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Pennsylvania House Committee on Health
Harrisburg, PA

Dear Committee Members:

I was asked for my opinion on the request for a moratorium on switching from chlorine disinfection of drinking water to an alternative disinfection process using chloramines. I am a scientist working in the area of drinking water disinfection by-products (DBPs) and I am a member of the National Science Foundation Center of Advanced Materials for the Purification of Water with Systems, University of Illinois. During the past decade my laboratory, in collaboration with scientists at academic, industrial and government institutions, established a systematic, quantitative analyses of drinking water DBPs and we have generated the largest dataset on this issue. At the onset I must state that the drinking water community continues to provide abundant, safe, tasteful water to 90% of the American population at reasonable cost. We must recognize that the disinfection of drinking water was one of the great public health achievements of the 20th Century. The goal continues to make good drinking water better.

At this time I recommend caution when considering converting from chlorine to chloramines disinfection methods by a water utility. We have the greatest experience with, and the highest level of understanding of, the toxic by-products generated by chlorine disinfection. The level of knowledge is lower with alternative disinfectants. Many scientists in the drinking water field are concerned that using alternative disinfection methods may lead to unintended adverse consequences because our level of knowledge on the long term effects of such actions on the public health and environment is limited. My basis of concern is founded on the following issues.

1. An example of an adverse unintended consequence related to changing from chlorine to chloramines disinfection caused the exposure of a large population to lead in Washington D.C. [1, 2]. Lead exposure during chloramines disinfection can now be controlled. However, the issue is that this problem came to light only after the change in disinfection practice which resulted in the exposure of a large population to a potent neurotoxin. This is not good policy and we should avoid repeating such policy errors.
2. Different disinfectants generate different levels of TOX (total organic halide) and generate different spectra of DBPs [3-5].
3. Research has demonstrated that water disinfection using chloramines generate iodinated DBPs (I-DBPs) [6], and nitrogen-containing DBPs such as carcinogenic nitrosamines (N-DBPs) [7].
4. These emerging DBPs occur in real drinking water that is consumed by real people [8].
5. Our laboratory demonstrated that I-DBPs are generally more toxic (both cytotoxic and genotoxic) than their brominated and chlorinated analogs [9-11]. Also we demonstrated that N-DBPs are more toxic than DBPs that do not contain nitrogen[9, 12].
6. We recently demonstrated that iodinated haloacetic acids are much more cytotoxic and genotoxic in embryonic human cells than regulated haloacetic acids and alter gene expression in important metabolic pathways that may lead to disease (cancer induction, adverse pregnancy outcomes and birth defects) [13].

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7. Working with scientists at the U.S. EPA and the Center for Disease Control (CDC) we discovered I-DBPs in chloraminated drinking water from 22 North American cities. Many of these cities were not located in coastal areas but had sufficient levels of iodine in the source waters that allowed the formation of I-DBPs via chloramines disinfection. Again this raises the issue that chloramines disinfection is generating increased levels of highly toxic agents in drinking water [14].
8. One cannot say with certainty that chloramines disinfected water poses an increased public health risk as compared to water disinfected by chlorine alone, however, the results from current science on the occurrence and toxicity of chloramine-related DBPs argue for caution.
9. Unless there is a serious problem with meeting the current Stage 2 Drinking Water Disinfection Rule [15] it may not be prudent for the utility to convert from chlorine-based disinfection.

I hope that the above information will aid you in your important decision.

Sincerely,

Michael Plewa, Ph.D.
University Scholar and Professor of Genetics

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Comparative Human Cell Toxicogenomic Analysis of Monohaloacetic Acid Drinking Water Disinfection Byproducts

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The monohaloacetic acids (monoHAAs), iodoacetic, bromoacetic and chloroacetic acids are toxic disinfection byproducts. *In vitro* toxicological end points were integrated with DNA damage and repair pathway-focused toxicogenomic analyses to evaluate monoHAA-induced alterations of gene expression in normal nontransformed human cells. When compared to concurrent control transcriptome profiles, metabolic pathways involved in the cellular responses to toxic agents were identified and provided insight into the biological mechanisms of toxicity. Using the Database for Annotation, Visualization and Integrated Discovery to analyze the gene array data, the majority of the altered transcriptome profiles were associated with genes responding to DNA damage or those regulating cell cycle or apoptosis. The major pathways involved with altered gene expression were ATM, MAPK, p53, BRCA1, BRCA2, and ATR. These latter pathways highlight the involvement of DNA repair, especially the repair of double strand DNA breaks. All of the resolved pathways are involved in human cell stress response to DNA damage and regulate different stages in cell cycle progression or apoptosis.

Introduction

The monohaloacetic acids (monoHAAs), iodoacetic acid (IAA), bromoacetic acid (BAA), and chloroacetic acid (CAA) are disinfection byproducts (DBPs) formed during the disinfection of drinking water (*1, 2*), wastewaters, and recreational pools (*3*). In the United States five haloacetic acids are regulated (maximum level of 60 μg/L) for the sum of BAA, CAA, dibromoacetic acid (DBAA), dichloroacetic acid (DCAA), and trichloroacetic acid (*4*). HAAs are formed by disinfection with chlorine, chloramines, chlorine dioxide, and ozone but are generally formed at the highest levels with chlorination (*1, 5, 6*). HAAs are genotoxic (*6, 7*). IAA, BAA, CAA, DBAA, and tribromoacetic acid were mutagenic in *Salmonella typhimurium* and induced genomic DNA damage in Chinese hamster ovary (CHO) cells (*8–12*).

As defined by Aardema and MacGregor (2002), toxicogenomics is "the study of the relationship between the structure and activity of the genome (the cellular complement of genes) and the adverse biological effects of exogenous agents". Toxicogenomics incorporates the modulation of cellular products controlled by the genome (*13*). Toxicogenomic analyses, when integrated with concurrent toxicological assays, provide insights into altered functional activity of biochemical pathways induced by toxins. Few studies have investigated the toxicogenomics of HAAs. In mice treated with drinking water that contained 2 g/L DCAA, altered gene expression was found in pathways that involved fatty acid metabolism, tissue remodeling/angiogenesis, and cellular damage response (*14*). Mice exposed to 8–216 mg/kg bromochloroacetic acid demonstrated altered gene expression involved in cell communication and adhesion, cell cycle and cell proliferation, metabolism, signal transduction, stress response, spermatogenesis, and male fertility (*15*). Recently, we found that in nontransformed human cells, 60 μM BAA altered transcriptome profiles for genes involved in DNA repair, especially repair of double strand DNA (dsDNA) breaks, and in cell cycle regulation (*16*).

The objective of this research was to integrate *in vitro* toxicological end points with pathway-focused toxicogenomic analyses of the monoHAAs and to evaluate the modulation of gene expression in normal nontransformed human cells. This study presents the first comparative toxicogenomic analysis of the monoHAAs. These data will aid in defining the biological impact and toxicity mechanisms of the monoHAAs.

Materials and Methods

Reagents. General laboratory reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma Chemical Co. (St. Louis, MO). Media supplies and fetal bovine serum (FBS) were purchased from Hyclone Laboratories (Logan, UT); human epidermal growth factor (EGF) was obtained from Sigma Chemical Co. (St. Louis, MO). The source and purity of the monoHAAs are listed in Table 1 of the Supporting Information. Stock solutions were prepared in dimethylsulfoxide (DMSO) and stored at −22 °C.

Human Small Intestine Epithelial Cells. Nontransformed human small intestine epithelial cells, line FHs 74 Int, were purchased from American Type Culture Collection (Manassas, VA); the cells were received at passage 12 and were used until passage 17 or 18. These cells are nontransformed intestinal cells isolated from a 3 to 4 month female fetus from a therapeutic abortion and did not show abnormalities (*17*). The cells express a normal female diploid karyotype and are histologically negative for PAS and keratin. Cell maintenance and growth conditions are presented in the Supporting Information.

Cell Viability. Concurrent with the genotoxicity analysis, the acute cytotoxicity of the cells was evaluated from a 1:1 (v/v) mixture of cell suspension and 0.05% trypan blue vital dye in phosphate-buffered saline (PBS) (*18*). As in our past work, genotoxicity data were not used if acute cytotoxicity exceeded 30% (*19*).

For the toxicogenomic experiments, cell viability was determined immediately after exposure or 24 h after exposure.

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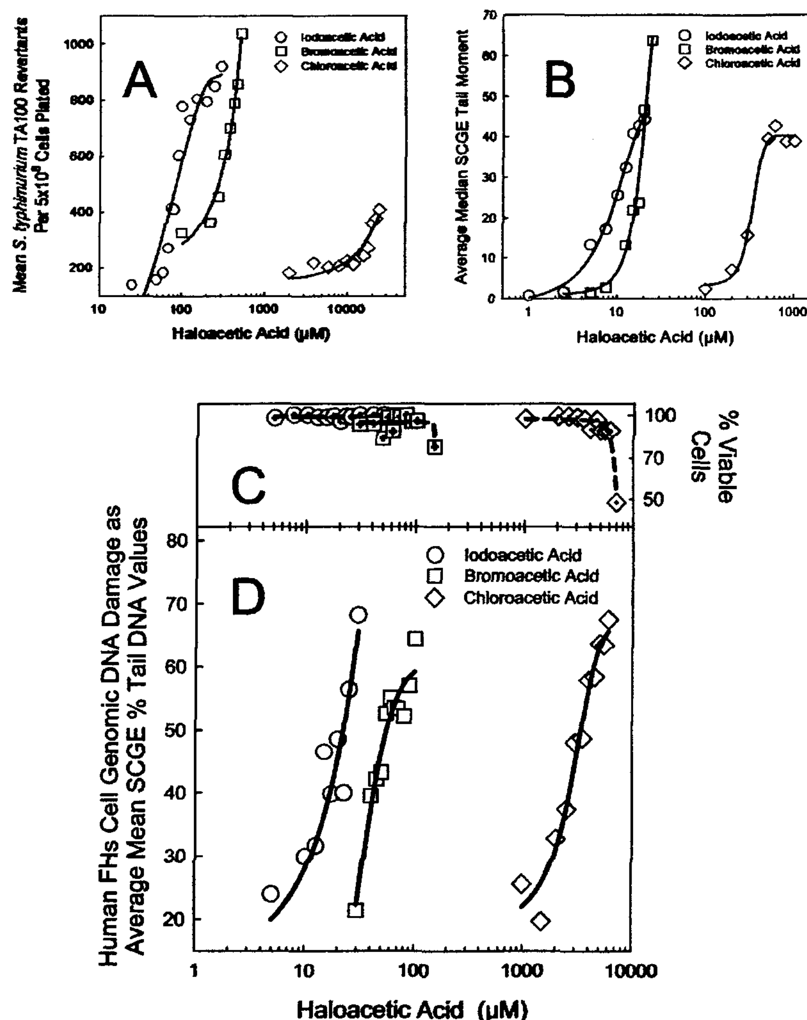

FIGURE 1. (A) ***Salmonella typhimurium*** **concentration–response curves illustrating the mutagenicity of IAA, BAA, and CAA (from ref** ***8*****). (B) CHO cell concentration–response curves showing the SCGE genotoxicity of IAA, BAA, and CAA (from ref** ***12*****). (C) The acute cytotoxicity induced by the monoHAAs in human FHs cells. (D) SCGE genotoxicity of the monoHAAs in human FHs cells.**

The concentrations of IAA, BAA, and CAA that induced equivalent biological responses were 22 μM, 57 μM, and 3.42 mM, respectively. FHs cells were exposed to the monoHAAs in microplates at a titer of 1×10^4 cells/well. The microplates were covered with sterile AlumnaSeal (RPI Corporation, Mt. Prospect, IL) and incubated for 30 min or 4 h at 37 °C. The cells were washed 3× with PBS, and cell viability was determined immediately after exposure with trypan blue. With parallel microplates, 200 μL of complete Hybri-Care medium were added to each well; these microplates were incubated for 24 h at 37 °C, 5% CO_2. The microplates were stained with the histological dye crystal violet and analyzed as previously published (*16*). Cell density was calculated as the percentage of the concurrent negative control. The positive control was 25% DMSO.

Single Cell Gel Electrophoresis (SCGE) Assay. The SCGE (or Comet) assay for genotoxicity was performed as described previously (*19*). The % Tail DNA was the metric used. The detailed procedures of this assay are presented in the Supporting Information.

MonoHAA **Toxicogenomic Analysis, RNA Isolation, and Purification.** A detailed description of the treatment of FHs cells with the monoHAAs, RNA isolation, and purification is presented in the Supporting Information. Four days prior to treatment, 4×10^5 FHs cells were seeded in each well in six-well plates. After a 30 min or 4 h exposure to each monoHAA, cells were washed, harvested, and centrifuged. Aliquots of each cell suspension were retained prior to centrifugation for acute cytotoxicity and SCGE analyses. The supernatant was removed and RNA isolated using a Qiagen RNeasy Mini Kit (Valencia, CA) following the recommended protocol. RNA quantity was determined using the Agilent 2100 Bioanalyzer (Santa Clara, CA). RNA Integrity Numbers (RIN) were determined for each treatment group and their concurrent controls (*20*); see Table 2 of the Supporting Information.

cDNA Synthesis. cDNAs were synthesized using the SuperArray RT[2] PCR Array First Strand Kit (Frederick, MD) according to the manufacturer's protocol. The detailed methods for cDNA synthesis are presented in the Supporting Information. After cDNA synthesis, the samples were diluted with nuclease-free water and stored at −20 °C.

Real Time PCR Analyses. A DNA damage signaling focused pathway-specific qRT-PCR array (SuperArray PAHS-029) was employed (*21*) according to the manufacturer's protocol. A detailed description of the qRT-PCR procedure is presented in the Supporting Information. The genes evaluated for their expression are listed in Table 3 of the Supporting Information. Real-time PCR analysis was conducted using a two-step cycling program on a Stratagene Mx3000p thermocycler (La Jolla, CA). Quality controls measuring genomic DNA contamination, reverse transcrip-

TABLE 1. Concentration of MonoHAAs That Induce Equivalent Levels of Genomic DNA Damage in Nontransformed Human FHs Cells

monoHAA	R^{2a}	SCGE[b] 20% Tail DNA (HAA molar concn)	SCGE 40% Tail DNA (HAA molar concn)	SCGE 50% Tail DNA (HAA molar concn)	ANOVA test[c]
IAA	0.89	5.90×10^{-6}	16.6×10^{-6}	21.9×10^{-6}	$F_{11,24} = 7.26$; $P < 0.001$
BAA	0.95	23.8×10^{-6}	43.4×10^{-6}	56.5×10^{-6}	$F_{12,25} = 38.5$; $P < 0.001$
CAA	0.98	1.04×10^{-3}	2.60×10^{-3}	3.42×10^{-3}	$F_{11,22} = 96.4$; $P < 0.001$

[a] R^2 is the coefficient of determination for the regression analysis upon which HAA concentration was calculated for each level of genomic DNA damage. [b] Genotoxicity metric as the percentage of DNA that migrated into the microgel from the nucleus under SCGE conditions. At all monoHAA concentrations, no acute cytotoxicity was observed. [c] Degrees of freedom for the between groups and residual associated with the calculated F-test result and the resulting probability value.

tion efficiency, and PCR amplification efficiencies were analyzed and were within satisfactory limits.

Safety and Data Handling. Manipulations of toxic chemicals were conducted in certified biological/chemical stage-2 safety hoods. qRT-PCR array data were analyzed using the RankProd algorithm of the Bioconductor package for R (*22, 23*) with a direct $P \leq 0.05$ considered as significant. Gene clustering was performed using the Unweighted Pair Group method with Arithmetic Mean in PAUP 4.0b10 (Sinauer Associates, Sunderland, MA) according to similarity of temporal expression patterns and with the SA Biosciences Gene Network Central program. The raw and normalized data are available in the Gene Expression Omnibus (GEO) database (*24, 25*) under the [NCBI tracking system #15759010] series accession number.

Results and Discussion

Toxicogenomics is a powerful tool to analyze the modulation of gene expression after exposure to a toxin. When compared to concurrent control transcriptome profiles, metabolic pathways involved in the cellular responses to toxic agents can be identified and provide insight on the biological mechanisms of toxicity. In much of the toxicogenomic literature, tumor cell lines are exposed to cytotoxic concentrations of a genotoxin to observe effects on gene expression (*26*). Tumor cell lines inherently exhibit aberrant gene expression. With cytotoxic concentrations, transcript profiles will reflect those of dead or dying cells. We avoided these approaches by using nontransformed human cells, concurrent negative controls at each treatment time and noncytotoxic concentrations. An additional concern is that much of the gene expression data is based on gene chip arrays without qRT-PCR confirmation. Our experimental design is based on the direct use of PCR gene arrays (*27*).

For this comparative human cell toxicogenomic analysis of DBPs we chose 3 monoHAAs (Table 1 of the Supporting Information). They represent a class of drinking water DBPs, they differ by a single halogen atom, and BAA and CAA are regulated.

Acute Cytotoxicity and Genotoxicity with FHs Cells. Acute cytotoxicity and genotoxicity concentration−response curves for the monoHAAs are presented in Figure 1C and 1D. Genotoxicity data were not used if the acute cytotoxicity (evaluated immediately after exposure) exceeded 30%. In FHs cells the rank order of genotoxic response was IAA > BAA ≫ CAA. The same rank order of response was demonstrated for mutagenicity in *Salmonella typhimurium* (*8*) (Figure 1A), genotoxicity in CHO cells (*11*) (Figure 1B), and teratogenicity in mouse embryos (*28*). Comparing the concentration−response curves presented in Figure 1B and 1D, the data indicate that CHO cells are more sensitive to monoHAAs than human FHs cells.

From the FHs cell concentration−response curves (Figure 1D) we calculated the monoHAA concentrations that generated equivalent genotoxic responses (Table 1). The distribution of individual nuclei for each monoHAA that induced an average SCGE damage of approximately 50% Tail DNA is presented in Figure 1 in the Supporting Information. BAA and CAA exhibited similar distributions; IAA expressed a broader distribution of genomic DNA damage with enhanced kurtosis (see Figure 1 in the Supporting Information).

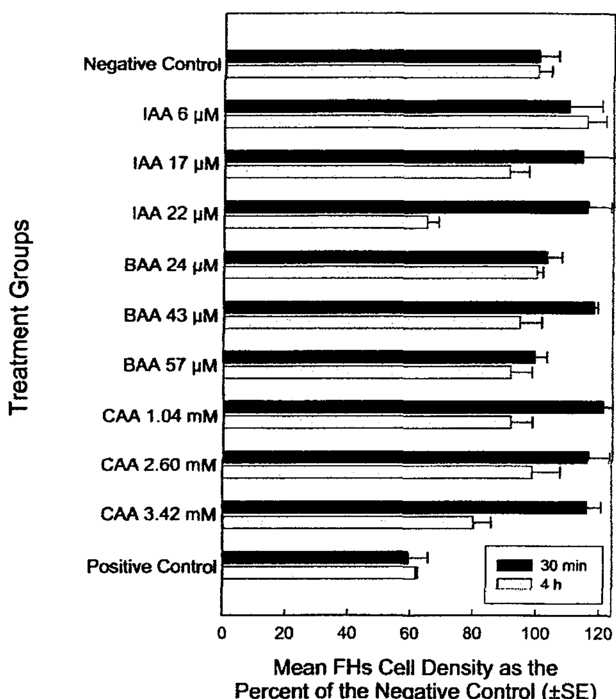

FIGURE 2. Cell density analysis of FHs cells exposed to monoHAAs for 30 min or 4 h, washed and incubated for 24 h. The positive control was 25% dimethylsulfoxide.

Cytotoxicity Measurements Associated with Toxicogenomic Experiments. We conducted a series of cytotoxicity studies with monoHAA concentrations that induced equivalent genotoxicity (20%, 40%, and 50% Tail DNA values) to ensure that the monoHAAs were not inducing high levels of cell killing. Cell viability was determined immediately after exposure and also after washing and additional 24-h incubation. For all monoHAA concentrations there was no increase in acute cytotoxicity (Table 1, Figure 1C). For the cells incubated 24 h after treatment, cell density was calculated as the percent of the negative control. In addition we microscopically investigated each well for floating (dead) cells. In all cases there was not an observable increase in detached cells as compared to the concurrent controls. The 24-h cell density data are presented in Figure 2. There was no decrease in relative cell density associated with a 30-min exposure followed by 24 h incubation. There was a reduction in cell density in 4-h treatments with IAA and CAA; the lack of detached

TABLE 2. Changes in Gene Expression from Concurrent Negative Controls after 30 Min of MonoHAA Exposure

altered gene expression	gene function	x-change BAA	P value BAA	x-change CAA	P value CAA	x-change IAA	P value IAA
		BAA, CAA, and IAA					
PPP1R15A	apoptosis, cell cycle arrest	−1.71	0.0041	−1.25	0.0290	−3.67	0.0023
XRCC3	dsDNA break repair	−2.86	0.0001	−1.36	0.0077	−2.64	0.0125
		CAA and IAA					
PNKP	damaged DNA binding, dsDNA break repair			−1.36	0.0023	3.79	0.0021
		Single MonoHAA					
HUS1	cell cycle arrest	−2.97	0.0001				
SEMA4A	damaged DNA binding	−2.27	0.0004				
MRE11A	dsDNA break repair	−1.78	0.0168				
ATM	dsDNA break repair	−1.58	0.0298				
PMS2L3	damaged DNA binding	−1.45	0.0367				
RAD9A	cell cycle arrest, DNA excision repair	−1.44	0.0399				
EXO1	DNA mismatch repair	1.25	0.0004				
XPC	damaged DNA binding, DNA excision repair			−1.35	0.0449		
RAD50	dsDNA break repair					−5.27	0.0001
PCBP4	apoptosis, cell cycle arrest, damaged DNA binding					−4.44	0.0001
IGHMBP2	damaged DNA binding					−3.53	0.0021
ERCC1	damaged DNA binding					−3.09	0.0108
FEN1	damaged DNA binding, DNA excision repair					−2.88	0.0224
MAPK12	cell cycle arrest					−2.58	0.0339
GADD45A	apoptosis, cell cycle arrest					−2.53	0.0243
MUTYH	base excision DNA repair, mismatch repair					−2.52	0.0267
SESN1	cell cycle arrest					1.33	0.0474
DDIT3	cell cycle arrest					1.50	0.0363
TREX1	DNA mismatch repair, dsDNA break repair					1.52	0.0394
GTSE1	cell cycle arrest					1.57	0.0227
MBD4	base excision DNA repair, DNA mismatch repair					1.59	0.0144
GTF2H1	DNA excision repair					1.71	0.0155
MLH1	DNA mismatch repair					1.79	0.0051
UNG	DNA excision repair					1.89	0.0037

cells suggests this may be due to cell cycle arrest rather than cell killing. Based on equivalent genotoxic responses (SCGE 50% Tail DNA), lack of acute cytotoxicity, and cell density data, we chose IAA, BAA, and CAA concentrations of 22 μM, 57 μM, and 3.42 mM, respectively, for the toxicogenomic experiments.

Comparative Analyses of Human Transcriptome Profiles. The qRT-PCR gene array employed focused on gene function groups related to damaged DNA binding, DNA repair, cell cycle regulation, and apoptosis. This DNA damage gene array was directly related to the concentration of the monoHAAs that induced a genotoxic response in the human FHs cells. The altered transcriptome profiles of monoHAA-exposed cells, as compared to their concurrent negative controls, expressed a remarkable level of similarity and provided insights into the biological mechanisms underlying their toxicity.

The changes in gene expression induced by the monoHAAs as compared to their concurrent negative controls are listed in Table 2 (30 min exposure) and Table 3 (4 h exposure). The effects of CAA and BAA on gene modulation were greater at 4 h, both in terms of numbers of genes and in fold-changes from their controls. IAA affected approximately the same number of genes at both time points.

The expression of two genes was modulated (downregulated) by all three monoHAAs with 30 min exposure. *XRCC3* encodes a protein involved in homologous recombination and the repair of double strand DNA (dsDNA) breaks (*29*); *PPP1R15A* (*GADD34*) is involved in response to DNA damage and cell cycle arrest (*30*) (Table 2). Interestingly, human polymorphisms in *XRCC3* have been linked with susceptibility to bladder cancer (*31, 32*) in which enhanced risk is associated with exposure to DBPs (*33*). Both CAA and IAA modulated *PNKP* that is involved in response to DNA damage and oxidative stress (*34*).

More genes exhibited altered expression after 4 h of exposure. Expression of 4 genes involved in the regulation of cell cycle and apoptosis were altered by all three monoHAAs (*MAP2K6* and *SESN1* (downregulated) and *DDIT3* and *IHPK3* (upregulated)). BAA and IAA expressed a similar pattern of gene expression changes when compared to CAA. Six genes were modulated by both BAA and IAA; these genes are involved in DNA repair (*BTG2*, *XPA*, and *DMC1*) and cell cycle regulation (*RBBP8*, *GADD45A*, and *PPP1R15A*). *DMC1* encodes for a protein involved in dsDNA break repair. Both BAA and CAA downregulated the expression of *XRCC2*, while CAA and IAA downregulated the expression of *PCBP4*.

Transcriptome profiles impacted by the monoHAAs were predominantly with genes involved in dsDNA break repair, cell cycle arrest, and apoptosis regulation (see Figure 2 in the Supporting Information). Genes modulated by structurally related genotoxins may increase our understanding of the type of DNA damage generated and subsequent DNA repair. Figure 3 illustrates the distribution of altered gene expression for each monoHAA within gene functional groups. The similarity of altered gene expression is striking. All three monoHAAs modulated the expression

TABLE 3. Changes in Gene Expression from Concurrent Negative Controls after 4 h of MonoHAA Exposure

altered gene expression	gene function	x-change BAA	*P* value BAA	x-change CAA	*P* value CAA	x-change IAA	*P* value IAA
		BAA, CAA, and IAA					
MAP2K6	cell cycle arrest	−5.98	0.0001	−6.22	0.0001	−4.55	0.0001
SESN1	cell cycle arrest	−3.84	0.0006	−1.63	0.0211	−3.31	0.0005
DDIT3	cell cycle arrest	1.53	0.0245	4.19	0.0001	2.60	0.0405
IHPK3	apoptosis, cell cycle arrest	3.04	0.0001	5.05	0.0001	2.06	0.0001
		BAA and CAA					
XRCC2	damaged DNA binding, dsDNA break repair	−3.59	0.0006	−1.61	0.0380		
		BAA and IAA					
BTG2	DNA damage repair, excision repair	−2.09	0.0148			−4.33	0.0001
XPA	damaged DNA binding	−2.08	0.0087			−1.91	0.0280
RBBP8	cell cycle checkpoint	1.60	0.0171			1.39	0.0186
GADD45A	apoptosis, cell cycle arrest	2.28	0.0007			1.91	0.0004
PPP1R15A	apoptosis, cell cycle arrest	2.79	0.0002			1.40	0.0163
DMC1	damaged DNA binding, dsDNA break repair	2.79	0.0002			1.47	0.0161
		CAA and IAA					
PCBP4	apoptosis, cell cycle arrest			−1.68	0.0424	−1.82	0.0374
		Single MonoHAA					
GTF2H2	DNA excision repair	−2.02	0.0126				
OGG1	damaged DNA binding, base excision repair	−1.91	0.0260				
BRCA1	damaged DNA binding, dsDNA break repair	−1.83	0.0373				
MRE11A	dsDNA break repair	−1.83	0.0401				
PMS1	DNA mismatch repair	−1.82	0.0374				
CHECK2	cell cycle checkpoint	1.63	0.0245				
SEMA4A	damaged DNA binding	2.70	0.0002				
XRCC3	damaged DNA binding, dsDNA break repair			−2.61	0.0006		
MUTYH	DNA excision repair, mismatch repair			−2.22	0.0010		
PNKP	dsDNA break repair, cell cycle arrest			−2.09	0.0045		
HUS1	cell cycle arrest			−1.98	0.0069		
LIG1	DNA damage repair			−1.78	0.0199		
IGHMBP2	damaged DNA binding			−1.69	0.0439		
FEN1	damaged DNA binding			−1.58	0.0477		
ABL1	apoptosis, cell cycle arrest			1.26	0.0471		
CDK7	cell cycle arrest, DNA damage repair			1.28	0.0443		
RAD9A	cell cycle arrest, DNA excision repair			1.29	0.0311		
TP73	apoptosis, cell cycle arrest			1.29	0.0404		
CCNH	cell cycle arrest			1.34	0.0252		
CRY1	cell cycle arrest			1.38	0.0146		
ANKRD17	damaged DNA binding			1.51	0.0035		
NBN	cell cycle checkpoint, dsDNA break repair					−3.13	0.0373
N4BP2	damaged DNA binding, dsDNA break repair					−2.88	0.0012
XPC	damaged DNA binding, excision repair					−2.10	0.0093
MAPK12	cell cycle arrest					−1.81	0.0405
GML	apoptosis, cell cycle arrest					1.30	0.0327
EXO1	DNA mismatch repair					1.69	0.0032
GTSE1	cell cycle arrest					2.19	0.0001

of genes involved in dsDNA break repair. Other types of DNA repair genes were impacted but with fewer numbers involved. The induction of oxidative stress may be one mechanism of HAA-associated genotoxicity (*10*); this is consistent with the altered expression of *PNKP* (Tables 2 and 3). Most oxidative stress-induced DNA lesions tend to be rapidly repaired except for dsDNA breaks (*35*). These lesions are very toxic/mutagenic and require more time for repair (*36*). Recently we determined the DNA repair kinetics for lesions induced by these monoHAAs (*37*); they required extended times for DNA repair as compared to lesions induced by ethylmethanesulfonate, H_2O_2, or bulky-adducts (*38*).

Repressing cell division is critical to repair genomic DNA damage (Figure 3). A longer treatment time was associated with increased numbers of genes with altered expression especially those involved in cell cycle regulation and apoptosis (Tables 2 and 3) (Figure 4). This was not due to cytotoxicity because the mRNAs were isolated from viable cells. Cell cycle arrest was implicated by the cell

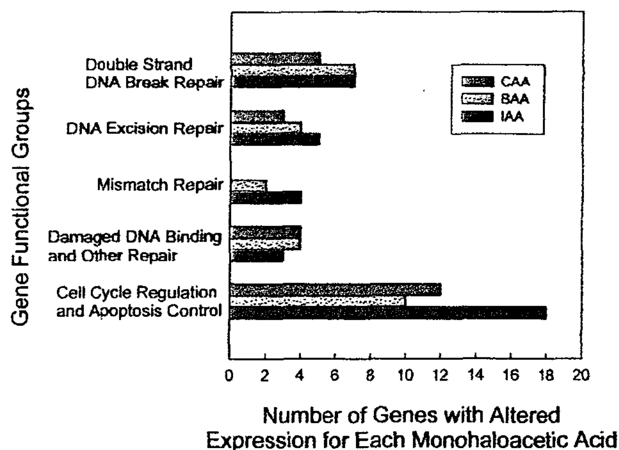

FIGURE 3. Changes in gene expression within gene functional groups in human FHs cells induced by BAA, CAA, or IAA.

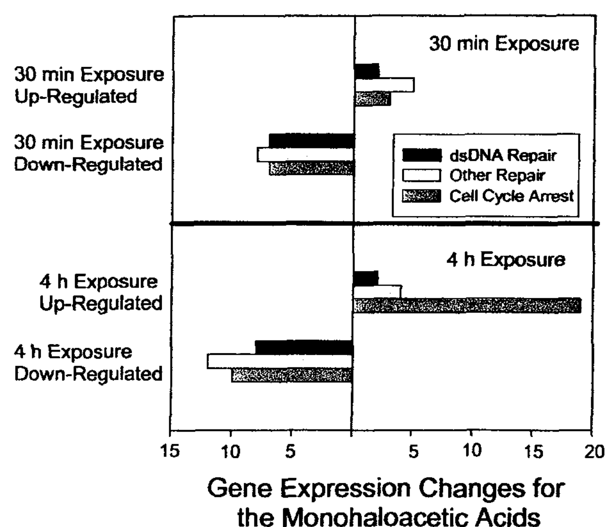

FIGURE 4. Changes in gene expression in human FHs cells induced by the monoHAAs as a function of treatment time.

density measurements of 4 h treatments with CAA and IAA (Figures 2 and 4).

We analyzed transcriptome profiles using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (*39*). The majority of the modulated genes were functionally categorized as genes responding to DNA damage or regulating cell cycle or apoptosis. Genes were assigned to different pathways as defined by Biocarta or the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Table 4). All of the treatments with one exception (CAA, 30 min) modulated genes involved in the ATM signaling pathway (*40*). The ATM signaling pathway is involved with tumor suppressor activity and the control of a broad network that includes the regulation of DNA repair and cell cycle regulation. Other modulated pathways include MAPK and p53 signaling (IAA 30 min and 4 h and BAA 4 h) and BRCA1, BRCA2, and ATR. MAPK signaling constitutes interrelated signal transduction networks that respond to cell growth factors, stress, cytokines, and inflammation. P53 function is to prevent the cell from progressing through the cell cycle in the wake of genomic DNA damage. BRCA1, BRCA2, and ATR pathways highlight the involvement of dsDNA break repair to monoHAA-induced genomic insult. Similar to the gene functional annotations, all of these

TABLE 4. MonoHAA-Induced Transcriptome Profiles Analyzed Using the Database for Annotation, Visualization, and Integrated Discovery (*39*)

pathway	BAA 30 min	CAA 30 min	IAA 30 min	BAA 4 h	CAA 4 h	IAA 4 h
ATM signaling pathway	X		X	X	X	X
cell cycle control				X	X	
cyclins and cell cycle regulation					X	
FC Epsilon RI signaling pathway						X
MAPK signaling pathway			X	X		X
p53 signaling pathway			X	X		X
role of BRCA1, BRCA2, and ATR in cancer susceptibility and dsDNA repair pathways	X		X	X	X	

pathways are involved in stress response to DNA damage and regulate different stages in cell cycle progression or apoptosis.

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Supporting Information Available

Additional information. This material is available free of charge via the Internet at http://pubs.acs.org.

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