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Haloacetonitriles vs. Regulated Haloacetic Acids: Are Nitrogen-Containing DBPs More Toxic?

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Haloacetonitriles (HANs) are toxic nitrogenous drinking water disinfection byproducts (N-DBPs) and are observed with chlorine, chloramine, or chlorine dioxide disinfection. Using microplate-based Chinese hamster ovary (CHO) cell assays for chronic cytotoxicity and acute genotoxicity, we analyzed 7 HANs: iodoacetonitrile (IAN), bromoacetonitrile (BAN), dibromoacetonitrile (DBAN), bromochloroacetonitrile (BCAN), chloroacetonitrile (CAN), dichloroacetonitrile (DCAN), and trichloroacetonitrile (TCAN). The cytotoxic potency (%C1/2 values) ranged from 2.8 μM (DBAN) to 0.16 mM (TCAN), with a descending rank order of DBAN > IAN ≈ BAN > BCAN > DCAN > CAN > TCAN. HANs induced acute genomic DNA damage; the single cell gel electrophoresis (SCGE) genotoxicity potency ranged from 37 μM (IAN) to 2.7 mM (DCAN). The rank order of declining genotoxicity was IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN. The accompanying structure−activity analysis of these HANs was in general agreement with the genotoxicity rank order. These data were incorporated into our growing quantitative comparative DBP cytotoxicity and genotoxicity databases. As a chemical class, the HANs are more toxic than regulated carbon-based DBPs, such as the haloacetic acids. The toxicity of N-DBPs may become a health concern because of the increased use of alternative disinfectants, such as chloramines, which may enhance the formation of N-DBPs, including HANs.

Introduction

Drinking water disinfection byproducts (DBPs) are formed unintentionally when a disinfectant reacts with natural organic matter and/or bromide/iodide that are present in the raw water (*1*). Although the acute benefits of drinking water disinfection are acknowledged, the health risks due to long-term DBP exposure are not well understood (*2*, *3*). Epidemiology studies provide moderate evidence for associations of DBPs with adverse pregnancy outcomes (*4*), and some DBPs are mutagens, carcinogens, teratogens, or developmental toxicants (*5*−*11*).

The Safe Drinking Water Act authorized the U.S. Environmental Protection Agency (EPA) to enforce drinking water regulations. In 2006, the Stage 2 Disinfectants/Disinfection Byproducts Rule was promulgated (*12*). In order to comply with this Rule, some utilities are switching from chlorine to chloramine disinfection, which may increase nitrogenous disinfection byproducts (N-DBPs). N-DBPs were cited as research priorities by the U.S. EPA (*13*, *14*). The haloacetonitriles (HANs) are an unregulated class of N-DBPs. From previous studies, specific HANs were determined to be toxic including bromoacetonitrile (BAN), chloroacetonitrile (CAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), and bromochloroacetonitrile (BCAN).

DCAN, BCAN, CAN, and TCAN were mutagenic in *Salmonella typhimurium* (*5*, *9*). HANs directly induced sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells (*5*). The rank order of the direct acting genotoxic activity was DBAN > BCAN > TCAN > DCAN > CAN, where brominated and di- and tri-halogenated HANs were more toxic. HANs produced DNA strand breaks in human lymphoblastic cells, with TCAN the most potent (*7*, *8*). Although HANs possessed direct acting alkylating activity, there was no correlation between DNA alkylation potential and their ability to produce DNA strand breaks (*8*). Using the single cell gel electrophoresis (SCGE) assay with HeLa S3 cells, CAN, BAN, DCAN, DBAN, and TCAN were positive; more halogenated HANs and brominated HANs caused greater amounts of damage (*9*). HANs directly interact with calf thymus DNA in the order of BAN > CAN > DCAN > TCAN. A DNA adduct was identified as 7-(cyanomethyl) guanine (*15*), and HANs bound to a nucleophilic trapping agent and formed a covalent bond to polyadenylic acid (*7*).

In vivo studies in rats demonstrated differential metabolism and excretion, with a rank order of CAN > BCAN > DCAN > DBAN > > TCAN (*8*). The HANs initiated skin tumors in mice with a rank order of DBAN > BCAN > CAN (*5*).

While broad comparisons of the relative toxicities of the HANs are possible from a literature review, it is impossible to rigorously and quantitatively analyze these N-DBPs and compare them with other classes of DBPs. The objective of our research was to quantitatively analyze the direct chronic cytotoxicity and the acute genomic DNA damaging capacity of seven HANs with CHO cell assays that have been extensively used in DBP research (*16*−*20*). This study presents an analysis of iodoacetonitrile (IAN), BAN, CAN, DBAN, DCAN, TCAN, and BCAN, and relates their mammalian cell cytotoxicity and genotoxicity with their structure function activity.

Materials and Methods

Reagents. General reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma Chemical Co. (St. Louis, MO). Media and fetal bovine serum (FBS) were purchased from Hyclone Laboratories (Logan, UT). The HANs were dissolved in dimethyl sulfoxide (DMSO) and stored at −22 °C in sterile glass vials. Sources and purities of the HANs are presented in Supporting Information Table 1.

Chinese Hamster Ovary Cells. Chinese hamster ovary (CHO) cells, line AS52, clone 11-4-8 were used (*21*). We employed this cell line in previous DBP toxicity studies (*16*−

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TABLE 1. Summary Comparison of the CHO Cell Chronic Cytotoxicity and Acute Genotoxicity of the Haloacetonitriles

chemical	lowest toxic concn. (M)[a]	%C1/2 (M)[b]	tox rank order[c]	lowest genotox. concn. (M)[d]	genotox. potency (M)[e]	GP rank order[f]
iodoacetonitrile	1.0×10^{-7}	3.30×10^{-6}	2	3.0×10^{-5}	3.71×10^{-5}	1
bromoacetonitrile	1.0×10^{-6}	3.21×10^{-6}	2	4.0×10^{-5}	3.85×10^{-5}	2
dibromoacetonitrile	1.0×10^{-6}	2.85×10^{-6}	1	3.0×10^{-5}	4.71×10^{-5}	2
bromochloroacetonitrile	7.0×10^{-6}	8.46×10^{-6}	4	2.5×10^{-4}	3.24×10^{-4}	4
chloroacetonitrile	5.0×10^{-5}	6.83×10^{-5}	6	2.5×10^{-4}	6.01×10^{-4}	5
dichloroacetonitrile	1.0×10^{-5}	5.73×10^{-5}	5	2.4×10^{-3}	2.75×10^{-3}	7
trichloroacetonitrile	2.5×10^{-5}	1.60×10^{-4}	7	1.0×10^{-3}	1.01×10^{-3}	6

[a] The lowest concentration of a specific haloacetonitrile that induced a significant cytotoxic response as compared to the negative control. [b] The %C1/2 value is the chemical concentration that induced a 50% reduction of the cell density as compared to the negative control [c] The cytotoxicity rank order for BAN, DBAN, and IAN was determined after statistical analysis; DBAN was significantly more cytotoxic than BAN or IAN. The rank order was based on the %C1/2 values. [d] The lowest concentration of a specific haloacetonitrile that induced a significant genotoxic response as compared to the negative control. [e] The SCGE genotoxic potency was the calculated concentration of the haloacetonitrile at the midpoint of the tail moment concentration−response curve with an acute cellular viability of >70%. [f] The SCGE genotoxic potency rank order for BAN, DBAN, and IAN was determined after statistical analysis; IAN was significantly more genotoxic than BAN or DBAN. The rank order was based on the genotoxic potency value.

20, *22*, *23*). The CHO cells were maintained in Ham's F12 medium containing 5% FBS at 37 °C in a humidified atmosphere of 5% CO_2.

CHO Cell Chronic Cytotoxicity Assay. This assay measures the reduction in cell density as a function of DBP concentration over a period of approximately 3 cell divisions (72 h). This assay has been calibrated, and the procedures have been published (*16*−*20*, *22*, *23*). A detailed procedure is presented in Supporting Information. Within each experiment, 10 HAN concentrations were analyzed and for each HAN concentration 8 replicate microplate wells were analyzed. Each experiment was repeated 2 or 3 times. Thus, the cytotoxicity of each HAN concentration was evaluated with between 16 and 24 individual cell culture responses.

Single Cell Gel Electrophoresis Assay. Single cell gel electrophoresis (SCGE) quantitatively measures the level of genomic DNA damage induced in individual nuclei of treated cells. This assay has been calibrated, and the procedures have been published (*16*−*20*, *22*, *23*). A detailed procedure is presented in the Supporting Information. CHO cells were exposed to a specific HAN for 4 h at 37 °C, 5% CO_2. Each experiment consisted of a negative control, a positive control (3.8 mM ethylmethanesulfonate), and 9 concentrations of a specific HAN. The concentration range was determined by the acute cytotoxicity of the test agent. In general, each HAN concentration was evaluated with 2 microgels, and 25 randomly chosen nuclei were analyzed per microgel. The experiments were repeated a minimum of 3 times which generated an analysis of 6 microgels per HAN concentration.

Structure−**Activity Relationship.** A quantitative, mechanism-based structure−activity relationship (SAR) analysis was used to interpret the CHO cell cytotoxicity and genotoxicity data of the HANs. We did not use a SAR model. The data were primarily compared to the relative S_N2 reactivity values obtained from the literature.

Results

Seven HANs were analyzed for their mammalian cell chronic cytotoxicity and genotoxicity (Table 1). All seven were cytotoxic over a concentration range that exceeded 3 orders of magnitude. Likewise, all of the HANs induced genomic DNA damage over a concentration range that exceeded 3 orders of magnitude.

Mammalian Cell Chronic Cytotoxicity. Data from individual experiments were normalized to the averaged percent of the negative control; these data were plotted as a concentration−response curve (Supporting Information Figure 1). A one-way analysis of variance test was conducted with the normalized data representing each microplate well. If a significant F value of $P \leq 0.05$ was obtained, a Holm−Sidak multiple comparison analysis was conducted. The lowest effective concentration and the ANOVA result for each HAN are presented in Supporting Information Table 2. The power of the test statistic (β) was ≥0.8 at $\alpha = 0.05$. Each concentration was from data derived from between 8 and 24 replicate wells. Regression analysis was conducted, and the coefficient of determination (R^2) was generated, from which the %C1/2 value for each concentration−response curve was calculated (Supporting Information Table 2). The %C1/2 value is the concentration of the HAN that induced a cell density of 50%, as compared to the concurrent negative control. There was no statistically significant difference between the %C1/2 values of IAN and BAN. The rank order for cytotoxicity based on the %C1/2 values was DBAN > BAN ≈ IAN > BCAN > DCAN > CAN > TCAN. The concentration−response curves are plotted in Figure 1.

Induction of Genomic DNA Damage. SCGE tail moment values are not normally distributed, thus the median tail moment value for each microgel was determined and averaged. Averaged median values express a normal distribution (*24*) and were used with an ANOVA test. If a significant F value of $P \leq 0.05$ was obtained, a Holm−Sidak multiple comparison analysis was conducted ($\beta \geq 0.8$ at $\alpha = 0.05$) (Supporting Information Table 3). The lowest significant genotoxic concentrations for the HANs are listed in Table 1 and in Supporting Information Table 3. Acute cytotoxicity was determined, and a concentration−response curve was generated within the range that contained ≥70% viable cells. The data were plotted and regression analysis was used to fit the curve. The SCGE genotoxic potency was calculated at the midpoint of the concentration−response curve (Supporting Information Figure 2). The rank order of the genotoxic potency was IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN (Table 1); concentration−response curves are presented in Figure 2.

Discussion

HANs are a class of unregulated nitrogenous DBPs that may be generated with chloramine, chlorine, or chlorine dioxide treatment. We analyzed 7 HANs for their mammalian cell chronic cytotoxicity and acute genotoxicity, and incorporated the data into a quantitative DBP toxicity database. As a class, under the conditions of these *in vitro* assays, the HANs are more toxic than regulated DBPs, such as the haloacetic acids.

Occurrence of Haloacetonitriles in Drinking Water. HANs have been measured in several occurrence studies

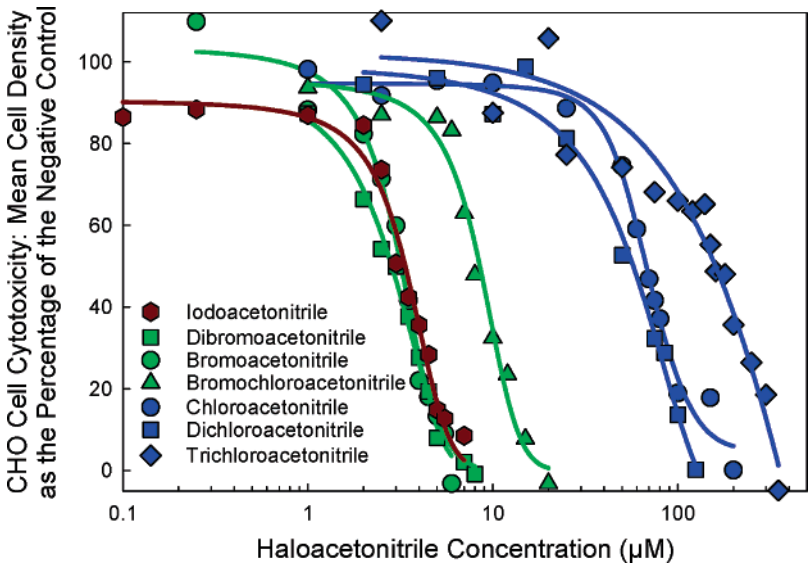

FIGURE 1. CHO cell chronic cytotoxicity concentration–response curves for the seven HANs analyzed in this study.

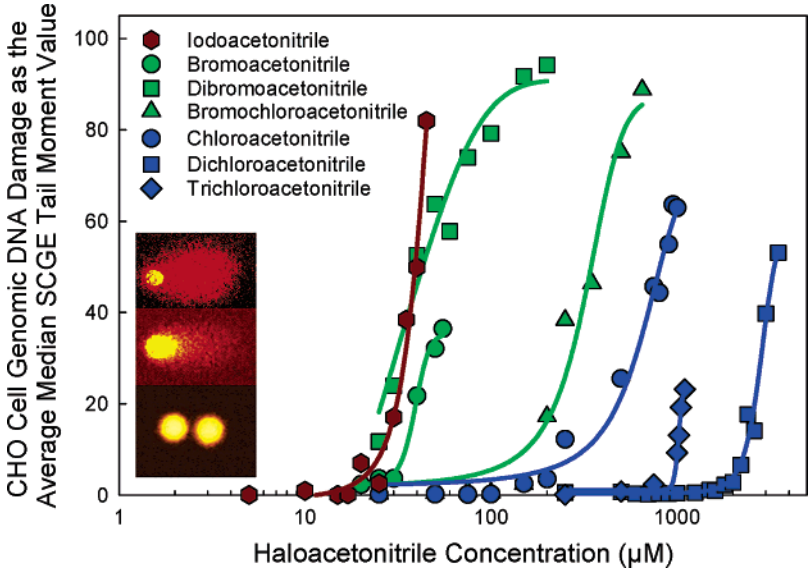

FIGURE 2. SCGE genotoxicity concentration–response curves for the seven HANs analyzed in this study. The images demonstrate the level of DNA damage in representative electrophoresed nuclei. The bottom panel illustrates nuclei from control cells, the middle panel shows a nucleus with a SCGE tail moment of ~30, and the upper panel shows a nucleus with a SCGE tail moment of ~60.

(*14*, *25*–*27*). CAN, BCAN, DBAN, and TCAN (HAN4) are the most commonly measured HAN species, and have been included in a survey of 35 U.S. water utilities (*26*), a survey of 53 Canadian water utilities (*25*), and the U.S. EPA's Information Collection Rule (ICR) effort, which involved 500 large drinking water plants in the United States (*27*). In the ICR, HANs ranged from <0.5 to 41.0 μg/L (HAN4), with a median of 2.7 μg/L for HAN4, and were generally 12% of the levels of the four regulated trihalomethanes (THM4, median of 23.3 μg/L) (*27*). These HANs were formed at plants that used chlorine, chloramine, chlorine dioxide, or ozone disinfection; plants using chloramines (with and without chlorine) had the highest levels in their finished drinking water. Higher HAN levels were found in distribution system waters treated with post-chloramination vs free chlorine. However, the increased HAN levels with chloramination may be a result of higher total organic carbon (TOC) levels in their source waters (*27*). HANs were frequently found in drinking waters from the Canadian survey, with DCAN in 97% of all samples (*25*). Although HANs are not regulated in the United States, the World Health Organization published a guideline of 70 μg/L for DBAN and a provisional guideline of 20 μg/L for DCAN (*28*).

Several other HANs were detected in a recent nationwide DBP occurrence study (*14*, *29*). These included BAN, bromodichloro-, dibromochloro-, and tribromoacetonitrile, plus the four ICR HANs listed above. Total HAN levels reached a maximum of 14 μg/L and were approximately 10% of the THM4 levels, although a maximum of 25% was observed. When bromide is present in the source waters, more brominated species were formed. This shift in speciation was observed in another study of high bromide waters in Israel (*22*), which also provided evidence that chlorine dioxide disinfection can form HANs (DBAN), as well as a new bromonitrile species (3-bromopropanenitrile).

Comparison of the Relative Chemical and Biological Activities of the Haloacetonitriles. A number of comparative HAN studies are summarized in Table 2. The data are normalized to the response expressed by CAN (*7*). Using a Pearson Product Moment Multiple Correlation statistic, we compared the relative activities of 6 HANs for metabolism, alkylation potential, DNA strand breaks, genotoxicity, and

TABLE 2. Comparison of the Relative Chemical and Biological Activities of the Haloacetonitriles[a]

HAN[b]	inhib. DMN DM[c]	extent met.[d]	alk. pot[e]	DNA strand break.[f]	SOS GT[g]	Newt MCN [h]	CHO SCE[i]	HeLa SCGE [j]	skin tumor ind.[k]	SOS tox[l]	newt tox[m]	CHO %C1/2[n]	CHO SCGE[o]
CAN	1	1	1	1	1	1	1	1	1	1	1	1	1
DCAN	450	0.7	0.07	2.1	1.3	0.48	1.05	10	0.4	10	5	1.19	0.22
TCAN	450	0.2	0.01	36.7	0.98	0.42	3.61	10	0.9	100	50	0.43	0.60
BAN					1.03	2.42		10		33.3	20	21.3	15.61
DBAN	3000	0.5	6.2	3.4	1.78	1.15	32.5	100	1.4	33.3	10	24.0	12.81
BCAN	2300	0.9	2.2	6.3	1.92	0.46	13.0		1.1	66.7	40	8.1	1.86

[a] Data normalized to CAN = 1.0. Adapted from refs *7* and *8*. Data from refs *5, 9, 30,* and *31*, and this paper. [b] Haloacetonitriles (HAN), abbreviations listed in Table 1 of Supporting Information. [c] Measured as the *in vitro* inhibition of rat hepatic microsomal dimethylnitrosamine-demethylase (*8*). [d] Extent metabolized measured as the percentage of the dose excreted as urinary thiocyanate (*30*). [e] Alkylation potential measured as the ability to alkylate 4-(*p*-nitrobenzyl) pyridine(*7*). [f] DNA strand breakage potential in CCRF CEM cells determined by the alkaline unwinding method (*7*).[g] SOS chromotest (*Escherichia coli* PQ37) (*31*). [h] *Pleurodeles* walt1 larvae micronuclei induction (*31*). [i] CHO cell sister chromatid exchange induction (*5*). [j] Single cell gel electrophoresis in HeLa S3 cells (*9*). [k] Measured as the total number of skin tumors per mouse after a topical dose of 2.4 g/kg of the HAN followed by 12-*O*-tetradacanoylphorbol-13-acetate for 20 weeks, data from (*5*); ranking from (*7*). [l] Threshold toxicity in the SOS chromotest (*31*). [m] Threshold toxicity in the newt micronucleus assay (*31*). [n] CHO cell chronic cytotoxicity %C1/2 values reported in this paper normalized to CAN = 1. [o] CHO cell SCGE genotoxic potency values reported in this paper normalized to CAN = 1.

toxicity in a number of assays with the CHO cell cytotoxicity and genotoxicity data of the present study (Table 2). The endpoints included inhibition of dimethylnitrosamine-demethylase (DMN-DM) (*8*), the extent metabolized in rats (*30*), alkylation potential (*7*), induction of DNA strand breaks (*7*), genotoxicity in the *Escherichia coli* SOS chromotest (*31*), clastogenicity in newt larvae (*31*), sister chromatid exchange (SCE) in CHO cells (*5*), DNA damage in HeLa cells (*9*), skin tumor induction (*5*), toxicity in *E. coli* and *Pleurodeles* newt (*31*), and, finally, the CHO chronic cytotoxicity and genomic DNA damage reported here. No significant correlation was observed among the metabolism of 5 HANs (*7*) and chronic cytotoxicity or genomic DNA damage in CHO cells. The *in vitro* inhibition of rodent microsomal DMN-DM was highly correlated with the alkylation potential, *E. coli* SOS genotoxicity, enhanced somatic chromosomal recombination (SCE) in CHO cells, genomic DNA damage in HeLa cells, and CHO cell chronic cytotoxicity ($r = 0.88$, $P < 0.05$; $r = 0.91$, $P < 0.03$; $r = 0.93$, $P < 0.02$; $r = 0.99$, $P < 0.002$; and $r = 0.91$, $P < 0.03$, respectively). HAN alkylation potential was highly correlated with measurements of mammalian cell genotoxicity: CHO SCE ($r = 0.98$, $P < 0.004$), HeLa cell SCGE ($r = 0.97$, $P < 0.03$), and CHO cell SCGE ($r = 0.97$, $P < 0.006$), as well as CHO cell cytotoxicity ($r = 0.99$, $P < 0.001$). Interestingly, DNA strand breakage using the alkaline unwinding procedure (*7*) did not demonstrate significant correlations with other measurements of DNA damage. However, a high correlation was observed between CHO cell SCGE and HeLa cell SCGE ($r = 0.99$, $P < 0.01$), CHO cell SCE and CHO cell SCGE ($r = 0.96$, $P < 0.01$), and HeLa cell SCGE and CHO cell SCE ($r = 0.99$, $P < 0.004$). The induction of SOS *E. coli* toxicity and newt larva toxicity were highly related ($r = 0.98$, $P < 0.001$), as was the induction of chromosome breaks in the newt and genomic DNA damage in CHO cells ($r = 0.87$, $P < 0.03$). Our data presented in this study expressed a significant and high correlation with other toxicity endpoints (Table 2). CHO cell chronic cytotoxicity was highly correlated with DMN-DM inhibition ($r = 0.91$, $P < 0.03$), alkylation potential ($r = 0.99$, $P < 0.001$), CHO cell SCE ($r = 0.99$, $P < 0.001$), HeLa cell SCGE ($r = 0.99$, $P < 0.006$), and CHO cell SCGE ($r = 0.96$, $P < 0.003$). CHO cell SCGE were also highly correlated with alkylation potential ($r = 0.97$, $P < 0.006$), CHO SCE ($r = 0.96$, $P < 0.001$), HeLa cell SCGE ($r = 0.99$, $P < 0.01$), and newt micronucleus induction ($r = 0.87$, $P < 0.03$). The induction of skin tumors expressed an association with alkylation potential, SCE induction, and induced genomic DNA damage in HeLa cells and CHO cells, as well as chronic cytotoxicity in CHO cells. However, this modest association ($r = 0.81$–0.74) was not statistically significant.

Structure–Activity Relationships (SARs) and Factors Affecting the Toxicity of Haloacetonitriles. The SARs of the HANs are interesting, but somewhat complicated. HANs have two potential electrophilic reactive centers: (a) displacement of a halogen atom at the α carbon by S_N2 reaction, and (b) addition at the partially positively charged carbon of the cyano group (*32*). Both reactions could contribute to the cytotoxicity or genotoxicity of the HANs.

The S_N2 reactivity of the HANs is dependent on the leaving tendency of the halogen and the degree of halogenation. The S_N2 reactivity of an alkyl iodide is 3–5× greater than alkyl bromide, which is 50× greater than alkyl chloride (*33*); a similar order is expected for monohaloacetonitriles. The leaving tendency of a halogen should decrease with increasing halogenation; therefore, the alkylating potential of the HANs should decrease with increasing halogenation. Both the alkylating potential (*7*) and interaction with calf thymus DNA (*15*) are consistent with the SAR expectation. However, the ability of HANs to bind to DNA may be significantly affected by the presence of glutathione (GSH) (discussed below).

The potential of the HANs to undergo nucleophilic addition at the partially positively charged carbon of the cyano group is dependent on the degree of halogenation. Polyhalogenation at the α carbon provides the ideal situation because (a) the halogens withdraw electrons away from the cyano carbon, making it more electrophilic, and (b) the halogens have lesser tendency to leave. Data showed that TCAN is the most potent in this respect (*32*). Analogous to halomethanes, the S_N2 reactivity should significantly contribute to the genotoxic potential of the HANs. In the present study, the observed relative order of IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN for SCGE genotoxic potency is generally in agreement with the S_N2 SAR expectation. The higher activity of TCAN versus DCAN may suggest that nucleophilic addition at the cyano carbon could also contribute to the genotoxicity. The relative order of DBAN > BAN ≈ IAN > BCAN > DCAN > CAN > TCAN for cytotoxicity observed in the present study is more difficult to explain. It appears that dihaloacetonitriles tend to be more cytotoxic than mono- or trihaloacetonitriles. This may be due to the potential of dihaloacetonitriles to act as crosslinking agents (which is conducive to cytotoxicity) and GSH-mediated activation.

The toxic and genotoxic consequence of HAN interaction with cellular macromolecules may be significantly affected by the presence of GSH compounds. For mono- and trihaloacetonitriles, GSH conjugation should be detoxifying because of elimination of reactive electrophiles. There is evidence that the toxicity and genotoxicity of these com-

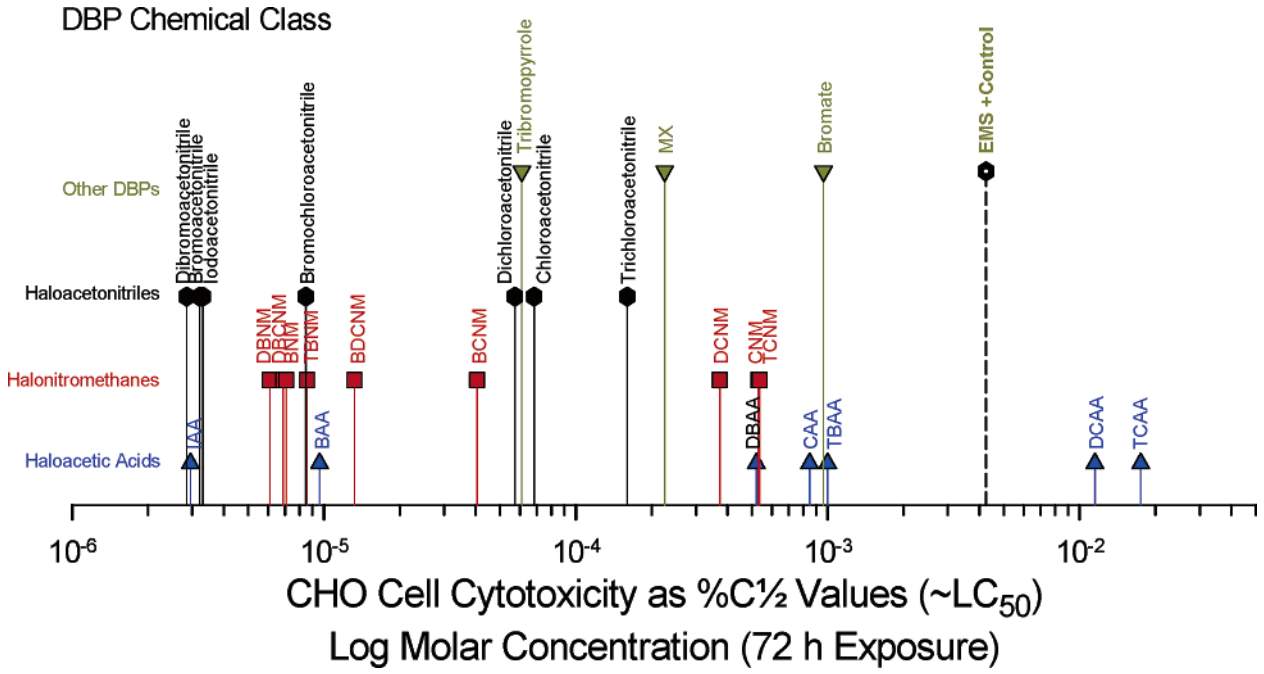

FIGURE 3. Quantitative comparison of the direct induction of CHO cell chronic cytotoxicity by different classes of DBPs. The data for the haloacetic acids are from refs *17* and *19*. MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2[*5H*]-furanone), ethylmethanesulfonate (EMS), and potassium bromate data are from ref *17*. Halonitromethanes data are from ref *18*; tribromopyrrole data are from ref *22*; and the HAN data are from this paper.

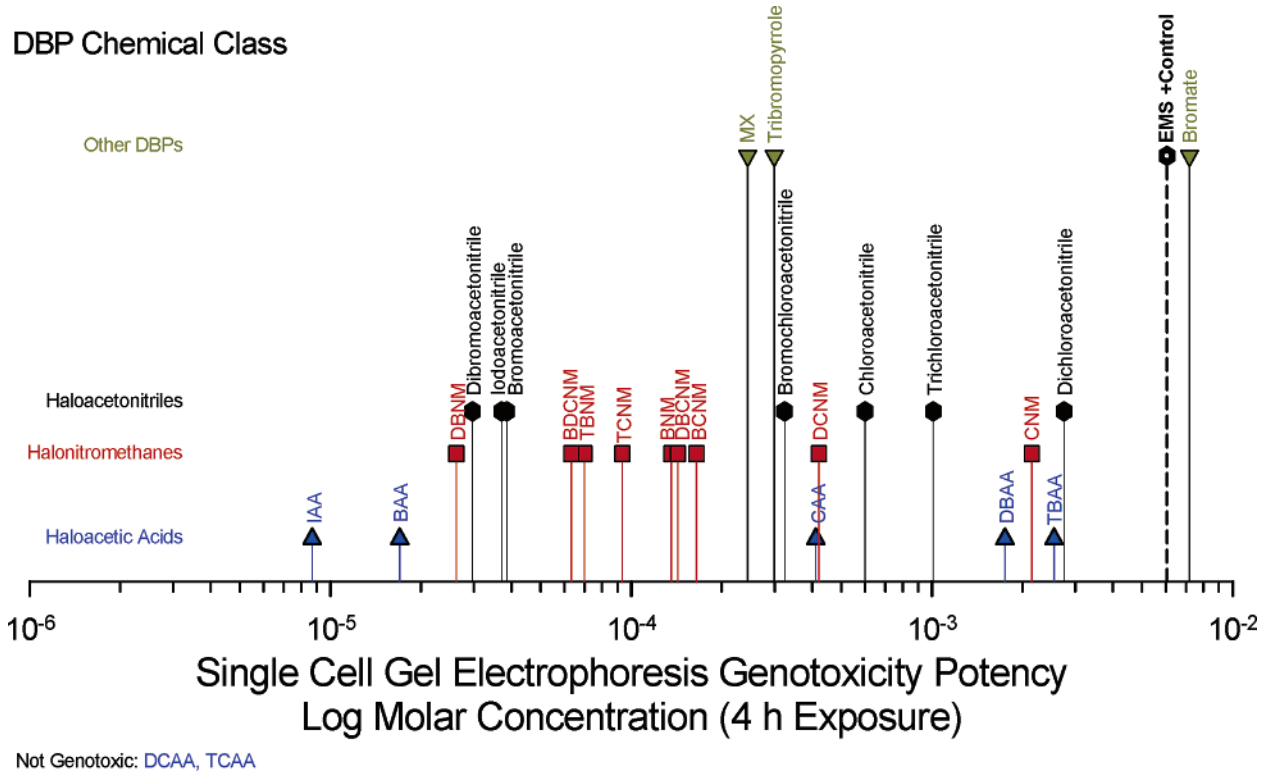

FIGURE 4. Quantitative comparison of the direct induction of CHO cell acute genomic DNA damage by different classes of DBPs. The data are from the sources listed in Figure 3.

pounds may not be fully manifested until the cellular GSH pool is depleted (*34*). For dihaloacetonitriles, GSH conjugation is only detoxifying if both halogens are displaced. If only one is displaced, GSH conjugation can become an activation pathway because the resulting intermediate (an α-halothioether) is a highly reactive electrophile. There are many examples of dihaloalkanes being activated by GSH conjugation (*35*). The relative importance of the GSH activation pathway for dihaloacetonitriles remains to be studied.

DBP Cytotoxicity and Genotoxicity Database. One goal of our research is to generate a quantitative and comparative *in vitro* mammalian cell database on emerging DBPs listed as priorities by the U.S. EPA (*13*, *14*). Using CHO cell microplate-based assays, we have published data on the

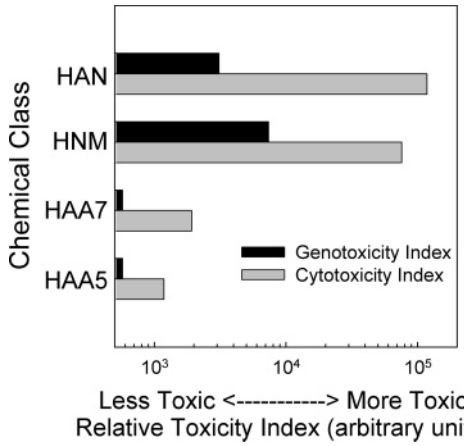

FIGURE 5. Log plot illustrating a comparison of the CHO cell cytotoxicity and genotoxicity for each DBP class, in which the reciprocal of the median %C1/2 values and the reciprocal of the median SCGE genotoxic potency values for each chemical class are represented. The DBP classes are the haloacetonitriles (HAN), halonitromethanes (HNM), the 7 haloacetic acids illustrated in Figures 3 and 4 (HAA7), and the 5 EPA regulated haloacetic acids: BAA, DBAA, CAA, DCAA, TCAA (HAA5).

cytotoxicity and genotoxicity of the haloacetic acids, halonitromethanes, and other DBPs (*17*−*19*, *22*). These data are directly comparable; the database for DBP-induced CHO cytotoxicity is presented in Figure 3, and the database for CHO genotoxicity is presented in Figure 4. The iodinated and brominated acetonitriles are among the most chronically cytotoxic DBPs, and the HANs rank in genotoxic potency with the halonitromethanes. With data derived from Figures 3 and 4, three DBP classes were compared for their CHO cell cytotoxicity and genotoxicity (Figure 5). The cytotoxicity and genotoxicity index values were calculated as the reciprocal of the median CHO cell cytotoxicity %C1/2 value for each DBP class or the reciprocal value of the median CHO SCGE genotoxic potency values for each DBP chemical class. Since both assays were based on molar units this allows us to use these toxicity index values to compare among the DBP classes. The least cytotoxic and genotoxic DBPs were the regulated haloacetic acids (HAA5: chloro-, bromo-, dichloro-, dibromo-, and trichloroacetic acid). The combined toxicity index value increased by 42% when iodoacetic acid and bromochloroacetic acid were included (HAA7). The halonitromethanes and the HANs were nearly 2 orders of magnitude more cytotoxic than the haloacetic acids. Likewise, the haloacetic acids were the least genotoxic of the three DBP classes, followed by the HANs; the most genotoxic were the halonitromethanes. Thus, this approach allows not only for a quantitative comparison among individual DBPs, but also among chemical classes of DBPs. In the future, the toxicity of N-DBPs may become a public health concern because of the increased use of alternative disinfectants that may enhance the formation of HANs and other N-DBPs.

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Supporting Information Available

Detailed methods for the CHO cell chronic cytotoxicity assay and the CHO cell SCGE genotoxicity assay; additional detailed data cited in the text are provided in tables and figures. (The reference citation numbers included in the Supporting Information refer to those listed in the Literature Cited Section.) This material is available free of charge via the Internet at http://pubs.acs.org.

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