Cranfield UNIVERSITY

Investigation of the Potential Formation of 2,4D from Fluoranthene

Project WT1290: Final Report

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1. Review any literature on the possible reactions of PAHs and any other potential 2,4-D precursors with chlorine

Observations by one UK water company have suggested that the pesticide 2,4-dichlorophenoxyacetic acid commonly known as 2,4-D can be formed as a disinfection by product (DBP) in water distribution systems. They have noticed that concentrations of 2,4-D have increased whilst concentrations of fluoranthene (a polycyclic aromatic hydrocarbon [PAH]) have decreased.
It has been hypothesised that 2,4-D is being formed as a DBP by the reaction of residual chlorine with fluoranthene. This transformation may involve microbial processes. Whilst no mechanism has been identified, a number of possible intermediates, such as phenol, and o- and p-hydroxybenzoic acids have been postulated. An alternative hypothesis is that other PAHs have may be leading to the formation of 2,4-D with the PAHs phenanthrene, fluorene and naphthalene proposed as precursors alongside dibenzofuran.

The aim of this review is to:

- Provide an evidence base for how 2,4-D formation may occur in the distribution system.
- Produce a list of possible precursors with reference to chemical oxidation and microbial activity.
- Gather response from other Water Companies on similar phenomena

1.1 Regulatory Position and Toxicity of 2,4-D

The Water Supply (Water Quality) Regulations 2000 (updated in 2007) provided by the Drinking Water Inspectorate state that individual pesticide levels must not exceed 0.10 µg/L (with the exception of Aldrin, Dieldrin, Heptachlor and Heptachlor epoxide which must not exceed 0.03 µg/L) with a total pesticide value not exceeding 0.50 µg/L at the consumers' tap (www.dwi.defra.gov.uk).

The chemical summary produced by the US EPA on 2,4-D provides the following information on the toxicity and exposure assessment of 2,4-D relating to children's health: "Health effects of chronic or acute 2,4-D exposure reported for adults included blood, liver, and kidney toxicity. Specific effects included a reduction in hemoglobin and red blood cell numbers, decreased liver enzyme activity, and increased kidney weight. Acute exposure can result in skin and eye irritation. Acute exposure to very high concentrations of 2,4-D can cause the following clinical symptoms: stupor; coma; coughing; burning sensations in lungs; loss of muscular coordination; nausea; vomiting; or dizziness. Experimental animal studies of chronic oral exposure have reported adverse effects on the eye, thyroid, kidney, adrenals, adrenals, and ovaries/testes. In addition, some experimental animal studies have reported teratogenic effects (birth defects) at high doses, including increased fetal death, urinary tract malformation, and extra ribs. When adult female experimental animals were exposed to 2,4-D during their pregnancy and lactation periods, their exposed offspring exhibited neurological effects, including delayed neurobehavioral development and changes in several neurotransmitter levels or binding activities and ganglioside levels in the brain. Delayed neurobehavioral development was manifested as delays in acquisition of certain motor skills such as the righting reflex." (http://www.epa.gov/teach/teachsummaries.html)

The US EPA stated: "Based on chronic studies on animals, 2,4-D has been classified as a Group D chemical, one that is not classifiable as to human carcinogenicity. Although 2,4-D continues to be the focus of epidemiological and laboratory studies, both EPA's review and the Scientific Advisory Panel have concluded that the available evidence is insufficient to classify 2,4-D as a human carcinogen" (www.epa.gov/iris/subst/0150.htm). A more recent review by the U.S. EPA in 2005 concluded "there is no additional evidence that would implicate 2,4-D as a cause of cancer". The World Health Organisation International Agency for Research on Cancer (IARC) has not evaluated 2,4-D and chlorophenoxy herbicides for carcinogenicity, and these herbicides are not listed as a priority for future evaluation (http://monographs.iarc.fr/ENG/Meetings/prioritylist.pdf).

If, as hypothesised, the pesticide 2,4-D is being formed in the distribution system as a DBP, it is important to understand this formation in order that regulations can continue to be met so that the health of the consumer is not compromised.

1.2 Structure and Properties

The structure of 2,4-D and the properties of the acid are given below (Table 1). Note that 2,4-D is applied as a salt which dissociates to acid in water.

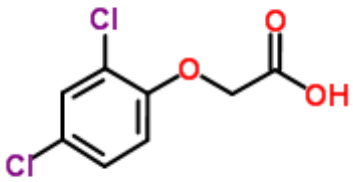


Table 1 Physical/Chemical Properties of 2,4-D

Active ingredient	2,4-D acid
Form	White to brown crystalline solid
Vapour pressure	1.9×10^{-5} Pa (1.4×10^{-7} mm Hg)
Henry's constant	8.6×10^{-6} atm.m^3/mol
Molecular weight	221
Solubility in water	pH 5: 29934 +/- 2957 mg/L pH 7: 44558 +/- 674 pH 9: 43134 +/- 336
Log K_{OW}	20.01 M solution pH 5: 2.14 pH 7: 0.177 pH 9: 0.102
K_{OC}	20-136

1.3 Manufacture and reaction mechanisms for formation of 2,4-D

The industrial scale synthesis of 2,4-D and similar compounds such as MCPA was traditionally carried out using the chlorination of phenol (or o-cresol in the case of MCPA) followed by the reaction of 2,4-dichlorophenol with monochloroacetic acid (Buckles and Wawzonek, 1948). A simplified small scale method has also been proposed by the reaction of phenol with monochloroacetic acid followed by chlorination of the phenoxy acetic acid by sodium hypochlorite. It was noted that poor yields are experienced if too much sodium hypochlorite is used in the second step (Buckles and Wawzonek, 1948). It therefore seems plausible that 2,4-D can be formed in water distribution systems which contain haloacetic acids (including monochloroacetic acid) as well as low levels of residual free chlorine providing the correct precursors are present. It should be noted that the process requires several adjustments to extreme pH values so the pathway for formation will be further explored by investigating the chlorination reactions of PAHs and products from coal tar lined pipes.

1.4 Chemical Oxidation of PAH with Chlorine

The reactivity of PAHs with chlorine and chlorine dioxide has been studied in the literature. A summary of the conditions during exposure to chlorine is presented (Table 2).

Table 2 Reactivity of PAHs with chlorine including conditions

PAH (ng/L, unless otherwise stated)	Reactive with chlorine?	pH (and time, h)	Temperature (°C)	Cl_2 Dose (mg/L)	Reference
Phenanthrene (200)	No	7 (24)	10	0.3	Merkel et al., 1998
Fluoranthene (200)	No	7 (24)	10	0.3	
Fluorene (200)	No	7 (24)	10	0.3	
Anthracene (200)	Yes	7 (24)	10	0.3	
Napthalene (100-5000)	Yes	7 (36)	20	2	Rav-Acha and Blits, 1985
Fluoranthene (100-5000)	Yes	7 (36)	20	2	
Pyrene (100-5000)	Yes	7 (36)	20	2	
Anthracene (100-5000)	Yes	7 (36)	20	2	
Napthalene (100-5000)	Yes	Nr	Nr	2-4	Ali and Tarek, 2009
Fluoranthene (6066)	Yes	Nr	Nr	2-4	
Pyrene (3278)	Yes	Nr	Nr	2-4	
Anthracene (1532)	Yes	Nr	Nr	2-4	
Phenanthrene (2688)	Yes	Nr	Nr	2-4	
Chrysene (4831)	Yes	Nr	Nr	2-4	
Fluorene (2110)	Yes	Nr	Nr	2-4	
Carbazole (10-100 mg/L)	Yes	5 (24)	20	200-2000	Onodera et al., 1989
Fluorene (10-100 mg/L)	Yes	5 (24)	20	200-2000	
Anthracene and derivatives (10-100	Yes	5 (24)	20	200-2000	

mg/L)					
1-methylnapthalene (~10 mg/L)	Yes	3 and 8	0.1-168	71	Oyler et al., 1982
Fluorene (~10 mg/L)	Yes		0.1-168	71	
Dibenzofuran (~10 mg/L)	Yes	3 and 8	0.1-168	71	
Anthracene (~10 mg/L)	Yes	3 and 8	0.1-168	71	
Phenanthrene (~10 mg/L)	Yes	3 and 8	0.1-168	71	
1-methylphenanthrene (~10 mg/L)	Yes	3 and 8	0.1-168	71	
Fluoranthene (~10 mg/L)	Yes	3 and 8	0.1-168	71	
Pyrene (~10 mg/L)	Yes	3 and 8	0.1-168	71	

Caution is advised when interpreting the results as PAH can be adsorbed onto the glass walls of bottles and can react with oxygen in the presence of light. Such losses can be up to 80% (Merkel et al., 1998).

In addition to the studies presented (Table 2), a study by Johnsen et al. (1989) investigated the chlorination by products from chlorination of fluoranthene, fluorene, anthracene and benzo(a)pyrene in humus poor and humus rich waters. They gave very little details of the conditions and concentrations but the reactivity from highest to lowest was anthracene, benzo(a)pyrene, fluoranthene and fluorene. For anthracene no chlorinated derivatives were found indicating oxidation rather than halogenation reactions. In the humus rich sample no chlorinated PAH were detected possibly due to the chlorine demand exerted by the organic matter.

In summary, PAHs are reactive with chlorine at high chlorine doses and at high PAH concentrations. PAHs are less reactive with chlorine at conditions found in water treatment. Additionally reactions at pH<6 tend to produce oxygenated products and reactions at pH>6 produce both oxygenated and chlorinated byproducts (Oyler et al., 1982). The conditions presented in the literature do not allow conclusions to be made on which species are more amenable to oxidation and although this information is available for the reaction of PAHs with ozone and other advanced oxidation processes (AOPs), it is not thought that this information is applicable here as chlorine can act as an oxidising agent as well as a halogenation agent. Also in the reaction with AOPs, free radicals are produced which are not present in the reaction with chlorine alone.

1.5 Influence of microbial activity

Microorganisms are known to play an important role in the conversion of chemical compounds present in water. Water distribution systems are inhabited with a great diversity of microbes that are well adapted to the prevailing conditions and concentrations of disinfectant residual. Applying cultivation-independent flow cytometry, Hammes et al. (2007) reported that microbial numbers in drinking water tend to be around 105-104 per

mL. The same group identified 504-939 operational taxonomic units (OTUs) belonging to 23 bacterial phyla found in samples from a water treatment works and the corresponding non-chlorinated distribution network (Lautenschlager et al., 2013). In a system alternating between chlorination and chloramination, up to 256 distinct OTUs were found (although with 1,206 sequences analysed, the real diversity can be expected to be higher (Hwang et al., 2012)).

It is not surprising therefore that the metabolic potential of the microbes in systems with chlorine-based disinfection comprises bacteria capable of degradation of HAAs from di- and tri-chlorinated species to monochlorinated species, i.e. monochloroacetic acid. Species with such capability include *Afipia sp, Pseudomonas spp. Delftia sp., Ultramicrobacterium sp.* and *Xanthobacter sp.* (Zhang et al., 2009). Although the extent is unknown, they are likely to contribute to decreases in HAA concentrations with increasing residence time of water in distribution systems (Speight and Singer, 2005). In fact, a PCR test targeting the deh and dehII genes (indicative for HAA degraders) was positive for 7 out of 13 tap water samples (54%) (Leach et al., 2009).

Whilst the first observation of HAA degradation was only observed recently, the conversion of polycyclic aromatic hydrocarbons (PAHs) by bacterial metabolic activity is well documented with excellent reviews of the process available (Cerniglia and Heitkamp, 1989, Kanaly and Harahama, 2000, Mrozik et al., 2002). Important species with the metabolic ability to degrade PAHs have been mainly found to belong to the genera *Pseudomonas* and *Mycobacterium* (Seo et al., 2009). The cometabolic biodegradation of the high molecular weight PAH fluoranthene and benzo[a]pyrene by stationary-phase cultures of *Pseudomonas* was reported as early as 1975 (Barnsley, 1975). Later research identified a number of bacteria capable of degrading fluoranthene, partly utilising the compound as a sole source of carbon and energy. Mueller et al. (1989) demonstrated that utilisation of fluoranthene by an enriched seven-member bacterial community (isolated from creosote-contaminated soil) resulted in an increase in bacterial biomass/protein concentration and disappearance of the compound from an aqueous solution. When measuring degradation of different PAHs (including fluoranthene at an initial concentration of 0.21 µg mL^{-1}), approximately 30% of the fluoranthene was degraded after 3 days, 48% after 5 days and 81.2% after 14 days. In a later study, *Pseudomonas paucimobilis* was found to be one of the members of the bacterial community able to utilise fluoranthene as a sole carbon source. Weissenfels et al. (1990, 1991) found *Alcaligenes denitrificans* strain WW1 was able to degrade fluoranthene via the dioxygenase pathway at a rate of 0.3 mg mL^{-1} per day. Other PAHs could be co-metabolised in both studies. *Mycobacterium sp.* strain PYR-1was found to degrade fluoranthene to more than 95% within 24 hours in mineral medium supplemented with organic nutrients with concentrations up to 15 mg L^{-1}not inhibiting microbial growth in water microcosms (Kelley and Cerniglia, 1991). Metabolic degradation resulted in a number of compounds including multi-ring PAH molecules (9-fluorenone-1-carboxylic acid; 9-hydroxyfluorene; 9-fluorenone; 1-acenaphthenone; 9-hydroxy-1-fluorenecarboxylic acid), but interestingly also 1-ring molecules (phthalic acid, 2-carboxybenzaldehyde; benzoic acid; phenylacetic acid (Kelley et al., 1993)). The formation of 2,4-D is therefore a definite possibility. The presence of Triton X-100 and other nonionic surfactants, especially in the presence of calcium, were found to substantially enhance the degradation of fluoranthene (Tiehm, 1994, Willumson et al., 1994). A reason might be found in the increasing apparent solubility of fluoranthene.

The study by Seo et al. (2009) proposed metabolic pathways for benzo(a)pyrene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene. Degradation products of fluorene and phenanthrene were 2-chromanone and coumarin respectively which are close in structure to 2,4-D. No indication of the timescale or conditions required for biodegradation to these products was provided.

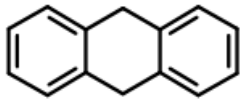
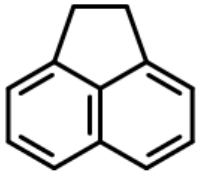
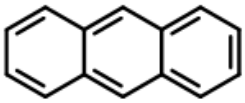
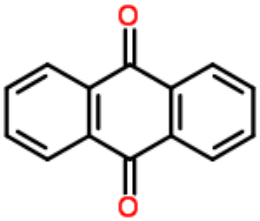
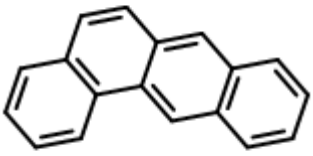
1.6 PAHs from coal tar identified in the literature

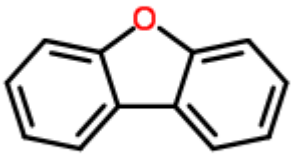
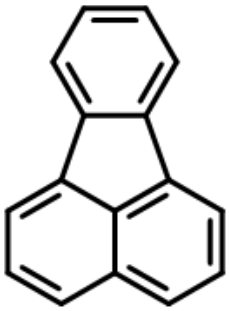
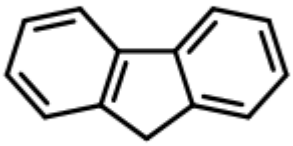
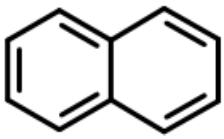
A study carried out by Alben (1980) investigated the PAHs present in coal tar leachate and chlorinated coal tar leachate. The leachate was obtained by exposing 40 L tap water at pH 9 to test panels coated with commercial coal tar. The resulting compounds were also measured after exposure to tap water containing 50 mg/L chlorine. Phenanthrene was found to be the dominant PAH but they found an additional 38 PAH compounds. When the leachate was chlorinated the distribution of PAHs was significantly modified with fluorene becoming the most prominent PAH with concentrations of fluoranthene and phenanthrene considerably diminished. Oxygen substituted PAHs such as dibenzofuran became more abundant and several new oxygenated compounds were found. Intermixed with the oxygenated compounds were low concentrations of chlorine- and bromine-substituted PAHs. The study concluded that the capacity of hypochlorite to initiate the oxidation or halogenation of PAHs was evident. It was also emphasised that the compounds identified in this work were obtained in base-neutral extracts. Chlorination of coal tar leachate is also expected to result in formation of PAHs with polar acidic functional groups.

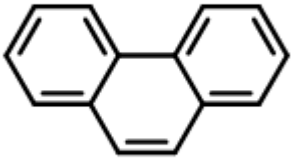
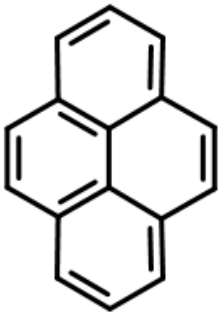
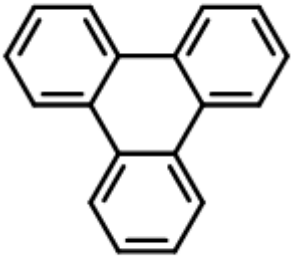
A recent study carried out in France (Tillner et al., 2013) developed a method for the simultaneous determination of PAHs and their chlorination by products in distribution systems. They also investigated pipe coatings and found all 17 targeted PAHs. The presence and magnitude of PAHs was indicative of coal tar lining as these can contain 1000 – 10000 more PAHs than asphalt. They concluded that the PAHs detected most frequently in water distribution systems are those with less than five aromatic rings concurrent with those previously identified in the literature, namely anthracene, benz(a)anthracene, fluoranthene, fluorene, phenanthrene, pyrene.

A list of possible precursors identified in the literature (Tillner et al., 2013, Maier et al., 2000, 1999, Merkel et al., 1998, Shiraishi et al., 1985, Alben, 1980)are presented (Table 3).

Table 3 Possible precursors from coal tar pipes

Possible Precursor	Structure	Measured by ALcontrol?
9,10-dihydroanthracene		No
Acenaphthene		Yes
Anthracene		Yes
Anthraquinone		No
Benz(a)anthracene		Yes
Dibenzofuran		Yes

		
Fluoranthene		Yes
Fluorene		Yes
Napthalene		Yes

Phenanthrene		Yes
Pyrene		Yes
Triphenylene		No

1.7 Chlorination byproducts of PAHs

A few studies have determined the chlorination byproducts when chlorinating PAHs. The chlorination of fluoranthene at conditions similar to those used in water treatment has shown halogenation of the otherwise unchanged structure and loss of an aromatic ring (Oyler et al., 1982, Tillner et al., 2013). However, there is little similarity of the products to the structure of 2,4-D with no oxygen bridging the aromatic rings such as that observed for dibenzofuran. Chlorination byproducts measured in real distribution systems included fluorenone, anthraquinone, cyclopenta[d,e,f]phenanthrenone, 3-chlorofluoranthene and 1-chloropyrene (Tillner et al., 2013). This shows that PAHs are readily halogenated by chlorine but not as susceptible to oxidation.

1.8 Discussion

It is likely that the loss of fluoranthene in distribution observed by the Water Company is caused by the reaction with chlorine to produce halogenated fluoranthene compounds such

as 3-chlorofluoranthene (Tillner et al., 2013). It is not clear if this is responsible for the formation of 2,4-D as the literature does not suggest a clear pathway for this reaction. It is possible that other PAHs would show the same loss on exposure to chlorine but these were not measured by the Water Company). Conversely an investigation into the role of biofilm in mobilisation of PAHs from coal-tar lining has shown that chlorination destabilises the biofilm which leads to elevated PAH concentrations which has been observed in laboratories and in distribution systems (Maier et al., 2000) but the study referred to the sum of 16 PAH and didn't investigate the individual PAHs. It is highly likely that microbial activity plays a part in reducing the PAHs to compounds that are more readily converted to 2,4-D as proposed by Seo et al. (2009).

1.9 Findings from Water Companies

All water companies in England and Wales have been contacted. Once information has been received and compiled it will be added to this review.

2. Conduct laboratory based DBP formation potential type studies using samples with potential precursors such as PAHs and chlorine.

This work involves two phases. The first is a scoping study to help define the protocol including sample timing for the main study. The second is the conducting of formation potential tests to determine the potential precursors of 2,4-D under sterile conditions.

2.1 Phase 1 tests

2.1.1 Work carried out

Treated water samples were collected after treatment but before chlorination for the tests. These samples were filter sterilised and chlorinated under sterile conditions using sterilised apparatus and solutions where possible. For each set of conditions, 7 samples will be subjected to chlorination. These will be spiked with the following compounds:

1. Fluoranthene
2. Phenanthrene
3. Naphthalene
4. Fluorene
5. Dibenzofuran
6. Anthracene
7. A mixture of compounds 1-6 above

These will be chlorinated at different concentrations (low and high) and the chlorine dose was determined by measuring the chlorine demand to ensure a residual of ~0.5 mg L^{-1} free chlorine after 1, 3 and 7 days similar to values experienced in UK water distribution systems. Chlorine demand tests will be carried out according to Standard Method 4500-Cl (APHA, 1998).

The Phase 1 Scoping Study conditions are outlined in Table 4 and the findings will be used to inform Phase 2 in terms of the test conditions. The number of samples produced in Phase 1 was 42 chlorinated samples plus 14 blanks (7 of untreated spiked samples and 7 procedural blanks). 29% of the samples were analysed in duplicate (16). Therefore the total number of samples was 72. All samples were measured for 20 acid herbicides including 2,4-D, 16 PAHs, dibenzofuran and 59 other SVOCs according to the methods detailed and validated by ALcontrol Laboratories. The chlorine demand and chlorination tests were carried out at Cranfield by Emma Goslan.

2.1.2 Experimental Work

Stock solutions of compounds 1-6 listed above were made at concentrations of ~3000 mg/L. The compounds 1-5 were chosen as these are most often encountered in distribution systems lined with coal tar (Tillner et al., 2013, Maier et al., 1999). Compound 6 was chosen as it had been shown to be more reactive with chlorine than compounds 1-5 as well as being found occasionally in coal tar mains (Merkel et al., 1998). The sample water was collected

from Ewden Water Treatment Works located in the Yorkshire Water region after treatment but before chlorination. This water is from a pristine moorland source and is known not to be impacted by pesticides. This water was been tested for its reactivity with chlorine and a dose of 1 mg/L Cl_2 has been shown to give a residual of ~0.5 mg/L Cl_2 after 7 days in water containing the added PAHs and dibenzofuran. The water was filter sterilised to 0.2 µm to remove all intact and dead cells. The samples were prepared directly in 1 L glass amber bottles to minimise the absorption of the PAHs and dibenzofuran to the glass. The concentrations were initially chosen as representative of levels found in real water distribution systems (Tillner et al., 2013, Maier et al., 1999). However these were later revised to higher levels to ensure that they would be measureable using the ALcontrol methods. The samples were prepared by adding 20 mL phosphate buffer (to maintain pH at 7) to 1 L amber bottles with PTFE lined lids. The spiked sample was then added to the bottle until almost full, the chlorine dosed and the bottle filled completely before capping and storing in the dark for 1, 3 and 7 days. After the required time has passed, an aliquot of 10 mL was removed to measure the free chlorine remaining and the chlorine remaining in the sample bottle was quenched using excess sodium sulphite and the samples transferred to the bottles provided by Alcontrol before being put in a cool box with ice packs for courier transport to Alcontrol laboratories.

Table 4 Phase 1 Scoping study conditions

Sample numbers	Concentration of PAH or dibenzofuran	pH	Cl_2 dose (mg L^{-1})	Time (days)
S1-S7	Low – 1000 ng/L	7	2	1
S8-S14	High – 10,000 ng/L	7	2	1
S15-S21	Low	7	2	3
S22-S28	High	7	2	3
S29-S35	Low	7	2	7
S36-S42	High	7	2	7

2.1.3 Results

All compounds showed some degradation with a corresponding reduction in chlorine. However no formation of 2,4-D was apparent with levels remaining below the LOD of 0.026 µg/L in all cases. The degradation of each compound and the chlorine reduction is shown (Figures 1-8). No other acid herbicides were found nor any SVOCs other than those added (PAHs and dibenzofuran).

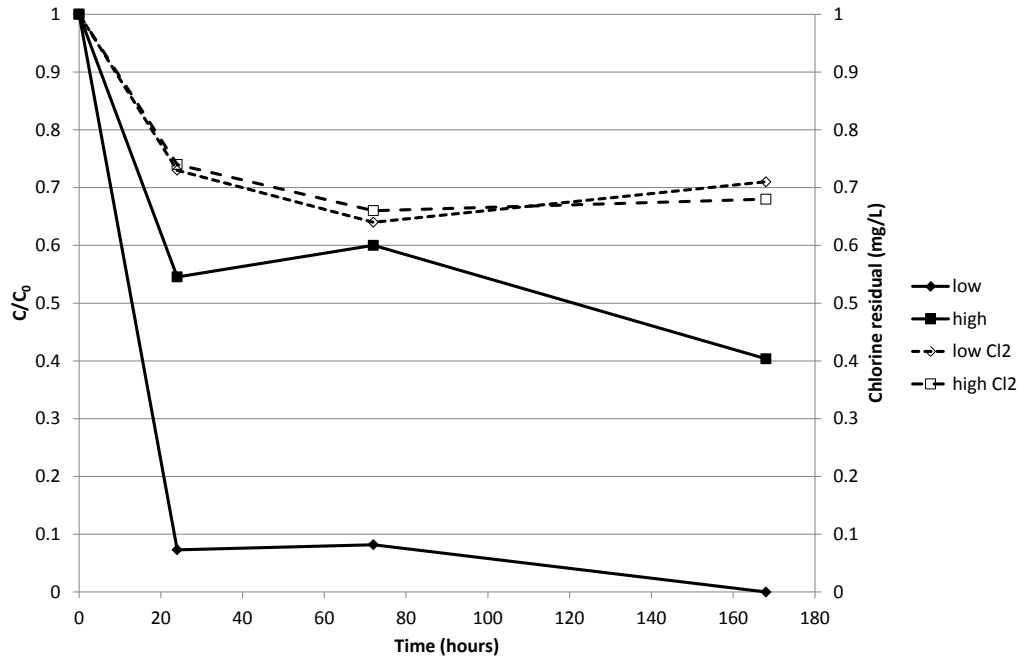


Figure 1 Degradation of Anthracene by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

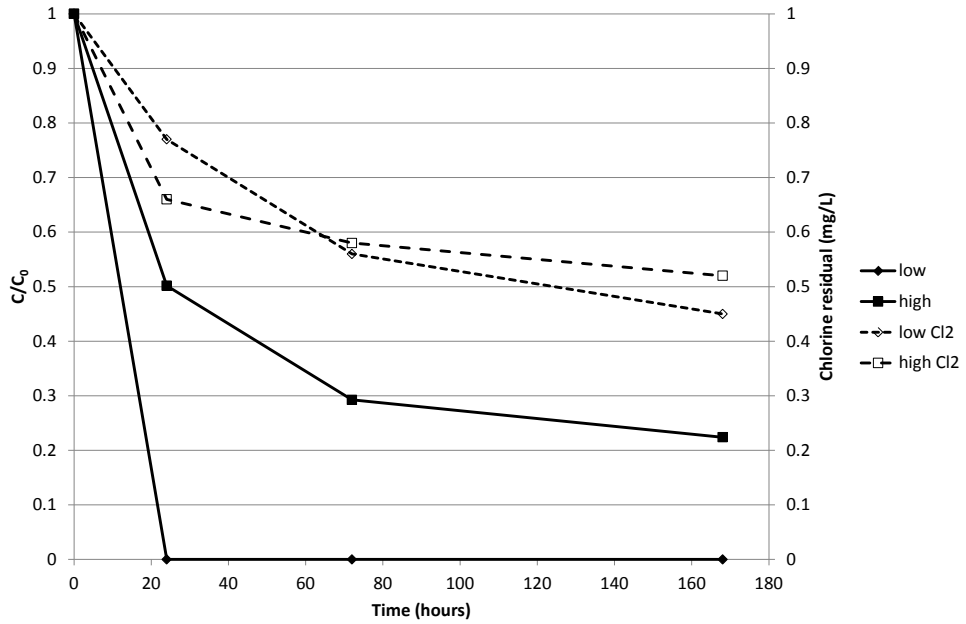


Figure 2 Degradation of Dibenzofuran by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

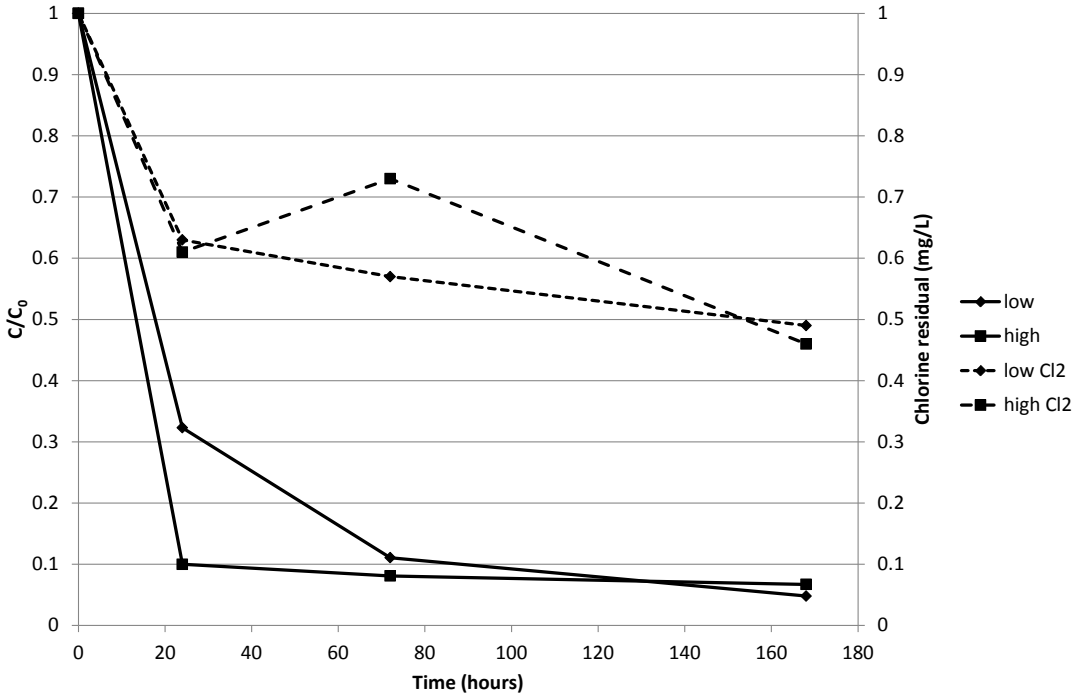


Figure 3 Degradation of Fluoranthene by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

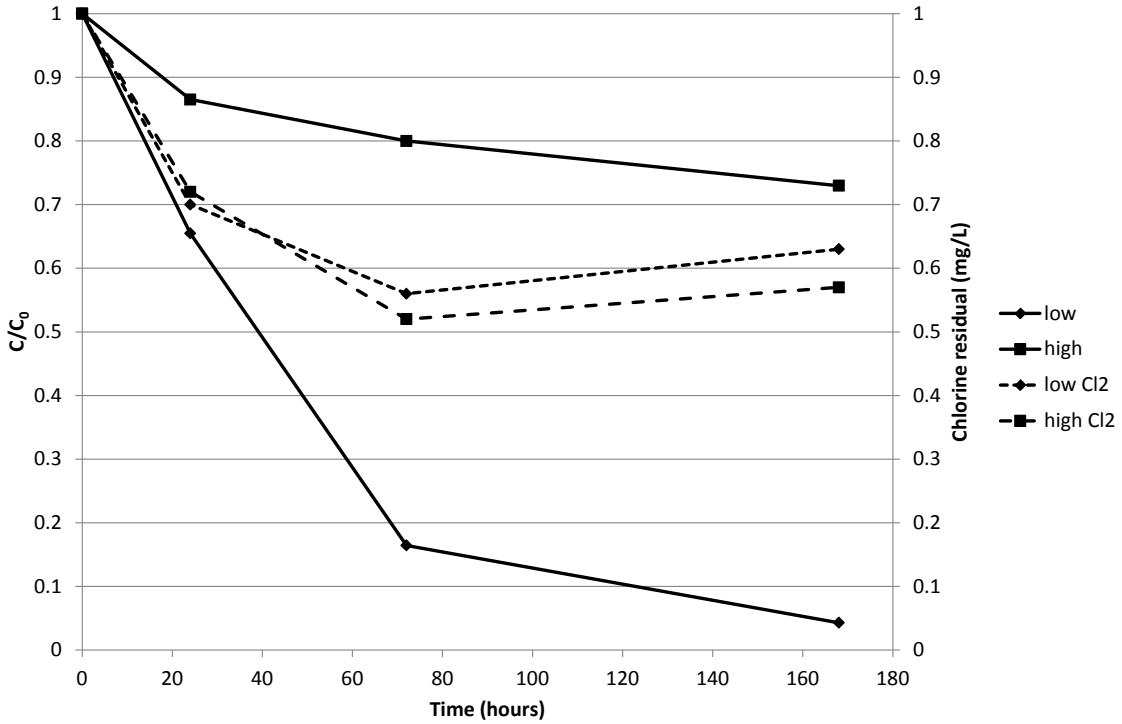


Figure 4 Degradation of Fluorene by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

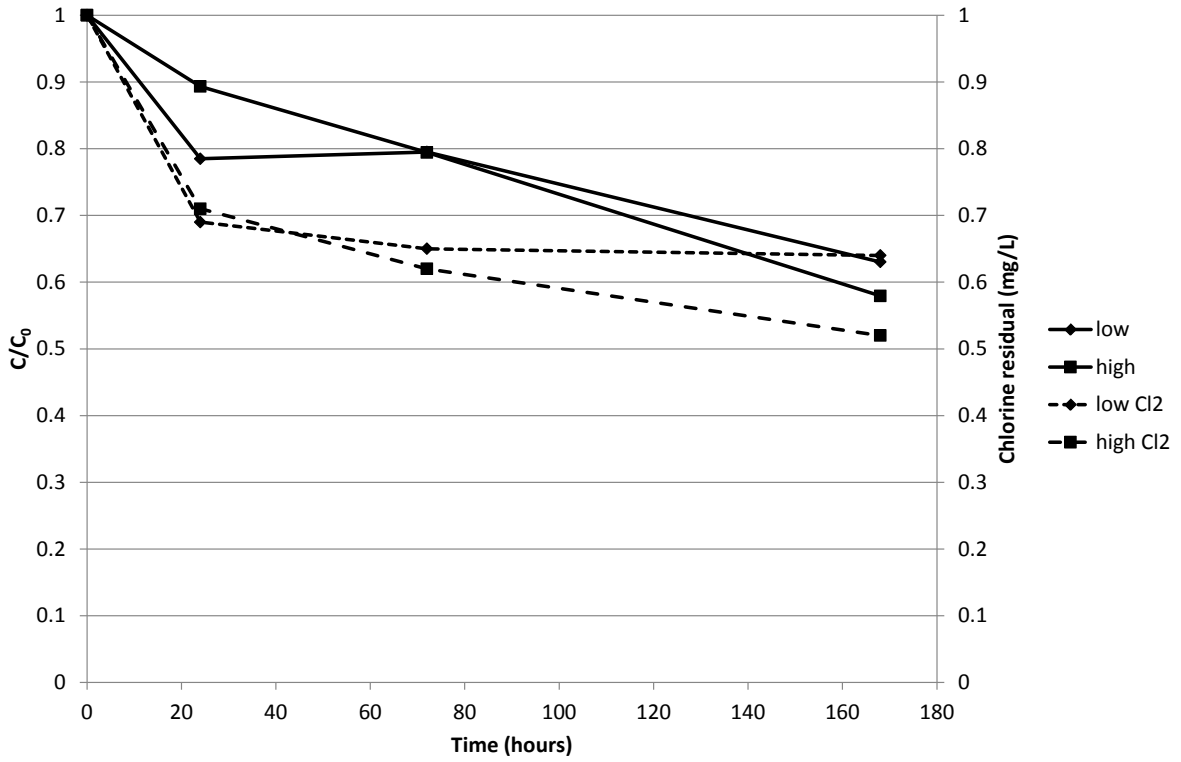


Figure 5 Degradation of Naphthalene by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

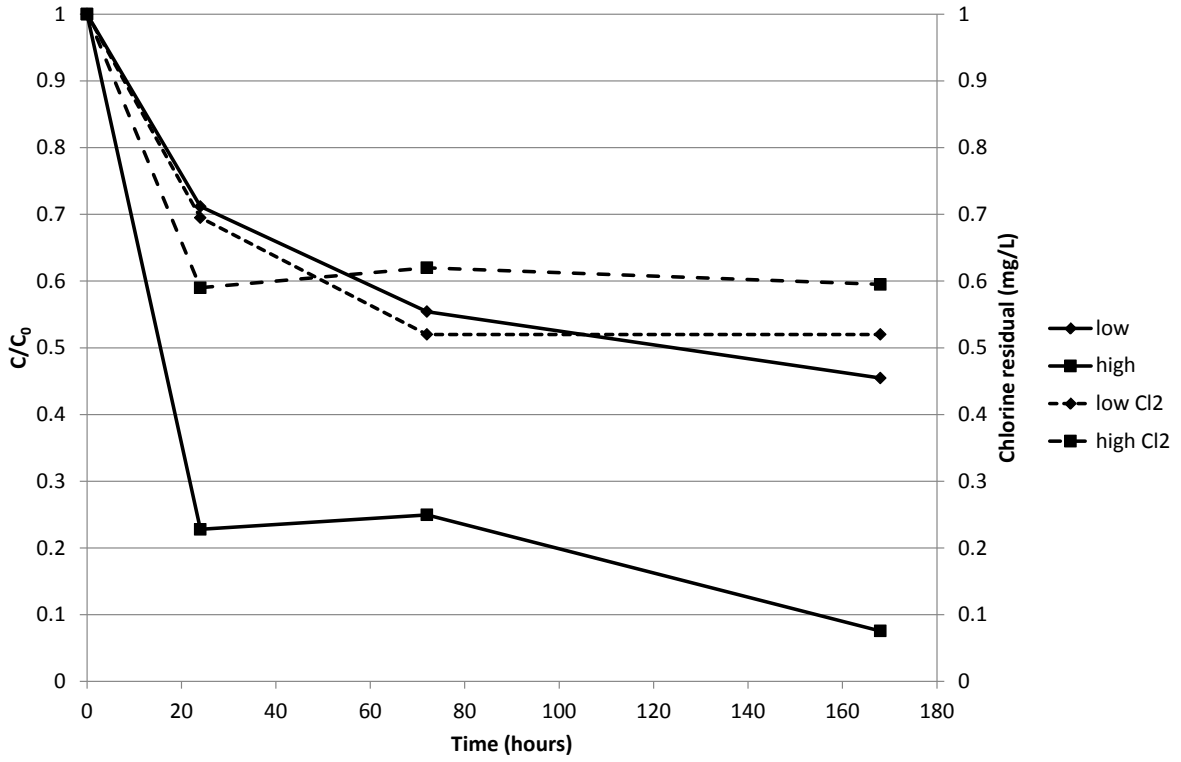


Figure 6 Degradation of Phenanthrene by chlorine at low (1000 ng/L) and high (10,000 ng/L) concentrations

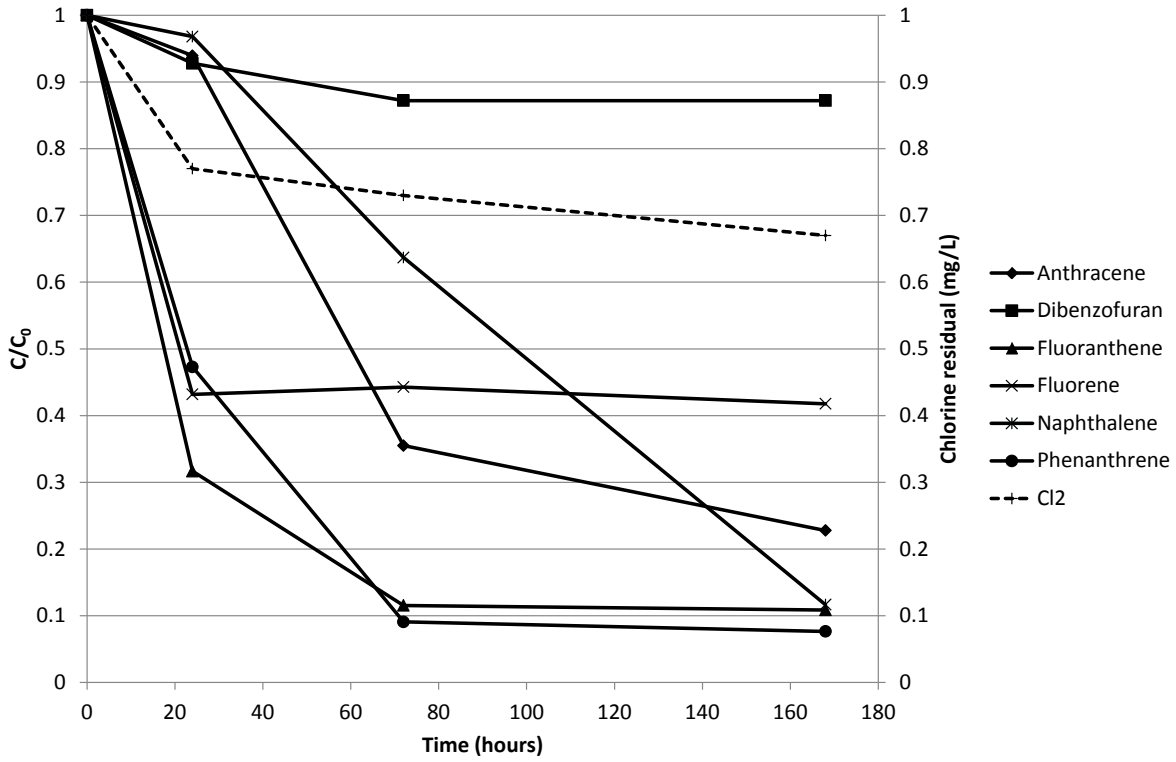


Figure 7 Degradation of Low Concentration mixture by chlorine

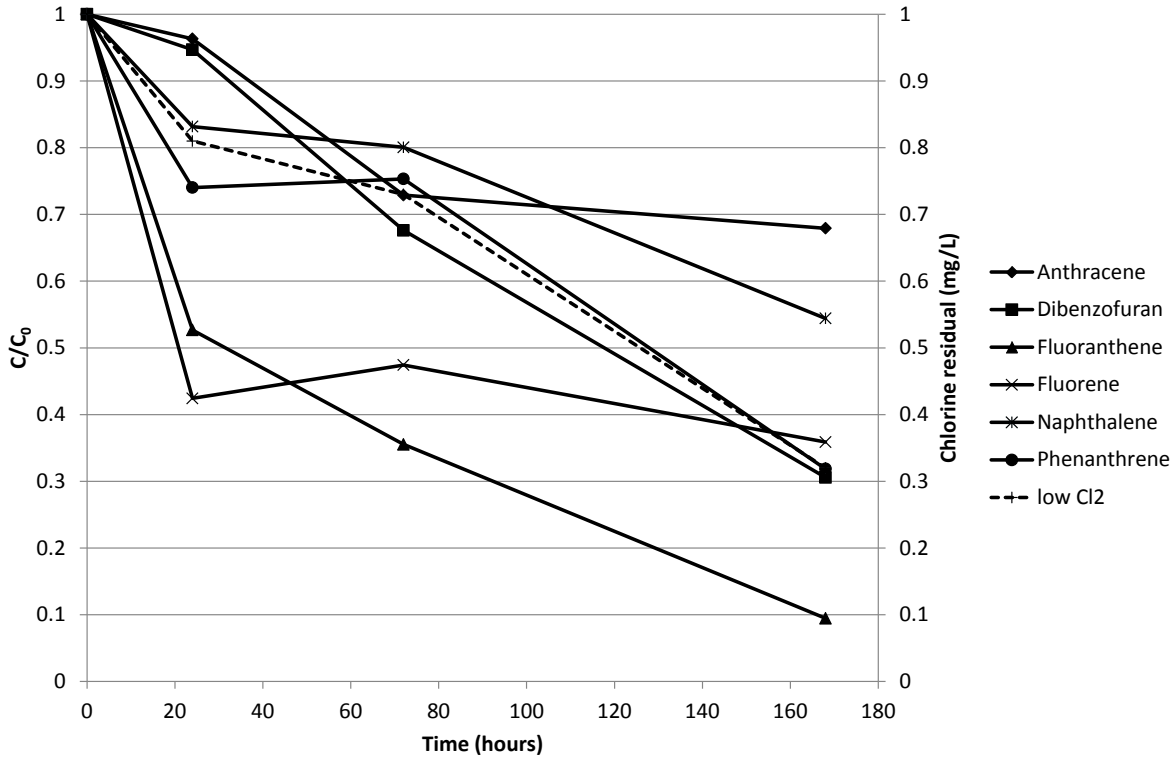


Figure 8 Degradation of High Concentration mixture by chlorine

2.2 Phase 2 tests

Given that phase 2 involves the “ideal” conditions from phase 1 but at different pH values and also given that the pH of the water in the distribution system was steady at pH 7-7.5, it was agreed to study the next experimental phase.

Conduct further studies using real waters in an attempt to determine whether microbes have a role to play on the formation of 2,4D.

The phase 3 experiments were planned to look at formation of 2,4-D from the chlorination of the following 3 samples spiked with PAH/dibenzofuran:

(1) non-sterilised unchlorinated drinking water containing natural microorganisms,

(2) tap water enriched with microorganisms: cells recovered from filtering 10L tap water will be resuspended in a small suitable volume, and

(3) tap water enriched with biofilm: a suitable amount of biofilm from a drinking water pipe will be resuspended in tap water.

Experiments (1) and (2) were carried out as outlined above but did not make any 2,4-D. It was not possible to carry out experiment (3) as it wasn’t possible to source a biofilm sample directly from a distribution pipe from Wessex. The piping the in the system has been replaced by a new one. The alternative to grow a representative biofilm (with mature microbes) in the lab was discarded since it would take a matter of months and it may not contain microbes that are used to living in coal tar lined pipes.

2.2.1 Work carried out

Treated water samples were collected after treatment but before chlorination for the tests. These samples were not filter sterilised or chlorinated under sterile conditions. For each set of conditions, 7 samples will be subjected to chlorination. These will be spiked with the following compounds:

1. Fluoranthene
2. Phenanthrene
3. Naphthalene
4. Fluorene
5. Dibenzofuran
6. Anthracene
7. A mixture of compounds 1-6 above

These will be chlorinated to ensure a residual of ~0.5 mg L^{-1} free chlorine after 7 days similar to values experienced in UK water distribution systems. Chlorine demand tests will be carried out according to Standard Method 4500-Cl (APHA, 1998).

The Phase 2 Study conditions are outlined in Tables 5 and 6. The number of samples produced in Phase 2 was 36 chlorinated samples plus 9 blanks (1 of unchlorinated spiked samples and 8 procedural blanks) including 39% of the samples were analysed in duplicate (14). Therefore the total number of samples was 45. All samples were measured for 20 acid herbicides including 2,4-D, 16 PAHs, dibenzofuran and 59 other SVOCs according to the methods detailed and validated by ALcontrol Laboratories. The chlorine demand and chlorination tests were carried out at Cranfield by Emma Goslan.

2.2.2 Experimental Work

a) Non-sterilised water

Stock solutions of compounds 1-6 listed above were made. The sample water was collected from Ewden Water Treatment Works located in the Yorkshire Water region after treatment but before chlorination. This water is from a pristine moorland source and is known not to be impacted by pesticides. This water was been tested for its reactivity with chlorine and a dose of 1 mg/L Cl_2 has been shown to give a residual of ~0.5 mg/L Cl_2 after 7 days in water containing the added PAHs and dibenzofuran. The samples were prepared directly in 1 L glass amber bottles to minimise the absorption of the PAHs and dibenzofuran to the glass. The samples were prepared by adding 20 mL phosphate buffer (to maintain pH at 7) to 1 L amber bottles with PTFE lined lids. The spiked- sample was then added to the bottle until almost full, the chlorine dosed and the bottle filled completely before capping and storing in the dark for 7 days. After the required time had passed, an aliquot of 10 mL was removed to measure the free chlorine remaining and the chlorine remaining in the sample bottle was quenched using excess sodium sulphite. The samples were transferred to the bottles provided by Alcontrol before being put in a cool box with ice packs for courier transport to Alcontrol laboratories. Two blanks were prepared and incubated alongside the samples.

Table 5 Phase 2 Study conditions – Non sterilised water

Sample numbers	PAH or dibenzofuran at 1000 ng/L	pH	Cl_2 dose (mg L^{-1})	Time (days)
1	Dibenzofuran	7	1	7
2	Dibenzofuran	7	1	7
3	Anthracene	7	1	7
4	Fluorene	7	1	7
5	Fluoranthene	7	1	7
6	Naphthalene	7	1	7
7	Phenanthrene	7	1	7
8	Phenanthrene	7	1	7
9	A mixture of all of the compounds above	7	1	7

b) Non-sterilised water with added tap water bacteria

Stock solutions of compounds 1-6 listed above were made. The sample water was collected from Ewden Water Treatment Works located in the Yorkshire Water region after treatment but before chlorination. This water is from a pristine moorland source and is known not to be impacted by pesticides. This water was been tested for its reactivity with chlorine and a dose of 1 mg/L Cl_2 has been shown to give a residual of ~0.5 mg/L Cl_2 after 7 days in water containing the added PAHs and dibenzofuran. The samples were prepared directly in 1 L glass amber bottles to minimise the absorption of the PAHs and dibenzofuran to the glass. The bacteria from Cranfield tap water was concentrated by filtering either 2 L, 5 L or 10 L tap water through a 0.2 µm nylon filter. The filter was then transferred to 50 mL of nonselective broth (peptone water [10 g/L], Sigma product no. 77185-500G) for 16-18h of incubation at 36°C ± 2°C (as described in Handbook of Water Analysis, edited by Leo M.L. Nollet, Leen S. P. De Gelder). The samples were prepared by adding 20 mL phosphate buffer (to maintain pH at 7) to 1 L amber bottles with PTFE lined lids followed by the 50 mL of enriched broth. The spiked sample was then added to the bottle until almost full, the chlorine dosed and the bottle filled completely before capping and storing in the dark for 7 days. After the required time had passed, an aliquot of 10 mL was removed to measure the free chlorine remaining and the chlorine remaining in the sample bottle was quenched using excess sodium sulphite. The samples were transferred to the bottles provided by Alcontrol before being put in a cool box with ice packs for courier transport to Alcontrol laboratories. Five blanks were prepared and incubated alongside the samples.

Table 6 Phase 2 Study conditions – Non sterilised water with added enriched bacteria

Sample numbers	PAH or dibenzofuran at 1000 ng/L	Tap water volume (L)			pH	Cl_2 dose (mg L^{-1})	Time (days)
		2	5	10			
1	Dibenzofuran	X					
2	Dibenzofuran		X				
3	Dibenzofuran		X				
4	Dibenzofuran			X			
5	Anthracene	X					
6	Anthracene		X				
7	Anthracene			X			
8	Anthracene			X			
9	Fluorene	X					
10	Fluorene	X					
11	Fluorene		X				
12	Fluorene			X			
13	Fluoranthene	X			7	1	
14	Fluoranthene	X					7

15	Fluoranthene		X				
16	Fluoranthene			X			
17	Naphthalene	X					
18	Naphthalene		X				
19	Naphthalene		X				
20	Naphthalene			X			
21	Phenanthrene	X					
22	Phenanthrene		X				
23	Phenanthrene			X			
24	Phenanthrene			X			
25	A mixture of all of the compounds above	X					
26	A mixture of all of the compounds above		X				
27	A mixture of all of the compounds above			X			
28	A mixture of all of the compounds above					0	

2.2.3 Results

All compounds showed some degradation from the initial value with a corresponding reduction in chlorine. However no formation of 2,4-D was apparent with levels remaining below the LOD of 0.026 µg/L in all cases. No other acid herbicides were found nor any SVOCs other than those added (PAHs and dibenzofuran).

2.3 Phase 3 tests

2.3.1 Work carried out

Experimental conditions in phase 2 study were repeating using water from the area where 2,4D has been detected to isolate the bacteria that were potentially producing the compound. Raw and treated samples were collected from Wessex, where the 2,4D has been detected.

Treated water samples were collected after treatment but before chlorination for the tests. For each set of conditions, 7 samples will be subjected to chlorination. These will be spiked with the following compounds:

1. Fluoranthene
2. Phenanthrene
3. Naphthalene
4. Fluorene
5. Dibenzofuran
6. Anthracene
7. A mixture of compounds 1-6 above

These will be chlorinated to ensure a residual of ~0.5 mg L^{-1} free chlorine after 7 days similar to values experienced in UK water distribution systems. Chlorine demand tests will be carried out according to Standard Method 4500-Cl (APHA, 1998).

The Phase 3 study conditions are outlined in Table 7. The number of samples produced in Phase 3 was 29 chlorinated samples plus 10 blanks (6 of unchlorinated spiked samples and 4 procedural blanks). All samples were measured for 20 acid herbicides including 2,4-D, 16 PAHs, dibenzofuran and 59 other SVOCs according to the methods detailed and validated by ALcontrol Laboratories. The chlorine demand and chlorination tests were carried out at Cranfield by Irene Carra Ruiz.

2.3.2 Experimental Work

a) Non-sterilised water with added tap water bacteria

Stock solutions of compounds 1-6 listed above were made. Untreated water was collected from Fovant Water Treatment Works located in the Wessex Water region. High levels of 2,4D have been detected in the distribution system after the work. This water was tested for its reactivity with chlorine and a dose of 2.5 mg/L Cl_2 has been shown to give a residual of ~0.5 mg/L Cl_2 after 7 days in water containing the added PAHs and dibenzofuran. The samples were prepared directly in 1 L glass amber bottles to minimise the absorption of the PAHs and dibenzofuran to the glass. Tap water from the distribution network in Fovant where the 2,4D has been detected was collected to concentrate the bacteria. The bacteria was concentrated by filtering either 2 L, 5 L or 10 L tap water through a 0.2 µm nylon filter. The filter was then transferred to 50 mL of nonselective broth (peptone water [10 g/L], Sigma product no. 77185-500G) for 16-18h of incubation at 36°C ± 2°C (as described in Handbook of Water Analysis, edited by Leo M.L. Nollet, Leen S. P. De Gelder). The samples were prepared by adding 20 mL phosphate buffer (to maintain pH at 7) to 1 L amber bottles

with PTFE lined lids followed by the 50 mL of enriched broth. The spiked sample was then added to the bottle until almost full, the chlorine dosed and the bottle filled before capping and storing in the dark for 7 days. After the required time had passed, an aliquot of 10 mL was removed to measure the free chlorine remaining and the chlorine remaining in the sample bottle was quenched using excess sodium sulphite. The samples were transferred to the bottles provided by Alcontrol before being put in a cool box with ice packs for courier transport to Alcontrol laboratories. The untreated water was also sent for analysis to measure the initial concentration of 2,4D.

Table 7 Phase 3 Study conditions – Non sterilised water with added enriched bacteria from where 2,4D has been detected

Sample	**PAH or dibenzofuran at 1000 ng/L**	**Tap water volume (L)**				**pH**	**Cl_2 dose (mg L^{-1})**	**Time (days)**
		2	**5**	**10**	**2 unchlorinated**			
1	Dibenzofuran	x						
2	Dibenzofuran	x						
3	Dibenzofuran		x					
4	Dibenzofuran			x				
5	Dibenzofuran				x			
7	Anthracene	x						
8	Anthracene		x					
9	Anthracene		x					
10	Anthracene			x				
11	Anthracene				x			
12	Fluorene	x						
13	Fluorene		x					
14	Fluorene			x				
15	Fluorene			x				
16	Fluorene				x			
18	Fluoranthene	x				7		
19	Fluoranthene	x						
20	Fluoranthene		x					
21	Fluoranthene			x				
22	Fluoranthene				x			
23	Naphthalene	x						
24	Naphthalene		x					
25	Naphthalene			x				
26	Naphthalene			x				
27	Naphthalene				x			
28	Phenanthrene	x						
29	Phenanthrene		x					
30	Phenanthrene		x					
31	Phenanthrene			x				
32	Phenanthrene				x			
34	Mixture	x					2.5	7

35	Mixture		x					
36	Mixture			x				
37	Untreated water							
38	Untreated water							

2.3.3 Results

The analysis indicated there was no 2,4D in the untreated water. All compounds showed some degradation from the initial value with a corresponding reduction in chlorine. However no formation of 2,4-D was apparent with levels remaining below the LOD of 0.026 µg/L in all cases. No other acid herbicides were found nor any SVOCs other than those added (PAHs and dibenzofuran).

2.3.4 Conclusions

The exact conditions to form 2,4D could not be reproduced during the experimental phase. It is believed that a microbial step is required, so further investigation should focus on taking non-sterilised sample from the distribution system where 2,4D has been detected after the new piping system has been installed for a few months to recreate the environment necessary to form the compound.

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