Use of Quantitative Structure-Toxicity Relationships (QSTRs) to Predict Mutagenicity and Developmental Toxicity of Haloacetic Acids

Raghuraman Venkatapathy[1]*, Robert Bruce[2] and Chandrika Moudgal[2]

1. DOE, Oak Ridge Institute for Science and Education, Oak Ridge, TN

2. NCEA, U. S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

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Introduction

- Chlorine containing chemicals such as chlorine gas, chlorine dioxide and monochloramine have long been used as disinfectants in drinking water systems to eliminate microbial contaminants.

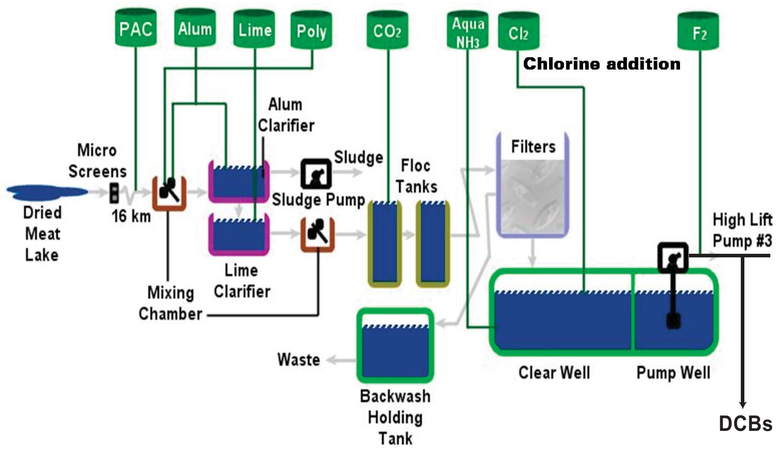
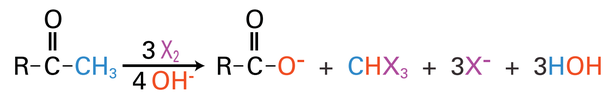


Figure 1.) Schematic of a water treatment plant for the City of Camrose, Canada. In the schematic, chlorine (Cl_2) is added to disinfect drinking water before it is sent for public consumption.

- Chlorine in the disinfectants and other halides react with humic acids and other natural organic matter to form halogenated disinfection by products (DBPs) such as trihalomethanes (THMs; Equation below) and halogenated acetic acids (HAAs).



- Various studies in the literature have shown that animal exposure to the DBPs may cause developmental, reproductive, neurotoxic and mutagenic/carcinogenic health effects (1).

- Experimental toxicity data is not available for a majority of DBPs.

- Quantitative Structure-Toxicity Relationships (QSTRs) can supplement this paucity of toxicological data.

Objectives

The objectives of this study were twofold:

- predict the mutagenicity and developmental toxicity of all possible halogenated acetic acids (HAAs) using QSTRs. Though fluorinated HAAs are generally not found in drinking water, they were included for comparison purposes.
- determine the cause of mutagenic and developmental toxicity of the HAAs.

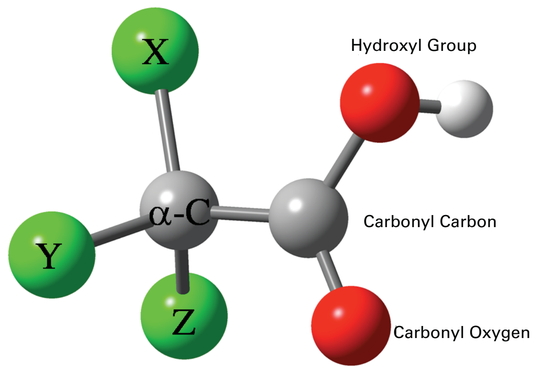
Methods

- The mutagenicity and developmental toxicities of acetic acid and the 34 halogenated acetic acids were evaluated using the Ames Mutagenicity and Developmental Toxicity Potential models in TOPKAT 6.1 (3), respectively.

- Physicochemical properties of the HAAs were calculated using Computer Aided Chemistry (4), CODESSA (5) and Molecular Connectivity (6).

- All statistical analyses in this study were performed using Microsoft Excel 2002 (7) and SAS (8).

Fragments in a Halogenated Acetic Acid



HAAs have seven 1- or 2-atom fragments: 3 halogens (X, Y, Z), 1 α-carbon, 1 carbonyl carbon, 1 carbonyl oxygen and 1 hydroxyl group. Each fragment may contribute positively or negatively towards the total toxicity of the HAA

Moiety Effect as Dependent Variables

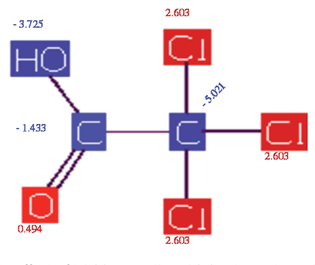


Figure 2.) Moiety effect of trichloroacetic acid due to mutagenicity

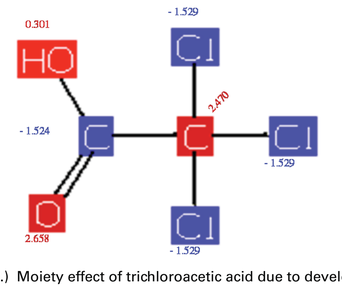


Figure 3.) Moiety effect of trichloroacetic acid due to developmental toxicity

- Illustration of the moiety effect (obtained from TOPKAT) of 1- and 2-atom fragments of trichloroacetic acid on its mutagenicity (left) and developmental toxicity (right).

- The value of the moiety effect is either positive or negative.

- Positive values imply that the given fragment contributes to the toxic effect and vice versa.

- The fragments are colored red if their value is positive, or blue if their value is negative.

- The magnitude of the numerical value is displayed by the darkness of the color: the larger the absolute value of the moiety effect, the darker the color.

Results and Discussion

TOPKAT Predictions

- Chlorine containing chemicals such as chlorine gas, chlorine dioxide and monochloramine have long been used as disinfectants in drinking water systems to eliminate microbial contaminants.

- For mutagenicity
  - all monohalogenated acetic acids were predicted positive
  - for dihalogenated acetic acids, the only positive predictions were those containing either bromine or iodine atoms or a combination of both
  - most trihalogenated acetic acids were predicted negative
- For developmental toxicity
  - all monohalogenated and trihalogenated acetic acids were predicted positive
  - all dihalogenated acetic acids were predicted negative

X	Y	Z	Mutagenicity	Developmental Toxicity
Monohalogenated Acetic Acids				
H	H	F	Positive	Positive
H	H	Cl	Positive	Positive
H	H	Br	Positive	Positive
H	H	I	Positive	Positive
Dihalogenated Acetic Acids				
H	F	F	Negative	Negative
H	F	Cl	Indeterminate	Negative
H	F	Br	Positive	Negative
H	F	I	Positive	Negative
H	Cl	Cl	Negative	Negative
H	Cl	Br	Positive	Negative
H	Cl	I	Positive	Negative
H	Br	Br	Positive	Negative
H	Br	I	Positive	Negative
H	I	I	Positive	Negative
Trihalogenated Acetic Acids				
F	F	F	Negative	Positive
F	F	Cl	Negative	Positive
F	F	Br	Negative	Positive
F	F	I	Negative	Positive
F	Cl	Cl	Negative	Positive
F	Cl	Br	Negative	Positive
F	Cl	I	Indeterminate	Positive
F	Br	Br	Negative	Positive
F	Br	I	Negative	Positive
F	I	I	Negative	Positive
Cl	Cl	Cl	Negative	Positive
Cl	Cl	Br	Negative	Positive
Cl	Cl	I	Negative	Positive
Cl	Br	Br	Indeterminate	Positive
Cl	Br	I	Indeterminate	Positive
Cl	I	I	Indeterminate	Positive
Br	Br	Br	Negative	Positive
Br	Br	I	Negative	Positive
Br	I	I	Negative	Positive
I	I	I	Negative	Positive

TOPKAT Structural Descriptors

- The impact of the α-carbon and the degree of halogenation of the acetic acids were ascertained by comparing the moieties of fragments to the total toxicity for the two endpoints.

- For Mutagenicity endpoint:
  - The carbon-halogen fragment may be responsible for mutagenicity among the monohalogenated acetic acids.
  - The results were not as clear for the di-halogenated and tri-halogenated acetic acids becuase of hidden correlations.
- For DTP endpoint:
  - The presence of a CHX fragment (carbon is attached to a single hydrogen atom and one or more halogen atoms) may prevent the occurrence of developmental toxicity.
  - The presence of CH_2X or CX_3 (carbon is attached to two hydrogens and one halogen, or is completely halogenated) may promote developmental toxicity.
  - The presence of CHX fragment in a chemical seemed to inhibit developmental toxicity in all aliphatics as evidenced by a negative TOPKAT prediction for 2-chloropropane (CH_3-$CHCl$-CH_3) and 1,1-dichloropropane (CH_3-CH_2-$CHCl$-Cl) versus a positive TOPKAT prediction for 1-chloropropane (CH_3-CH_2-CH_2Cl).

Toxicity Mechanism elucidation using QSTR Equations

- QSTRs were developed with the moiety of the α-carbon and the halogens as the dependent variable to elucidate cause of mutagenicity.

- Four QSTRs were generated for each class of HAA: sum of moieties of the three halogens, maximum moiety among the three halogens, average moiety of the three halogens and the moiety of the a-carbon as the dependent variables.

- The best correlated QSTR among the four mentioned above was used to elucidate the toxicity mechanism for the three classes of HAAs: mono-, di-, and tri-halogenated acetic acids.

Monohalogenated Acetic Acids

- Equation (1) illustrates the correlation between the moiety of the halogen and the Electrophilic frontier Density (EFD) of the halogen for monohalogenated acetic acids.

- The EFD is a measure of the susceptibility of a chemical to attack by an electrophile (positively charged species).

- The slope between the moiety of the halogen and the EFD of the halogen (=2.81; Equation 1) indicates that the moiety increases with an increase in the EFD of the halogen.

- The results thus seem to indicate that the EFD of the halogen-substituted α-carbon (CH_2X) is the cause behind the mutagenicity of monohalogenated acetic acids.

Equation 1.) Moiety of halogen = 2.81 (± 0.110) [EFD-halogen] + 7.84 (± 0.0986)

N=4, r^2= 0.997; r^2_{adj}=0.995; F=614.8; q^2= 0.928

Dihalogenated Acetic Acids

- The sum of moieties of halogens is correlated with the number of strong hydrogen bond acceptors in a HAA (nHBa; Equation 2).

- Hydrogen bonding occurs between a hydrogen atom, which is called a hydrogen bond donor, and a strongly electronegative heteroatom such as oxygen or nitrogen, which is called a hydrogen bond acceptor.

- All acetic acids have a minimum of two hydrogen bond acceptors, namely: the carbonyl group and the hydroxyl group.

- Fluorines and chlorines are considered as strong hydrogen bond acceptors while bromines and iodines are not.

- Difluoroacetic acid has 4 hydrogen bond acceptors while diiodoacetic acid has 2 hydrogen bond acceptors.

- The moiety of halogens increases with size (F < Cl < Br < I).

Equation 2.) Summation of moieties of halogens = - 4.48 (± 0.482) [nHBa] + 9.83 (± 0.458)

N = 10; r^2= 0.983; r^2_{adj}= 0.981; F = 458.6; q^2= 0.983

Trihalogenated Acetic Acids

- The moiety of α-carbon is related to the number of fluorine atoms, the maximum partial charge on a chlorine atom and the density of the Lowest Unoccupied Molecular Orbital (LUMO) (Equation 3).

- The LUMO density is on the α-carbon or one of the halogens for all trihalogenated acetic acids (data not shown).

- The LUMO densities on the α-carbon, halogens, and a maximum partial charge on a chlorine atom generally promotes nucleophilic reactions (attack by a negatively charged species), with an attack on the α-carbon or the halogen (2).

- Since the monohalogenated acetic acids appear to be mutagenic due to electrophilic reactions (attack by a positively charged species) between the DNA and the halogenated acetic acids, this could explain why the monohalogenated acetic acids are Ames-positive while trihalogenated acetic acids are Ames-negative.

Equation 3.) Moiety of α-carbon = - 0.360 (± 0.240) [LUMO density] - 1.91 (± 0.241) [Number of F atoms] + 1.25 (± 0.209) [Maximum partial charge for a Cl atom] - 5.70 (± 0.180)

N = 20; r^2= 0.959; r^2_{adj}= 0.952; F = 125.6; q^2= 0.961

Conclusions

- TOPKAT predicted that a majority of mono- and di-halogenated acetic acids were mutagenic while most trihalogenated acetic acids were non-mutagenic.

- TOPKAT also predicted all mono- and tri-halogenated acetic acids to be developmental toxicants while dihalogenated acetic acids were predicted to be non-toxic.

- The presence of a >CHX fragment seems to inhibit developmental toxicity while the presence of -CH_2X or -CX_3 fragments seemed to promote developmental toxicity.

- The results of the QSAR analysis seem to indicate that the mutagenicity of the HAAs is dependent on their ability to undergo electrophilic reactions with specific fragments in the DNA or form hydrogen bonds with hydrogen bonding donor sites on DNA, thereby suggesting that an electron donating ability is essential for these chemicals to be mutagenic.

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* Corresponding author; contact address: NCEA-USEPA, MS-117, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, e-mail: venkatapathy.raghuraman@epa.gov; Phone: (513) 569 7077; Fax: (513) 569 7475