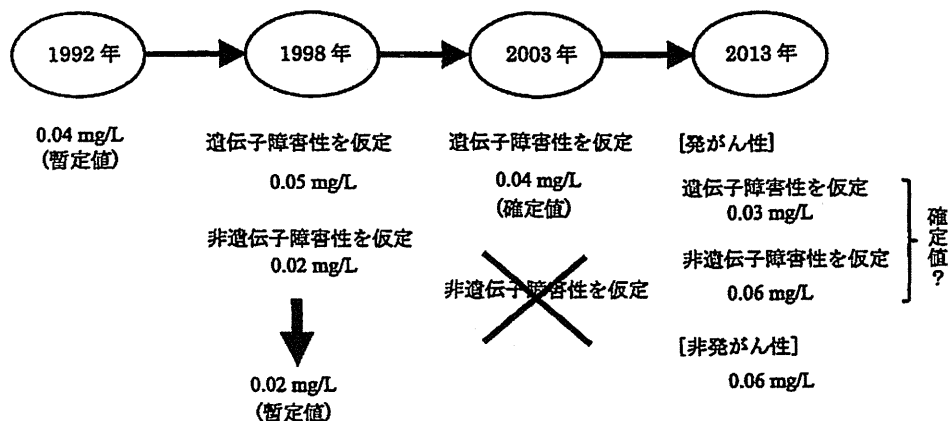


図12 ジクロロ酢酸の指針値および基準値設定の経緯

1に示した)分類が変化し、これによって全く異なる方法で評価値が算出されている。2003年の評価では、遺伝子障害性があると仮定するのが安全側の評価になるとの観点からVSD法のみで算出が行われた。一般的にこの考え方は誤りではない。しかし、1998年時の算出結果をみてみよう。VSD法にしたがった場合の値は0.05 mg/Lであるのに対し、TDI法にしたがうと0.02 mg/Lとなり、逆にTDI法の方が安全側の値となっている。このようにVSD法の方が値が小さく出るという保証があるわけではない。

2013年(平成25年)に行われた評価では、発がん性を指標として、2003年に評価対象としたのと同じの実験結果に対し遺伝子障害性を仮定した計算と非遺伝子障害性を仮定した計算の2通りを行っている。それぞれ0.03 mg/L、0.06 mg/Lが得られたが、ここで0.03 mg/Lを採用した場合、確定値とはいえず暫定値とするのが従来の扱い方である。しかし現在は、この2つの計算方法によって得られた小さい方の値を確定値とする立場に変化しているのである(なお、非発がん毒性を指標として得られた0.06 mg/Lと比較して安全側の値を得るプロセスは、毒性評価法の確定の問題とは質が異なるため、得られた値は確定値とすることができない)。

なお、遺伝子障害性を仮定した計算と非遺伝子障害性を仮定した計算の2通りを行って、それぞれ

0.03 mg/L、0.06 mg/Lを得、安全側として0.03 mg/Lを選択したように見えるが、このような方法を使った場合、実は前者の方が後者より必ず2倍小さい値が得られ、結果的に、必ず遺伝子障害性を仮定した計算結果が採用されることになるのである。以下にそのカラクリを説明しよう。

今回の場合、上記2つの値はベンチマーク用量法によって算出されている。図13に概要を示す。まず、動物実験から得られるデータに適切な用量反応曲線をあてはめ、これに基づいて、ある影響量(例えば、影響が10%過剰に発生)に対応する用量(ED_{10} , Estimated dose to 10% response)を求める。ついで安全のため、95%信頼限界の下限值(LED_{10} , Lower limit of estimated dose to 10% response)を算出する。これがベンチマーク用量である。これをNOAELとみなし、不確実係数で除してTDIを算出する。

また、この方法は、閾値をもたないと考えられる発がん物質にも適用される。すなわち、図13に示すように LED_{10} を求めた後、これを出発点(Point of departure)として、低濃度域に対し閾値を前提とせず直線で原点まで外挿する。これによって発がんリスク増分が 10^{-5} となる用量を算出し、これをVSDとする。

ベンチマーク用量とは10%のリスク増分レベルすなわち 10^{-1} レベルであり、これより 10^{-5} レベルに相当する値を求めるためには、単に比例計算するだけなの

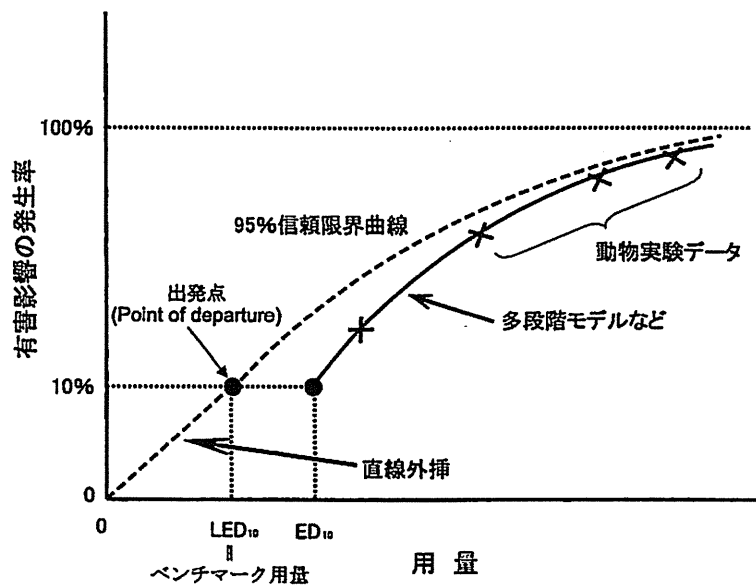


図13 ベンチマーク用量法の概要

で、VSD はベンチマーク用量の1万分の1の大きさとなる。

一方、非遺伝子障害性を仮定する場合、ベンチマーク用量はNOAELとみなされる(現在の「経口発がんリスク評価に関する手引き」²⁰⁾では、疫学研究または動物実験から発がん性に関するNOAELを得ることができない場合には、ベンチマーク用量法の適用を考慮することとされている)。不確実係数は、種内差×種間差の100に対し、発がんという重篤性を考慮して10を追加し1000とする。したがって、TDIはベンチマーク用量/1000となる。さらに評価値を算出する場合、飲用寄与率は、本稿で述べたように消毒副生成物に対しては20%を用いる。平均体重50kgと摂取水量(2L/日)を用いて、結局評価値は次式によって求められることになる。

$$\text{評価値} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\text{ベンチマーク用量} \left(\frac{\text{mg}}{\text{kg}} \right)}{10 \times 10 \times 10} \times 50 \text{ (kg)} \times 0.2 \div 2 \left(\frac{\text{L}}{\text{日}} \right) \quad (6)$$

この式を見ればわかるように、ベンチマーク用量に結局1万分の2を乗じている。VSDを得た後に使用する平均体重と摂取水量は同一であるから、VSDを経て求める値の方が常に2倍小さな値となるわけである。

なお、もし消毒副生成物ではなく一般の化学物質の場合、飲用寄与率は10%に設定するから、両者の計算結果は必ず一致することになる。

ベンチマーク用量法が導入される以前は、TDI法とVSD法による評価値はオーダーが異なって算出される可能性もあった²¹⁾。算出メカニズムが全く異なるのだからありうることである。これに対し、ベンチマーク用量法が使用されるようになったことによって、飲用寄与率が10%の発がん物質の場合には両者は必ず一致(飲用寄与率20%の消毒副生成物の場合には2倍の差)するようになったということもできる。

また、先述したように、2003年時と2013年時で評価対象とした動物実験結果は同じものである。同一の実

験データを用いながら、計算方法が変わることで基準値も変わる結果となっていることにも注目しておきたい。

4.1.2 DCAA, TCAAともに基準値が0.03 mg/Lとなることについて

DCAAとTCAAを比較すると、歴史的に、DCAAの方がTCAAよりも毒性が強いことが示されてきたと考えられる。今回の評価書^{21,22)}の中でも、例えば、亜急性毒性に関するNOAELの最大値は、DCAA 75(60) mg/kg/日、TCAA 312 mg/kg/日とTCAAの方が大きい値が得られている。生殖・発生毒性に関するLOAELも、DCAAの値の方が小さく、TCAAの値の方が大きい傾向が認められる(ただし、慢性毒性及び発がん性に関するNOAELの最大値は、DCAA 94 mg/kg/日、TCAA 78 mg/kg/日と同程度)。

しかるに、評価結果をみると、DCAAでは、非発がん毒性に対するTDIとして12.5 μg/kg/日、発がん性に対するTDIとして12.8 μg/kg/日(発がん性に対しては、別にユニットリスク7.8×10⁻³/ (mg/kg/日))を与えており、TCAAでは、非発がん毒性及び発がん性ともにTDIとして6 μg/kg/日を与えている。数値の上ではTCAAの方がDCAAよりも毒性が強いことを意味し、従来の試験結果にみられる毒性の大小関係が逆転する結果となっている。

次に、これらの値を用いて導出される水道水質基準値は、DCAAについては上述のように0.03 mg/Lを採用することとなる。一方、TCAAのTDI 6 μg/kg/日から算出される評価値は0.03 mg/Lとなる。こうして、本来、毒性の大小関係が認められる両物質であるにもかかわらず、それらの評価値は偶然にも一致するという現象がおきてしまう。

もちろん、2つの評価書は、現行の評価ルールに従ってそれぞれ作成されたものであることは理解できる。しかし、評価対象物質に関する毒性情報の全体を俯瞰するとともに、類縁物質間で比較検討しその整合性をチェックするという作業も同時に必要と考えるがいかがであろうか?(以上の内容は、筆者によるパブリックコメントとして提出した。)

さらに付け加えれば、本稿では、表5に示したようにDCAAとTCAAに対する飲用寄与率として、それぞれ40%、30%を推奨した。DCAAは遺伝子障害性発

がん物質を仮定した場合の値を採用しているため、飲用寄与率は使用されず基準値には変化はない。一方、TCAAに推奨値30%を使用して計算すると0.05 mg/Lが得られる。このようにすれば、MCAA, DCAA, TCAAの基準値はそれぞれ、0.02 mg/L, 0.03 mg/L, 0.05 mg/Lとなり、本来の毒性の強さの順序とかがうじて整合性を保つことができる。

4.2 消毒処理水の全体毒性と米国 EPA プロジェクト

1970年代にトリハロメタン問題が提起されて以来、塩素処理水中の副生成物を検出し同定する試みが世界中で行われてきた。この結果、現在までに実験室レベルで確認されたものを含めると600~700種類にも及ぶ塩素処理副生成物が同定されている²⁸⁻³⁰⁾。それにもかかわらず、これらは生成する全有機ハロゲン (Total Organic Halides; TOX) の50%程度でしかない。さらに、動物実験にもとづいて毒性評価が行われ、基準値や目標値が設定できる物質は20にも満たない。

筆者らがバイオアッセイによって塩素処理水に対する個別副生成物の寄与率を推定した結果、TCM, DCAA, TCAAの3物質の合計でも、染色体異常誘発性に対する寄与率は2.9%、形質転換誘発性に対する寄与率は1.4%であった。また、MX (但し培養細胞を用いた結果) や臭素酸イオンについては0.1%未満であり、その寄与はほとんど無視できた³⁾。このように、個別物質の毒性の寄与率は低く、現在水質基準として規制されている物質以外の物質の毒性が大半を占めている。個別物質ごとに基準値を設定して水質管理を行う現在の体制では不十分といわざるをえない。個別物質の基準だけでなく、消毒副生成物全体としての毒性を把握し、それを制御するための総括指標の導入を本来は検討すべきと指摘できる。

水道水中の有機ハロゲン化合物の総量を表す指標としてTOXが用いられている。上述のように、個別物質の規制では限界があるため、TOXを用いて毒性を総括的に把握し、その許容レベルの提示を試みた³⁾。この結果、いくつかの仮定の下ではあるが許容TOXレベルは0.022 mgCl/L (=22.0 µgCl/L) となった。わが国の水道水の一般的なTOXの生成量は80~100 µgCl/Lと想定され、その毒性がいかに大きいか分かる。水質基準の設定リスクレベル 10^{-6} に照らして考える

と、TOXは現状の1/4から1/5しか許容できないということになる。

このような消毒副生成物のガイドライン値や基準値が果たす役割とその限界に関する認識に基づき、筆者は2009年世界保健機関 (WHO) に滞在し、「WHO 飲料水水質ガイドライン」の編集責任者 (当時) である Jamie Bartram 氏 (現在ノースカロライナ大学チャペルヒル校教授) らとともに、2011年にレビュー論文を発表した^{31,32)}。

そこでは筆者らの研究成果が多くレビューされ、まずは、副生成物の生成と処理水本来の毒性との乖離例をいくつか示した。それらは、上述の個別物質の全体毒性に対する寄与度のほか、全体毒性に対する有機臭素化合物と臭素酸イオンの寄与^{33,34)}、塩素処理水のイニシエーション活性・プロモーション活性の変化とMXの指標性³⁵⁾、二酸化塩素処理水の変異原性の安定性と塩素処理水との比較³⁶⁾、塩素処理水のエストロゲン様作用の構造³⁷⁻³⁹⁾などである。

このような研究例を見ると、規制下にある指標副生成物は、安全な水道水を供給するために有用であるが、他の副生成物に由来する毒性を把握できないという限界もあることがうかがえる。しかし、水道事業者は、設定された基準値を超過しないことに関心を集中させるので、水道水の安全性全体から見ると不適切あるいは誤った対応をとってしまうことさえある。例えば、水道事業者の中には、前もって塩素を注入して有機臭素化合物を生成させることで原水中の臭化物イオンを削減し、その後のオゾン処理における臭素酸イオン生成量を抑制しようとする事例がある。しかし、最終的にできる塩素処理水の有害性に対する臭素酸イオンの寄与は無視できるほど小さく、有機臭素化合物の寄与の方がはるかに大きいので、このプロセスは危険な技術である可能性がある。安全な水道水を届けるために水質基準を設定したのに、本来の水の安全性が見過され、逆により有害な水道水を生産してしまう可能性さえあるのである。水道水の全体の安全性をみる必要があることを教えてくれる好例といえよう。

水質基準は (少なくとも消毒副生成物については) 全能なものではなく、参考資料と考える方がよい。今後は消毒処理水としての安全性全体を考慮する方向へ考え方をシフトしていかなければならない。

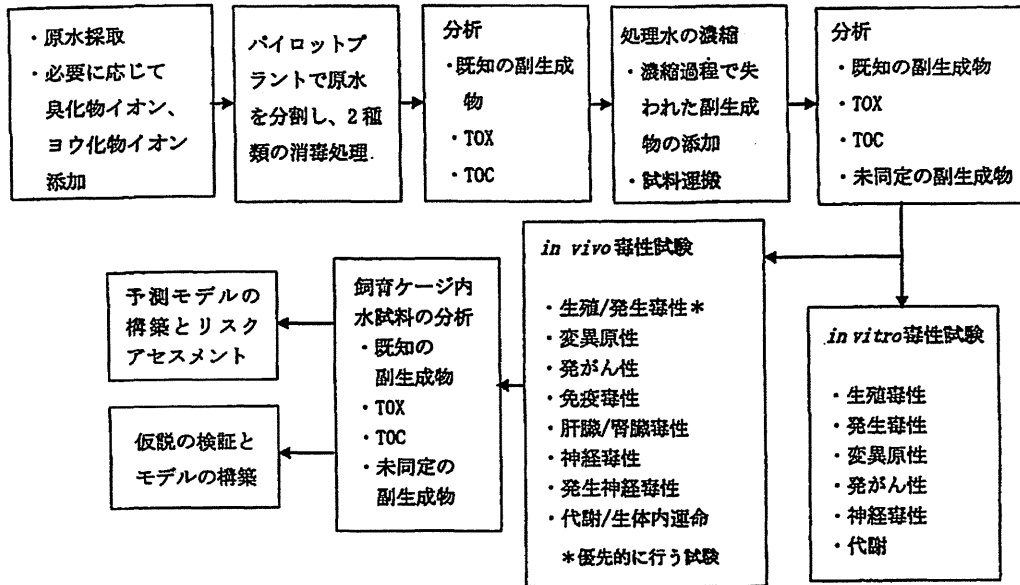


図14 米国 EPA における消毒処理水の毒性評価フロー⁴⁰⁾

一方、本レビュー論文に引用された筆者らの研究は、いずれも *in vitro* バイオアッセイの結果から考察したものであり、動物個体に対する毒性に関して結論が得られるわけではないという制約がある。これまでのところ、塩素処理水そのものの *in vivo* 試験を行った例は極めて少ない。今後は、例えば TOX の水質基準値を導出できるような毒性試験が必要であると考えられる。実際、米国 EPA には、まさにこの目的のためのプロジェクトが存在する⁴⁰⁾。計画のフローを図14に示す。塩素及びオゾン処理水に対して、*in vitro* バイオアッセイも行うが、*in vivo* での毒性試験を、発がん性のみに限定せず系統的に行うとしている点に極めて大きな意義がある。内分泌攪乱の影響に関連した生殖・発生毒性、免疫毒性、神経毒性などの毒性も重要な評価対象となっている。

本プロジェクトでは、逆浸透膜による濃縮技術の整備⁴¹⁾、実験動物が飲水可能な濃縮水の調製⁴²⁾、濃縮水の安定性⁴³⁾などいくつかの課題が容易に想像できるが、異なる分野の専門家が試験計画を立案しプロジェクトを進めており注目に値する。プロジェクトでは、生殖・発生毒性に関する試験を優先的に行うとしており、これまでのところ、塩素処理水およびオゾン/塩素処理水ともに、130倍までの濃縮試料から発生毒性は検出されなかった⁴²⁾などの結果が得られている。発がん性試験については、2年間に及ぶ動物実験

に必要な濃縮試料水を得るのが容易ではないことから優先順位が下げられている⁴⁴⁾。また、このプロジェクトでは、二酸化塩素やクロラミンによる処理水の毒性試験は計画されていない。本プロジェクトの進展を強く期待したい。

以上述べた内容を背景として、筆者らは、国際機関や各国の基準値設定委員会は、消毒処理水の全体の毒性に関する情報収集を進めるべきであると勧告した。規制へ向けた有用な結果、すなわち、例えば塩素処理水の TOX に関する評価値の導出が可能となる結果が得られるか否かは、*in vivo* 試験が成功するかどうかにかかっている。国際的に、優先度を高めた取り組みが強く期待される。

4.3 疫学調査結果と消毒処理水の毒性

4.3.1 疫学調査の状況と動物実験結果との相違

1970年代に、米国でがん死亡率と水道水飲用との関係に関する疫学調査の結果が報告された。この THMs 問題の提起以来、多年にわたり多くの疫学調査が行われてきた⁴⁵⁻⁴⁷⁾。表7に検出または示唆されてきた代表的な健康影響や疾患の種類を示す。

もちろん、この表に記載した健康影響と塩素処理水の消費との間の因果関係は立証されるには至っていない。これはそもそも塩素処理した水道水の例えば発がん性がそれほど強いわけではないことが主たる原因と

表7 疫学調査によって検出または示唆された主な健康影響の種類

区分	健康影響の種類
発がん性	膀胱がん 結腸がん 直腸がん
生殖・発生毒性	流産 死産 低体重児の出生、胎児の生育不良 神経毒性 各種先天異常、奇形

いえる。疫学調査の結果、その因果関係が立証されないとしても本来不思議ではない。しかしながら、それでもなお、塩素処理された水道水によって表に示した健康影響が（すべてではないが）実際に起きているというのが現在の一般的な認識となっている。

がん発生率の増加が有意にみられたとする調査例は多い。検出されるがんの種類としては、膀胱がんに関するものが最も多い。ついで検出事例が多いものに結腸がん、直腸がんがある。その他、塩素処理された水道水の消費との関係を示した、または示唆した報告例のあるがんとしては、膵臓がん、食道がん、乳がん、脳腫瘍がある。ただ、統計的に有意に増加したとはいえない結果もあり統一的とはいえない。さらに、腎臓がん、肝臓がん、肺がんとの関係についても検討事例は存在する。

さて、実はここに、疫学調査で検出されるがんの種類と、動物実験で生成するがんの種類が異なるという大きな問題が存在する。これは従来から専門家の間では認識されてきた長い間の悩みであった。表7に示すように、疫学調査で検出されるがんは、主に膀胱がんであり、ついで結腸がん、直腸がんである。これに対して、THMsやHAAsといった多くの消毒副生成物によって、げっ歯類で生起するのは主に肝臓がんである。この他、BDCM、TCM、臭素酸イオンでは腎臓がん、BDCM、TBMでは大腸がん、MXでは多臓器にがんが生成する。これらはいずれも、疫学調査で検出されるがんとは異なるのである。基準値の設定は動物実験を基に行うが、実際に生起する健康影響とは異なるエンドポイントで評価してしまっているのではないかと疑念が生じることになる（ただし、THMsの場合、発がん性を根拠とするよりも、他の慢性毒性を

根拠として評価値を算出する方が安全側であることから、現行基準値は非発がん影響をエンドポイントとして設定されている）。

これに対する最近の理解は次のようになっている⁴⁸⁾。

疫学調査結果から膀胱がんを生起する要因として、以下の3つが抽出された。1) 水道水中 THMs 濃度、2) シャワー / 入浴 / 水泳による曝露量（吸入 / 経皮曝露）、3) 遺伝子型 (*GSTT1-1* (glutathione-S-transferase-theta) 遺伝子の有無)。このうち、水道水中 THMs 濃度よりも、シャワー / 入浴 / 水泳による曝露量（吸入 / 経皮曝露）の方が重要なファクターであり、膀胱がんの多くは、経口摂取よりも吸入 / 経皮摂取によると考えられるとされた。また、*GSTT1-1* 遺伝子を持っている人（米では75%の人が持つ）のリスクが高かった。これは、*GSTT1-1* 酵素によって、(TCM以外の) THMs が変異原性物質へ活性化されるためと説明されている。

このように、疫学調査と動物実験で検出されるがんの種類が異なる原因は、曝露経路の違いである可能性がある。つまり、膀胱がんの多くは、経口摂取よりは吸入 / 経皮摂取によって生成していると考えられる。つまり、水道水を飲むことによってがんができるのではなく、いったん気相に移行した物質を吸入してがんができるということになる（他に経皮摂取もあり）。

例えば、経口摂取された THMs は、直接体内に分配されるのではなく、まず肝臓に届き、そこで解毒・排泄作用を受けることになる。一方、吸入 / 経皮摂取の場合には、THMs は、血液中に直接取り込まれていくことになり、血中濃度、および標的臓器に対する用量は大きいと考えることができる。これらの現象を示した測定結果も存在する。

以上のことは、例えば TCM の場合、歴史的に、経口による毒性よりも吸入による毒性の方が強いとされてきたこととも符合する。例えば、米国 EPA が評価した発がんスロープファクターを見ると、吸入摂取の方が13倍大きな値となっている⁴⁹⁾。わが国でも、経口による耐容1日摂取量は645 µg/日 (TDI 12.9 µg/kg/日²⁵⁾ × 50 kg) であるのに対し、吸入による耐容1日摂取量は270 µg/日⁵⁰⁾と、吸入による値の方が2.4倍厳しくなっている。

今後は、げっ歯類で生起するがんと、ヒトの疫学調

査結果の不一致を埋めるための調査研究が必要である。それらは、遺伝子型に関する研究、曝露経路に着目した研究（吸入/経皮曝露）が含まれよう。また、経口摂取よりも吸入摂取による影響の方が主体であるとするなら、動物実験も、飲水投与だけではなく吸入投与という方法も考慮する必要があるかもしれない。

4.3.2 生殖・発生上の影響について

塩素処理副生成物による健康影響として、発がん性の次に注目されてきているものに生殖・発生毒性がある。周知のように、1990年代後半、内分泌攪乱化学物質（環境ホルモン物質）の問題に世界の大きな関心が集まった。これにともない、消毒副生成物についてもそのような作用があるか否かが関心事となったのである。米国EPAが設置した「内分泌攪乱化学物質のスクリーニングと試験法に関する諮問委員会」は、1996年に最終報告書⁵¹⁾を示したが、その中で個別物質の試験に加えて、6種類の混合物についても試験を行うことを勧告した。そしてこの6種類の中に消毒副生成物が含まれている。

もちろん生殖・発生毒性に関する調査研究は従前から行われていたが、内分泌攪乱化学物質問題が顕在化する中、Wallerらの調査研究⁵²⁾が注目された。米カリフォルニア州において妊婦（妊娠3ヶ月余まで）を対象とした調査の結果、水道水によるTHMs摂取量が多いグループでは流産率が増大したことを報告したのである。この他、流産・死産の増加、低体重児の出生および胎児の生育不良、神経毒性、心臓、泌尿器、呼吸器などにおける先天異常や奇形の発生などが報告または示唆されている⁴⁷⁾。一方、早産などの影響は報告されていない。

内分泌攪乱化学物質を取り上げた調査研究にみられる特徴のひとつは、いずれも対象物質がビスフェノールA、フタル酸類、アルキルフェノール、17β-エストラジオールなどの個別の化学物質に限定されていることであった。これに対して疫学調査では、塩素消毒された水道水の消費と生殖・発生毒性との関係が議論されている。そこでは当然、フミン物質を中心とする天然有機物から生成した有機ハロゲン化合物などの副生成物に関心事とすべきところである。筆者らは、この観点から水道水のエストロゲン様作用の構造と制御に関する研究を進めていった³⁷⁻³⁹⁾。

上述の疫学調査の報告の中には、弱い関係しか認められないものや、明確な結論は得られず示唆にとどまるものも多い。しかし、現在、塩素消毒された水道水の消費によって生殖・発生上の影響が実際に起きていると認識されている。Wallerらの調査研究は注目され、わが国でも新聞報道されたが、1970年代に大問題となった発がん影響に次ぐ健康影響問題として、社会的にもっと注目されてよい問題と考える。

5. おわりに

本稿では消毒副生成物を取り上げて、飲用奇与率の設定問題をはじめとして、基準値設定上の課題を論じた。そして、それは水道水の安全性をいかに把握し管理すべきかという本質的な議論に発展することとなった。

水道水質基準に限らず、基準値を所与のものと考えてはいけない。水質基準とは科学的根拠に基づいて設定されるというのが建前なのだが、本稿で論じたように様々な課題を抱えているのであって、「科学的根拠」といえるほど確かなものがあるわけではない。それは現在の科学の限界というべきであろう。

水質基準が一旦定められてしまうと、基準値を超えた水道水は危険な水とみなされ、逆に、特に水道事業者などは、基準値以下の水を配っているからうちの水は絶対安全な水、というように、水の安全性について白黒をつける判断材料として使われてしまう傾向がある。

しかしながら、現在の「科学的根拠」の頑健さに照らして考えると、基準値は不確実性を有しているのであって、今後その値が変化していく可能性も大きい。また、消毒副生成物に関する規制項目と水の本来の安全性との関係について論じたように、水質基準とは水質のある側面を切り取って見た、まさに「水質指標」であるということができる。

総じて、基準値を絶対視し、これのみに縛られ、またそれを満たせばそれで事足りるとするのは水質管理のあるべき姿とはいえない。むしろ基準値とは、水質管理上の「目安」あるいは重要な参考資料として「活用」すべき性格のものとして位置付けるのが望ましい。

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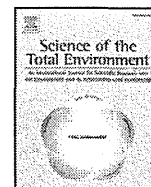
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Contribution of tap water to chlorate and perchlorate intake: A market basket study[☆]



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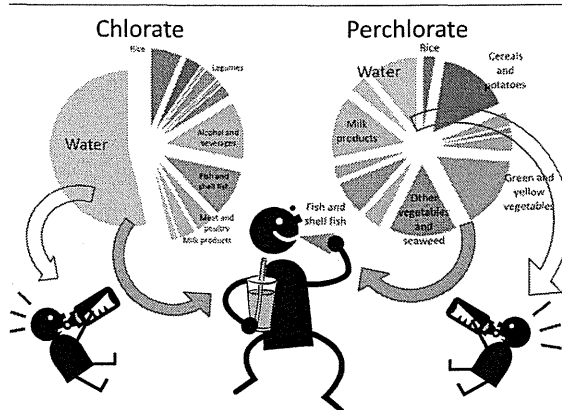
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HIGHLIGHTS

- Tap water contribution to total chlorate intake was first revealed.
- Cooking with tap water significantly influenced total chlorate intake.
- Chlorate intake from water is important especially when rice is major food.
- Total perchlorate intake was higher than the previous U.S. study due to vegetables.
- Total chlorate and perchlorate intake from baby formulas and water were high.

GRAPHICAL ABSTRACT



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ABSTRACT

The contributions of water to total levels of chlorate and perchlorate intake were determined using food and water samples from a market basket study from 10 locations in Japan between 2008 and 2009. Foods were categorized into 13 groups and analyzed along with tap water. The average total chlorate intake was 333 (min. 193–max. 486) $\mu\text{g}/\text{day}$ for samples cooked with tap water. The contribution of tap water to total chlorate intake was as high as 47%–58%, although total chlorate intake was less than 32% of the tolerable daily intake, 1500 $\mu\text{g}/\text{day}$ for body weight of 50 kg. For perchlorate, daily intake from water was 0.7 (0.1–4.4) $\mu\text{g}/\text{day}$, which is not high compared to the average total intake of 14 (2.5–84) $\mu\text{g}/\text{day}$, while the reference dose (RfD) is 35 $\mu\text{g}/\text{day}$ and the provisional maximum tolerable daily intake (PMTDI) is 500 $\mu\text{g}/\text{day}$ for body weight of 50 kg. The highest intake of perchlorate was 84 $\mu\text{g}/\text{day}$, where concentrations in foods were high, but not in water. The contribution of water to total perchlorate intake ranged from 0.5% to 22%, while the ratio of highest daily intake to RfD was 240% and that to PMTDI was 17%. Eight baby formulas were also tested – total chlorate and perchlorate intakes were 147 (42–332) $\mu\text{g}/\text{day}$ and 1.11 (0.05–4.5) $\mu\text{g}/\text{day}$, respectively, for an ingestion volume of 1 L/day if prepared with tap water.

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1. Introduction

Chlorate and perchlorate are known as micropollutants in water. Chlorate is an impurity in sodium hypochlorite used as a disinfectant, and was added to the Japanese drinking water quality standard in November 2007 (in effect from April 2008) (MHLW, 2007). One of the known health effects of chlorate is oxidative damage to red blood cells, and the tolerable daily intake (TDI) was set to 30 µg/kg/day (MHLW, 2003). Chlorate is mainly thought to be present in water and its intake via the atmosphere seems unlikely (WHO, 2011). Therefore, 80% of TDI was allocated to drinking water, and the standard value of 600 µg/L was derived and established (MHLW, 2007). At present, 80% of TDI is also allocated to water in the guidelines issued by World Health Organization (WHO); due to the differences in standard body weight, the value in the WHO guideline is 700 µg/L (WHO, 2011).

Perchlorate is utilized for various purposes, including as a propellant for rocket fuel, explosives, fireworks, and airbags, and is also included in sodium hypochlorite (Kosaka et al., 2011). Perchlorate is known to suppress the uptake of iodine in the thyroid gland. In February 2005, the U.S. National Academy of Sciences (NAS) announced a perchlorate reference dose (RfD) of 0.7 µg/kg/day (NRC, 2005). In the same year, the U.S. Environmental Protection Agency (USEPA) announced a drinking water equivalent level (DWEL) of 24.5 µg/L (EPA, 2009) (when 100% contribution rate is assumed as 2 L of water a day consumed by an adult of body weight 70 kg). In 2008, the USEPA issued an Interim Health Advisory Level (HAL) of perchlorate of 15 µg/L (EPA, 2008). The USEPA has decided to regulate perchlorate under the Safe Drinking Water Act (SDWA) and is now developing a proposed national primary drinking water regulation for perchlorate and anticipates publication of the proposed rules for public review and comment in 2013 (EPA, 2012). JECFA (2011) established a the provisional maximum tolerable daily intake (PMTDI) of 0.01 mg/kg based on human data and including an uncertainty factor of 10 considering potentially vulnerable subgroups, such as pregnant women, fetuses, newborns and young infants.

In 2011, the Water Supply Division, Ministry of Health, Labour and Welfare, Japanese government, posed an evaluation value of 25 µg/L for perchlorate, assuming that the allocation of TDI to perchlorate in water is 10%, while it has been included in “the items for further study” as supplementary items to the Japanese Drinking Water Standard (MHLW, 2011).

Previously, we measured the concentrations of chlorate and perchlorate in bottled water and commercial beverages (Asami et al., 2009a), and also reported that the concentration of chlorate in some tap water exceeded 600 µg/L before the standard for chlorate was in effect (Asami et al., 2008). The highest chlorate concentration, 2900 µg/L was found due to high dose of sodium hypochlorite in 2006. Chlorate was present in degradation byproducts of sodium hypochlorite added in water purification process as an oxidative chemical and/or disinfectants (Kosaka et al., 2007). We also investigated the current state of chlorate and perchlorate in the Tone River basin in Japan, a main water source for Tokyo. The results indicated that perchlorate was present in environment water, drinking water, and water supplies in a wide region in Tone River basin due to environmental contamination mainly attributable to industrial effluents from few specific sources including a chlorate and perchlorate manufacturer and a facility conducting electrolysis processes. Takatsuki et al. (2009) made a survey on perchlorate contamination in leafy vegetables sold in Tokyo. Among 82 leafy vegetable samples tested, perchlorate was detected in the range of 0.3 ng/g and 29.7 ng/g in 79 samples. It was found at relatively high concentration in spinach and leafy green vegetables. Although it is not clear why they have higher concentration, it may have been possibly affected from water used for cultivation, as one of the sources of perchlorate.

In setting the guideline value for chlorate, the allocation ratio to water is 80% as one of disinfection byproducts, which is much higher

than the default assumption of 10%–20% for other items, since chlorate was not considered to be exposed from other sources than water (WHO, 2011). To authors' knowledge, there is no data on total chlorate intake, therefore, intake survey through food is important, especially when considering tap water.

In 2008, the U.S. Food and Drug Administration reported the results of a perchlorate intake survey in the USA (Murray et al., 2008). However, the intake in Japan has yet to be investigated where cooking (boiling) and eating of rice at home is common, which seems a little different from that in the USA and European countries and which might be affected by constituents in tap water. The contribution of tap water is important to consider the allocation ratio to water based on the diet and, to our knowledge, there have been no previous surveys of chlorate intake.

In addition, the diet of infants differs from that of adults. The WHO guideline specifies the amount of drinking water for children weighing 10 kg as 1 L per day, and that for infants weighing 5 kg as 0.75 L per day (WHO, 2011). The concentration of perchlorate in milk was investigated in both Japan and the USA (Dyke et al., 2007). A relatively high concentration of perchlorate was detected in Japan. As infants rely on breast milk or baby formula for their daily nutritional intake, data regarding the amounts of chlorate and perchlorate in breast milk and formula are required to allow estimation of their intake levels by infants; however, no such data have been reported in Japan.

In the present study, we analyzed samples for the Total Diet Study (TDS) in cooperation with the National Institute of Health Sciences, Japan, and local health science institutes, estimated the amounts of chlorate and perchlorate intake, and compared the levels of their intake derived from food and tap water. In addition, a purchase survey was conducted on baby formula.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared from ultrapure water obtained using a Gradient A10 water purification system (Millipore, Bedford, MA). Calibration standards were prepared by diluting 1000 mg/L certified standard solutions of chlorate (Kanto Chemical, Tokyo, Japan) and perchlorate (GFS Chemicals, Columbus, OH) in ultrapure water.

2.2. Test samples

From 2008 to 2009, foods were purchased and collected using the market basket method at 10 different locations across Japan, and were prepared at each location for use as test samples. Locations were selected to cover throughout Japan considering geographical and cultural differences as in the National Nutrition Survey (MHLW, 2005). At each sampling location, about 150 kinds of food were purchased from local grocery stores according to the methods used by the National Nutrition Survey (MHLW, 2005). Local custom and preference were taken into account when food items were selected, so the composition of the composite food samples was a little different to each other. Based on the amount of food intake according to the survey, and using the same method as described previously in detail (Ohno et al., 2010), a wide variety of foods were divided into 13 different groups (Table 1). After appropriate pre-cooking (normally boiling or baking), the foods were mixed and homogenized to make composite samples. Tap water was used for the pre-cooking at six locations, and pure water was used at other four locations. Representative intake levels for each food group in this study are shown in Fig. 1. The 14th group consisted of tap water from municipal drinking water treatment plants that used surface water as the source. The amount of water intake was not included in the above-mentioned national survey, so the level is represented as 2 L based on normal assumptions

Table 1
Food groups in the survey.

Food group	Contents
1	Rice
2	Cereals and potatoes
3	Sugar and confectioneries
4	Oil and fats
5	Legumes
6	Fruits
7	Green and yellow vegetables
8	Other vegetables and seaweed
9	Alcohol and beverages
10	Fish and shellfish
11	Meat and poultry
12	Milk products
13	Seasoning
14	Water

used in establishing drinking water quality standards. The pure waters used for cooking in four locations and the travel blank samples were also analyzed. Food samples were stored at $-20\text{ }^{\circ}\text{C}$ and thawed at room temperature immediately before analysis.

For eight baby formula samples, powdered milk and an ionized water (a mineral water for infants) were purchased from a market in the Tokyo area then used as samples in the test (Table 2). Baby formulas were evaluated for their concentration based on the manufacturers' indications.

2.3. Analytical method: pretreatment and analysis

Chlorate and perchlorate concentrations were analyzed by ion chromatograph (IC)–tandem mass spectrometry (MS/MS) as described elsewhere (Asami et al., 2009a; Kosaka et al., 2007). As an internal

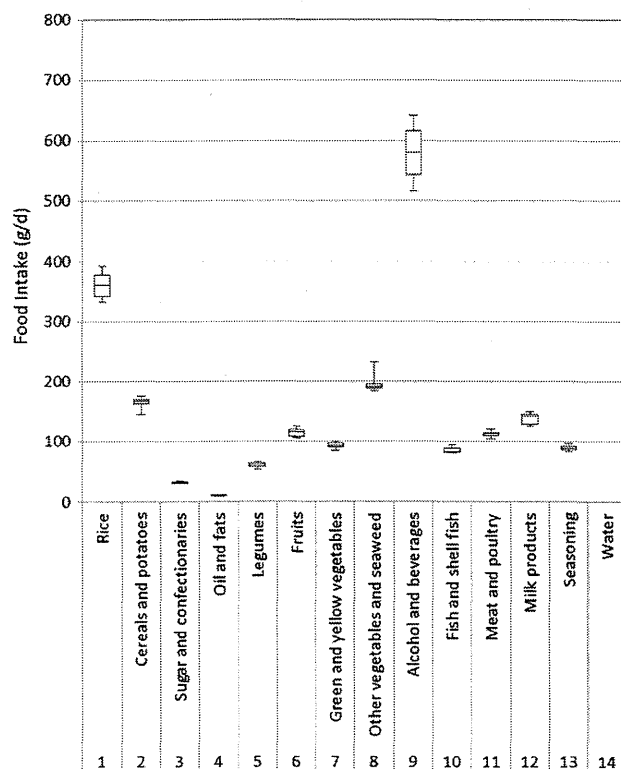


Fig. 1. Intake of each food group as determined by the methods of the National Nutrition Survey (MHLW 2004). Boxes represent 25%–75% quartile, while the center bars represent medians, and whiskers are maximum and minimum values. No data were available for group 14.

Table 2
Powdered milk.

No.	Manufacturer
I	WA
II	WA
III	MO
IV	MO
V	ME
VI	BS
VII	ME
VIII	ME

standard for perchlorate, ^{18}O -enriched NaClO_4 (Cambridge Isotope Laboratories, Andover, MA) was added to the samples immediately prior to analysis.

Samples of 1–5 g of each food group were placed in 50-mL tubes, and a standard solution of chlorate was added to a final concentration of 0, 1, or 2 $\mu\text{g/L}$. In addition, $^{18}\text{O}_4$ -perchlorate was added as an internal standard to a final concentration of 1 $\mu\text{g/L}$. After adding 20 mL of solvent (mixed solution of distilled water: acetonitrile, 1:1 v/v), the samples were mixed and shaken at 75 rpm for 2 h. After shaking, the samples were separated by centrifugation (2000 rpm for 15 min), and 10 mL of the supernatant was collected and put into cartridges pretreated with methanol and distilled water (SepPak C18 1g; Waters, Milford, MA). The eluate was then filtered using hydrophilic polytetrafluoroethylene (PTFE) syringe filters (pore size 0.2 μm or 0.45 μm ; Advantec, Dublin, CA), and the resulting solution was used as the test solution. For chlorate, the solution was further diluted by 10-fold and used as the test solution. For some food groups, preparation cartridges (OnGuardII Ba/Ag/H; Dionex) were used to avoid the effects of coexisting anions.

As pretreatment to determine the concentration of chlorate in baby formula, samples of 1 g were collected and the standard solution of chlorate was added to a final concentration of 0, 1, or 2 $\mu\text{g/L}$. Pretreatment to determine the concentration of perchlorate in baby formula was the same as described for food samples, and $^{18}\text{O}_4$ -enriched perchlorate was added as an internal reference to a final concentration of 1 $\mu\text{g/L}$. Then, 10 mL of distilled water was added to dissolve the formula, after which 10 mL of 1% acetic acid solution was added and mixed thoroughly. Then, 20 mL of acetonitrile was added, mixed, and filtered using folded filter paper (2 V; Whatman, Kent, UK) that had been pretreated with distilled water. The filtrate was added to C18 cartridges and the samples for chlorate were diluted 10-fold, and then filtered again using anion-exchange cartridges or PTFE syringe filters.

In the rice cooking test, 50 g of rice was boiled with 100 mL of tap or pure water. Tap water contained 93.7 $\mu\text{g/L}$ of chlorate and pure water contained no chlorate. The boiled rice was then treated the same as the rice sample described above and compared with the calculated value adjusted by wet weight.

2.4. Analytical method: equipment used

Separation was performed using an IC system (ICS-2000; Dionex, Sunnyvale, CA) with an eluent generator (EG50; Dionex), guard column (IonPac AG20 column $2 \times 50\text{ mm}$; Dionex), separation column (IonPac AS20, $2 \times 250\text{ mm}$; Dionex), and suppressor (ASRS Ultra II, 2 mm; Dionex). The eluent was potassium hydroxide (KOH) at a flow rate of 0.25 mL/min with an injection volume of 100 μL . As a post-column solvent, a mixture of acetonitrile (high-performance liquid chromatography grade; Wako Chemical, Osaka, Japan) and ultrapure water (90/10 v/v) was supplied to the eluent at a flow rate of 0.2 mL/min.

An API 3200QTrap (Applied Biosystems, Foster City, CA) was used for MS/MS. Selected multiple reaction monitoring (MRM) transitions were set at m/z 83/67 (quantification) and 85/69 (confirmation) for

chlorate; m/z 99/83 (quantification) and 101/85 (confirmation) for perchlorate, and 107/89 for ^{18}O -enriched perchlorate.

The limits of quantification (LOQ) for the aqueous samples were set as 0.05 $\mu\text{g/L}$ and 0.01 $\mu\text{g/L}$ for chlorate and perchlorate, respectively. The LOQ for food samples varied for each group and each location depending on the collection quantity and concentration, and those for chlorate and perchlorate ranged between 0.2 and 10 ng/g-wet-weight and 0.05 and 0.2 ng/g-wet-weight, respectively. The LOQ for chlorate was higher than that for perchlorate because the former was measured using the standard addition method. Nevertheless, the concentration of chlorate was higher than the LOQ, and we were able to determine the concentration in all food groups. The concentrations of chlorate and perchlorate in the pure waters and the travel blank samples were all below LOQ.

With regard to accuracy, samples were analyzed twice for several food groups ($n = 2$). The relative standard deviation (RSD, %) for repeat accuracy was 0.86% minimum and 37.2% maximum for chlorate, while those for perchlorate were 0.6% and 8.5%, respectively. Samples were pretreated such that the additive recovery rate would be above 60% for chlorate and perchlorate. For some dairy products and baby formula samples, additive recovery rates of internal standard substance of chlorate and perchlorate did not reach 50%, however, their linearity and duplicability were high. Therefore, the standard addition method was employed for all samples to minimize the analytical errors.

3. Results and discussion

3.1. Concentrations of chlorate and perchlorate in tap water samples

The chlorate and perchlorate concentrations in tap water are shown in Table 3. Chlorate concentrations in tap water at the sampling locations were in the range of 34–140 $\mu\text{g/L}$. Chlorate is contained in sodium hypochlorite used for disinfection in the majority of water treatment processes. This range was almost the same level as our previous study on water supply, 25–120 $\mu\text{g/L}$ contained in treated water (Asami et al., 2008). The concentration of perchlorate ranged from 0.03 to 0.23 $\mu\text{g/L}$ at all except one location, which had a much higher concentration than the others (2.2 $\mu\text{g/L}$). As the degree of water contamination in the area including this location was previously shown to be high (Kosaka et al., 2007), perchlorate concentration in tap water represents source water contamination by perchlorate contamination in that area which may be affected by river water contamination.

3.2. Chlorate and perchlorate concentrations in food and water samples

The concentrations of chlorate and perchlorate in each group for food samples and water are shown in Figs. 2 and 3, respectively. Measured concentrations in food samples were converted for each sample as the concentration per kilograms wet weight.

Chlorate concentration varied from <0.02 $\mu\text{g/kg}$ to 920 $\mu\text{g/kg}$. Among food sample groups, legumes (group 5), seafood (group 10), and meat and poultry (group 11) had rather high average concentrations of chlorate. The concentration of chlorate in sugar and confectioneries (group 3) was specifically high at one location, 150 $\mu\text{g/kg}$.

The concentration in rice (group 1) was low (between 0.30 and 8.0 $\mu\text{g/kg}$) at locations A, B, G and I where food samples were cooked with pure water. In other locations, the values varied between 28 and 91 $\mu\text{g/kg}$, probably because tap water was used for cooking these samples. The concentrations of chlorate in cereals and potatoes (group 2) were between 30 and 110 $\mu\text{g/kg}$, which were similar to those in rice cooked with tap water. The cereals and potatoes group included potatoes and noodles, which require tap water for cooking. The concentrations in the legumes (group 5) were relatively high, ranging between 70 and 140 $\mu\text{g/kg}$. This group included many foods that may have used tap water during processing in commercial factories, such as tofu.

In general, the concentration of perchlorate was lower than that of chlorate (Fig. 3), but was highest at 350 $\mu\text{g/kg}$ in vegetables and seaweed (group 8) at location B, followed by green and yellow vegetables (vegetables rich in colors, group 7) at location B with 110 $\mu\text{g/kg}$. The vegetables and seaweed group (group 8) included a total of 16 items: vegetables with light colors, such as lettuce, bean sprouts, Chinese cabbage, eggplant, as well as Japanese radish, onions, mushrooms, and seaweed. It is not considered that these concentrations were affected from water since tap water was not used in location B. The concentration in the cereals and potatoes (group 2) was 2.8–28 $\mu\text{g/kg}$, that in sugar and confectioneries (group 3) was 1.3–27 $\mu\text{g/kg}$, and that in fish and seafood (group 10) was 1.3–23 $\mu\text{g/kg}$, all of which were relatively high. In these groups, the concentrations of some food samples varied greatly depending on the locations. The concentrations were the highest in location B followed by location D.

3.3. Comparison of food items cooked using pure water and tap water

In this study, pure water was used for cooking at 4 of 10 locations (A, B, G and I) and tap water was used at 6 locations. When chlorate concentrations of each sample were divided into two groups; those cooked with pure water and those cooked with tap water, the measured chlorate concentrations were higher in locations that used tap water for cooking compared to the other locations where pure water was used (Fig. 2). The difference between samples prepared with pure water and tap water was statistically significant in rice and green and yellow vegetables (group 1 and 7, $P < 0.05$).

For perchlorate, it seems that the difference was found among foods themselves, not in waters used for cooking, because perchlorate concentration was higher in foods cooked with pure water.

In food groups of 2, 7 and 8, perchlorate concentrations in food cooked with pure water were mostly higher than those in cooked with tap water. Based on the fact that the perchlorate was not contained in pure water, perchlorate concentrations in some food items were considered higher at some locations.

These observations suggested that chlorate in tap water used for cooking affected the food concentration. It was not clear how each of these groups were prepared or cooked, such as boiling or baking. The way of cooking varies among food groups and location, and they should have been cooked in a 'common' way at each location. Among these food groups, it seems rice is Japan's most staple food and should have prepared with water at home as a rice cooker is found in most Japanese households. Therefore chlorate concentration in rice and that in tap water was compared. Among the 6 locations

Table 3
Chlorate and perchlorate in tap water in the study areas.

	A*	B*	C	D	E	F	G*	H	I*	J
	($\mu\text{g/L}$)									
Chlorate	130	59	45	83	80	130	34	140	56	67
Perchlorate	0.05	0.21	0.16	2.2	0.13	0.18	0.03	0.23	0.13	0.12

* Pure water was used for food preparation all of which contained no chlorate (<0.05 $\mu\text{g/L}$) or perchlorate (<0.01 $\mu\text{g/L}$).

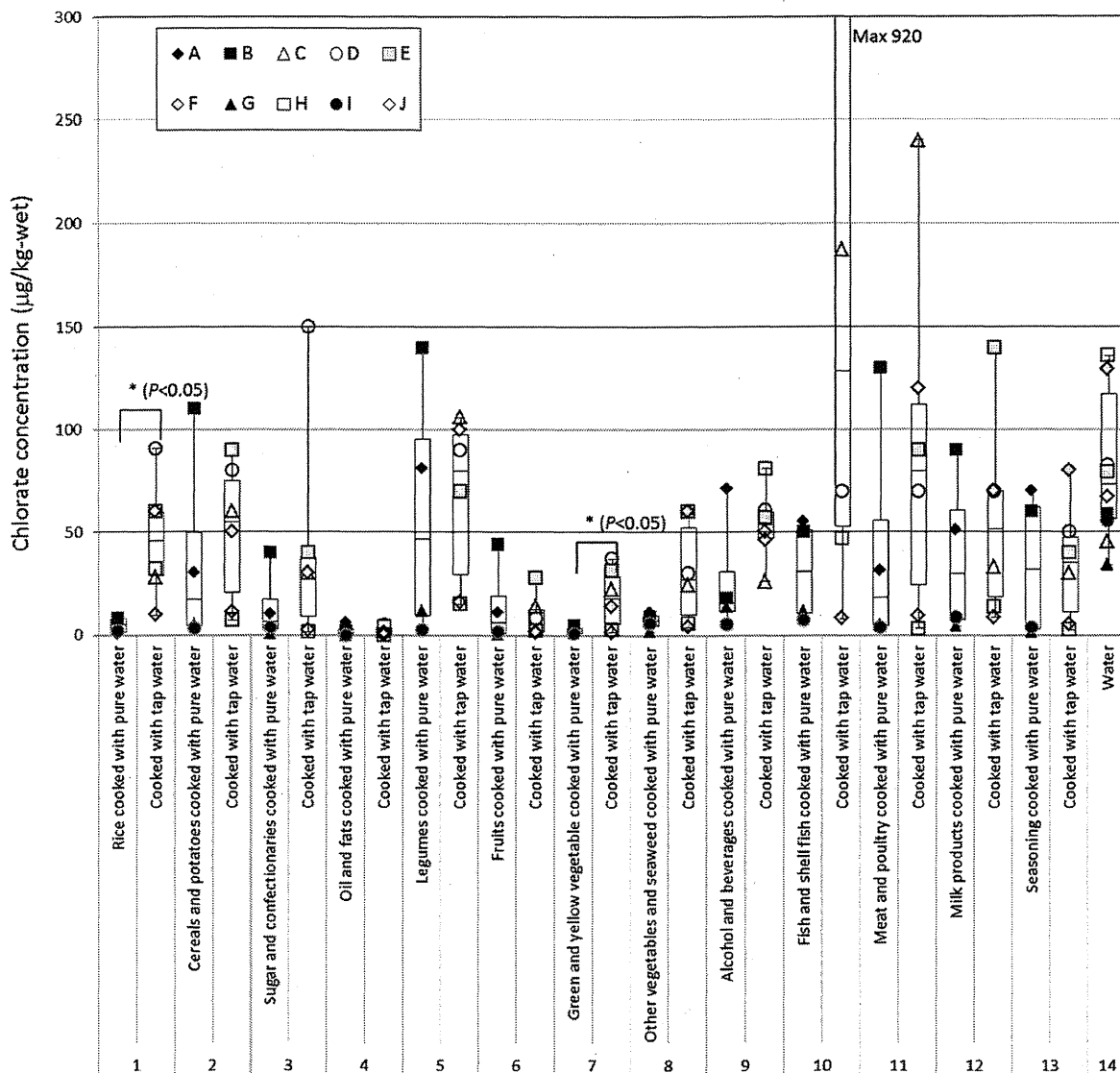


Fig. 2. Chlorate concentrations of each food group. Boxes represent 25%–75% quartile, the center bars represent medians, and whiskers are maximum and minimum values; * indicates significant difference between samples cooked with pure water and those with tap water at $P < 0.05$.

where tap water was used for cooking, the chlorate concentration in rice increased/decreased in proportion to that in tap water (Fig. 4). Chlorate in cooked rice correlated with cooked with tap water ($r^2 = 0.498$, $n = 6$).

3.4. Rice cooking test

Rice cooking test was separately conducted in the lab using ultrapure water and tap water. The levels of chlorate and perchlorate were lower in rice cooked with pure water than with tap water. Neither chlorate nor perchlorate was detected in rice samples cooked with pure water. Chlorate concentration in rice cooked with tap water with a content of 93.4 µg/L was 55.6 µg/kg, where rice contains 71% water. This value was close to the estimated chlorate concentration in rice of 57.1 µg/kg calculated from the tap water concentration. The same relationship was found for perchlorate, where the concentration was 2.3 µg/kg in cooked rice and the estimated concentration was 2.0 µg/kg. The above observations indicated that cooking food with tap water directly affects the levels of chlorate and perchlorate

intake from food, because neither is present in rice but both are stable in water and non-volatile.

3.5. Daily intake of chlorate and perchlorate from food and water

Table 4 shows the average daily intakes of chlorate and perchlorate from food by multiplying the measured concentrations of the two compounds by the average daily intake of each food group based on the national nutrition survey (Fig. 1). The amount of water intake was not included in the above national survey, so the level is represented as 2 L based on normal assumptions used in establishing drinking water quality standards. Although this amount was presumed to include water used for cooking, it was used because no data are currently available. Authors are now conducting a national survey on water intake and it is tentatively elucidated 2 L can be considered to err on the side of safety for majority of population (in preparation).

The results indicated that the sum of daily chlorate intake from food (without water) at each location was within the range of 47–84 µg/day

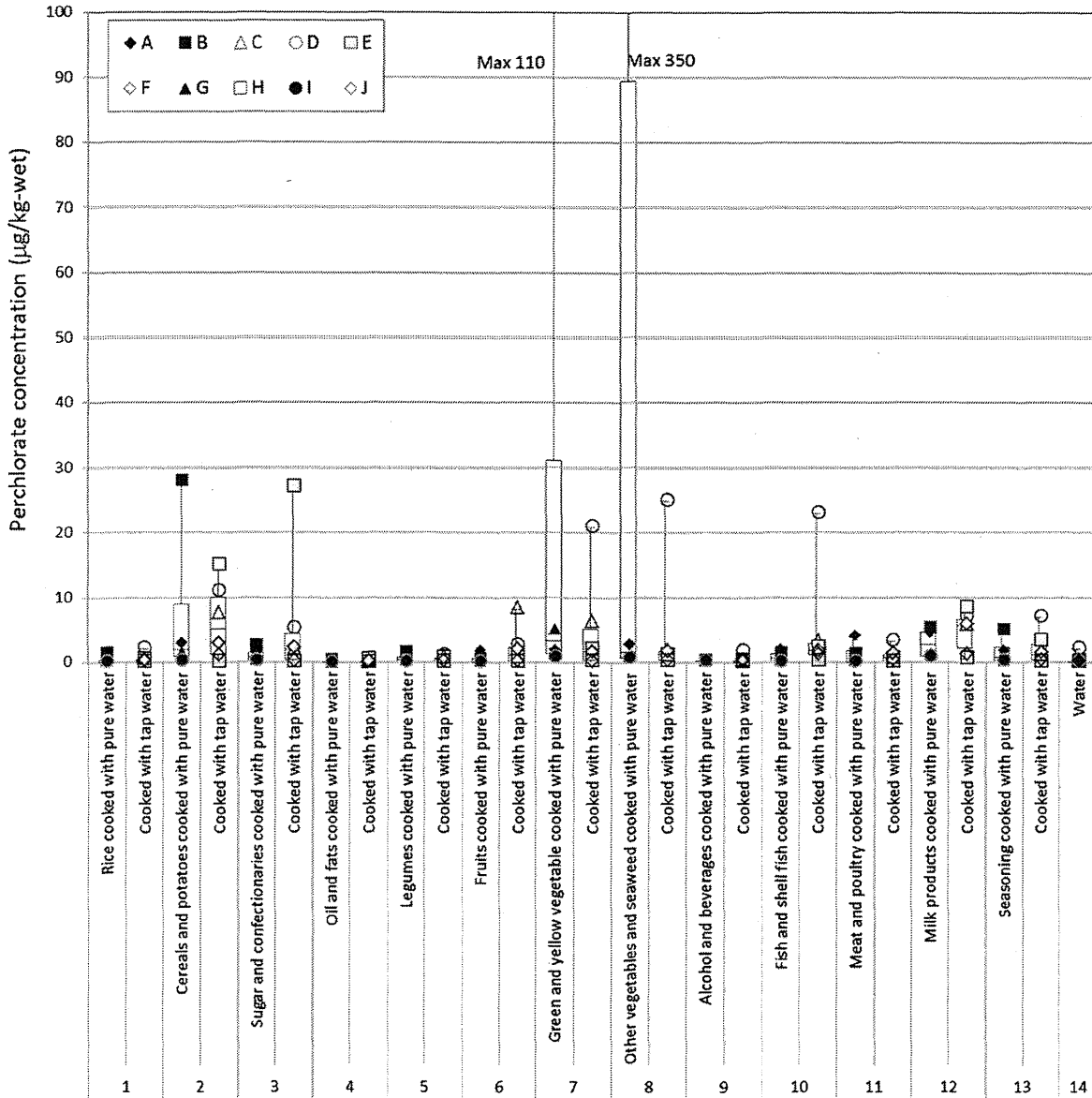


Fig. 3. Perchlorate concentrations of each food group. Boxes represent 25%–75% quartile, the center bars represent medians, and whiskers are maximum and minimum values.

for locations using pure water for cooking, and 102–214 µg/day for locations using tap water for cooking. The results of daily intake at each location were shown in Fig. S1. Among the food groups in average,

rice (group 1), cereals and potatoes (group 2), alcohol and beverages (group 9), fish and shellfish (group 10), and meat and poultry groups (group 11) had high levels of intake.

For rice, the amount of chlorate intake was higher in locations C, D, E, F, H, and J (9.6–32 µg/day) compared to other locations, which was considered to be because tap water was used to cook rice at these locations. In the cereals and potatoes group (group 2), the chlorate intake ranged from 3.2 to 19 µg/day, while in the fish and seafood group (group 10) the intake ranged from 4.1 to 81 µg/day, and that in the meat poultry group (group 11) ranged from 3.4 to 29 µg/day. These observations indicated differences in chlorate intake depending on location, but not on water used for cooking as a result of statistical analysis (Fig. 2). The sugar and confectioneries group (group 3) and legumes group (group 5) showed relatively high measured concentrations, but intake levels were not markedly high compared to other food groups when measured concentrations were multiplied by the daily intake. The preferred beverage group – alcohol and beverages (group 9) – showed relatively high intake values at all locations ranging from 5.3 to 81 µg/day.

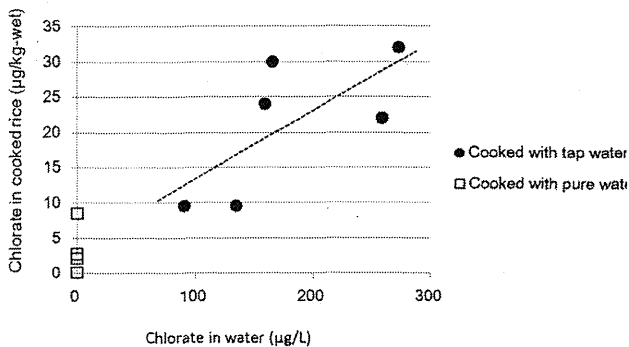


Fig. 4. Relationship of chlorate concentration in tap water to that in cooked rice. Chlorate in cooked rice had correlation with cooked with tap water ($r^2 = 0.498, n = 6$).

Table 4

Daily intakes of chlorate and perchlorate from 13 food groups and water.

Food groups	All locations			Cooked with pure water			Cooked with tap water		
	Average	Min	Max	Average	Min	Max	Average	Min	Max
<i>(a) Chlorate ($\mu\text{g}/\text{day}$)</i>									
1 Rice	14.1	0.1	32.0	3.4	0.1	8.5	21.2	9.6	32.0
2 Cereals and potato	9.7	3.2	19.0	7.9	3.2	19.0	10.9	7.2	15.0
3 Sugar and confectionaries	1.8	0.3	4.8	1.5	0.3	3.7	2.0	0.9	4.8
4 Oil and fats	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1
5 Legumes	8.0	2.4	16.0	6.8	2.4	12.0	8.8	4.7	16.0
6 Fruits	1.7	0.2	5.0	2.0	0.2	5.0	1.6	0.2	3.4
7 Green and yellow vegetables	1.7	0.3	4.0	0.6	0.3	1.2	2.4	1.2	4.0
8 Other vegetables and seaweed	5.7	1.7	14.0	2.9	1.7	5.7	7.5	3.9	14.0
9 Alcohol and beverages	31.1	5.3	81.0	18.6	5.3	45.0	39.5	16.0	81.0
10 Fish and shell fish	22.1	4.1	81.0	6.9	4.1	12.0	32.2	5.9	81.0
11 Meat and poultry	9.8	3.4	29.0	6.6	3.5	14.0	12.0	3.4	29.0
12 Milk products	9.7	4.5	17.0	8.2	4.5	13.0	10.7	4.7	17.0
13 Seasoning	4.3	1.7	7.8	4.1	1.7	6.2	4.5	2.7	7.8
14 Water	163.6	68.0	272.0	139.2	68.0	260.0	179.8	90.4	272.0
Total	283.2	135.2	486.2	208.6	135.2	339.2	333.0	192.8	486.2
Ratio of water (%)	57.8	46.9	76.7	63.9	50.3	76.7	53.1	46.9	57.8
Ratio of water and rice (%)	62.7	51.9	76.7	66.1	56.6	76.7	59.4	51.9	66.9
Water ratio compared to TDI (%)	10.9	4.5	18.1	9.3	4.5	17.3	12.0	6.0	18.1
Water and rice ratio compared to TDI (%)	11.8	5.1	20.3	9.5	5.1	17.3	13.4	6.7	20.3
Total ratio compared to TDI (%)	18.9	9.0	32.4	13.9	9.0	22.6	22.2	12.9	32.4
<i>(b) Perchlorate ($\mu\text{g}/\text{day}$)</i>									
1 Rice	0.2	0.0	0.7	0.2	0.0	0.5	0.2	0.0	0.7
2 Cereals and potato	1.4	0.1	4.8	1.7	0.2	4.8	1.2	0.1	2.5
3 Sugar and confectionaries	0.2	0.0	0.8	0.1	0.0	0.3	0.2	0.1	0.8
4 Oil and fats	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 Legumes	0.1	0.0	0.3	0.1	0.0	0.3	0.0	0.0	0.1
6 Fruits	0.2	0.0	1.0	0.1	0.0	0.2	0.3	0.1	1.0
7 Green and yellow vegetables	1.9	0.0	9.4	3.9	0.2	9.4	0.5	0.0	2.1
8 Other vegetables and seaweed	7.6	0.2	67.0	17.3	0.5	67.0	1.1	0.2	5.2
9 Alcohol and beverages	0.2	0.1	0.9	0.2	0.1	0.3	0.3	0.1	0.9
10 Fish and shell fish	0.4	0.1	1.9	0.2	0.1	0.2	0.6	0.1	1.9
11 Meat and poultry	0.2	0.0	0.5	0.2	0.0	0.5	0.2	0.1	0.4
12 Milk products	0.9	0.6	1.1	0.8	0.6	1.1	0.9	0.6	1.1
13 Seasoning	0.3	0.1	0.7	0.3	0.1	0.4	0.3	0.1	0.7
14 Water	0.7	0.1	4.4	0.2	0.1	0.4	1.0	0.2	4.4
Total	14.1	2.5	83.9	25.2	2.9	83.9	6.8	2.5	19.7
Ratio of water (%)	4.9	0.5	22.3	2.8	0.5	6.8	11.4	4.4	22.3
Ratio of water and rice (%)	6.2	1.0	25.9	4.0	1.0	7.2	14.1	6.5	25.9
Water ratio compared to RfD (%)	2.0	0.2	12.6	0.6	0.2	1.2	2.9	0.7	12.6
Water and rice ratio compared to RfD (%)	2.5	0.5	14.6	1.1	0.5	2.5	3.5	0.8	14.6
Total ratio compared to RfD (%)	40.4	7.2	239.6	71.9	8.4	239.6	19.4	7.2	56.2

Group 9 included alcoholic beverages, bottled teas, juices, and instant coffee and the referred beverages contribute a high percentage of the daily intake. As the consumption of soft drinks has been increasing in recent years (Asami et al., 2009b), surveys of chlorate and perchlorate contents shall be continued for these soft drinks for future studies.

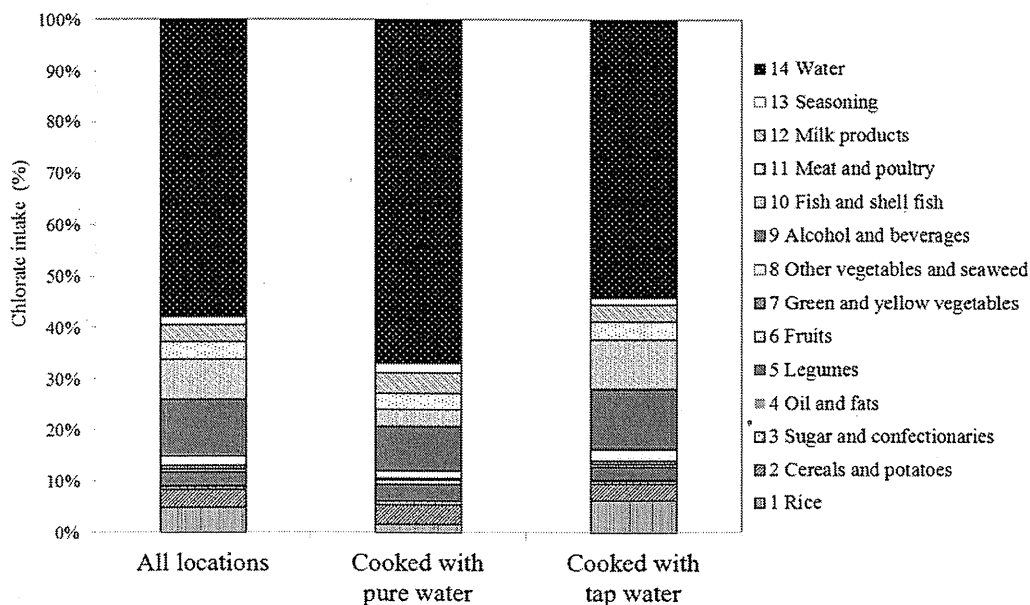
High concentrations of chlorate were detected in some bottled waters for various reasons; e.g., chlorinated water may be used as the raw source to produce the bottled water, or there may be certain factors that cause high concentration of chlorate in the production process, such as antiseptic washing and disinfection.

Fig. 5(a) shows the average proportions of daily chlorate intakes based on data of locations where used tap water for preparation of foods and the daily intake of tap water set as 2 L. As the contribution of water to food showed significant difference between samples cooked with tap water and those with pure water, the average proportions were calculated using data for all locations, tap water and pure water. The daily intake of chlorate ranged from 193 to 490 $\mu\text{g}/\text{day}$ in these locations cooked with tap water and the percentage intake from tap water at each location ranged from 47% to 58% (Table 4). When the amount of water used to cook rice was considered as part of the intake from tap water, the value ranged from 52% to 78%. Besides the contribution of tap water was large, the sum of daily intake of chlorate ranged from 13% to 32% of the TDI proposed by JECFA,

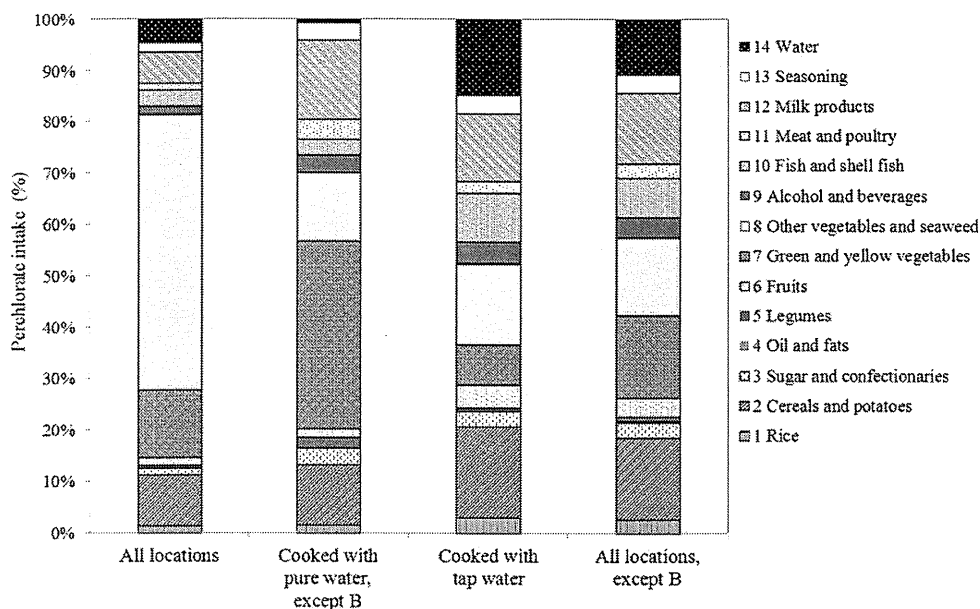
i.e. 1500 $\mu\text{g}/\text{day}$ for an adult with a body weight of 50 kg, and was much lower than the TDI.

Fig. 5(b) shows the proportion of daily perchlorate intakes. The sum of daily intake of perchlorate at each location was within the range of 2.5–20 $\mu\text{g}/\text{day}$ for all location, except one location, B, 84 $\mu\text{g}/\text{day}$, where other vegetables and seaweed showed high concentrations of perchlorate. The ratio of water contribution to the total intake ranged from 0.6% to 22%, while it was 0.5%, when excluding the location B. Because the location B contained extraordinary high sample, average proportion of all locations excluding B was also shown in the figure. Among all food groups, highest intake was observed for the other vegetables and seaweed group. Compared to chlorate, the daily intake of perchlorate varied markedly depending on location but there was no direct relationship with water.

As shown in Table 5, the perchlorate intake from food was specifically high at location B, and the perchlorate intake from tap water was high at location D compared to other locations. The perchlorate intake at location B was relatively high, because the food samples, especially in "other vegetables and seaweed". As location B hasn't been known as a contaminated area, to the author's knowledge and the samples were analyzed in composite for each group, we couldn't identify in this study what and how food was contaminated. For location D, it was suspected vegetables grow in this area might have perchlorate contamination, because leafy vegetables in this



(a) Proportion of daily chlorate intake



(b) Proportion of daily perchlorate intake

Fig. 5. Average proportion of daily chlorate (a) and perchlorate (b) intakes (%) from each food group. (a) Proportion of daily chlorate intake. (b) Proportion of daily perchlorate intake.

area were previously reported to contain relatively high concentration of perchlorate (Takatsuki et al., 2009). Among all locations, the daily intake from food was higher than that from tap water for perchlorate. At 9 of the locations, except the location B, the daily intake was less than 35 $\mu\text{g}/\text{day}$, the RfD (EPA, 2009b), i.e. for adults with a body weight of 50 kg; however, it exceeded this value by twofold at location B. Although the total perchlorate intake was 17% of the PMTDI value prepared by JECFA (2011), the total intake in this study (2.5–84 $\mu\text{g}/\text{day}$) is higher than the estimated dietary exposure mentioned in JECFA (2011), i.e. 0.7 $\mu\text{g}/\text{kg}/\text{day}$ (highest) and 0.1 $\mu\text{g}/\text{kg}/\text{day}$

(mean), or 35 $\mu\text{g}/\text{day}$ (highest) and 5 $\mu\text{g}/\text{day}$ (mean) assuming the body weight as 50 kg.

3.6. Chlorate and perchlorate concentrations in baby formula and water

Eight samples of baby formula were purchased from 4 major manufacturers for testing. Of the 8 samples, only VII was a liquid electrolyte. Measured concentrations in each sample other than the sample VII, were converted to the concentration in 1 g of dry weight of the sample powder. The measured concentration of chlorate was

Table 5

Chlorate and perchlorate daily intake from 13 food groups.

Food groups	A*	B*	C	D	E	F	G*	H	I*	J
1) Chlorate ($\mu\text{g}/\text{day}$)										
1 Rice	0.1	2.7	9.6	30.0	24.0	22.0	8.5	32.0	2.1	9.6
2 Cereals and potatoes	4.4	19.0	10.0	14.0	15.0	8.3	5.1	7.2	3.2	11.0
3 Sugar and confectionaries	0.3	1.3	1.1	4.8	1.2	0.9	0.7	1.5	3.7	2.3
4 Oil and fats	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1
5 Legumes	5.2	7.5	6.1	5.4	4.7	5.8	12.0	15.0	2.4	16.0
6 Fruits	1.2	5.0	1.7	1.0	3.4	0.2	0.2	2.2	1.5	1.0
7 Green and yellow vegetables	0.3	0.4	2.0	4.0	3.0	1.2	1.2	2.6	0.6	1.3
8 Other vegetables and seaweed	2.1	1.7	4.4	6.3	14.0	11.0	1.9	5.6	5.7	3.9
9 Alcohols and beverages	45.0	9.9	16.0	33.0	30.0	27.0	14.0	81.0	5.3	50.0
10 Fish and shell fish	4.4	4.1	15.0	5.9	36.0	81.0	12.0	47.0	7.2	8.2
11 Meat and poultry	3.5	14.0	29.0	7.8	9.4	13.0	5.4	3.4	3.6	9.2
12 Milk products	6.4	13.0	4.7	9.6	17.0	10.0	4.5	14.0	8.7	8.7
13 Seasoning	6.2	5.0	2.8	4.7	3.5	7.8	1.7	2.7	3.6	5.2
14 Water	2260.0	117	90.4	1165.4	1159.0	2258.0	68.0	272.0	111.2	134.2
Total	3339.2	2201.3	1192.8	2292.0	3320.2	4446.2	1135.2	486.2	158.8	260.6
2) Perchlorate ($\mu\text{g}/\text{day}$)										
1 Rice	0.064	0.45	0.17	0.70	0.16	0.075	0.16	0.09	0.018	0.036
2 Cereals and potatoes	0.41	4.8	1.3	1.9	2.5	0.46	1.3	0.07	0.24	0.93
3 Sugar and confectionaries	0.039	0.080	0.067	0.17	0.83	0.067	0.32	0.06	0.14	0.05
4 Oil and fats	0.002	0.003	0.002	0.001	0.004	0.001	0.003	0.0009	0.002	0.0027
5 Legumes	0.045	0.080	0.04	0.066	0.047	0.035	0.28	0.04	0.043	0.048
6 Fruits	0.17	0.057	1.0	0.33	0.084	0.22	0.05	0.06	0.043	0.067
7 Green and yellow vegetables	0.17	9.4	0.58	2.1	0.19	0.13	5.1	0.15	0.79	0.036
8 Other vegetables and seaweed	0.51	67	0.33	5.2	0.26	0.29	0.91	0.19	0.78	0.16
9 Alcohols and beverages	0.13	0.15	0.12	0.92	0.069	0.21	0.17	0.37	0.25	0.1
10 Fish and shell fish	0.14	0.11	0.26	1.9	0.21	0.14	0.24	0.21	0.11	1.1
11 Meat and poultry	0.45	0.13	0.16	0.37	0.063	0.13	0.17	0.1	0.048	0.15
12 Milk products	0.56	0.76	0.83	0.96	1.0	0.83	1.1	0.63	0.9	1.1
13 Seasoning	0.14	0.42	0.10	0.67	0.29	0.13	0.23	0.08	0.22	0.23
14 Water	0.10	0.42	0.32	4.40	0.26	0.36	0.06	0.46	0.26	0.24
Total	2.9	83.9	5.3	19.7	6.0	3.1	10.1	2.5	3.8	4.2

* Pure water was used for food preparation all of which contained no chlorate ($<0.05 \mu\text{g}/\text{L}$) or perchlorate ($<0.01 \mu\text{g}/\text{L}$).

highest in VIII (1400 ng/g dry weight) followed by VI (810 ng/g dry weight). The daily intake was calculated by multiplying the measured concentration by the amount of formula per feeding, and the daily intake in 1 L of formula was calculated. The daily intake of chlorate was within the range of 8.4–196 $\mu\text{g}/\text{day}$ for each sample. The measured concentration of perchlorate was high in I (22 ng/g dry weight) followed by II and VIII (each 10 ng/g dry weight). The daily intake of perchlorate was calculated in the same way as for chlorate, and ranged from 0.02 to 2.3 $\mu\text{g}/\text{day}$.

Using the measured concentration of TDS sample tap water to make the formula, the amounts of chlorate and perchlorate intake were as shown in Tables 6 and 7. The average daily intake of chlorate was 147 (42–332) $\mu\text{g}/\text{day}$ for an ingestion volume of 1 L/day if prepared with tap water. In comparison to the TDI value of 150 $\mu\text{g}/\text{day}$ for infants and toddlers calculated weighing 5 kg, some samples had higher daily intake levels.

The average daily intake of perchlorate was 1.1 $\mu\text{g}/\text{day}$ ranged from 0.05 to 4.5 $\mu\text{g}/\text{day}$ also for an ingestion volume of 1 L/day if

prepared with tap water. There was no correlation between samples with high levels of chlorate intake and those with high perchlorate intake. The amounts were almost lower compared to the RfD value of 3.5 $\mu\text{g}/\text{day}$ for infants and toddlers weighing 5 kg, but if tap water from location D with high intake was used to prepare the formula, total intake exceeded the RfD value (some samples were for toddlers weighing over 8.5 kg, and the daily intake also changes as infants grow), although it is sufficiently below PMTDI.

Using the combination of the mean, minimum and maximum concentration of chlorate and perchlorate in tap water examined in this study, the contribution of water for baby intake were calculated as 56% (15–94) for chlorate and 31% (1.3–99) for perchlorate. The range greatly depends upon the concentration of the sample baby formula and the concentration of water. It is difficult to conclude the contribution of water, because the number of sample baby formula and water was limited in this study, but the intake greatly depends on concentration of chlorate and perchlorate in water.

4. Discussion

The present study was performed using samples from the total diet study to evaluate the concentrations of chlorate and perchlorate

Table 6

Chlorate and perchlorate in baby formula.

		Intake ($\mu\text{g}/1 \text{ L}$)	
		Chlorate	Perchlorate
I	WA	33.6	2.3
II	WA	15.6	0.98
III	MO	50.7	0.57
IV	MO	88.2	0.42
V	ME	63.8	0.04
VI	BS (liquid)	113.4	0.60
VII	ME	8.4	0.02
VIII	ME	196	1.05
	Av.	71.2	0.75

Table 7

Chlorate and perchlorate in baby formula.

	Chlorate intake ($\mu\text{g}/\text{day}$ as 1 L)			Perchlorate intake ($\mu\text{g}/\text{day}$ as 1 L)		
	Av.	Min.	Max.	Av.	Min.	Max.
Baby formula*	65	8	196	0.77	0.02	2.3
Water	82	34	136	0.34	0.03	2.2
Total	147	42	332	1.11	0.05	4.5

* Average is calculated excluding sample VI.

in food and tap water from 10 locations and calculate the total levels of intake. In most cases, the concentrations of chlorate and perchlorate from the various food groups were higher than the lower limit of quantification.

For the chlorate ion, the percentage contribution of tap water to total intake was as high as 47%–77%, and the level of intake in some food samples probably included chlorate originating from tap water. For example, chlorate in the tap water used for boiling and simmering of rice, potatoes, and noodles may have affected the amount of chlorate in food. The sum of the intakes from food and tap water for adults was less than approximately 32% of TDI.

A survey of the total intake of perchlorate and iodine was performed by the FDA in the USA, and the presence of perchlorate was confirmed in a wide variety of foods, as also demonstrated in the present intake survey (Murray et al., 2008). In the USA, the main sources of perchlorate intake were milk and dairy products, as well as vegetables. The intake from vegetables was relatively high in both countries. The survey in the USA classified the subjects into 14 categories according to age group and gender, and the amounts of intake for each category were compared. The intake of perchlorate for a 2-year-old was approximately 50% of the RfD value, and it seemed that younger age groups had higher intake ratios. As the study in the USA indicated that the average concentration of perchlorate in breast milk is 5 times higher than that in cow's milk (Kirk et al., 2005), it may be necessary to investigate the intake of perchlorate from breast milk in future studies.

In the present study, the measured concentration of perchlorate in dairy products was relatively high; however, when converted to the amount of intake, the levels were not high. Vegetables, seaweed, cereals and potatoes showed specifically high perchlorate levels at certain locations. Location D, which had high levels of perchlorate intake from tap water, had relatively high total intake corresponding to 56% of the RfD, and this was considerably higher than at the other locations. There was no obvious relationship whether tap water was used to prepare food, but further studies are required regarding the concentrations in both tap water and food from the viewpoint of total intake.

The perchlorate intake from "other vegetables and seaweed" at location B was relatively high. Because the food samples were analyzed in composite for each group, this study was not able to further reveal which individual food(s) were actually of high perchlorate concentration. But it should be noted seaweed, which is generally known to contain high perchlorate and iodine (Martinelango et al., 2006, for example), was included in location B and other locations. Japanese diet normally includes a high proportion of seafood especially seaweeds, such as Wakame (*Undaria pinnatifida*). Although it is known that perchlorate interferes with iodine uptake, adverse health effect from iodine deficiency might not be occurred if iodine intake from foods is high and if perchlorate intake is relatively low. Because iodine uptake from Japanese normal diet is relatively higher than other countries (Kikuchi et al., 2008), so evaluation of both perchlorate and iodine intake should be conducted in future when considering perchlorate and iodine uptake.

To our knowledge, no detailed data are available regarding water consumption for drinking, cooking use, both only boiling and making soup, ice, and other uses for ingestion. Therefore, we are currently engaged in a national survey of water consumption.

The results of the present study revealed the total intake, as well as the percentage intake from water, of chlorate and perchlorate in Japan. With the studied areas being selected from across Japan, the percentage contribution of chlorate and perchlorate from water to the total amount of intake was first proved. The contribution of water used for preparation of food was also found as an important factor in the present study. The contribution of total daily intake of chlorate originated from water including water used for preparation of food in this study was lower than the current TDI allocation of chlorate to water (i.e., 80%) and also the total intake of chlorate was much

the less than the TDI. The contribution of perchlorate and total perchlorate intake ranged widely, therefore it needs further studies when considering the current allocation (i.e., 10%).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.05.097>.

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