INTRODUCTION

In work sponsored by the Next-Generation Fire Suppression Technology Program, the Center for Global Environmental Technology at University of New Mexico and GlobeTech, Inc. are identifying promising tropodegradable bromocarbons and evaluating their performance as replacements for Halon 1301. While a number of candidate tropodegradable compounds have been identified from the alkene, ether, and amine families the current interest is primarily on bromofluoroalkenes and bromofluoro-ethers. The current project, though only partially complete, is focused on improving the methods for estimating the cardiac sensitization properties of chemicals of interest to the halon replacement effort and has identified some potentially promising approaches.

The recently announced results of a cardiac sensitization study by the Advanced Agent Working Group (AAWG) on 2-bromo-3,3,3-trifluoropropene indicate that its LOAEL and NOAEL are respectively, 1.0% and 0.5% by volume. Though there remain some questions that will be resolved once the final test report is made available, these values indicate that 2-bromo-3,3,3-trifluoropropene is equivalent to Halon 1211 insofar as cardiac sensitization is concerned. As the goal of the NGP program is to identify a replacement compound for Halon 1301, additional compounds must be selected from the promising chemical families.

The equivalence of 2-bromo-3,3,3-trifluoropropenes' cardiac sensitization to that of Halon 1211 does provide some promise of eventual success for the search for halon replacements in the bromofluoro-alkene family of compounds, Table 1. While several bromofluoroalkenes have been prepared only one has been subject to a standard cardiac sensitization test. It is expected that differences in fluorine and bromine substitution patterns as well as the overall degree of
fluorination represented within the bromofluoro-propenes' family of compounds will exhibit a wide range of cardiac sensitization responses in dog exposure studies.

Table 1. Representative LOAEL and NOAEL values for fire suppressing agents.

<table>
<thead>
<tr>
<th>CHEMICAL</th>
<th>FORMULA</th>
<th>LOAEL (V%)</th>
<th>NOAEL (V%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halon 1301</td>
<td>CF$_3$Br</td>
<td>7.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Halon 1211</td>
<td>CF$_2$ClBr</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Trifluoromethyl Iodide</td>
<td>CF$_3$I</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>2-Bromo-3,3,3-trifluoropropene</td>
<td>C$_3$F$_3$H$_2$Br</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Currently no method exists for the estimation of cardiac sensitization performance and a single test costs approximately $70,000, not including the chemical. This cost effectively prohibits the testing of more than a few compounds by the established method and leaves the halon replacement research community with limited options. The inability to efficiently screen compounds using the established dog exposure test method also precludes the ready development of useful trends to guide in compound selection and requires that new cardiac sensitization performance estimation methods be identified or developed to fill this role. These new methods of ranking compounds may utilize the results of single test protocols or combinations of test methods. Regardless, short of a dramatic increase in the resources for toxicity testing, a new approach to the estimation and ranking of a compound's estimated cardiac sensitization properties is essential to continued progress in the area of halon replacement.

Past work on toxicological assessments of halon replacement compounds employed QSAR studies to predict toxicity, identified trends based on limited data relating composition and structure as well as substitution patterns to toxicity, and in limited cases employed chemical reactivity estimates[1]. All of these methods have in the past proven useful in estimating unknown toxicological, biological or physical properties of chemicals from known information on related chemicals or chemical families. QSAR approaches often employ correlation equations, determined by linear regression analysis techniques using general, steric,
hydrophobic, electronic, and molecular structure properties of known chemicals, to form the basis of the toxicity analysis.

Molecular connectivity based QSAR’s require that indices reflecting structure be determined, and generally the amount of data needed is less than that for property-activity QPAR’s. In both cases, sufficient toxicity data must be available with which to develop correlations capable of providing toxicity estimates of unknown substances.

Toxicity estimation methods involving in vitro test protocols are now in widespread use. Methods for evaluating the impacts of chemicals on target organs (kidney, liver, skin, heart, etc.) have in many cases become well developed. The potential for employing a modified in vitro method as a means for evaluating the relative potential of a series of chemicals to induce arrhythmia is readily apparent. As such, the identification of an existing in vitro (cell or tissue) based protocol or the development of a new protocol enabling the ranking of compounds with respect to induction of cardiac arrhythmia could greatly enhance the selection of optimal compounds.

**GOALS: IDENTIFICATION OF IMPROVED SCREENING METHODS**

This project is intended to address the need for improvements in existing methods and development of new methods and approaches to the estimation of cardiac sensitization. The approaches being taken in this project to improving the existing QSAR methods, locating additional exposure information, and identifying promising in vitro approaches are described briefly below.

a) A review of compound attributes and physical properties not currently employed in QSAR predictions of cardiac sensitization.

b) A review of anesthesiology research to identify and collate relevant test data, SAR methods and any attributes that might be employed in evaluating the cardiac sensitization properties or establishing a relative ranking of the cardiac sensitization properties of a series of brominated fluorocarbons.
c) A search for possible in vitro methods applicable to the low cost assessment of the cardiac sensitization potential (or relative performance) of a candidate halon replacement chemical or series of such chemicals. This effort will identify and list existing research centers employing in vitro methods to study cardiac muscle and involve contacts with medical researchers to determine additional sources of information on cardiac sensitization related topics.

**IN VITRO METHODS**

In vitro methods represent a very promising approach to the screening of chemical candidates of interest to the halon replacement research community. The in vitro method refers to the use of tissue or cells derived from living organisms. In general, cell lines are grown in a manner analogous to the growth of bacterial cultures. The cells and tissues employed come from a wide range of donor species including humans, rats, mice, and monkeys.

There are several phenomena which can be monitored in in vitro studies at present. In myocytes (heart cells) cell cultures and single cell suspensions as well as tissue samples or isolated muscle, rhythmic cellular contraction occurs naturally. Observations of changes in rhythm or the onset of arrhythmia following chemical exposure have been reported in many research papers.

A prior review of in vitro methods and their limitations provides a useful summary of this technology and a good indication of its potential for providing a means to give ranking information useful to the research community[2].

Dog ventricular myocytes isolation methods [3], based on procedures for preparing adult rat heart cells [13] have been reported. Subcellular organelles have been used to investigate mechanisms of cardiac function [4] and may lend themselves to the development of a ranking method. Human heart tissue preparations have been used in studies of in-situ signal conduction [14]. Preparations of rat and guinea pig atrial tissues have been described by a number of researchers [5-8].

Each of these methods yield unique observations, have specific limitations and inherent difficulties. In the final analysis it is not unreasonable to employ not one but several of these in vitro methods in a testing program involving an establishment of a baseline response to existing halons and compounds whose cardiac sensitization are documented.
Table 2. In vitro Cardiac Cell Systems.

<table>
<thead>
<tr>
<th>Model</th>
<th>Life stage</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial cells</td>
<td>Fetal</td>
<td>Human</td>
<td>Goldman (9)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Neonatal</td>
<td>Rat</td>
<td>Mark and Strasser (10)</td>
</tr>
<tr>
<td>Ventricular Cardiomyocytes</td>
<td>Neonatal</td>
<td>Rat</td>
<td>Schanne (11)</td>
</tr>
<tr>
<td>Ventricular Cardiomyocytes</td>
<td>Neonatal</td>
<td>Dog</td>
<td>Liu (12)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Adult</td>
<td>Rat</td>
<td>Vahouny (13)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Adult</td>
<td>Rabbit</td>
<td>Dani (14)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Adult</td>
<td>Dog</td>
<td>Youker (15)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Adult</td>
<td>Feline</td>
<td>Woosley (16)</td>
</tr>
<tr>
<td>Ventricular Cardiomyocytes</td>
<td>Adult</td>
<td>Guinea Pig</td>
<td>Failli (17)</td>
</tr>
<tr>
<td>Ventricular Cardiomyocytes</td>
<td>Adult</td>
<td>Rat</td>
<td>Eid (18)</td>
</tr>
</tbody>
</table>

The results of such a study will help establish the repeatability of the method, give confidence in the ability of the method to "rank" compounds, as well as provide further information useful to the eventual understanding of the mechanism of cardiac sensitization. This method must be:

- Sensitive to variations in air concentrations in the range of interest (1% to 7.5%)
- Reflect the cardiac sensitization results of known compounds (Halons 1211 & 1301, CF3I, HFC’s, etc.)
- Result in a ranking of compounds by air concentration

**QUANTITATIVE STRUCTURE PROPERTY RELATIONSHIPS (QSAR)**

QSPR-based estimates of the compound properties of potential halon replacement compounds have been performed and reported by Mainstream Engineering Corporation[1]. This effort modeled boiling point, flame extinguishment, global warming potential (GWP), ozone depletion potential (ODP), tropospheric lifetime, cardiac sensitization, and LC_{50} values. A major hindrance to all QSAR efforts is the lack of known toxicity data points for related compounds or chemical fragments. Without data upon which to establish relationships between structure and toxicity, the basis for subsequent predictions become tenuous at best. In this regard, in vitro studies may well help by providing data for the onset of adverse effects for specific chemical concentrations under a variety of in vitro methods. Incorporating the results of in vitro exposure
studies on cardiac myocytes, tissues, perfused organs, and associated nerve tissues into existing QSPR could prove of great value to the identification of predictive trends.

CHEMICAL STRUCTURES OF INTEREST

Tropodegradable chemical structures of greatest interest incorporate unsaturated carbon-carbon bonds. These sites of unsaturation are vulnerable to attack by OH radicals and ozone with the result that the compound decomposes into polar species that “rain out” of the atmosphere. While the simplest unsaturated species are those based on the ethene molecule, Figure 1, the propenes, Figure 2, represent the greatest hope for the eventual identification of a Halon 1301 replacement.

![Ethene](image1.png)

"Ethene"

Figure 1. Flat structure of ethene molecule

![Propene](image2.png)

"Propene"

Figure 2. Flat structure of propene molecule.

The bromofluoro propenes of greatest interest are the bromo-difluoropropanes, bromo-trifluoropropanes, and the bromo-tetrafluoropropanes. Together these compounds represent a very large number of unique structural isomers. For example, the site of the bromine in the general group of bromodifluoropropanes can be on any of the three carbons.
In addition, for those structural isomers where bromine is bonded to the terminal carbon of the ethene fragment, there are several unique bonding patterns for the two fluorines; i.e. 1-Bromo-3,3-difluoropropene*, 1-Bromo-2,3-difluoropropene*, 1-Bromo-1,3-difluoropropene*, and 1-Bromo-1,2-difluoropropene (* indicates “cis” or “trans” isomers possible). Each of these seven structures will exhibit differences in chemical reactivity, polarity, solubility, and stereochemistry (shape). Likely structural isomerism effects on the properties of a specific chemical are listed below:

- Polarity of the compound – Solubility
- Boiling Point
- Reactivity
  - Chemical
  - “Lock and Key” reactivity - blocking enzymatic sites
  - Decomposition in vivo
- Toxicity (inhalation, cardiac etc.)

The bromofluoro propene, trans-1-bromo-3,3,3-trifluoropropene, induced notable muscular effects (tremor) in the tissues of the exposed rats following acute inhalation toxicity tests. These effects were noted during post exposure necropsy. The tested compound's structure was assigned based on proton-to-fluorine NMR spin coupling as the “trans” isomer shown below with the “cis” isomer of this compound, Figure 3. These two isomers are expected to differ in reactivity and polarity due to the relative positions of the bromine and CF3 group and are not therefore expected to necessarily have the same effect on muscle.

![Figure 3. “Trans” and “Cis” structural isomers of 1-bromo-3,3,3-trifluoropropene.](image)

Similarly, 2-bromo-1,3,3,3-tetrafluoropropene exists as "cis" and “trans” structural isomers, Figure 4, and again its cis and trans structural isomers would be expected to have distinctly different toxicity and physical properties.
CUP-BURNER METHOD IMPROVEMENTS

Typically, to test a chemicals cup-burner extinguishment performance using a full scale cupburner requires approximately 500g to 1000g of the test chemical. Where research chemicals are concerned this is usually prohibitively expensive. Costs for the synthesis of new compounds for which viable.syntheses and occasionally purification processes must be developed are typically in the range of $2,500 for 10g to 50g. This situation lead to the early development of the NMERI Standard cup-burner which requires only approximately 50g to run a modified cup-burner test and provide a minimum of three tests of extinguishment. As research into the synthesis of new compounds continues, costs per gram are increasing, in some cases quite dramatically. This has necessitated an effort to further refine the sample introduction methods being employed in the cup-burner method. NMERI tested and now employs a sample introduction and dispersion method based on well established nebulizer technology widely employed by inductively coupled plasma (ICP) analytical instruments, Figure 5. In the analytical application the nebulizer aspirates the sample and disperses it as a very fine mist into the hot torch of the ICP. In our cup-burner, we have replaced aspiration with a syringe pump and 2.5cc Gastight™ syringe. In addition, because we have found that higher boiling compounds condense onto cool surfaces resulting in loss of agent from the air stream and elevated cup-burner extinguishment values, we employ a pre-heater column, Figure 6. In practice, the entire cupburner base and the evaporation/mixing column are wrapped with heating tape and insulating material to prevent the condensation loss of agents under test due to cool

Figure 4. Cis and trans 1-bromo-1,3,3,3- tetrafluoropropene
glass surfaces. Some experimentation is required to develop the heating required to prevent condensation while not overheating the apparatus.

The nebulizer as supplied requires a nominal air flow of 1L/min. - this is established using a regulated pressure source for air and appropriate valving. As the NMERI Standard cupburner requires a nominal 10L/min. total flow a "make-up" stream of air is introduced around the nebulizer tip.

This modified sample introduction method allows three sequential extinguishment tests to be run on the same sample. Sample may be introduced over a period of several minutes and the flow rate ramped up in a very reproducible and controlled fashion.

Advantages and additional details of this method are itemized below.

- ICP Nebulizer – liquid agents uniformly misted, wide range of flow rates. ~5gm of agent needed.
- Heated nebulizer zone and cup-burner base prevents condensation, vaporizes higher boiling compounds; low BP compounds are chilled.
- Syringe pump accurately controls liquid agent flow rate.
- Air flow monitored via mass flow meter.
- Barometric pressure and room temperature corrections applied to calculate actual air concentrations.
Figure 5. Cup-Burner (ICP) Nebulizer

Figure 6. Evaporation/Mixing Column for Cup-Burner.
CONCLUSIONS

The prospects for developing a screening method based on in vitro toxicity testing methods are promising. However, such a screening method will be of limited use and will potentially fail to lead to the identification of the ideal candidate if only a few halon replacement candidate compounds can be tested. Therefore both compound acquisition efforts and cardiac sensitization screening method development need to proceed in parallel.

ACKNOWLEDGMENTS

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