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IDENTIFICATION AND CHARACTERIZATION OF CONSERVATIVE ORGANIC TRACERS FOR USE AS HYDROLOGIC TRACERS FOR THE YUCCA MOUNTAIN SITE CHARACTERIZATION STUDY

PROGRESS REPORT

APRIL 1, 1993 to JUNE 30, 1993

DOE Cooperative Agreement

No. DE-FC 08-90NV10872

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The Tracer Toxicity Report has been completed and a copy is attached. Because of the toxicologist's comments we have decided to split the report into two, one for the fluorinated benzoic acids and one for the fluorinated aliphatic acids. The assumptions made in the report regarding the amount of tracer that will be used, dilution of the tracer during the test and the length of exposure (if any) to individuals drinking the water were made by the authors. These assumptions must really come from the USGS hydrologists in charge of the c-well tracer testing program. A copy of the report, with a request to make his own calculations has been sent to M. J. Umari.

Accurate estimates of dilution of the tracer during the test are also important because of solubility limitations of some of the tracers. Three of the difluorobenzoic acids have relatively low solubilities and may not be usable if the dilution estimates are large.

The toxicologist that reviewed the document agreed with our conclusion that the fluorinated benzoic and toluic acids do not represent a health hazard if used under the conditions as outlined in the report. We are currently testing 15 of these compounds, and if even if three difluorobenzoic acids cannot be used because of solubility limitations we will still have 12 tracers.

The toxicologist felt that the aliphatic fluorinated acids potentially present more of a health risk than the aromatic. This assessment was based on the fact of a known allergic response to halothane anesthetic. This risk, although minimal, is known and he felt that was enough reason to recommend against their use. The authors feel that the toxicologists interpretation of this risk was overly conservative, however, we will not go against his recommendation at this time for the following reasons. First, without the aliphatic compounds we still have 12 to 15 fluorinated aromatic acids which, according to M.J. Umari should be enough for the c-well tests. Second, to get a permit to use aliphatic compounds would undoubtedly require a hearing which could be quite lengthy. Without the aliphatic acids the DOE may be able to have the existing permit amended to include all of the fluorobenzoic acids. This may not require an additional hearing.

PLANNED WORK FOR THE NEXT QUARTER

Continue stability and soil column work.
Develop HPLC-MS methods for the aromatic acids.

Tonya Dombrowski, who has been doing most of the tracer work and all the HPLC-MS analyses, will be leaving at the end of July to start a PhD program at the University of Kansas. This will impact the level of activity on this project. Plans have been made to hire a replacement.

ORGANIC TRACER TOXICITY REPORT
FLUORINATED BENZOIC ACIDS

DOE Cooperative Agreement

No. DE-FC 08-90NV10972

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INTRODUCTION

Ground water tracers are solutes dissolved in or carried by ground water to delineate flow pathways. Tracers provide information on direction and speed of water movement and that of contaminants which might be conveyed by the water. Tracers can also be used to measure effective porosity, hydraulic conductivity, dispersivity and solute distribution coefficients. They can be naturally occurring compounds or elements, or they can be completely foreign to the environment. The latter is generally preferred. For most applications tracers should be conservative, that is, move at the same rate as the water and not adsorb to aquifer materials. Aquifer materials are generally negatively charged, so anionic tracers will not adsorb and will move with the water.

A tracer should behave as follows:

1) Be water soluble.
2) Not adsorb on the aquifer material (i.e., be conservative).
3) Be chemically and biologically stable for the duration of the test.
4) Be foreign to the environment.
5) Have excellent analytical sensitivity.
6) Have a low toxicity.

Eighteen fluorinated organic acids (table 1) are presently being studied as possible tracers for the Yucca Mountain Site Characterization Project. There are two additional isomers of trifluorobenzoic acid, 2,3,6-trifluorobenzoic acid and 2,4,6-trifluorobenzoic acid, that are not presently being studied, but were included in this review of currently available toxicological information. Since these compounds are not used in large quantities in industrial or other processes, only limited data are available, especially human toxicology information.

It should be pointed out here that analytical sensitivity and stability are important adjuncts to the issue of toxicity. Virtually any element or compound that is introduced into an aquifer will cause some degradation of the water quality if the concentration is high enough. Tracers with high analytical sensitivity will permit lower input concentrations and therefore reduce the potential for degradation of the water quality. For example, a difference in sensitivity between 100 ppm and 100 ppb may mean introducing only 1 kg of tracer into the aquifer instead of 1,000 kg.

All organic chemicals dissolved in water, given enough time, will be degraded to carbon dioxide and inorganic ions (if any are contained in the parent compound) by bacterial action. The time required for this to occur depends on many factors, including lethality to indigenous flora, initial concentration, the capability of the microorganisms to utilize the compound and the probability of contact between a molecule and a degrading organism. Since the majority of microorganisms in the subsurface are associated with the host rock and not the water, strong anions will have limited contact with them, thus increasing their longevity in the environment. The fluorinated organic acids, suggested here as potential tracers, are generally quite resistant to chemical and biological activity. Although not totally unreactive, as anions they will have reduced probability of interaction with bacteria.

POTENTIAL for EXPOSURE to TRACER-CONTAINING WATER

Concerns regarding exposure to pure tracer and the various tracer solutions will depend on the procedures used for transportation, injection and recovery during the tracer tests. For the tracer tests at the c-wells, we anticipate that laboratory space will be available at the site. Therefore, we intend to transport pure tracer to the site and prepare highly concentrated, almost saturated, injection solutions at the site. These highly concentrated solutions will be diluted to approximately 0.1% to 2% solutions for injection in the tracer injection apparatus. The concentration of the injection solution will depend upon the analytical sensitivity of the tracer and the estimated dilution that will take place on its migration to and into the recovery well, which will be from 100 to 200 feet away. The actual dilution will depend on several aquifer parameters, which will be measured by the tracing test. Estimated peak tracer recovery concentrations are expected to range from 1 to 20 ppm.

Location	Physical Form	Amount	Volume	Concentration
Laboratory	Solid	1 kg	–	–
Lab & Test Well	Liquid	1 kg	200 L	5,000 ppm (0.5%)
Groundwater	Liquid	1 kg	10^6 L	1.0 ppm average 10. ppm peak

There are three exposure scenarios for humans and the environment which may result from the use of these tracer compounds. The first is direct contact with or accidental loss of the pure liquid or solid tracer compound. This would happen if the workers spilled the solid (or pure liquid) material or used poor safety practices in the laboratory. The second scenario would occur if the 200 liters of 0.5% solution were spilled in the laboratory or on the ground before the solution was introduced into the injection wells. In both of these cases steps can be taken immediately to rectify or remediate the occurrence. The third scenario of contact is the long-term exposure to very low concentrations of the tracer which are not recovered during the study and which migrate out of the study area. In this case, exposed persons may not be aware of their contact with these compounds.

In this report we will address all three scenarios, provide as much toxicity information as is available and give realistic estimates of the probability of contact with the proposed tracers. In the first two scenarios, the exposure will be acute and the actions will be supervised by technical personnel. In the third scenario, the exposure will be sub-chronic and will involve the greatest risk to the general population.

PURE TRACER

Current estimates are that 100 g to 3 kg of tracer will be used per test. Injection solutions will be prepared on site to minimize possibilities of accidental spills during transport. Handling of the pure tracer to prepare the concentrated injection solution will be limited to trained chemistry personnel. Standard laboratory precautions will be used when these materials are handled. This includes wearing appropriate apparel, safety glasses and respirators if necessary. All warnings noted in the material safety data sheets (MSDS) will be observed.

If the pure tracer is accidentally released into the environment, remediation will depend upon the physical state of the tracer chemical. If the tracer is a solid, remediation will entail removal of the chemical from the ground and any soil that was in contact with the tracer. Since the solid chemical will not react with or penetrate the soil, this should entail only small amounts of soil which may have to be removed. This will be done primarily to prevent contamination so that the tracer can be used without background interference. Liquid tracers may react with and penetrate the soil; this will require more extensive clean-up to prevent contamination.

Potential health effects will be limited to acute toxicity resulting from a single exposure. Since the personnel will be wearing protective clothing and the compounds under consideration have a low volatility, exposure via the normal pathways (ingestion, inhalation or dermal absorption) is expected to be very small.

CONCENTRATED INJECTION SOLUTIONS

The concentrated tracer solutions which will be injected into the wells will vary between 0.1% and 2%. Even though the tracers are acids, the injection solution will be basic to increase the solubility of the tracer and to be closer to the ground water pH. Accidental spills of the injection solution will present problems similar to those described above for the liquid tracers, except there will be larger volumes. The larger volumes, 50 to 200 liters, will prevent complete removal of the material if all or a large portion is spilled. The use of that tracer may then be lost to the project, especially if the spill occurs in the immediate area surrounding one of the wells. Over time, bacterial degradation in the root zone will degrade the tracer to fluoride and carbon dioxide and rainwater will eventually carry some of the compound to groundwater.

Accidental ingestion of the tracer injection solutions by humans or animals are highly unlikely due to the limited access to the area and because the tracer injection apparatus will be a closed system. Exposure from other routes is expected to be very small because of the use of protective clothing. Potential health effects will be limited to acute toxicity resulting from a single exposure.

LOW CONCENTRATION SOLUTIONS

During the tracing test, the tracer will migrate from the injection well to the pumping

well, a distance of approximately 100 to 200 feet. It is anticipated that the tracer injection solution will be reduced in concentration by three to six orders of magnitude during its migration. This will result in estimated peak concentrations of 20 ppb to 20 ppm at the pumping well. The amount of tracer recovered will depend upon the length of the tracing test, as well as specific aquifer conditions. Tracer recoveries can be as high as 70 to 80 percent, but typically average 50% or less. Recoveries of two fluorinated benzoic acids that were used as ground water tracers at the Waste Isolation Pilot Project (WIPP) site in New Mexico ranged from 15 to 53 percent (Kelly and Pickens, 1986), and when thiocyanate was used as a tracer in Hanford Washington the recovery was 60% (Thompson, 1982).

If the minimum percent recovery value (15%), and the maximum injection mass (3.0 kg) are used, then 2.55 kg of tracer will remain in the aquifer per test. The concentration of the tracer, which will have been diluted 3 to 6 orders of magnitude during the tracer test, will be further reduced as the tracer is dispersed as a result of the natural ground water movement. By the time it migrates off-site, or reaches a Nevada Test Site well that is being used to supply drinking water, the tracer concentrations will be at incredibly low levels if it has not already been degraded by bacteria.

Potential human health effects would be limited to acute or chronic effects resulting from sub-chronic exposure. The worst case exposure would probably result from the use of the recovery well water for drinking water. Because of the limited access to this site, it is not possible that persons living nearby could use well water for domestic drinking water but we will determine the risk from such a scenario in case other test sites are chosen in the future. The exposure would be sub-chronic because there is a single injection of the tracer which then moves with the groundwater flow (50 - 150 L/min). For persons using water from a well (which is fixed in space), exposure to the tracer would exist during the few weeks required for the tracer plume to pass the well.

CHEMISTRY

Fluorinated organic compounds were selected as potential tracers because of their long term environmental stability and low reactivity. The carbon-fluorine bonds are the shortest of the carbon-halide bonds and are therefore expected to be the strongest and least reactive. Low reactivity and low toxicity are characteristics of numerous carbon-fluorine compounds. For example, Teflon (polytetrafluoroethylene) is extremely inert and is used in a variety of products that humans are in contact with every day, from containers to Gore-Tex. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and isofluorane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) are anesthetics that are used throughout the world. Halons are used as fire extinguishers and freons have been used for many years as refrigerants and propellants because of their stability in the lower atmosphere and low human toxicity.

As a general rule, it can be stated that the greater the number of halogen atoms on a molecule, especially fluorine, the greater its stability. Perfluorinated acids such as pentafluoro-benzoic acid (PFBA) and trifluoroacetic acid (TFAA) are therefore extremely resistant to chemical attack. Because fluorine is a strong electron withdrawing atom, it also

follows that the more fluorine atoms on an organic acid molecule, the stronger that acid becomes.

The acid functional group (carboxyl group), is completely oxidized, difficult to reduce and does not undergo other reactions in aqueous (ground water) solutions. Essentially all of these acids are ionized at typical ground water pH values. The weakest acids being evaluated have dissociation constants (pK_a) values of approximately 4.5, which means they will all be completely ionized and in their anionic form at water pH values of six or higher (Freiser and Fernando, 1963). The increasing number of halogens on a compound increase the ionizability, and consequently, the water solubility.

Ionized compounds are generally not able to pass through the microbial cell membranes and therefore are not readily utilized by these systems. However, when they are utilized, the reactions are almost identical to the non-fluorinated parent compound.

In addition to the chemical and microbial stability, the carbon-fluorine bond is also difficult for mammalian enzyme systems to break down, and compounds that contain these bonds are generally excreted without loss of fluoride rather than metabolized. This tends to make such compounds less toxic. These fluorinated acids are among the most polar of the halogenated organic compounds. They contain a polar functional group, the acid or carboxyl group, and the presence of that group facilitates their excretion as the parent compound directly, or as conjugates of the parent compound.

ENVIRONMENTAL BIODEGRADATION of CONTAMINANTS

Microorganisms are capable of degrading aromatic hydrocarbons and the aromatic fraction can be used to sustain bacterial growth. Utilization of organic compounds occurs via catabolic pathways catalyzed by a series of enzymes. The product formed may then be utilized by another population of bacteria or the resulting compounds may be resistant to further degradation.

Environmental factors that affect the rate and extent of biodegradation of a chemical tracer are its concentration, the population of microorganisms, presence of other nutrients (including trace metals), dissolved oxygen, pH, temperature salinity and contact time. Another factor which may affect degradation is prior exposure of the microbial population to the tracer or to a similar compound. One must be careful about assumptions made because of similarities between two chemicals or environmental conditions. Differences such as positional isomers, type of halogen or activity of the microbial population may alter the expected results.

The fluorinated aromatic acid tracers have pK_a values of 4.5 or less, making them anions at normal ground water pH. Ionized compounds are not able to pass through the microbial cell membrane and therefore are not generally utilized. Some bacteria however have acquired or can acquire the ability to transport such compounds as the result of mutations. It is possible then, during extended tracer tests of 7 to 30 days to lose a small percentage of tracer as the result of bacterial activity. During long tracer tests (1 to 1.5 years) all of the tracer may be degraded.

The success of microbial degradation is also related to previous and/or low level chronic exposure to the compound. Chronic sublethal exposure may increase the tolerance of microorganisms and allow for adaptation of the biological community. The microbial population would then more rapidly respond to the presence of that and/or similar compounds, produce the necessary enzymes, and degradation rates would increase. If the compound is chemically analogous to a natural substrate a similar situation may occur with the microbial population readily producing enzymes necessary for degradation. Some chemicals can also act as initiators. These compounds may or may not be degraded in the process, but their presence gives bacteria the ability to degrade compounds of similar chemical structure. Without the presence of the initiator degradation may not take place.

The concentration of the organic compound is also important in determining the rate of biodegradation. Mineralization rates have been shown to be directly proportional to the concentration of the compound over a wide concentration range. The compound may also be in a concentration too low for degradation to occur. The lowest concentration of a compound that will support microbial growth is termed the threshold. At concentrations too low, degradation produces only enough energy for maintenance of the microbial population and growth is absent. Under these conditions degradation rates are retarded due to lack of an actively growing population. At higher concentrations, diffusion provides molecules to the cell surfaces at a rate sufficient to meet the needs of energy of maintenance and cell growth. The microbial population is stimulated and the number of microbes increase with time and degradation rates are enhanced. Thresholds vary with the microbial population involved. If the population is oligotrophic in nature (grows and survives in an environment with a low concentration of available carbon) a lower threshold may exist.

It is not practical to perform field experiments to attempt to estimate the stability of these tracers in the environment. Laboratory stability tests show the mono-fluorinated benzoic acids are not stable for more than 30 days, while some di- and poly-fluorinated benzoates were stable for more than one year (Thompson and Stetzenbach, 1980). Actual field use has also shown that some isomers are more stable than others (Bowman, 1984) and that some are stable for more than one year under saturated (Jones et al, 1992) and unsaturated (McCray et al, 1983) conditions. However, prolonged use of numerous isomers in an area may enhance degradation (Barackman, 1986), presumably by bacteria.

MICROBIAL STUDIES OF FLUOROBENZOIC ACIDS

All the information related to the microbial degradation of fluorinated benzoic acid is for the mono- and di-fluorobenzoic acid isomers.

A comparison was made of the breakdown of fluorobenzoic acid by *Pseudomonas sp. B13*, and a similar degradation of benzoic acid. Only ordinary enzymes of the benzoate pathway were detected in 2-fluorobenzoate, 3-fluorobenzoate, and 4-fluorobenzoate-grown cells. The production of these enzymes indicates that the compounds were not treated any differently than the non-substituted acid. These compounds were recognized by the bacteria as benzoic acid (Schreiber, 1980). These studies demonstrate that the substitution of a fluorine for a hydrogen atom can create relatively innocuous analogues that proceed along normal metabolic pathways (Goldman, 1967). More recent research using a difluorobenzoic

acid yielded similar results. In one study, the biological degradation of both the fluoro-, and chloro-substituted acids was followed using the release of labeled carbon-dioxide as a marker of bacterial degradation activity. In soil containing bacteria known to utilize benzoic acid as a food source, the chlorinated compounds were degraded much more slowly than the fluorinated compounds (Nimmo, 1990). This difference in rate was most likely due to the fact that the much larger chlorine atoms created some stearic hindrance to breakdown, while the smaller fluorine atoms more closely resembled the hydrogens present in the unsubstituted benzoic acid.

Research conducted on the degradation of 2-fluorobenzoic acid by *Pseudomonas species* isolated from Potomac River mud, showed that over 80% of the fluorine in the 2-fluorobenzoic acid was released as fluoride during the growth of the organism (Goldman, 1967). Similar research using 4-fluorobenzoic acid indicated that nearly 100% of the organic fluorine was released into the culture medium as fluoride (Harper, 1971), indicating that the fluorobenzoate was utilized by the bacteria the same as benzoic acid.

Similar studies have also been done using *Pseudomonas putida* bacteria to monitor the degradation of difluoro compounds. Monofluorobenzoates release fluoride when they are degraded by *P. putida*, and although the reactions involved are considerably more complex for difluorobenzoates, about 85% of the fluorine was mineralized. The degradation potential of *P. putida* was evaluated for six different isomers of difluorobenzoate, and most of the fluorine was eliminated from both positions as F^-, leaving a catechol and a hydrodiol as major products (Cass, 1987; Milne, 1968).

LITERATURE REVIEW OF TRACER TOXICITY

No detailed information on the toxicity of the fluorinated benzoic acids was found. Most of the references to toxicology of these compounds were to the mono-fluorinated benzoic acids, which are not being considered as tracers, and some difluorinated benzoates which are being considered. Many references exist for the parent compound, benzoic acid, since it is used extensively as a preservative and antifungal compound. This information, as well as some studies that show similarities in toxicity between fluorinated and non-fluorinated benzoates, are presented as possible evidence of the relatively low toxicity of the fluorinated benzoates.

In the absence of specific toxicology information about fluorinated benzoic acids, some health effects information will be presented for chlorinated benzoic acids even though they are much more toxic compounds. This will permit us to examine a range of potential toxic effects and the dose-response relationships for worst case scenarios. If there is a low risk for the exposure conditions expected from the tracer experiments for the more toxic compounds, then we will have confidence that there will be a low risk from the less toxic compounds.

BENZOIC ACID

Benzoic acid is a compound of relatively low toxicity and is used in numerous food

(Casarett and Doull, 1980) and pharmaceutical (Tester-Daldrup, 1982) products. It is also found naturally in most berries in concentrations as high as 0.05% (Merck, 1989). It has an LDLO of 500 mg/kg (Sigma-Aldrich MSDS) and doses as high as 20 g have been given to human subjects in toxicological studies (Stewart 1960). A case has been reported of a 67 kg man ingesting 50 g doses without ill effects (Gosselin et al. 1984). The lethal dose to 50% of the test population (LD_{50}) in rats has been reported as 1.7 g/kg (Merck, 1989). If a "standard adult human" (SAH) weighing 70 kg (or about 155 pounds) were used, this would amount to a dosage of 119 grams.

Benzoic acid is used as a preservative and an antifungal compound. The acid is used in antifungal preparations, while the sodium or potassium salt, the benzoate ion, is used as a preservative. Benzoic acid is a broad spectrum microbial inhibitor, and is used as a food preservative in a great variety of products ranging from baked goods to jellies to soft drinks. Acid or benzoate concentrations in these products do not exceed one tenth of one per cent or 1,000 ppm (Cassarett and Doull, 1980). The daily acceptable intake is up to 5 mg/kg of body weight of either the acid (Remington, 1985) or the anion (Casarett and Doull, 1980). Benzoic acid is also used as a preservative in many cosmetics, where concentrations range from 0.1 to 0.5% (Remington, 1985). The Food and Drug Administration has classified benzoic acid as GRAS (Generally Recognized as Safe) as an antimicrobial (21CFR 181.23) and as a food additive (21CFR 184.1733) with appropriate levels given in 170.3 subparagraph O subparagraphs 2 and 12.

The effectiveness of benzoic acid as an antifungal and antimicrobial agent is due to the undissociated form. It is therefore effective at a pH of 4.0 or lower, but relatively inactive above a pH of 5.0 (Remington, 1985; Krebs, 1983). Benzoic acid is an active ingredient in topical ointments used for the treatment of fungal infections of the skin such as ringworm and athlete's foot. The concentrations of the acid in these ointments can range from 0.5% to 6.0% (Martindale, 1989).

Benzoic acid is not significantly oxidized in vertebrates. Depending on the species, it conjugated with glycine or glucuronic acid to form hippuric acid or benzoylglucuronic acid which is then excreted in the urine (Neal, 1980). This conjugation with amino acids (Caldwell, 1978) makes the new compound a stronger acid and it is more readily cleared by the kidneys (Jenner, 1980). In humans, benzoic acid taken by mouth is absorbed from the gastro-intestinal tract. It is conjugated with glycine in the liver to form hippuric acid which is rapidly excreted in the urine. Humans can also eliminate benzoic acid as benzoylglucuronic acid (Remington, 1985). In a study, with humans, 96% of a 20 g dose was eliminated as hippuric acid within 12 hours (Stewart, 1960). In a study with rabbits, 83% of the ingested benzoic acid was excreted as the glycine conjugate, 15% as the glucuronide and only 1% as the free acid (Caldwell, 1978).

The finding that benzoic acid is excreted as a conjugate is confirmation that benzoic acid can enter cells in the body, presumably by anion transport mechanisms. Thus, benzoic acid and its derivatives have the potential for further metabolism. It also confirms the finding that benzoic acid itself is a poor substrate for the anion secretion pathway in the kidney (Ullrich et al., 1988) and must be conjugated to the hippurate before being significantly excreted from the body.

Benzoic acid is readily degraded by microorganisms into CO_2. In stability studies,

where benzoate solutions are equilibrated with soil, the compound rapidly disappears from solution (Thompson and Stetzenbach, 1980), and when used as a ground water tracer, it was found to readily be degraded by microorganisms (Barackman, 1986).

METABOLIC PRODUCTION OF BENZOIC AND METHYL-BENZOIC ACIDS

Several chemical compounds are oxidized by the normal metabolic processes to benzoic or toluic acid. By examining their toxicity, additional information can be inferred for the toxicity of benzoic acid. In man, the chief metabolite of inhaled toluene is benzoic acid. Several studies have been done which list the efficiency of this oxidization process at from 62% (Piotrowski, 1967) to 72% (Sbrova, 1952) to 80% (Baselt, third ed.) of the total toluene inhaled. Of the urinary benzoic acid measured, 10 to 20% is conjugated with glucuronic acid, and the remainder is eliminated in the form of hippurate. The excretion of conjugated benzoic acid is rapid, with an excretion half-time of between 2 and 3 hours. The urinary concentration, which is dependant on initial exposure levels, has been measured as 8650 mg/L (Baselt, third ed.) with no reported ill effects. At approximately 24 hours after exposure the levels of the metabolite decline to pre-exposure values (Piotrowski, 1977).

Xylene in the human body undergoes oxidation, which leads to the formation of toluic acids. Three isomers are formed, 2-, 3-, and 4-toluic acid. Two of these compounds, 3- and 4-toluic acid are conjugated almost exclusively with glycine to form 3- and 4-methyl hippuric acids. The 2-toluic acid undergoes preferential conjugation with glucuronic acid. These processes are highly efficient, and in the case of the 3- and 4-toluic acids, oxidation is on the order of 90%, with all the toluic acid bound to glycine (Piotrowski, 1977).

Toluene and xylene have a very low order of toxicity in humans or in animals. The most common finding is central nervous system (CNS) toxicity after inhalation of high concentrations (Casarett and Doull, 1991), a biological effect shared by all volatile organic solvents. It is not possible for benzoic acid or its congeners to demonstrate this type of toxicity because of their low volatility. In vitro tests with these compounds do not show any potential genotoxic effects.

HALOGENATED BENZOIC ACIDS

Research done on the relative lethalities of halogenated compounds was conducted using benzoic acid and halogenated benzoic acids. It was shown that the toxicities of benzoic acid and its 4-fluoro substituted derivative are not significantly different. The chloro-, bromo-, and iodo-substituted acids all exhibit similar toxicities, which are greater than those of the unsubstituted or fluoro-substituted acids. This research also demonstrated that a fluorine atom located in the para (4) position on a benzoic acid molecule had no significant effect on its acute lethality in white rats, but a chlorine, bromine, or iodine atom in the para position, almost doubled the toxicity of the original benzoic acid compound (Hager and Starkey, 1943). The substitution of a fluorine atom for a hydrogen atom on the compounds evaluated is therefore shown to be the least toxic of all possible halogen substitutions.

Benzoic acids which have a halogen atom in the ortho (2) position on the ring often exhibit herbicidal and/or fungicidal properties (Engesser, 1980). The insecticide 'Dimilin' contains diflubenzuron, which is degraded readily in various agricultural soils and hydro-soils to 2,6-difluorobenzoic acid (Nimmo, 1984). Trichlorobenzoic acid is also used to some extent as an herbicide (Martindale, 1989).

Substantial toxicity data exists for the chlorinated benzoic acids which have found use as herbicides: 2-methoxy-3,6-dichlorobenzoic acid (Dicamba) and 3-amino-2,5-dichlorobenzoic acid (Chloramben). Reference doses (RfD) for a lifetime exposure are 3E-2 mg/kg/day
(2.1 mg/SAH/day for Dicamba (IRIS, 1993) and 1.5E-2 mg/kg/day (1.05 mg/SAH/day for Chloramben (IRIS, 1993). Unfortunately, these values are used to compute a recommended lifetime dose limit for exposure to these compounds. A more realistic exposure parameter would be a 10-day or longer term Health Advisory (HA) value but neither of these two compounds have such a recommended value. The critical effect used for Dicamba was maternal and fetal toxicity in a rabbit developmental study and a No Observed Adverse Effect Level (NOAEL) of 3 mg/kg/day (210 mg/SAH/day) was used. A 90 day feeding study in the rat showed a NOAEL of 250 mg/kg/day and a critical effect of a slight decrease in body weight and food consumption. The critical effect for Chloramben was hepatocyte degeneration in an 18 month mouse feeding study and a Lowest Observed Adverse Effect Level was 15 mg/kg/day (1050 mg/SAH/day). No shorter term studies were reported.

FLUORINATED BENZOIC ACIDS

The fluorinated benzoic acids that are being considered as tracers for the C-well complex fall into two categories. The poly-fluorobenzoates (table 1) have 2,3,4 or 5 fluorine atoms that replace an equivalent number of ring hydrogens. There are sixteen possible isomers of these compounds, fourteen of which are commercially available. (Twelve of these are currently being evaluated, but this report covers all fourteen acids available commercially). The other group is the trifluoromethyl substituted benzoates or trifluorotoluic acids (table 1). These compounds substitute a CF_3 group for a ring hydrogen. There are only three isomers of trifluorotoluic acid.

The number of compounds that have fluorine substituted rings have been increasing rapidly over the last ten to fifteen years, primarily due to their use as precursors in antibiotics and pesticides. (Schreiber, 1980; O'Reilly, 1990; Domagala, 1991). The metabolic breakdown products from these compounds quite often include some of the acids shown on table 1. This has generated some interest in the biodegradation of these compounds. Very little information exists on the fate of these compounds in man. Some work with animals has been done, but most of the research has been with microbial degradation of the fluorobenzoates.

ANIMAL STUDIES

Studies with rabbits (Caldwell, 1978) exposed to benzoic acid and 2- and 4-fluorobenzoic acids show that the fluorinated analogs are excreted as free acids at significantly higher rates than benzoic acid. This is probably due to their somewhat lower pKa values (Benson; Bowman, 1992; Walter, 1982) and their being better substrates for the anion secretion pathway in the kidney (Ullrich et al., 1988). In the study described by Caldwell (1978), 99% of the benzoic acid dose was recovered; 83% as the glycine conjugate and 15% as the glucuronide. The 2-fluorobenzoic acid (o-FBA) and 4-fluorobenzoic acid (p-FBA) were excreted with almost identical ratios: 43% as the glycine conjugate, 9% as the glucuronide, and 34% as the free acid for o-FBA and 35% for p-FBA. The fate of the other 13 or 14% is unknown. It could have lost F^-, and become benzoic acid as was described for mixed function oxidase enzymes in Pseudomonas bacteria (Goldman, 1967). In another study, rats that were dosed with 100 mg/kg (intraperitoneal) of 4-trifluoromethylbenzoic acid (p-TFMBA) produced the glucuronide as the main urinary metabolite (Ghauri, 1990).

In a comparative toxicity study by Harger and Starkey using white rats, benzoic acid and para substituted fluoro-, chloro-, bromo- and iodo-benzoic acids, it was shown that the toxicities of benzoic acid and its p-fluoro substituted derivative were not significantly different. In this study solutions of the benzoic acids were injected intravenously and the acute lethality was determined. The LD_{50} value for benzoic acid was 1.714 +/- 0.037 g/kg, while that for p-fluorobenzoic acid was 1.542+/-0.107 g/kg. The LD_{50} values for the chloro-, bromo-, and iodo-substituted acids were 0.838+/-0.033, 0.812+/-0.042 and 0.786+/-0.037 g/kg respectively. The values for the latter three halogenated acids are essentially the same, but they are twice as toxic as benzoic acid or the fluoro-substituted acid. Generally, acute toxicity determined after intravenous injection shows compounds to be more toxic than would be determined after oral administration because the blood levels reach higher values after injection. Oral LD_{50} values would be expected to require more compound to cause death than those shown above.

ESTIMATED RISK ASSOCIATED WITH FLUOROBENZOIC ACID EXPOSURE

A risk analysis is composed of three elements; **Exposure** to a **Hazard** results in a **Risk**. In this project, it has been difficult to construct exposure scenarios. **Exposure** to the pure tracer and concentrated injection solutions will be minimized by good industrial hygiene practices and by the fact that the proposed tracer chemicals are not volatile or present in water or food consumed by the workers. Any exposure will be from accidents which might result in acute exposure episodes and will occur to trained personnel who are being supervised by knowledgeable persons.

Likewise, exposure to the tracer chemicals in the groundwater is unlikely because that water will reach the surface at the pumping well. This water will also be handled by the trained personnel. We will assume that this water might be consumed and the potential risk estimated. Also, we will postulate that another well 100 - 200 feet down gradient from the pumping well might be drilled and water used from that well for drinking water purposes.

The **hazard** has been difficult to determine because health effects of these proposed

tracer chemicals have not been studied. The fact that these are uncommon chemicals makes them desirable as tracers because there will be no background levels to interfere with the interpretation of the experimental results. We have attempted to bracket the toxicity of the tracer chemicals by choosing similar compounds which are expected to be less toxic and others which are expected to be more toxic.

Risk from Acute Exposure

Exposure due to accidents could result in acute effects if the chemical spills on the skin or is accidently ingested. Exposure via skin absorption cannot be estimated for these compounds but their ionic nature makes it unlikely that much will be absorbed. As mentioned earlier, the LDLo of benzoic acid is 500 mg/kg (35.0 grams per SAH), doses as high as 20 grams have been given to adults without ill effects (300 mg/kg) and the LD_{50} in rats is 1.7 g/kg (120 grams per SAH). The fluorinated benzoic acid analog was shown to have the same LD_{50} as benzoic acid itself after intravenous injection. The daily dose of benzoic acid allowed for adults is 5 mg/kg (350 mg per SAH).

Based on the evidence presented, a single dose of tracer of 5 mg/kg (350 mg per SAH) should present a minimal risk. To derive that the LDLo of 500 mg/kg was adjusted by a factor of 10 for sensitive human populations and another factor of 10 to adjust for the extrapolation of animals to humans. This is the equivalent of ingesting 70 mL of the concentrated injection solution. The chances of someone ingesting concentrated tracer solution are extremely small, as all personnel present would be trained in the proper handling and disposal precautions for these compounds, and the injection apparatus is a closed system. However, this evidence seems to indicate a very low toxicity level for these compounds, even at extremely high concentrations.

Risk from Sub-chronic Exposure

If the pumping well water was used for drinking water, the exposure would not be for an entire lifetime. Exposure to groundwater would occur only as long as the time it would take for the tracer plume to pass the well. Groundwater moves at the rate of 0.14 to 3.4 m/day (Claassen and Cordes 1975) and it is expected that persons would be exposed for less than 6 months if they drank from a well which had been used for a tracer test. The average tracer concentration would be 1 ppm based on a conservative dilution factor of 5,000.

If another well was drilled in the pathway of the tracer gradient (approximately 100 - 200 feet from the pumping well), then another dilution factor of 1,000 should be applied. In this case, groundwater from the well would contain 1 ppb tracer concentration.
The maximum daily dose for a lifetime exposure to benzoic acid is 5 mg/kg/day (350 mg/SAH/day); this equates to a drinking water level of 175 ppm for a water consumption of 2 liters per day. For the chlorinated benzoic acid herbicides, the maximum daily dose for a lifetime exposure is
2.1 mg/SAH/day for Dicamba and 1.05 mg/SAH/day for Chloramben. These equate to drinking water levels of 1 ppm for Dicamba and 0.5 ppm for Chloramben.

The concentration of fluorinated benzoic acids in drinking water that would result in minimal risk lies between 0.5 - 175 ppm based on the probable toxicity of the compounds. This level has been computed for a lifetime (70 years) exposure to these compounds. To adjust this level for a six month exposure, the upper concentration would be increased by 140 times, resulting in a possible highest range concentration of 24500 ppm. It is concluded that the ingestion of water from the pumping well (expected concentration ≈1 ppm) or from another well 100 - 200 feet away (expected concentration ≈1 ppb) would not result in a significant risk for any adverse health effects.

CONCLUSIONS

Several other conclusions can be drawn from the body of literature cited above:

(I) Benzoic and methyl-benzoic acids are widely used in many pharmaceutical preparations and as preservatives in many food products and cosmetics, often at concentrations which exceed those predicted to occur during tracer testing by several orders of magnitude. With such wide spread use and application, especially by the medical industry, any problems associated with exposure to these compounds should be well documented. To date, no serious health problems have been associated with exposure to benzoic and methyl-benzoic acids.

(II) The fluorinated benzoic and methyl-benzoic acids appear to be fairly non-toxic compounds at low to moderate concentrations. Studies have shown that the mono- and di-fluorinated derivatives of benzoic acid are not significantly more toxic to living systems than the original parent compounds.

(III) Benzoic and methyl-benzoic acids are natural metabolites of toluene and xylene exposure, and even at extremely high concentrations, they have caused no known health problems. Mammalian systems have a well-established pathway using glycine and glucuronic acid conjugation to form hippuric and benzoylglucuronic acids, which are rapidly excreted in the urine. No known health problems have been associated with high concentrations of these acid conjugates.

(IV) Studies of the degradation of fluorinated benzoic acids in soil have shown that defluorination occurs, and nearly all of the organic fluorine is eliminated as fluoride, leaving degradation products which are then broken down further to CO_2. 2,6-Difluorobenzoic acid has been proven to be stable in a number of tracing experiments, yet it can also be degraded by soil bacteria. This indicates that even highly stable tracers will eventually be degraded.

The above mentioned conclusions, coupled with the fact that potential exposure to these compounds from tracer tests would occur at ultra trace levels, lead to the conclusion that the fluorinated benzoic and methyl-benzoic acids would be relatively safe for use as ground water tracers.

RECOMMENDATIONS

These conclusions and recommendations were made using a "worst case scenario" exposure assessment of a tracing test conducted in a *densely* populated area. The application of this point of view to the actual conditions existing in the proposed tracer testing area should be duly noted when any review of this evaluation is performed.

Before any of these compounds are used in populated areas where sub-chronic exposure is likely, further toxicity studies should be done. These studies should be of 90 day duration as a minimum or of 6 months duration. They should involve two animal species with one species being the rat so the results can be compared with similar compounds. They should be done at three dose levels with one level being low enough to insure that a no effect level would be found.

The existing toxicity data for the proposed compounds are so sketchy that there is a low level of confidence in the levels proposed if repeated human exposure is likely.

Benzoic Acid Reference List:

AHFS Drug Information Formulary, 1992 ed., American Society of Hospital Pharmacies, 1992, pp. 1230, 1263, 1355.

Atlas, R.M.; Bartha, R. *Microbial Ecology: Fundamentals and Applications,* Second ed., Benjamin/Cummings, Menlo Park, CA, p 370.

Barackman, M.L. *Diverging Flow Tracer Tests in Fractured Granite; Equipment Design and Data Collection,* Masters Thesis, University of Arizona, Tucson, 1986.

Baselt, R.C.; Cravey, R.H. *Disposition of Toxic Drugs in Man,* Third ed., Year Book Medical, Chicago, IL, pp 812.

Benson, C.F.; Bowman, R.S. Acid Dissociation Constants of Fluorinated Benzoic Acids Used as Water Tracers, *(in submission).*

Bowman, R.S. *Soil Sci. Soc. Am. J.*, 1984, *16*, 250.

Bowman, R.S.; Gibbens, J.F. Difluorobenzoates as Nonreactive Tracers in Soil and Ground Water, *Ground Water*, 1992, *30*, No. 1, 8.

Caldwell, J. *Conjugation Reactions in Drug Biotransformation,* Aitio, A. Ed.; Elsevier/North Holland Biomedical, 1978, pp 111.

Casarett and Doull's Toxicology, Second ed.; Doull, J.; Klaassen, C.D.; Amdur, M.O.; Eds.; Macmillan, New York, 1980, Chapter 23 and p 72.

Cass, A.E.G.; Ribbons, D.W.; Rossiter, J.T.; Williams, S.R. Biotransformation of Aromatic Compounds: Monitoring Fluorinated Analogues by NMR, *Federation of European Biochemical Societies,* 1987, *220,* No. 2, 353.

Claassen, H.C.; Condes, E.H.; Two-well Recirculating Tracer Test in Fractured Carbonate Rock, Nevada, *Hydrological Sciences Bulletin,* 1975, *Vol XX,* No. 3, 367.

Concepts in Drug Metabolism (part A), Jenner, P.; Testa, B. Ed.; Drugs and the Pharmaceutical Sciences, Marcel Dekker, New York, 1980, pp 53-176, 211.

Domgala, J.M.; Bridges, A.J.; Culbertson, T.P.; Gambino, L.; Hagen, S.E.; Karrick, G.; Porter, K.; Sanchez, J.P.; Sesnie, J.A.; Spense, F.G.; Szotek, D.; Wemple, J. Synthesis and Biological Activity of 5-Amino- and 5-Hydroxyquinolones, and the Overwhelming Influence of the Remote N_1-Substituent in Determining the Structure-Activity Relationship, *Journal of Medicinal Chemistry,* 1991, *34*, 1142.

Engesser, K.-H.; Schmidt, E.; Knackmuss, H.-J. Adaption of Alcaligenes eutrophus B9 and Pseudomonas sp. B13 to 2-Fluorobenzoates as Growth Substrate, *Applied and Environmental Microbiology,* 1980, *January,* 68.

Freiser, H.; Fernando, Q. *Ionic Equilibria in Analytical Chemistry,* John Wiley and Sons, New York, 1963, Chapter 5.

Ghauri, F.Y.K.; Wilson, I.D.; Nicholson, J.K. ^{19}F and ^{1}H-NMR Studies of the Metabolism of 4-Trifluoromethylbenzoic Acid in the Rat, *Methodological Surveys in Biochemistry and Analysis,* 1990, *20,* 321.

Goldman, P.; Milne, G.W.A.; Pignataro, M.T. Fluorine Containing Metabolites Formed From 2-Fluorobenzoic Acid by Pseudomonas Species, *Archives of Biochemistry and Biophysics,* 1967, *118,* 178.

Gosselin, R.E.; Smith, R.P.; Hodge, H.C.; Braddock, J.E.; Eds.; *Clinical Toxicology of Commercial Products,* Fifth ed.; Williams and Wilkins, Baltimore MD, 1984, p II-203.

Hager, G.P.; Starkey, E.B. Fluorine Substituted Aromatic Acids, *Journal of the American Pharmaceutical Association,* 1943, *32,* 44.

Harper, D.B.; Blakley, E.R. The Metabolism of p-Fluorobenzoic Acid by Pseudomonas sp., *Canadian Journal of Microbiology,* 1971, *17,* 1015.

Jones, T.L.; Kelly, V.A.; Pickens, J.F.; Upton, D.T.; Beauheim, R.L.; Davies, P.B. *Interpretation of Results of Tracer Tests Performed in the Culebra Dolomite at the Waste Isolation Plant,* Sandia National Laboratories: Albuquerque, NM, 1992, SAND92-1579, UC-721.

Kelly, V.A.; Pickens, J.F.; *Interpretation of the Convergent-Flow Tracer Tests Conducted in the Culebra Dolomite at the H-3 and H-4 Hydropads, Waste Isolation Pilot Plant (WIPP) Southeastern New Mexico,* Sandia National Laboratories: Albuquerque, NM, 1986, SAND86-7161.

Krebs, H.A.; Wiggins, D.; Stubbs, M. Studies on the Mechanism of the Antifungal Action of Benzoate, *Journal of Biochemistry,* 1983, *214,* 657.

Martindale: The Extra Pharmacopia, 29th ed., Reynolds, J.E.F. Ed.; Pharmaceutical Press, London, 1989, pp 1355-1356, 1430.

Matthews, H.B.; Kato, S. The Metabolism and Disposition of Halogenated Aromatics, *Health Effects of Halogenated Aromatic Hydrocarbons,* Annals of the New York Academy of Sciences, Nicholson, W.J.; Moore, J.A. Eds.; New York Academy of Sciences, New York, 1979, *vol. 320,* 271.

McCray, J.G.; Nowatzki, E.A.; Van Zyl, D.; Thompson, G.M.; Armstrong, G.; *Low-Level Nuclear Waste Shallow Land Burial Trench Isolation;* NUREG/CR-3570; Nuclear Regulatory Commission: Washington D.C., September 1983.

Milne, G.W.A.; Goldman, P.; Holtzman, J.L. The Metabolism of 2-Fluorobenzoic Acid, *Journal of Biological Chemistry,* 1968, *243,* No. 20, 5374.

Moffett, R.B.; Tang, A.H. Skeletal Muscle Stimulants. Substituted Benzoic Acids, *Journal of Medicinal Chemistry,* 1968, *11,* 1020.

Neal, R.A. Metabolism of Toxic Substances (chapter 4),*Casarett and Doull's Toxicology,* Second ed.; Doull, J.; Klaassen, C.D.; Amdur, M.O.; Eds.; Macmillan, New York, 1980, Chapter 4, pp 56.

Nimmo, W.B.; de Wilde, P.C.; Verloop, A. The Degradation of Diflubenzuron and its Chief Metabolites in Soils. Part I. Hydrolytic Cleavage of Diflubenzuron, *Pesticide Science,* 1984, *15,* 574.

Nimmo, W.B.; Joustra, K.D.; Willems, A.G.M. The Degradation of Diflubenzuron and its Chief Metabolites in Soils. Part III. Fate of 2,6-Difluorobenzoic Acid, *Pesticide Science,* 1990, *29,* 39.

O'Reilly, N.J.; Derwin, W.S.; Fertel, L.B.; Lin, H.C. An Expedient Route to the Quinolone Antibacterial Intermediate, 2,4,5-Trifluorobenzoic Acid, *Synlett,* 1990, *October,* 609.

Piotrowski, J. Quantitative Evaluation of Exposure to Toluene in Men, *Med. Pracy,* 1967, *18,* 213.

Piotrowski, J.K. *Exposure Tests for Organic Compounds in Industrial Toxicology,* U.S. Department of Health, Education and Welfare, Cincinnati, OH, 1977, pp 48.

Prescott, L.F. *Side Effects of Drugs Annual 1* (Antipyretic Analgesics), Dukes, M.N.G. Ed.; Excerpta Medica, Amsterdam, 1977, Chapter 8.

Remington's Pharmaceutical Sciences, 17th ed., Gennaro, A.R. Ed.; Mack, Easton PA, 1985, pp B1230, B1263.

Schreiber, A.; Hellwig, M.; Dorn, E.; Reineke, W.; Knackmuss, H.-J. Critical Reactions in Fluorobenzoic Acid Degradation by Pseudomonas sp. B13, *Applied and Environmental Microbiology,* 1980, *January,* 58.

Sittig, M. *Handbook of Toxic and Hazardous Chemicals and Carcinogens,* Second ed.; Noyes, Park Ridge, New Jersey, 1985, p 117.

Srbova, J.; Teisinger, J. Absortion and Elimination of Toluene in Man, *Prac. Lek,* 1952, *4,* 41.

Tester-Dalderup, C.B.M. *Side Effects of Drugs Annual 6* (Antihistamines), Dukes, M.N.G.; Elis, J. Eds.; Excerpta Medica, Amsterdam, 1982, Chapter 16.

The Merck Index, Eleventh ed., Budavari, S. Ed.; Merck, Rahway, NJ 1989, pp. 170, 242.

Thompson, G. *Ground Water and Transport Characteristics of Flood Basalts as Determined from Tracer Experiments,* Unpublished results, 1982.

Thompson, G.; Stetzenbach, K. *Assessment and Advances in Tracer Technolgy*, A Topical Report to the Nuclear Regulatory Commission, October 1980.

Walter, G.R. *Theoretical and Experimental Determination of Matrix Diffusion and Related Solute Transport Properties of Fractured Tuffs from the Nevada Test Site,* Los Alamos National Laboratories: Los Alamos, NM, 1982, LA-9471-MS, UC-70.

Wolff, M.S. Body Clearance of Halogenated Hydrocarbons: Workshop Summary, *Health Effects of Halogenated Aromatic Hydrocarbons,* Annals of the New York Academy of Sciences, Nicholson, W.J. Moore, J.A. Eds.; New York Academy of Sciences, New York, 1979, *vol. 320,* 271.

TABLE 1

LIST OF POTENTIAL TRACERS COMPOUNDS for the C-WELL TESTS

FLUORINATED BENZOIC ACIDS

2,3-Difluorobenzoic acid
2,4-Difluorobenzoic acid
2,5-Difluorobenzoic acid
2,6-Difluorobenzoic acid
3,4-Difluorobenzoic acid
3,5-Difluorobenzoic acid

2,3,4-Trifluorobenzoic acid
2,4,5-Trifluorobenzoic acid
3,4,5-Trifluorobenzoic acid

2,3,4,5-Tetrafluorobenzoic acid
2,3,5,6-Tetrafluorobenzoic acid

Pentafluorobenzoic acid

ortho-Trifluoromethylbenzoic acid (2-trifluoromethyltoluic acid)
meta-Trifluoromethylbenzoic acid (3-trifluoromethyltoluic acid)
para-Trifluoromethylbenzoic acid (4-trifluoromethyltoluic acid)

ORGANIC TRACER TOXICITY REPORT
PERFLUORINATED ALIPHATIC ACIDS

DOE Cooperative Agreement

No. DE-FC 08-90NV10972

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Eighteen fluorinated organic acids (table 1) are presently being studied as possible tracers for the Yucca Mountain Site Characterization Project. These have been divided into two separate categories: fluorinated aromatic compounds (poly-fluorinated benzoic acids), and fluorinated aliphatic compounds. Fluorinated organic compounds were selected as potential tracers because of their long term environmental stability and low reactivity. Low reactivity and low toxicity are characteristics of numerous carbon-fluorine compounds. As a general rule, it can be stated that the greater the number of halogen atoms on a molecule, especially fluorine, the greater its stability. Perfluorinated acids such as pentafluoro-benzoic acid (PFBA) and trifluoroacetic acid (TFAA) are therefore extremely resistant to chemical attack.

A complete report detailing the available literature references of the stability and toxicity of the fluorinated aromatic acids, as well as an exposure risk assessment for all tracing compounds during, and immediately prior to the actual tracing tests has been submitted separately. Please refer to this report for further information on these topics.

ALIPHATIC ACIDS

In addition to the fluorinated aromatic acids mentioned previously, several fluorinated aliphatic acids are being evaluated for use as ground water tracers. These compounds are: trifluoroacetic acid (TFAA), pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA).

The parent compounds of these acids occur naturally in, or are present as an additive in many food products. Aliphatic acids such as acetic acid (vinegar) and propionic acid are present in many areas of the food industry. The calcium and sodium salts of propionic acid are used effectively against filamentous fungi in breads, cakes and some cheeses. They are also used to inhibit rope formation in bread dough and milk products. Propionates are formed naturally during the production of Swiss cheese, and act as a natural preservative.

Acetic acid is commonly used as an inhibitor of fungal and bacterial growth to preserve meat products, mayonnaise, and catsup. It is also a main ingredient in many salad dressings, tomato sauces, salsas, and relishes.

Butyric acid occurs naturally in butter as an ester at concentrations of up to 4 or 5%. Magnesium butyrate is used in the manufacture of esters which are used in artificial flavorings (Merck, 1989).

FLUORINATED ALIPHATIC ACIDS

For the three perfluorinated aliphatic acids being considered, toxicity data is available

for TFAA only. TFAA is the perfluorinated derivative of acetic acid. It is also a metabolite of anesthetics such as halothane and fluoroxene in both animal and human systems.

ANIMAL STUDIES WITH TFAA

Studies have been done in which TFAA was administered to animals directly, either with an injection into the blood stream or muscle tissue, or in their diet. TFAA at 2.1 mmol concentrations (neutralized pH) was diluted to 5% in water and administered intravenously through the tail vein to 10 rats. Serum concentrations of TFAA after 24 hours were found to be 1.71 mmol. The intestinal mucosa was analyzed after 24 hours, and TFAA was found to be present at 1.02 micromol/gram. The intestinal mucosa was selected because it is an area where rapid proliferation and cell growth occurs, and therefore would show the effects of toxicity sooner than less rapidly growing tissues. No toxic effects were observed in any of the rats studied when compared to control groups (Fraser, 1988). TFAA (neutralized to pH 7.0) at concentrations of up to 25mg/kg of body weight, was given to male rats in their water. These rats were followed over a course of 3 days, for morphological changes in testicular tissues (an area of rapid cell proliferation). No biologically significant changes were seen in the tissue samples at the dosage concentrations studied, compared to control groups (Lloyd, 1988).

Studies done on mice and guinea pigs show the LD_{50} for TFAA to be greater than 2000 mg/kg (intraperitoneal injection) and 1200 mg/kg intravenous administration (table 2). This same research reports that the TFAA does not appear to block the Krebs cycle like the more toxic monofluoroacetic acid (Airaksinen, 1968). Research to date seems to confirm that TFAA is less harmful when taken orally than intravenously because, at physiological pH, it is ionized and therefore unlikely to penetrate the cellular membranes (Cohen, 1978; Cohen, 1975). Mice were not killed by intraperitoneal doses of 5,000 mg/kg of sodium fluoroacetate (Blake, et al., 1969) and no effects were seen after 100 mg/kg of trifluoroethanol ip daily for 18 days except for a failure to gain weight. Histopathological examination did not show any differences between test and control groups in this 18 day study. Trifluoroethanol is more acutely toxic than TFAA (LD_{50} = 350 mg/kg by oral and intraperitoneal routes) and is partially converted to TFAA (about 15%) using the same enzymes which metabolize ethanol (Blake et al., 1969).

CELLULAR EFFECTS OF TFAA

Research has also been done on the effect of TFAA on cellular systems. Studies were done using C6-murine glioma cells (in vitro) where the TFAA concentrations in the cell media exceeded that of the rodent research listed above. The results indicated that the TFAA had a relatively mild impact on the C6 cells, and only minimally affected the cell's energy metabolism (Ma, 1990). Similar studies performed with the anti-influenza virus showed that the virus activity was not affected by TFAA (Harada, 1991).

METABOLIC TFAA IN ANIMALS

A large body of work has also been done on TFAA as a metabolite in the body.

Such a catabolic pathway is known to occur with exposure to certain anesthetics, the ingestion of trifluoroethanol, and the inhalation of certain chlorofluorocarbons.

The anesthetic compounds studied for eventual breakdown to TFAA include halothane, fluoroxene, and desflurane, and isoflurane. Until recently, Halothane was the most widely used anesthetic in the world with the possible exception of nitrous oxide (Greene, 1968). It has been replaced by isoflurane, desflurane, and fluoroxene. All of these anesthetics are commonly referred to as halothane anesthetics, or halothanes. TFAA is the principle oxidative metabolite of halothane anesthesia, and is very stable. As the metabolism of halothane occurs mainly in the liver, concentrations of TFAA in the blood, renal, and hepatic systems reach their peak from 5 to 16 hours after exposure, and then gradually taper off (Ma, 1990).

Recent research on beagle dogs exposed to halothane anesthetics showed that over 80% of all fluoride inhaled was excreted in the urine as organic fluoride, of which TFAA was a major constituent. As a major metabolite, TFAA is distributed from the liver into either the blood or the bile. TFAA in the blood serum is filtered by the kidneys, and subsequently concentrated in the urine. TFAA which enters the bile can be secreted into the duodenum and be reabsorbed. The TFAA which was in the bile and subsequently reabsorbed would also enter the liver, and, since it is not metabolized further, would go into the kidneys, and then be excreted in the urine. The major percentage of the TFAA produced through the metabolism of halothane anesthetics is therefore eventually excreted in the urine (Sakai, 1991).

Similar research done on guinea pigs (Nakao, 1991), rats (vanDyke, 1965), and rabbits (Steir, 1964; Steir, 1968) also show the major metabolite of halothane anesthetics to be TFAA. Analyses done on mice and dogs (Blake, 1967) exposed to fluoroxene anesthetics show the major metabolite to be TFAA also.

TFAA has also been studied as a metabolite of 2,2,2-Trifluoroethanol (TFE) in rats. TFE is the initial metabolite of the anesthetic agent fluoroxene, which is then further broken down into TFAA. TFE and trifluoroacetaldehyde (TFAld) were administered to rats intravenously, and the blood serum, and intestinal mucosa were monitored for TFAA. TFAA was detected at approximately 1 hour after initial exposure, and levels continued to increase over the next 16 hours, and remained at their peak concentration for another 8 hours. It was noted that within the metabolic pathway from fluoroxene to TFAA, there is a toxic compound formed. However, specific research on this problem has demonstrated that the toxic moiety is a metabolic intermediate and not TFAA. Thus, the toxicity of TFE is mediated by its ultimate metabolism to TFAA (Fraser, 1988, 1987), which is then excreted to a large degree in the urine (Blake, 1969). Administration of TFAA directly did not produce any evidence of toxicity in the bone marrow or small intestine, even though it was shown to be distributed to both the blood serum and small intestine mucosa in similar concentrations to those occurring from administration of TFE and TFAld. This result precludes TFAA or any TFAA conjugate from being the toxic metabolite (Fraser, 1988).

Several of the studies cited above (Fraser, 1988; Lloyd, 1988; Airaksinen, 1968; and Blake, 1987) explored the effects of TFAA as a directly introduced compound, and as a metabolite of TFE. These studies showed that TFAA produced no toxic effects when given in a single dose, and no histological or morphological changes in the analyzed tissues

compared with control groups at the concentrations studied (Fraser, 1988; Lloyd, 1988; Airaksinen, 1968). However, there have been no long term toxicity studies performed and the hazard of TFAA cannot be assessed from this data.

METABOLIC TFAA IN HUMANS

Studies of exposure to halothane anesthetics in humans has shown TFAA to be the major metabolic product (Rehder, 1967; Blake, 1972; Cohen, 1975; Witte, 1977). The fact that operating room personnel are continuously exposed to low level concentrations of halothane anesthetics, coupled with the much higher doses received by patients undergoing anesthesia, suggest that the halothane anesthetics are relatively safe at the dosages applied. However, in a small number of cases (1:10,000) a condition called "halothane hepatitis" occurs and 50% of those people die from the disease (Goodman and Gilman, 1990). This response may be related to an immune reaction after a protein adduct has formed with some component of the halothane. Possibilities for this component could be the trifluoroacetaldehyde or the trifluoroacetic acid metabolites formed from halothane. In several preliminary studies, most notably Fraser, 1988; TFAA was shown to have no toxic effects in this metabolic pathway. An oxidative intermediate on the pathway between TFAA and trifluoroacetaldeh de is suspected as the toxic moiety; because this intermediate precedes TFAA in the metabolic process, TFAA is not expected to have a toxic capacity in this hepatic condition according to this research.

Initial research using the body fluids of people exposed to halothane anesthesia show a sharp increase in blood and urine TFAA co ncentrations over a period of about 8 hours, then a gradual increase over 48 hours, where maximum concentration levels are attained at roughly 48 hours after exposure, and then decline slowly over 11 t) 14 days. Maximum concentration values ranged from 130 to 300 ug/ml of TFAA in urine, and 75 to 150 ug/ml of TFAA in blood serum samples (Witte, 1977). Earlier research shows this same pattern of excretion, with roughly the same concentrations and time frame (Rehder, 1967).

The biliary excretion of TFAA in infants was studied using two babies, one 5 months old, and one two months old. Anesthesia was administered prior to performing a surgical procedure; bile, urine, and faeces samples were collected continuously for five days following surgery and TFAA concentrations were measured. The concentrations monitored in these samples showed that all of the TFAA was excreted in the bile and urine. No TFAA was measured in the faecal samples, which shows an enterohepatic circulation for this metabolite (Wark, 1991).

Desflurane and isoflurane anesthetics also produce TFAA as the primary metabolite. The major difference between these anesthetics and Halothane is the extent to which they are metabolized. Halothane is metabolized to between 20 and 30%, while desflurane and isofluorane are metabolized to between 1 and 2%, with the bulk of the administered anesthetic being exhaled unmetabolized.

Thirteen volunteers, used as a healthy metabolism control group, and twenty six patients, that did not necessarily have healthy metabolism, were exposed to desflurane anesthesia. The blood serum showed TFAA concentrations around 40 mg/L, and a urinary excretion rate of about 20 ug per hour TFAA, 24 hours after exposure. The levels of TFAA in the

blood serum and urine, after similar exposure to isoflurane are approximately 10 times higher than the desflurane results above (Sutton, 1991). It is apparent from the research cited that TFAA is a metabolic byproduct of these anesthetics that is excreted normally by the body without any further metabolism. Metabolism of these anesthetics leads to significant concentrations of TFAA in the body but little is known about the toxic effects of the anesthetics in humans.

The structural similarity of certain chlorofluorocarbons to halothane anesthetics lead to a study of the metabolic products of HCFC-124, a refrigerant substitute. Results indicate that in human subjects, the major metabolites are TFAA and F^- (Olsen, 1991). This same pathway is thought to extend to other, structurally similar refrigerant compounds currently in use.

CHLORINATED ACETIC ACIDS

The chlorinated analog of TFAA, trichloroacetic acid, has been studied because it is a metabolite of two carcinogenic solvents, trichloroethylene and tetrachloroethylene. Although it might be expected to be more extensively metabolized than the fluoroacetic acids, it provides a reference for comparison.

Trichloroacetate was administered to B6C3F1 mice at concentrations of 1 or 2 g/L for up to 52 weeks (Bull et al., 1990). Trichloroacetate induced hepatoproliferative lesions in male mice, including hepatocellular nodules, adenomas and hepatocellular carcinomas within 12 months. The induction of these lesions was linear with respect to dose. Trichloroacetate did not cause cellular necrosis but rather appeared to increase lipid peroxidation. This suggests that the production of radicals may cause its effects.

ESTIMATED RISK ASSOCIATED WITH TRIFLUOROACETIC ACID EXPOSURE

Risk from Acute Exposure

Exposure due to accidents could result in acute effects if the chemical spills on the skin or is accidently ingested. Exposure via skin absorption cannot be estimated for these compounds but their ionic nature makes it unlikely that much will be absorbed. Oral doses of trifluoroacetic acid have an LD_{50} in excess of 5,000 mg/kg in mice and have LD_{50}'s in the range of 1,000 - 2,000 mg/kg after systemic administration.

Based on the evidence presented, a single dose of tracer of 5 mg/kg (350 mg/Standard Adult Human (SAH)) should present a minimal risk. To derive that the LD_{50} of 5,000 mg/kg was adjusted by a factor of 10 for sensitive human populations, a factor of 10 to adjust for the extrapolation of animals to humans and another factor of 10 to adjust for using an LD_{50} value instead of an LDL_0. This is the equivalent of ingesting 70 mL of the concentrated injection solution. The chances of someone ingesting concentrated tracer solution are extremely small, as all personnel present would be trained in the proper handling and disposal precautions for these compounds, and the injection apparatus is a closed system. However, this evidence seems to indicate a very low toxicity level for these

compounds, even at extremely high concentrations.

Risk from Sub-chronic Exposure

If the pumping well water was used for drinking water, the exposure would not be for an entire lifetime. Exposure to groundwater would occur only as long as the time it would take for the tracer plume to pass the well. Groundwater moves at the rate of 0.14 to 3.4 m/day (Claassen and Cordes 1975) and it is expected that persons would be exposed for less than 6 months if they drank from a well which had been used for a tracer test. The average tracer concentration would be 1 ppm based on a conservative dilution factor of 5,000. If another well was drilled in the pathway of the tracer gradient (approximately 100 - 200 feet from the pumping well), then another dilution factor of 1,000 should be applied. In this case, groundwater from the well would contain 1 ppb tracer concentration. The closest value to a repeated dose toxicity study was the 100 mg/kg/day (700 mg/SAH/day) of trifluoroethanol that was administered to mice for 18 days. That was partially converted (20%) to TFAA; this would be equivalent to 20 mg/kg/day (140 mg/SAH/day) of TFAA. The daily dose for 6 months would have to be adjusted by a factor of 10,000 to provide any confidence in the number. This would result in a 0.070 ppm drinking water level.

The experience in humans with halothane was extensive and involved people who were not healthy but anesthesia is not given on a repeated dose basis. Operating room personnel would be exposed on a daily basis but the dose would be low. The possibility of an immune response is of concern with the halothane data.

The use of the trichloroacetic acid data involves a carcinogenic endpoint. If other compounds were available for use, as is the case with tracer molecules, they would be chosen instead of one which has the potential to be carcinogenic.

CONCLUSIONS

Several conclusions can be drawn from the body of research cited above:

(I) TFAA appears to be a fairly non-toxic compound at low to moderate concentrations, with an LD_{50} several orders of magnitude above the concentrations expected at the downstream end of the tracing tests.

(II) Ionized compounds do not penetrate cell membranes easily, and at groundwater pH values, TFAA will be highly ionized. This will make the compound more water soluble, and therefore more easily excreted by mammalian systems. If TFAA in the water was ingested by people or animals down gradient of the test injection area, it would most likely be excreted in the urine, rather than being metabolized.

(III) Significant concentrations of TFAA are present in patients who have undergone halothane anesthesia, or have been exposed to a number of fluorinated hydrocarbon compounds. There was a low incidence of toxicity (1:10,000) for halothane hepatitis but there was a 50% likelihood of death in those who developed the illness. On that basis, halothane is no longer generally used as an anesthetic agent.

(IV) Parallels can be drawn from the TFAA to the PFPA and HFBA because of the similar structural properties and ionic character of these compounds.

(V) Trifluoroacetic acid and its congeners may not be the compounds of choice for organic tracer use in cases where sub-chronic or chronic exposure may occur unless further toxicity work is done. Its use in situations which may result in single dose exposures is acceptable.

RECOMMENDATIONS

These conclusions and recommendations were made using a "worst case scenario" exposure assessment of a tracing test conducted in a *densely* populated area. The application of this point of view to the actual conditions existing in the proposed tracer testing area should be duly noted when any review of this evaluation is performed.

Before any of these compounds are used in populated areas where sub-chronic exposure is likely, further toxicity studies should be done. These studies should be of 90 day duration as a minimum or of 6 months duration. They should involve two animal species with one species being the rat so the results can be compared with similar compounds. They should be done at three dose levels with one level being low enough to insure that a no effect level would be found.

The existing toxicity data for the proposed compounds are so sketchy that there is a low level of confidence in the levels proposed if repeated human exposure is likely.

TOXICITY REPORT
TFAA Reference List:

Airaksinen, M.; Mammisto, T. LD_{50} and Some Metabolic Effects of Trifluoroethanol and Trifluoroacetic Acid in Mice and Guinea Pigs, *Annales Medicinae Experimenatalis et Biologiae Fenniae,* 1968, *46,* 242.

Blake, D.; Rosman, R.; Cascorbi, H.; Krantz, J. Biotransformation of Fluroxene - Metabolism in Mice and Dogs In-vivo, *Biochemical Pharmacology,* 1967, *16,* 1237.

Blake, D.; Cascorbi, H.; Rozman, R.; Meyer, F. Animal Toxicity of 2,2,2-Trifluoroethanol *Toxicology and Applied Pharmacology,* 1969, *15,* 83.

Blake, D.; Barry, J.; Cascorbi, H. Qualitative Analysis of Halothane Metabolites in Man *Anesthesiology,* 1972, *36,* 255.

Claassen, H.C.; Condes, E.H.; Two-well Recirculating Tracer Test in Fractured Carbonate Rock, Nevada, *Hydrological Sciences Bulletin,* 1975, *Vol XX,* No. 3, 367.

Cohen, E.; Trudell, J.; Edwards, H.; Watson, E. Urinary Metabolites of Halothane in Man, *Anesthesiology,* 1975, *43,* 392.

Cohen, E. Toxicity of Inhalation Anesthetic Agents, *British Journal of Anesthesia,* 1978, *50,* 665.

Fraser, J.M.; Kaminsky, L.S. Metabolism of 2,2,2-Trifluoroethanol and Its Relationship to Toxicity *Toxicology and Applied Pharmacology,* 1987, *89,* 202.

Fraser, J.M.; Kaminsky, L.S. 2,2,2-Trifluoroethanol Intestinal and Bone Marrow Toxicity: The role of It's Metabolism to 2,2,2-Trifluoroacetaldehyde and Trifluoroacetic Acid, *Toxicology and Applied Pharmacology,* 1988, *94,* 84.

Greene, N. *Halothane and Metabolism,* Clinical Anesthesia - Halothane, FA Davis, Philadelphia, 1968, p 182.

Lloyd, S.C.; Blackburn, D.M.; Foster, P.M.D. Trifluoroethanol and It's Oxidative Metabolites: Comparison of In-vivo Effects in Rat Testis, *Toxicology and Applied Pharmacology,* 1988, *92,* 390.

Ma, T.G.; Ling, Y.H.; McClure, G.D.; Tseng, M.T.; Effects of TFAA, A Halothane Metabolite, on C-6 Glioma Cells, *Journal of Toxicology and Environmental Health,* 1990, *31,* 147.

Rehder, K.; Forbes, J.; Alter, H.; Hessler, O.; Steir, A. Halothane Biotransformation in Man - A Quantitative Study, *Anesthesiology,* 1967, *28,* 711.

Steir, A. The Biotransformation of Halothane, *Anesthesiology,* 1968, *29,* 388.

Steir, A. TFAA as a Metabolite of Halothane, *Biochemical Pharmacology,* 1964, *13,* 1544.

vanDyke, P.; Chenoweth, M. Metabolism of Volatile Anesthetics, *Anesthesiology,* 1965, *26,* 348.

Witte, L.; Nau, H.; Fuhrop, J.; Doenicke, A.; Grote, B. Quantitative Analysis of TFAA in Body Fluids of Patients Treated With Halothane, *Journal of Chromatography - Biomedical Applications,* 1977, *143,* 329.

TABLE 1

LIST OF POTENTIAL TRACERS COMPOUNDS for the C-WELL TESTS

FLUORINATED BENZOIC ACIDS

2,3-Difluorobenzoic acid
2,4-Difluorobenzoic acid
2,5-Difluorobenzoic acid
2,6-Difluorobenzoic acid
3,4-Difluorobenzoic acid
3,5-Difluorobenzoic acid

2,3,4-Trifluorobenzoic acid
2,4,5-Trifluorobenzoic acid
3,4,5-Trifluorobenzoic acid

2,3,4,5-Tetrafluorobenzoic acid
2,3,5,6-Tetrafluorobenzoic acid

Pentafluorobenzoic acid

ortho-Trifluoromethylbenzoic acid (2-trifluoromethyltoluic acid)
meta-Trifluoromethylbenzoic acid (3-trifluoromethyltoluic acid)
para-Trifluoromethylbenzoic acid (4-trifluoromethyltoluic acid)

PERFLUORINATED ALIPHATIC ACIDS

Trifluoroacetic acid
Pentafluoropropionic acid
Heptafluorobutyric acid

END

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