Australia's National
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Identification and screening for potential physical hazards to human health from coal seam gas activities at a study site in the Surat Basin, southern Queensland.

Health 2 Extension: Chemical interactions with soil and groundwater in the Health 2 study site

Technical brief., 2022.

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This report details a short research task that aimed to examine the microbiological potential to degrade eight chemicals identified in the preceding GISERA Health 2 project. The project obtained two water and two soil samples from the study site used in the GISERA Health 2 project. Quantitation of chemicals was used to assess the microbiological degradation and to determine whether further assessment of the chemicals was required. The key results from the project are summarised below:

- In the present study, the chemicals tested were, in general, more readily degraded in soil microcosms than in groundwater microcosms. This result is consistent with prior work from GISERA projects in both South Australia (W15) and the Northern Territory (W17).
- The chemicals tributyl tetradecyl phosphonium chloride, tetrakis (hydroxymethyl) phosphonium sulphate, nonylphenol ethoxylates, and possibly the tracer 4-fluorobenzoic acid, were more persistent in both soil and groundwater samples and will require further assessment.
- The ready degradation of acrylamide and the isothiazolinone biocides indicate that these chemicals should be a lower priority for human health impacts.
- Glutaraldehyde readily interacts with organic matter forming cross linkages that alter the compound, removing its biocidal activity. The compounds resulting from glutaraldehyde cross linkages are readily degradable by microbes commonly found in soil and groundwater. In the present study, glutaraldehyde was undetectable in soils almost immediately after being added. In groundwater microcosms, glutaraldehyde persisted throughout the 90-day incubation, however, this is likely to be due to the absence of reactive materials in the groundwater. In the aquifer, glutaraldehyde will rapidly react with any organic material, and some inorganic material, rendering it inactive. The transport of glutaraldehyde over long distances through the aquifer seems highly improbable.
- Based on the results of this study, further assessment of TTPC, THPS, NPE, and the tracer 4-FBA is required through completing the assessment framework created by GISERA Health 1 and 2.

Introduction

The GISERA Health 2 project indicated that eight chemicals warranted further assessment for hazard potential to human health impacts. These eight chemicals had exposure pathways to humans via groundwater pathways. Four of these chemicals were of significant concern and were designated as chemicals of potential concern (COPC), and the other four chemicals required further information to determine their hazard potential to human health at the Health 2 study site. Specifically, the COPCs were: nonylphenol ethoxylates (NPE), tetrakis (hydroxymethyl) phosphonium sulphate (THPS), methylisothiazolinone (MIT) and methylchloroisothiazolinone (CMIT) (see Table 1). The chemicals requiring further information were: (poly)acrylamide (PAM), glutaraldehyde (GA), 4-fluorobenzoic acid (4-FBA), and tributyl tetradecyl phosphonium chloride (TTPC) (see Table 1).

The Health 2 extension work was a research task that aimed to examine the microbiological potential to degrade these eight chemicals by microbes found in soil and groundwater samples from the study site used in the GISERA Health 2 project. Chemical quantitation was used to assess microbiological degradation and to determine whether further assessment of the chemicals was required. Any chemical requiring further assessment would be progressed to the GISERA Health 3 project.

More than half of these chemicals are used in industrial applications as biocides to control microbial activity. Furthermore, four of these biocides fall into two broad groups, the first are the isothizolinone biocides MIT and CMIT which are structurally similar chemicals (Figure 1).

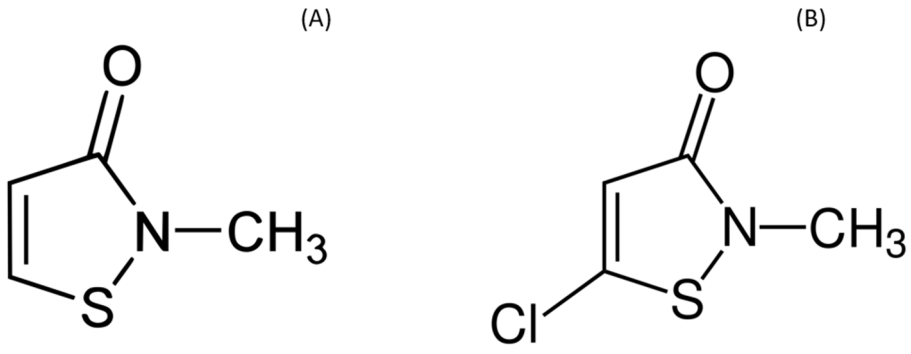

Figure 1: The chemical structure of the methylisothiazolinone (MIT; A) and methylchloroisothiazolinone (CMIT; B) biocides.

The second group of biocides both include a phosphonium group, though they differ in strucure (Figure 2), with TTPC featuring a long alpiphatic tail and longer branches around the phosphonium group.

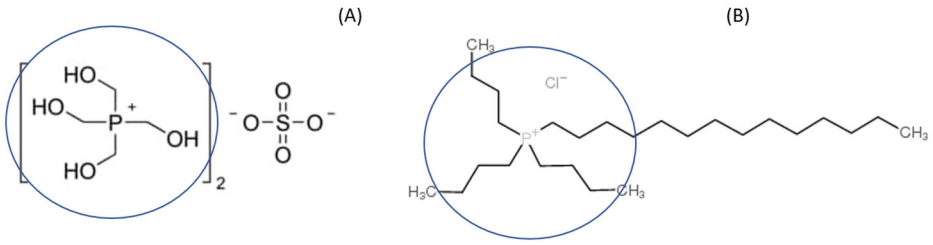

Figure 2: The chemical structure of the phosphonium biocides: tetrakis (hydroxymethyl) phosphonium sulphate (THPS; A) and tributyl tetradecyl phosphonium chloride (TTPC; B).

Blue circles denote phosphonium groups.

Glutaraldehyde, another biocide, is known to be a very reactive compound, with the double-bonded oxygen groups at each end of the molecule (Figure 3) readily interacting with a range of organic molecules to "cross-link" organic components.

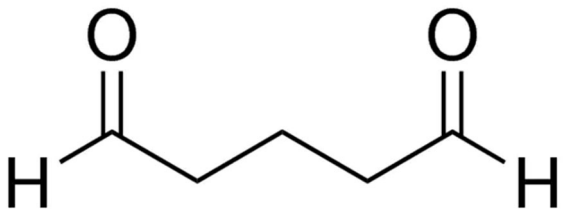

Figure 3: The chemical structure of glutaraldehyde.

That five of the eight COPCs being examined here are biocides is unsurprising, as these compounds are known to/designed to have strong, negative interactions with living organisms.

Nonylphenol ethoxylates (NPE) and nonylphenols (NP) are both used as surfactants, detergents, emulsifiers, wetting agents, industrial cleaners, metalworking fluids, plastics additives, and numerous other applications. These chemicals are wetting agent additives used in drilling and completion fluids. NP are phenols with a nine-carbon tail in the *para*-position, however, the nonyl group can be in the *ortho*-position and the nonyl tail itself can be branched or linear (Figure 4). NPE are ethoxylated versions of NP with an additional carbon chain interspersed with ether bridges and terminating in a hydroxyl group (Figure 4). This ethoxylated carbon chain can vary in length. In the present study, only NPE was considered, and NP will be progressed to the GISERA Health 3 project.

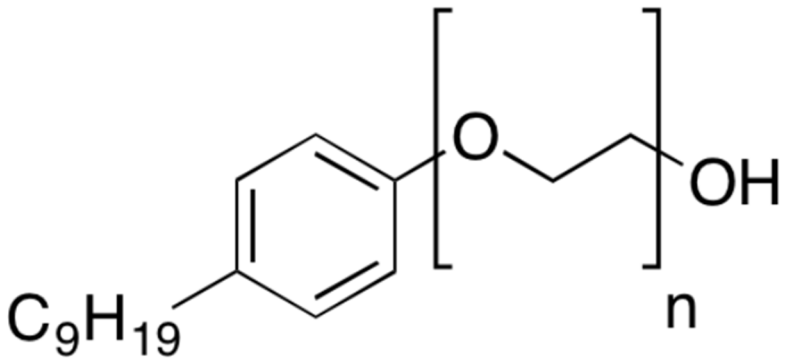

Figure 4: The chemical structure of nonylphenol ethoxylate.

Fluorobenzoic acids (FBAs) are synthetic chemicals not naturally found occurring in the environment and are commonly used as tracers in water systems. In the coal seam gas (CSG) industry, FBAs are used in hydraulic fracturing to understand fluid flow behaviours in reservoirs and flow pathways. Chemically, FBA refers to three isomers (*ortho*-, *meta*- and *para*-) of fluorinated benzoic acid, however, the CSG industry, and other industries, refer to a range of related tracers as FBAs. This includes di, tri, tetra fluorinated benzoic acids, along with trifluoromethylated forms and occasionally chlorinated benzoic acids. The present study used 4-FBA, the *para*-position of fluorobenzoic acid (Figure 5). The remaining commercial FBA tracers used by industry will be progressed to the GISERA Health 3 project.

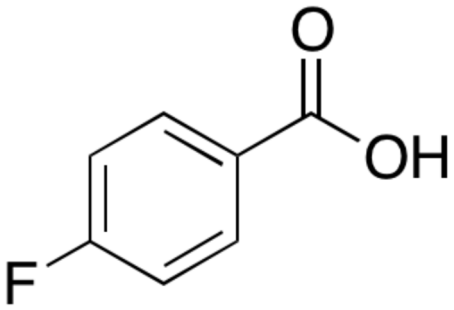

Figure 5: The chemical structure of 4-fluorobenzoic acid.

Methods

Groundwater and soil sampling

A sampling program was undertaken in the GISERA Health 2 study site (Figure 6) to collect two matched soil and groundwater samples. For the purposes of reporting, the soil samples will be referred to as S1 and S2; and the groundwater samples will be referred to as W1 and W2. Samples were collected with the assistance of a local landowner within the GISERA Health 2 study site. Soil samples were taken within 10m from water bores used for groundwater sampling.

Soils were sampled using sanitised spades into a sanitised polypropylene bucket (approximately 12kg). Field sanitisation was undertaken by rinsing and wiping with methylated spirits. The surface litter was removed prior to collection. Samples were sealed and transported back to laboratories in Lindfield, NSW.

For groundwater samples, bores were allowed to purge for 10 minutes to ensure samples were collected from the aquifers. Bulk groundwater samples of ~15L were collected and stored in polypropylene containers for transport. In addition to the bulk groundwater samples, anoxic samples of ~1L volumes were collected (under a CO_2 atmosphere). Water was bubbled vigorously with CO_2 prior to being sealed in Schott bottles with silicone rubber seals. Sealed bottles were transported inside eskies back to the CSIRO laboratory at Lindfield, NSW. On receipt at Lindfield, the bottles were vented inside an anaerobic chamber where the atmosphere comprised ~95% argon, 1-2% hydrogen and the balance nitrogen (~3-4%). These samples were used as inoculum in groundwater microcosm experiments.

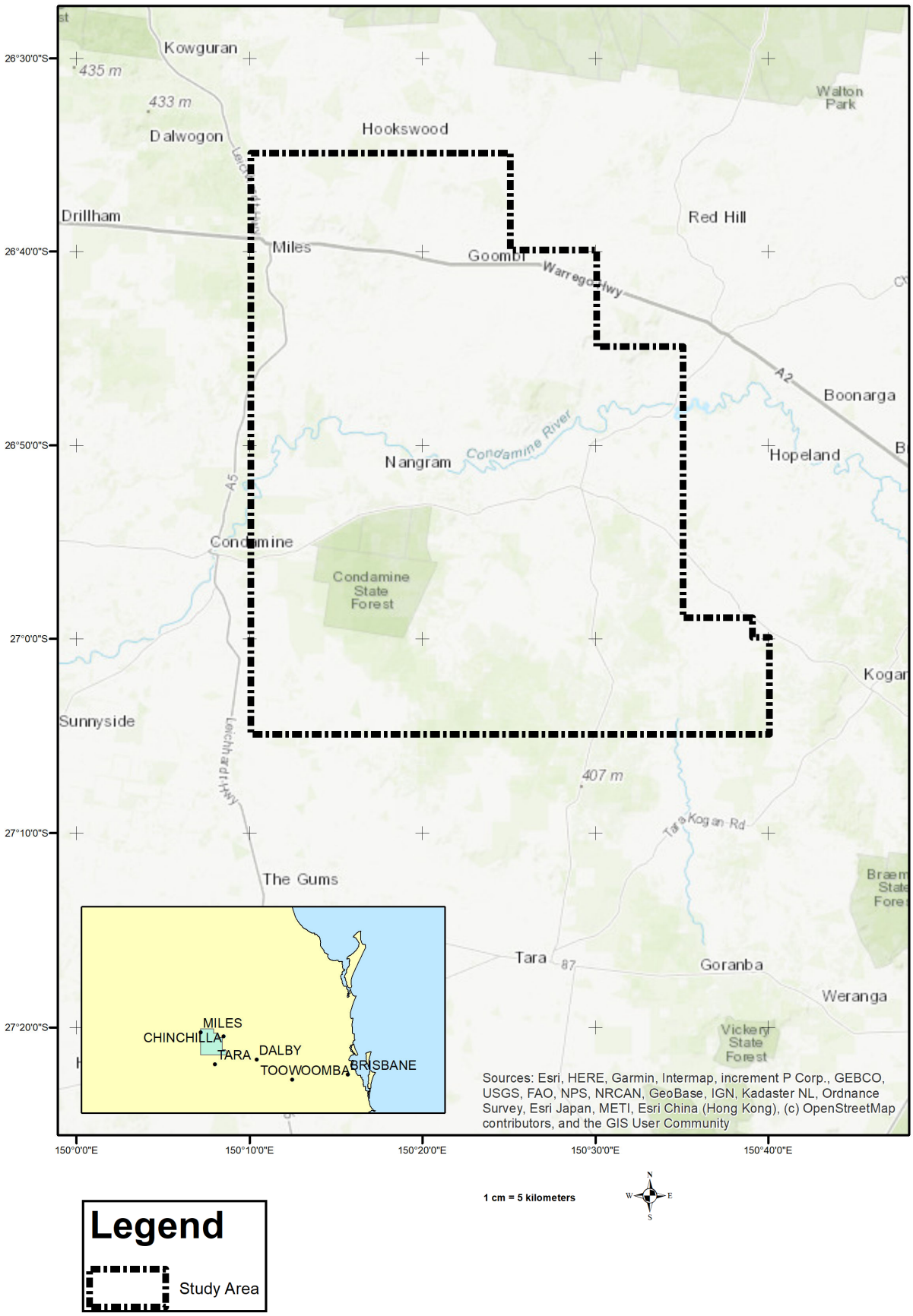

Figure 6: GISERA Health 2 study site.

Soil and groundwater chemistry

The soil and groundwater chemistry were both measured by Australian Laboratory Services (ALS) Environmental using a range of standard methods in a NATA-accredited laboratory environment. The schedule of physical and chemical tests included pH, EC, moisture content, metals (Al, As, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Hg, Pb, Mn, Mo, Ni, Sb, Se, Sr, Sn, V, Zn), total inorganic and organic carbon, total nitrogen and phosphorus, major cations and anions, total fluoride and bromide.

Soil microcosm establishment

On return to the Lindfield laboratories, the soil samples were coarsely sieved through a 6mm mesh, prior to extensive mixing. For S1, larger soil particles were crushed prior to being sieved. All soil samples were stored in the dark prior to use in experiments.

In order to establish microbial degradation of chemicals, each soil microcosm was setup with ~200mL fresh soil and 4mL sterile reverse osmosis water for wetting. Chemicals were added to a final target concentration of 10mg/kg. All microcosm experiments were established in sets of four. Two of these replicates were frozen at -80°C after establishment (within 1-2 hours) and were used for chemical quantitation at zero time (see below). Two replicates were incubated for 30 days at 22°C with day/night lighting (10hr light with 14 hr dark). Microcosm lids were loose to provide air exchange.

Groundwater microcosm establishment

All groundwater microcosms were established in 165mL glass serum vials under an anoxic atmosphere that comprised ~95% argon, 1-2% hydrogen and the balance nitrogen. Each vessel contained 110mL of filter-sterile groundwater. This was inoculated with 30mL of groundwater collected under a CO_2 atmosphere and chemicals were added to achieve a target final concentration of 10mg/L**Error! Reference source not found.**. All groundwater microcosm experiments were established in sets of four. Two of these replicates were frozen at -80°C after establishment (within 1-2 hours) and were used for chemical quantitation at zero time (see below). Two replicates were incubated for 90 days at 22°C in the dark.

Chemical quantitation

To determine the initial and final chemical concentrations, microcosms were sampled immediately and at the end of the incubation period for analytical analysis by a NATA accredited laboratory, ACS Laboratories (Australia) located in Melbourne, Victoria. All microcosms were transported frozen to ACS Laboratories. Analysis of all microcosm samples and standards was performed by UPLC-MS-MS on a Waters Acquity Xevo-TQS micro. Separation was performed on a reverse phased C18 column and acquisition was by positive mode electrospray (ESI+). Identification was based on compound specific retention times and metastable reaction monitoring.

Table 1: Chemicals of potential concern tested in this project

Chemical	Abbreviation	CAS	Chemical formula	Drinking water guideline mg/L	Target final concentration in microcosms
Nonylphenol ethoxylates	NPE	9016-45-9	$C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$	0.5	10 mg/L
Tetrakis(hydroxymethyl) phosphonium sulphate	THPS	55566-30-8	$(C_4H_{12}O_4P)_2SO_4$		10 mg/L
Methylisothiazolinone	MIT	2682-20-4	C_4H_5NOS	0.6	10 mg/L
Methylchloroisothiazolinone	CMIT	26172-55-4	$C_4H4ClNOS$	0.6	10 mg/L
(Poly)acrylamide	PAM	9003-05-8	$(CH_2{:}CHCONH_2)_n$	0.0002	10 mg/L
Glutaraldehyde	GA	111-30-8	$OHC(CH_2)_3CHO$	0.14	10 mg/L
4-Fluorobenzoic acid	FBA	456-22-4	$FC_6H_4CO_2H$		10 mg/L
Tributyl tetradecyl phosphonium chloride	TTPC	81741-28-8	$C_{26}H_{56}P{\bullet}Cl$	0.03	10 mg/L

Results and Discussion

Two soils were collected from two locations within the Health 2 study site (Figure 6). Both samples were topsoil samples taken from the top 10 cm of the soil. The first, S1, was taken from pasture used for grazing cattle. S1 was a heavy clay loam that formed hard, large peds (Figure 7). The S1 soil was mildly acidic in pH, and compared to the other soil in the study, was moderately saline (though compared to saline soils it was relatively non-saline). This mild salinity in S1 is evident in its electrical conductivity and elevated levels of both sodium and chlorine (Table 2). The other soil sample, S2, was collected from pasture that was not used for grazing. In contrast to the S1, S2 was neutral in pH and had negligible conductivity (Table 2). In addition, S2 was a more sandy, open soil that did not readily form peds (Figure 7). Both soils had appreciable concentrations of nitrogen, ~1000 and ~500 mg per kg for S1 and S2, respectively. Similarly, the soils both had relatively high concentrations of phosphorus >100mg per kg (Table 2).

Table 2: Soil chemistry of the two soil samples collected within the Health 2 study site.

Analyte	S1	S2
Soil type	Clay loam	Sandy loam
pH (pH units)	5.4	6.9
Electrical Conductivity (µS/cm)	1770	20
Moisture Content (%)	10.5	7.8
Bromide (mg/kg)	6.31	<0.05
Fluoride (mg/kg)	<0.5	0.24
Bicarbonate Alkalinity as $CaCO_3$ (mg/kg)	260	277
Total Alkalinity as $CaCO_3$ (mg/kg)	260	277
Sulfate as $(SO_4)^{2-}$ (mg/kg)	830	<10
Chloride (mg/kg)	2750	10
Calcium (mg/kg)	170	<10
Magnesium (mg/kg)	190	<10
Sodium (mg/kg)	1360	20
Potassium (mg/kg)	20	<10
Aluminium (mg/kg)	5330	2720
Barium (mg/kg)	40	40
Boron (mg/kg)	<50	<50
Chromium (mg/kg)	13	29
Cobalt (mg/kg)	3	10
Iron (mg/kg)	12900	8230
Lead (mg/kg)	5	<5
Manganese (mg/kg)	158	581
Molybdenum (mg/kg)	<2	12
Nickel (mg/kg)	4	65
Strontium (mg/kg)	33	13
Vanadium (mg/kg)	44	24
Zinc (mg/kg)	6	<5
Uranium (mg/kg)	0.4	0.2
Lithium (mg/kg)	0.4	0.6
Ammonia as N (mg/kg)	60	<20
Nitrite as N (Sol.) (mg/kg)	<0.1	0.2
Nitrate as N (Sol.) (mg/kg)	81.3	0.4
Nitrite + Nitrate as N (Sol.) (mg/kg)	81.3	0.6
Total Kjeldahl Nitrogen as N (mg/kg)	1140	450
Total Nitrogen as N (mg/kg)	1220	450
Total Phosphorus as P (mg/kg)	182	122
Total Organic Carbon (mg/kg)	1.06	0.64
Total Carbon (mg/kg)	1.17	0.75
Total Inorganic Carbon (mg/kg)	0.11	0.11

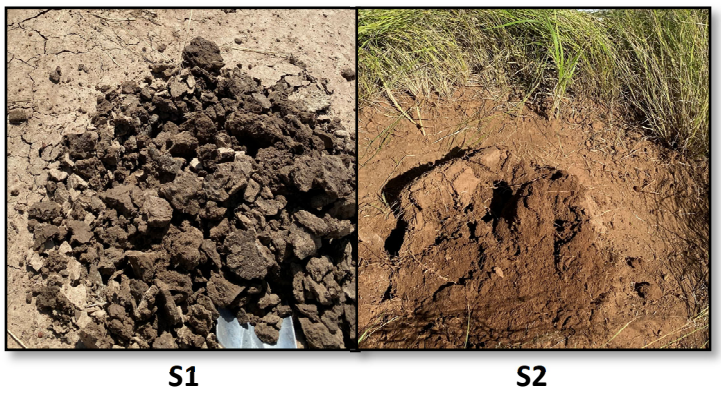

Figure 7: Soil samples S1 and S2.

The two groundwater samples for the present study were collected from bores that were ~90m (W1) and ~250m (W2) subsurface and both are reported on completion reports as intersecting the Kumbarilla Beds. The Kumbarilla Beds encompass multiple aquifers and are characterised by Na-HCO_3-Cl-type groundwater chemistry with a large range of values for chemical analytes (including pH, salinity, sulfate, and chloride) depending upon the depth within the sequence (Ransley et al., 2015). There are distinctions between the various formations of the Kumbarilla Beds in the subsurface, however, for many of the water bores within the Kumbarilla Beds these distinctions have not been used in assigning aquifer intercepts (Ransley et al., 2015).

The chemistry of the two groundwaters differed considerably (Table 3). In brief, W1 was neutral in pH and was moderately brackish, having a comparatively high electrical conductivity (~15000µS/cm). In contrast, W2 was more alkaline, having a pH over 8.5 but had much lower conductivity ~2000µS/cm (and was therefore non-brackish freshwater). As is common in groundwaters, both W1 and W2 contain only very small amounts of phosphorus and nitrogen (Table 3). The chemistries of W1 and W2 are broadly consistent with previous bore water samples from the Kumbarilla Beds (Ransley et al., 2015).

Table 3: Water chemistry of the two groundwater samples collected within the Health 2 study site.

Analyte	W1	W2
pH Value (pH units)	7.25	8.62
Electrical Conductivity (µS/cm)	15400	2040
Bromide (mg/L)	8.5	0.46
Carbonate Alkalinity as $CaCO_3$ (mg/L)	<1	58
Bicarbonate Alkalinity as $CaCO_3$ (mg/L)	181	848
Total Alkalinity as $CaCO_3$ (mg/L)	181	907
Sulfate as SO_4 – Turbidimetric (mg/L)	92	<1
Chloride (mg/L)	5510	142
Calcium (mg/L)	190	2
Magnesium (mg/L)	216	<1
Sodium (mg/L)	2780	552
Potassium (mg/L)	13	2
Aluminium (mg/L)	0.11	<0.01
Arsenic (mg/L)	0.001	<0.001
Beryllium (mg/L)	0.004	<0.001
Barium (mg/L)	0.179	0.036
Cadmium (mg/L)	0.0003	<0.0001
Cobalt (mg/L)	1.06	<0.001
Copper (mg/L)	0.028	<0.001
Lead (mg/L)	0.007	<0.001
Lithium (mg/L)	0.105	0.032
Manganese (mg/L)	1.42	0.005
Nickel (mg/L)	0.833	<0.001
Strontium (mg/L)	6.78	0.116
Zinc (mg/L)	0.102	0.015
Boron (mg/L)	0.4	2.74
Iron (mg/L)	83.2	0.72
Fluoride (mg/L)	0.1	2.5
Ammonia as N (mg/L)	0.09	0.63
Total Kjeldahl Nitrogen as N (mg/L)	0.5	0.6
Total Nitrogen as N (mg/L)	0.5	0.6
Total Phosphorus as P (mg/L)	0.11	0.1
Reactive Phosphorus as P (mg/L)	<0.01	0.04
Total Anions (mg/L)	161	22.1
Total Cations (mg/L)	148	24.2
Ionic Balance (mg/L)	4.02	4.39
Total Inorganic Carbon (mg/L)	58	218

The microbiome within soils and groundwaters is typically able to degrade a large range of compounds due to the diversity of microbes in these environments and the catabolic (i.e., the part of metabolism associated with breaking down compounds) potential contained in the collective genomes of the different species present (Cho and Kim, 2000; Dagley, 1978; Díaz, 2004; Feris et al., 2004; Franzmann et al., 2002; Haack et al., 2004; Johnson et al., 2004; Pieper et al., 2004). Different environments have vastly different numbers of microbial species and cell numbers, for instance, soils are known to have between 5000-8000 species per gram of soil and somewhere between 10^8 and 10^{10} cells per gram of soil (Griebler and Lueders 2009; Rosselló-Mora and Amann 2001; Whitman et al. 1998). In contrast, groundwaters have relatively low microbial diversity, with typically much less than 1000 species per millilitre of water, and cell numbers of 10^2-10^5 per millilitre of water (Griebler and Lueders 2009; Whitman et al. 1998). Regardless, even accounting for relatively low diversity, the catabolic potential of hundreds of microbial species is relatively high and can degrade various compounds (Dagley, 1978).

One constraint on possible degradation of compounds in soils and groundwaters are the absence of other important macronutrients (Jayaramaiah et al., 2022; Kuppusamy et al., 2017). One useful parallel can be drawn from composting. Typically composting requires the use of both brown materials (containing mostly carbon in the form of cellulosic compounds and lignin e.g., wood and sticks) along with green material (nitrogen/phosphorus rich materials e.g., fresh leaves). In the absence of these green materials, brown materials are typically slow to degrade due to a shortage of nitrogen and phosphorus which are required to use the carbon contained within the brown material. Similarly, chemicals in soil or groundwater which are mostly carbon can only be degraded in the presence of significant sources of nitrogen. Thus, the degradation of a particular compound is heavily dependent on two factors:

1) the microbial community present and their genetic potential to degrade this compound.

2) the nutrient availability, particularly of nitrogen or phosphorus, required for microbial catabolic activity.

The biodegradation potential of soil microbial communities has been reported to be highly depended on both initial microbial diversity and nutrient availability (Jayaramaiah et al., 2022).

In addition, toxicity of a specific compound at a given concentration may be too high to allow degradation and may instead be bactericidal in these environments, as ever '*the dose makes the poison*', is particularly true for biocides, chemicals whose designated purpose is to kill bacteria and other microbes.

In the current experiments, the concentration of all chemicals was fixed at 10 mg per litre or kilogram of groundwater or soil, respectively. The only differences between the soil and groundwater experiments were their (1) time of incubation and (2) atmosphere. Soil microcosm experiments were incubated with the chemicals for 30 days. In contrast, the groundwater microcosms were incubated with the chemicals for 90 days. This increase in time allowed the groundwater microbial community, which is typically slower acting, to interact with all chemicals tested. Regarding atmosphere, the soil microcosms were incubated under oxic conditions (i.e., they were exposed to air), while the groundwater samples were incubated under an anoxic atmosphere that consisted primarily of argon.

In the current study, microcosm experiments were established in sterile laboratory vessels which contained either soil or groundwater. These microcosms were supplemented with the chemcials (at 10 mg per litre or kg) examined in the present study. No additional nutrients or chemicals were added to the microcosms.

One of the chemicals examined in the present study was polyacrylamide. Polyacrylamide is used by the CSG industry to manage the viscosity of other liquids as it forms a gel like material in water (Smith and Oehme, 1991; Xiong et al., 2018). Polyacrylamide itself is nontoxic (Farkas et al., 2020; Hansen et al., 2019; McCollister et al., 1965), however, the compound is created through the polymerisation of acrylamide, a known neurotoxic compound (King and Noss, 1989; Smith and Oehme, 1991). The release of acrylamide is thus the primary concern regarding polyacrylamide. The degradation of polyacrylamide has been well studied, particularly in soil (Caulfield et al., 2002; Kay-Shoemake et al., 1998; Nyyssölä and Ahlgren, 2019; Xiong et al., 2018). Microorganisms can biodegrade the polymer to utilise the amide group as a nitrogen source and/or the carbon backbone as a carbon source (Nakamiya and Kinoshita, 1995; Wen et al., 2010). The process is largely thought to proceed via very rapid deamination of the polymer (i.e., the removal of the $-NH_2$ groups from the compound) to form a compound known as polyacrylate (Nyyssölä and Ahlgren, 2019), followed by quite slow degradation of polyacrylates (Hayashi et al., 1994; Liang et al., 2018). For example, Wilske et al., (2014) found polyacrylate degraded in soil at rates of 0.12–0.24% per six months. It is notable, however, that polyacrylates are generally shown to be of moderate to low toxicity ("Acrylic acid polymer, neutralized, cross-linked," 2012). Furthermore, the rapid deamination of polyacrylamide in soils renders the release of acrylamide (a compound that contains amide groups) highly improbable. In the present study, no acrylamide was released when polyacrylamide was added to either soil S1 or S2 (Figure 8), and after the 30 day incubation acrylamide was undetected (Figure 8). This is consistent with the literature which suggests acrylamide cannot be liberated from polyacrylamide via oxic microbial degradation (Nyyssölä and Ahlgren, 2019).

In contrast to its degradation in soils, a small number of studies have reported acrylamide release during anoxic degradation of polyacrylamide (Dai et al., 2014; Wang et al., 2018). It should be noted that residual acrylamide can also be trapped within the polymeric matrix that forms during polymerisation of acrylamide to make polyacrylamide (Wang et al., 2018). This trapped, residual acrylamide can be released when the matrix is degraded or is agitated in water. This property complicates microbial studies that use polyacrylamide as it is difficult to distinguish "microbially released" acrylamide from residual acrylamide that was trapped and released during polyacrylamide degradation. Furthermore, it seems improbable from a thermodynamic perspective that a high-energy compound like acrylamide could be reformed from polyacrylamide, especially under anoxic conditions where energy is at a premium (Reber et al., 2007). As described

above, such production of acrylamide, from polyacrylamide would require significant energy input and the energetic advantage such a process would have to microbes is unclear.

Furthermore, there is evidence that anaerobic microbes can use both acrylamide and polyacrylamide as sole sources of carbon and nitrogen, with the former being degraded to acrylic acid (Caulfield et al., 2002; Kay-Shoemake et al., 1998; Nyyssölä and Ahlgren, 2019; Xiong et al., 2018). The process for acrylamide appears to mirror the process for polyacrylamide and starts with deamination followed by digestion of the carbon backbone. In the present study, trace amounts of acrylamide were evident in the groundwater samples hours after the polyacrylamide additions (maximum detected concentration 0.1 mg L^{-1}; Figure 8), most probably due to the release of residual acrylamide within the polymeric matrix of polyacrylamide. After 90 days of incubation however, no acrylamide was detected in groundwater microcosms (Figure 8). This indicated that any residual acrylamide that was initially released had been degraded.

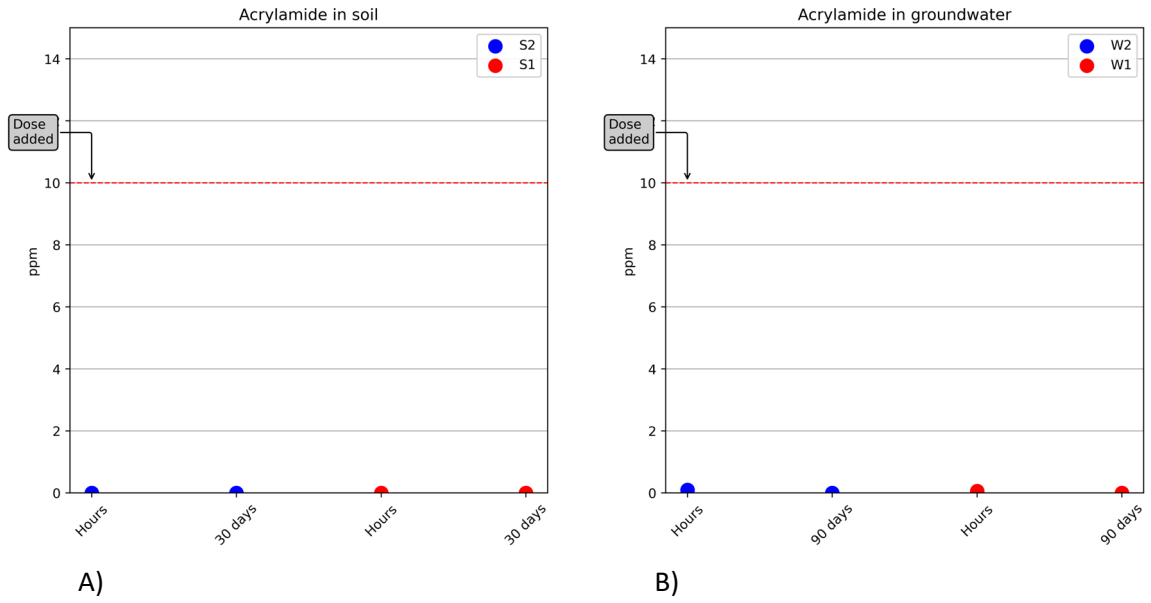

Figure 8: Acrylamide concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Glutaraldehyde is another chemical examined in this study. Structurally, this chemical is a dialdehyde, and its activity as a biocide is due to the double-bonded oxygen groups that occur at each end of the molecule (Russell and Hopwood, 1976). These oxygen groups are highly reactive and will interact with a range of biological and abiotic components within the environment (Migneault et al., 2004). When these aldehydic groups interact with proteins or other suitable chemicals, ether bridges are formed (Cheung and Nimni, 1982a, 1982b). This reaction links the glutaraldehyde backbone to organic matter it is interacting with. The chain-like structure of glutaraldehyde (being a straight chain of five single-bonded carbons) is such that it can link two, previously unconnected components, and is the mode by which glutaraldehyde acts as an antimicrobial compound (McGucken and Woodside 1973). Furthermore, this behaviour explains the scientific use of glutaraldehyde as a cross-linking agent (Pal et al., 2013; Payne, 1973; Salem et al., 2010). From a toxicological perspective, it is important to note that once these bonds are formed, glutaraldehyde no longer exists, instead what remains is a pentane-like compound, linked by diether bonds to other components.

Microbially, glutaraldehyde is degraded via one of two known pathways, both via the carboxylic acid glutarate (glutaric acid). In the anaerobic pathway, glutaraldehyde is first converted to 1,5-pentanediol, while this step is omitted in the aerobic pathway. Glutarate is subsequently degraded via oxidation and eventually enters the TCA cycle (in those organisms that possess this pathway), where its carbon is recycled into common cellular components or CO_2. Microbes can also attack the ether bridges that link the pentane-like backbone of glutaraldehyde, yielding compounds like 1,5-pentanediol, in order to access the carbon contained within the carbon backbone.

In the present study, glutaraldehyde was unaffected in the groundwater microcosms and did not degrade (Figure 9). This is most probably due to the lack of compounds for GA to interact with in the groundwater. In contrast, within a few hours virtually all GA was consumed within soil samples (Figure 9). Indeed, only 20 µg/kg of the 10 mg/kg of GA added being detectable in the time it took to setup the experiment (a few hours; see Methods: Soil microcosm establishment), indicating its high reactivity. Even though GA was present after incubation for 90 days in the groundwater, it seems highly likely that its high reactivity would mean that during its transport through various aquifers and soils, it would likely encounter biofilms, clays, moribund organic matter that would render it neutralised. For these reasons it seems an unlikely risk to human health and will not be substantively investigated in the upcoming GISERA Health 3 programme of work.

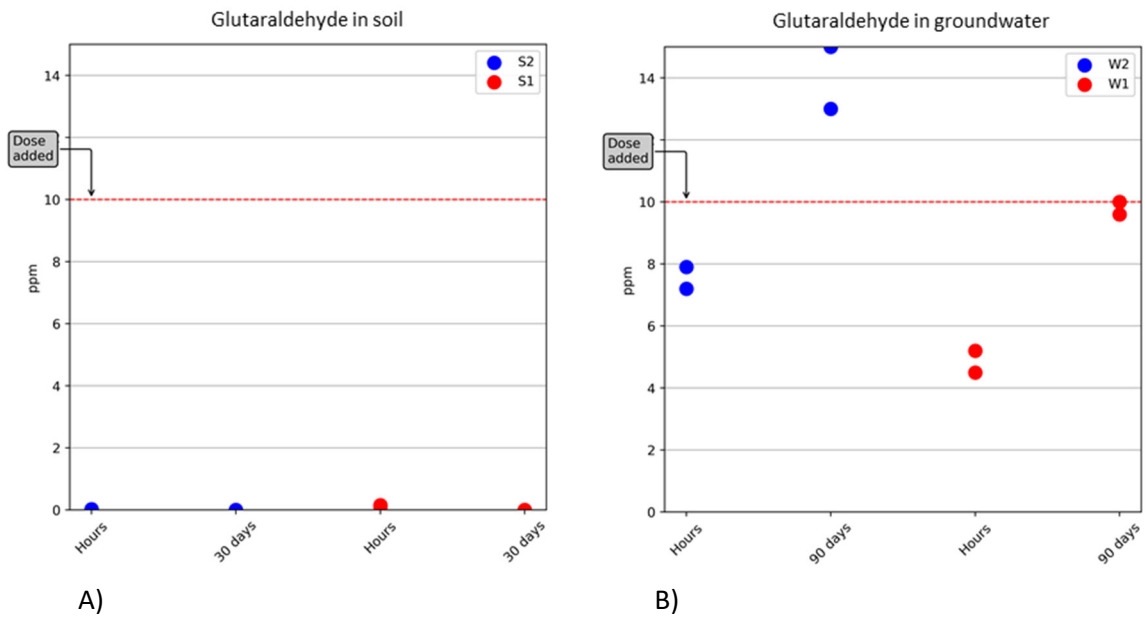

Figure 9: Glutaraldehyde concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

As described in the introduction, nonylphenols (NP) and their ethoxylated derivatives nonylphenol ethoxylates (NPE) were used by industry for a variety of purposes. It should be noted that both NP and NPEs can represent groups of related compounds. Furthermore, there is a substantial, but incomplete literature on their microbial degradation. For example, studies using a single strain of *Psuedomonas putida* demonstrate that the ethoxylated chain is readily degraded by sequential, exo-acting attack that result in production of nonylphenol diethoxlate regardless of the initial length of the ethoxylate side group. This is presumably due to steric interference caused by the phenolic ring closer to the centre of the molecule. It is also likely the source of the suggestion that nonylphenol is generated as the by-product of NPE degradation (Mao et al., 2012). The literature, however, provides numerous examples of complete degradation of NP and NPE by a range of microbes including numerous common genera e.g., pseudomonads of various kinds. This occurs under both oxic and anoxic conditions. In the present study, one of the soils (S2) degraded NPE (Figure 10), though this study did not determine the presence of intermediates like NP. Interestingly, in the other soil (S1) the NPE did not degrade appreciably over the course of the 30-day incubation (Figure 10). This may be due to interactions of the ethoxylate side chain (which is more hydrophilic) with sheet minerals like clays which are more abundant in S1 than in S2. In the groundwater microcosm experiments, NPE did not appreciably degrade after a 90-day incubation

with groundwater (Figure 10). As such, NPE, and other chemicals in this group (NP), will be progressed to the upcoming GISERA Health 3 programme of work.

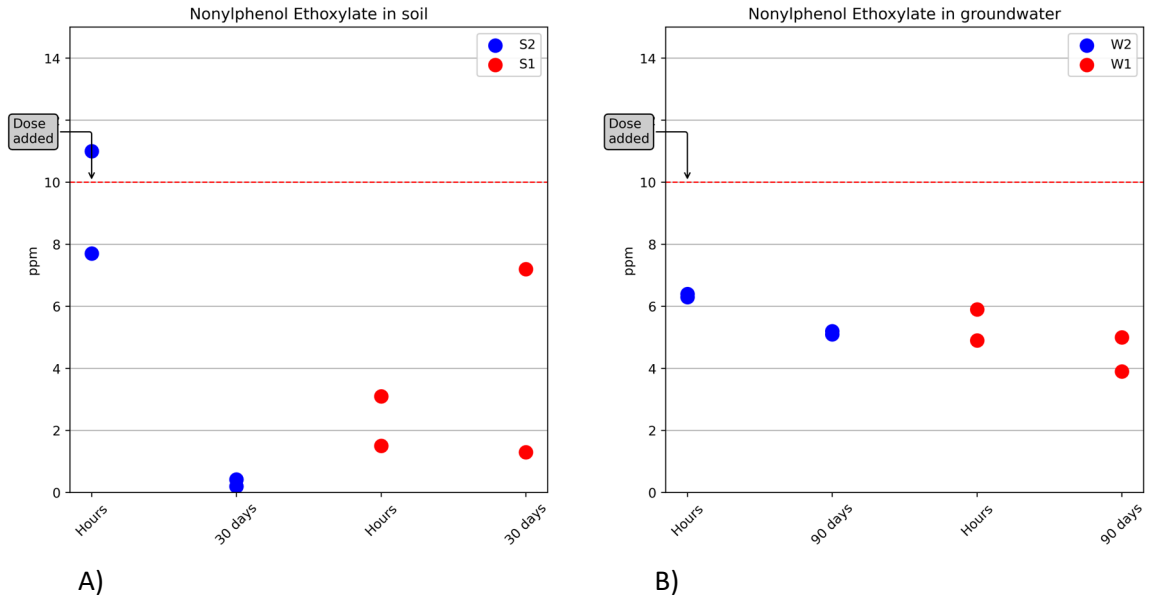

Figure 10: Nonylphenol ethoxylate concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Similarly to nonylphenols and their ethoxylates, fluorobenzoic acids represent specific chemicals and, more broadly, a group of related chemicals. The gas industry typically groups these compounds together as “fluorobenzoic acids” (FBA) but it should be noted that there is variability within this group and there are between 14-20 commercially available FBAs for use primarily as tracers (Hu and Moran, 2005). Chemical variation in the FBAs is due to the number and position of the fluorine atom (or of the fluorinated-methyl group) (Hu and Moran, 2005). In the present study, just one isomer of fluorobenzoic acid (*sensu stricto*) was examined, namely 4-fluorobenzoic acid, and will be referred to as 4-FBA for reporting. There have been numerous studies demonstrating that FBAs are readily used as a sole source of carbon, particularly under oxic conditions (Boersma et al., 2004; Engesser et al., 1990, 1980; Kiel and Engesser, 2015; Oltmanns et al., 1989; Schennen et al., 1985; Schlömann et al., 1990). In these oxic reactions, degradation proceeds via the intermediate catechol, which is fluorinated in the case of FBAs (Carvalho et al., 2006; Murphy, 2010).

In the present study, 4-FBA was degraded in both soils, with S2 degrading more of the dosed 4-FBA than S1 (Figure 11). In contrast, in both groundwater samples 4-FBA appeared to be recalcitrant to degradation (Figure 11). This is in keeping with its use in the subsurface as a tracer compound. Despite this, there is evidence of anaerobic metabolism of FBAs (Schennen et al., 1985; Song et al., 2000; Vargas et al., 2000). These degradations proceed using the benzoyl-coA reductase pathway (Tiedt et al., 2018). Regardless, the limited catabolism of 4-fluorobenzoic acid in S1 and in the groundwater samples requires this compound to be progressed to the GISERA Health 3 programme of work.

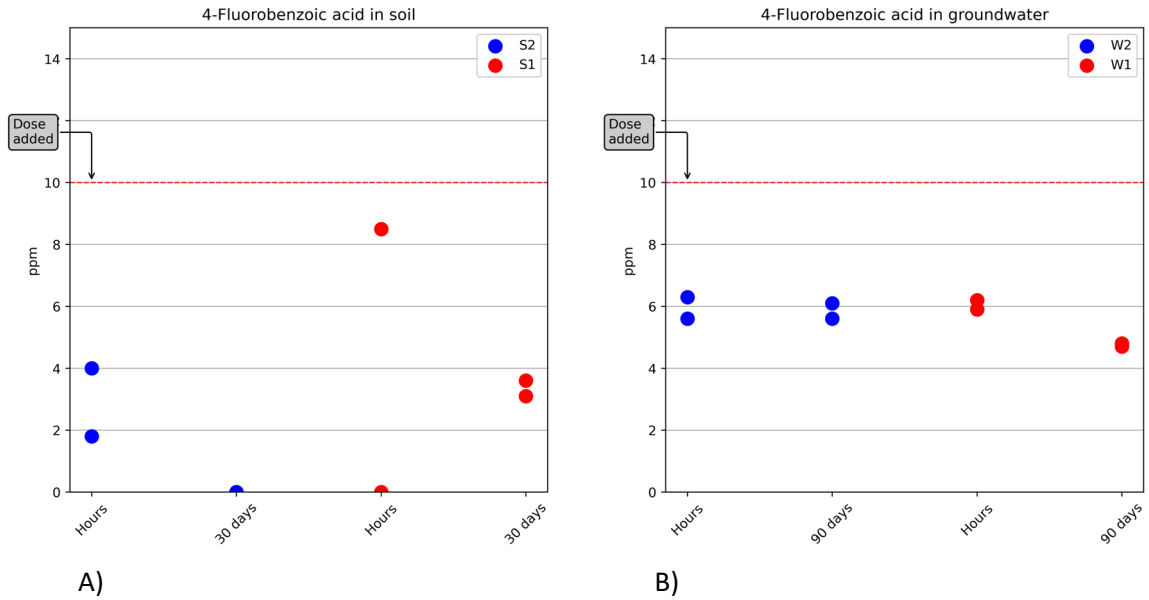

Figure 11: 4-Fluorobenzoic acid concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Isothiazolinone biocides are used as drilling additives and during hydraulic fracturing processes. These compounds include a range of derivatives including the two COPCs progressed from the Health 2 study: methylisothiazolinone (MIT) and methylchloroisothiazolinone (CMIT). While it has been demonstrated that these biocides have activity against prokaryotic microbes and fungi, numerous fungi also appear to have acquired high tolerance to these compounds. For example, Gomes et al., (2018) demonstrated that numerous fungi including both asco- and basidiomycetes were able to grow on very high concentrations (up to 40 g/L) of MIT. That many of these fungi were isolated from wall paint containing isothiazolinones suggests that these fungi were selected for tolerance of high concentrations of these biocides. Gomes and co-workers note that while the

fungi they isolated are extremely tolerant to isothaizolinones, this is, in their opinion, a somewhat uncommon situation and many taxa are likely sensitive to these compounds. In the present study, MIT was almost completely degraded in both soils and partially degraded in one groundwater (W2) (Figure 12).

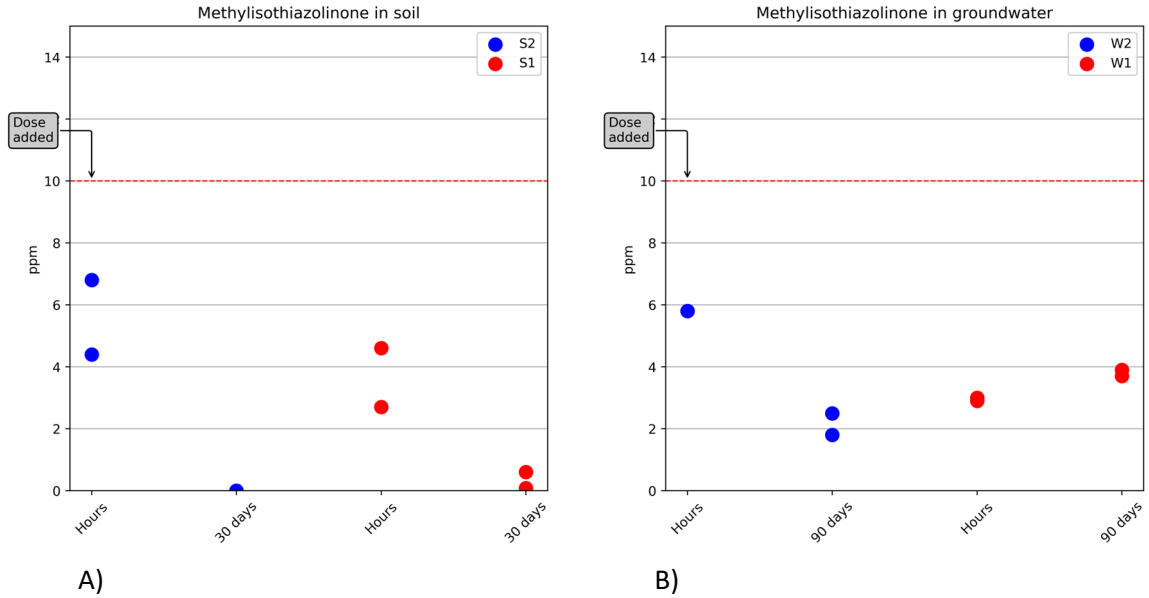

Figure 12: Methylisothiazolinone concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

The addition of the chlorine to methylisothiazolinone increases the biological activity and decreases the rate of degradation of methylchloroisothiazolinone. For example, in river water and a simulated sewerage system, methylchloroisothiazolinone was determined to be significantly slower to degrade compared to methylisothiazolinone (Krzeminski et al., 1975a, 1975b). As for other biocides, the dose is critical, with lower doses being more readily degraded as, presumably, they do not kill the organisms capable of their degradation. Various degradation products are formed including: N-methylmalonamic acid, malonamic, malonic, acetic, and formic acids along with 5-chloro-2-methyl-4-isothiazolin-1-oxide, N-methylglyoxylamide, ethylene glycol, and urea (Krzeminski et al., 1975b). Taken together, the distribution and dilution of isothiazolinones in a hypothetical aquifer or soil render the environmental and human health risk of these compounds through aquifer pathways improbably low (Krzeminski et al., 1975a).

In the present study, CMIT was completely degraded in both soils and almost completely degraded in both groundwaters (Figure 13).

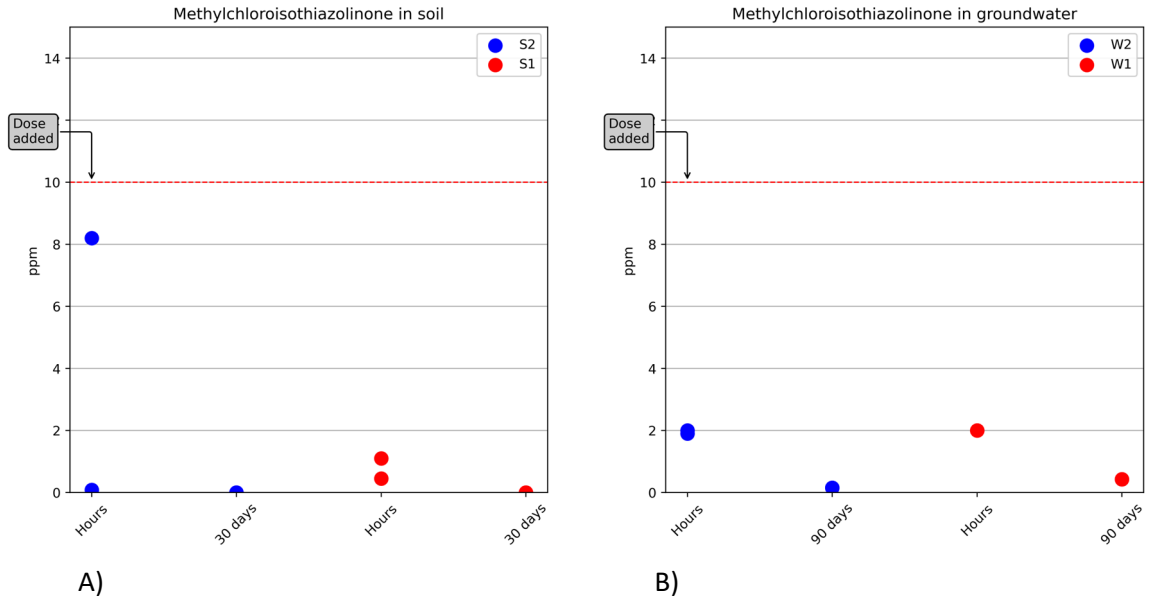

Figure 13: Methylchloroisothiazolinone concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Tetrakis phosphonium sulphate (THPS) is a biocide used in hydraulic fracturing fluids at low concentrations (usually 0.0001% w/v) to control bacterial growth, and the CSG industry commonly uses THPS to specifically target bacteria responsible for H_2S production and corrosion of well tubing, casing and equipment. THPS is a chemically reactive biocide causing denaturing of proteins, damaging membranes and interrupting proton flux and energy cycling (Okoro, 2015).

In the current study, THPS was persistent in one soil (S1; Figure 14) and was degraded in both of the groundwaters (Figure 14). Based on the degradation results from the microcosm experiments, the authors of the present study would normally recommend excluding THPS from further study in upcoming GISERA projects. In this instance, however, there is relatively little information on its degradation in soils and anoxic environments and few studies exist where microbes are shown to grow on THPS as a sole source of carbon or phosphorus. As such, it is recommended that THPS be progressed to GISERA Health 3 for further assessment.

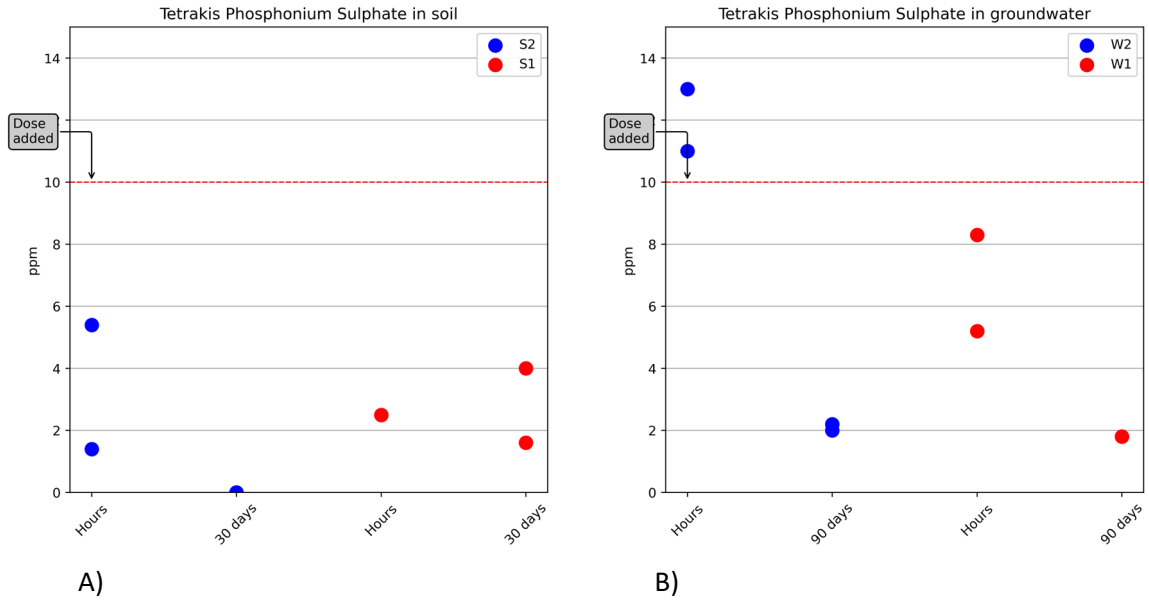

Figure 14: Tetrakis phosphonium sulphate concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Very limited prior research exists looking at the microbial degradation of tributyl tetradecyl phosphonium chloride (TTPC) in soils and groundwaters, or its persistence or bioaccumulative potential (Kahrilas et al., 2015). TTPC is a lytic biocide with surface-acting properties (Kahrilas et al., 2015; Kim and Park, 2015) and may remain bioactive (Harney et al., 2009). In the present study, TTPC was found to be persistent across all soil and groundwater microcosm experiments (), and thus will be progressed to the GISERA Health 3 programme of work.

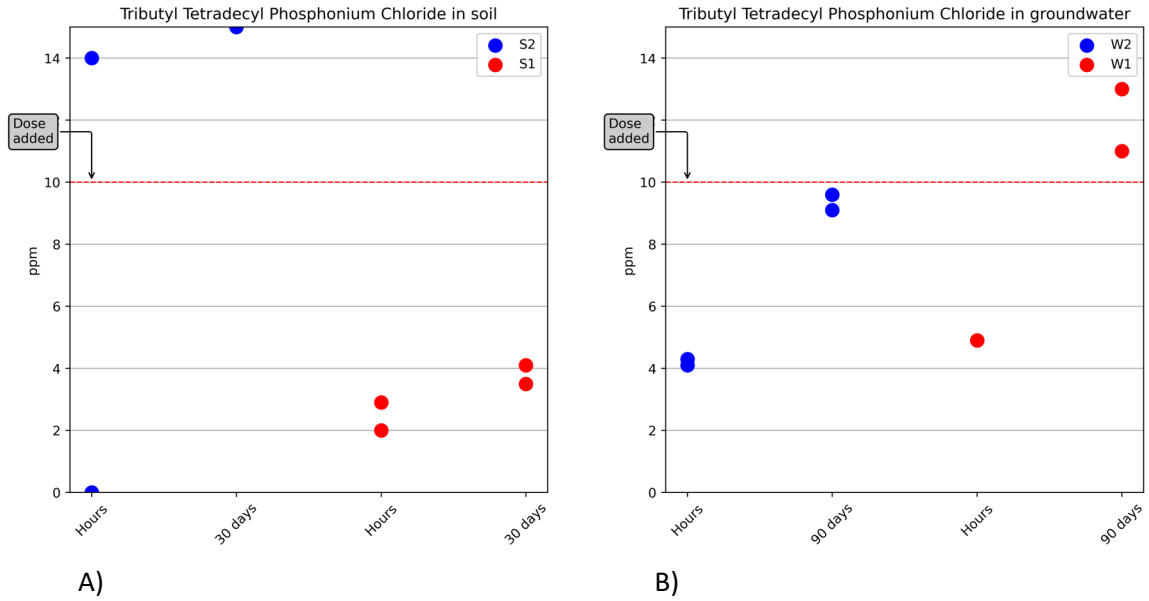

Figure 15: Tributyl tetradecyl phosphonium chloride concentration in soil (A) and groundwater (B) microcosms.

Concentrations were analysed after two time periods: hours and after 30 days for soil microcosms and 90 days for groundwater microcosms. Polyacrylamide was added at the start of the experiment to a concentration of 10 mg/kg in soil microcosms and 10 mg/L in groundwater microcosms.

Health 2 extension caveats and limitations

- The current study assumes that microbial degradation has been the main mechanism of loss of the chemicals in the soil microcosm experiments. Potentially, other factors may be of equal or greater importance in the loss of detectable chemicals. Most notably of these factors is adsorption of chemicals to soil components. Regardless, binding of chemicals to soil components would likely render these chemicals immobile (these chemcials were not detected in the current study despite vigorous solvent extraction) and thus unlikely to present a risk to human health.
- The current study did not measure degradation products of chemicals. For instance, polyacrylamide is rapidly deaminated in soil to form polyacrylate. This study did not test for polyacrylate. Similarly, nonylphenol ethoxylates can sometimes be only degraded to nonylphenol, though we did not test for nonylphenol in the present study. Furthermore, with the exception of nonylphenols, most chemicals degrade to less toxic intermediates.
- The current study tested a limited number of soil and groundwater samples. Despite this, the present study provides evidence of loss of detectable chemicals for four of the eight chemicals examined. Further samples may demonstrate less, the same or greater degradative potential. Combined with evidence from other GISERA projects and the literature, the microbes involved in the degradation of the chemicals for the most part tend to be common, widespread and relatively well studied taxa. This suggests that the results from the present study are broadly indicative of the study site.
- For NPE and FBA, only single chemicals were evaluated in this study. The remaining chemicals in these groups will be progressed into the GISERA Health 3 project.

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