Polychlorinated biphenyls (PCB) have attracted much attention over the last 20 years due to their recalcitrance, ability to bioaccumulate and implication in a range of toxicological investigations. The many aspects of their synthesis, analysis, properties and biodegradation have been reviewed elsewhere [1]. PCB were synthesized for approximately 60 years from the early 1920s until they were banned in many countries during the late 1970s. Their uses varied from closed-system applications in capacitors and transformers to open-system applications in the manufacture of adhesives, textiles and printing. Obviously, such a plethora of uses has facilitated their ubiquity in the environment and this has been compounded by the complexity of the commercial mixtures that were synthesized. Mixtures, made by the direct chlorination of biphenyl using anhydrous chlorine at 150°C, were manufactured by a variety of manufacturers in the U.S.A., Europe and Japan. The best known of these was the Aroclor range of PCB manufactured by Monsanto plc. These were named on the basis of their 12 carbon atoms and the percentage by weight of chlorine (e.g. Aroclor 1242 contains 42% chlorine). Their method of manufacture gave a complex product that contained anything up to 80 different congeners, of a possible 209, with the number and positioning of the chlorine dependent upon the contact time of the chlorine and the biphenyl used.

It is estimated that $10 \times 10^7$ kg of PCB still reside in the biosphere [2] and the revised Waste Management Paper Number 6 [3] suggests that in the U.K., of the 12000 tonnes of PCB waste in the U.K., 4000-4500 tonnes have been destroyed. Moreover, a study into the deposition of PCB in the U.K., which used archived vegetation samples covering the period 1965–1989, showed a significant reduction of air contamination with PCB. However, it was also noted that, in soils, the rate of deposition exceeded that of removal [4].

It is clear that several factors contribute to PCB being a ‘worse-case pollutant’ when researching novel methods of (bio)remediation. These are best summarized as follows:

- PCB are complex, hydrophobic mixture containing halogen xenophores;
- bioremediation probably requires both anaerobic and aerobic growth regimes;
- matrix effects are site-specific and heavily influenced by ageing.

Each of these will be discussed in relation to their effect on the (bio) remediation of PCB, although it must be realized that they are not mutually exclusive. Ultimately, a novel method of remediation of historically contaminated soils ex situ will be presented.

### PCB biochemistry

The microbial degradation of PCB is known to occur via two main routes: highly chlorinated PCB congeners may be dechlorinated under anaerobic conditions to form lower chlorinated congeners, which are more susceptible to aerobic degradation. Lower chlorinated congeners can be degraded by aerobic bacteria via a well-documented pathway as outlined in Scheme 1 [5].

Aerobic metabolism of PCB involves oxygen insertion at adjacent unsubstituted carbons in the least chlorinated ring, followed by ring cleavage to form a chlorinated benzoate. Consequently, the metabolism of commercial mixtures of PCB, containing up to 80 congeners, may be expected to result in the formation of only nine mono- and dichlorinated benzoates.

### Process considerations

In collaboration with E.U. partners a two-stage process for the remediation of soils contaminated with PCB has been investigated. It was anticipated that the isolation of anaerobes capable of PCB dechlorination (Wageningen Agricultural University, Netherlands) would furnish di- and trichlorinated biphenyls which could serve as substrates for aerobic constructs able to mineralize PCB (Bergische Universität, Wuppertal, Germany). Unlike many other workers who have used microbial consortia, this process used transconjugants which have the potential to mineralize the widest range of PCB congeners.

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**Abbreviations used:** PCB, polychlorinated biphenyl; HMPA, hexamethylphosphoramide; SACRED, samarium catalysed reductive dechlorination.
Xenobiotic Pollution and Recovery by Natural Systems

Scheme 1
Schematic pathway for the aerobic microbial metabolism of PCB

These constructions have been described in detail elsewhere [6,7]. At the University of Kent at Canterbury Burkholderia cepacia strain JHR22 has been the most studied and its construction is outlined in Scheme 2.

Our investigations have shown there to be several limitations to the sequential anaerobic-aerobic reactor configuration for the mineralization of PCB, whether in soils, sediments or simply supplied in aqueous culture.

(1) Incompatibility in hydraulic retention times
Although it was demonstrated that anaerobic systems could dechlorinate a variety of PCB it was clear that the development of a process would be limited by the inability to isolate the catalyst(s) in the absence of sediment. Additionally, the incubation time required for the anaerobic dechlorination was in the order of weeks to months. Neither of these were compatible with the development of an ex situ process where the subsequent aerobic mineralization would occur in a time-frame of hours to days.

(2) Influence of bioavailability
Sorption of PCB has been shown to be major factor in the retardation of bioremediation [8]. Using B. cepacia JHR22, it has been shown that the potential to mineralize various monochlorinated biphenyls is dependent upon the matrix and also on the positioning of the chlorine atoms. Moreover, soil microcosm experiments have shown that ageing of the soil was hugely influential in the biodegradation of the chlorobiphenyls [9].

(3) Presence of metabolic intermediates
The key intermediates in PCB metabolism are the chlorinated benzoates. These occur between the upper and lower pathway and are characteristic intermediates in the co-metabolism of PCB. B. cepacia JHR22 was constructed to metabolize all monochlorobiphenyls and all monochlorobenzoates. However, as outlined above, it would be expected that the metabolism of a typical Aroclor mixture would result in the formation of all mono- and dichlorinated benzoates. Investiga-
tions have shown that *B. cepacia* JHR22 is only able to grow on 2,4- and 3,5-dichlorobenzoic acid, leaving four further intermediates which would be formed, but not metabolized, by the transconjugant. Although it is true that indigenous microflora may well transform or mineralize these intermediates, it detracts from the utility of the construct in the bioremediation of PCB-contaminated soils. We have also shown that, of the non-metabolized dichlorobenzoates, the presence of 2-, 3- and 3,4-dichlorobenzoate inhibits the metabolism of all monochlorobiphenyls [10]. Therefore, it is possible that the metabolism of Aroclor 1242 by *B. cepacia* JHR22 could be self-limiting, both by the production of dead-end metabolites and the inhibition of the PCB upper pathway.

(4) **Incomplete mass balance**

The aerobic metabolism of PCB usually results in the production of (chlorinated) benzoates as described above. However, it is clear in the studies undertaken that the stoichiometric release of chloride from the PCB does not always occur. This may result from the production of a dead-end metabolite, identified as 4-chlorodihydroxybenzoate by our colleagues [6], or be a chlorinated C-5 fragment which arises when the initial ring fission of chlorobiphenyl occurs. However, it was not possible to identify this in the microcosm studies using *B. cepacia* JHR22.

Though the aim was to design an ex situ process it was clear that the problems encountered and outlined above would preclude the operation of a sequential anaerobic–aerobic reactor for the treatment of historically contaminated soils. Three main factors were identified as the major limitations in the design of a generic process. Firstly, bioavailability should be increased irrespective of the matrix and history of the site. Additionally, the feed for the aerobic mineralization stage using *B. cepacia* JHR22 must be produced more rapidly and more reproducibly than could be expected if using the non-characterized anaerobic consortia.

**Hybrid chemical–biological process**

Work in this laboratory has sought to circumvent these problems by investigating the possibility of coupling a chemical and a biological process together. Several chemical processes have been proposed for the treatment of PCB including the use of Fentons reagent [11], alkali metal polyethylene glycolate [12] and photochemical processes for liquid effluents [13]. Although these processes transform PCB they result in the formation of hydroxylated haloaromatics which may have increased mobility and toxicity and would not be expected to be mineralized by isolates such as *B. cepacia* JHR22. The aims were: (i) to mimic the anaerobic dechlorination of the higher chlorinated PCB, i.e. reductively transform the higher chlorinated PCB to lower chlorinated PCB; and (ii) to provide a reproducible feed of biphenyl, mono-, di- and trichlorinated biphenyls which could be mineralized by *B. cepacia* JHR22.

It had been reported previously that titanocene dichloride in conjunction with sodium borohydride and an amine could reductively dechlorinate aryl halides at elevated temperatures [14,15]. Additionally, during the course of the work a report appeared relating to a process that had been developed from this technology but no details were provided [16]. This patented process [17] was, therefore, evaluated as a means of dechlorinating PCB in soils, not only for the transformation of the parent molecules but to provide a feed for subsequent microbial mineralization. The essential requirements of this process were titanocene dichloride, sodium borohydride, triglyme and pyridine under an inert atmosphere at a temperature of approximately 150°C. It was shown that the end product of the chemical reaction was the required lower chlorinated biphenyls, irrespective of the presence of soil, with a suggestion that the process may have a preference for ortho substituted PCB [18]. The latter was especially noteworthy as 85% of the congeners present in Aroclor 1242 possess ortho substitutions. The rate and extent of the dechlorination was also shown to be dependent on the basicity of the amine used in the process, with pyridine proving optimal. To demonstrate the compatibility of the chemical and biological stages a study was undertaken using the components of the metalloene-catalysed reaction in conjunction with *B. cepacia* JHR22. The results demonstrated that, although sodium borohydride and pyridine are inhibitory to biphenyl metabolism, the post-dechlorination product can be fed at a rate which causes minimal inhibition of the biocatalyst (Table 1).

Further chemical dechlorinations were carried out on 50,000 ppm Aroclor 1242. After neutralization with HCl a sample of the product was added at 3% (v/v) to a mixture of transconjugants and an isolated pyridine degrader. After 24 h incubation the majority of the biphenyl
Table I

The effect of various reagents used in metallocene-catalysed dechlorination on the metabolism of biphenyl by B. cepacia JHR22

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical dechlorination product</td>
<td>39 (v/v)</td>
</tr>
<tr>
<td>Triglyme</td>
<td>0 (v/v)</td>
</tr>
<tr>
<td>Titanocene dichloride</td>
<td>0 (v/v)</td>
</tr>
<tr>
<td>Sodium borohydride</td>
<td>100 (v/v)</td>
</tr>
<tr>
<td>Pyridine</td>
<td>100 (v/v)</td>
</tr>
</tbody>
</table>

and all of the 4-chlorobiphenyl had been utilized. Unfortunately, the 3-chlorobiphenyl produced had not been metabolized. In summary, the metallocene-catalysed process was compromised by the presence of water, which would have necessitated soil drying, and the requirement for high temperatures and an inert atmosphere. Furthermore, for commercial reasons, the process would also have been limited by the need to recycle the triglyme, the most costly reagent used in the process.

A second chemical catalyst was investigated to alleviate some of these problems, facilitating its application in the field. Kagans reagent has been used extensively in organic synthesis [19] and there were some precedents for its use in the reduction of organohalogens [20], especially the debromination of 4-bromobiphenyl [21]. However, to our knowledge, this reagent had never been used for environmental remediation. The SACRED process (SAmarium Catalysed REductive Dechlorination) employs Kagans reagent (Samarium [II] iodide) in the presence of hexamethylphosphoramide (HMPA) in tetrahydrofuran for the dechlorination of higher chlorinated congeners of Aroclor 1242. We have demonstrated that SACRED can be applied to the treatment of Aroclors whether in the presence or absence of soil and irrespective of age [22]. Unlike the metallocene-catalysed process, SACRED operates at ambient temperature and will tolerate a moderate (<5% w/v) amount of water. It yields mainly biphenyl, monochlorobiphenyl and traces of di- and trichlorobiphenyls, providing a predictable feedstock for the subsequent microbial mineralization. Of the solvents employed in SACRED only HMPA has a high boiling point, limiting its recovery. HMPA is thought to co-ordinate the samarium diiodide such that the electron transfer required for dechlorination is highly effective. Alternative, environmentally benign reagents have been investigated and it is possible that these could be used without compromising the yield and composition of the product.

Conclusions

We have shown that the rapid remediation ex situ of PCB-contaminated soils is a realistic possibility if a hybrid chemical–biological process is used. The complexity of the commercial mixtures of PCB coupled with the heterogeneity of soils and sediments and the requirement for both anaerobic and aerobic metabolism precludes the sole use of bioremediation. Although only the preliminary results using SACRED coupled with aerobic mineralization have been presented here it is clear that it offers an opportunity to develop a continuous, cost effective process for the treatment of PCB contaminated soils ex situ. Moreover, the nature of the Kagans reagent suggests it will have broader utility for the remediation of soils contaminated with haloorganics.

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Biomonitoring in the aquatic environment: use of molecular biomarkers

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Introduction

The aquatic environment is under increasing threat from chemical pollution. Contamination of this compartment of the biosphere derives from not only accidental spillages, with which the public is familiar, but also the continuous entry of material from industrial, agricultural and waste disposal activities. Chemical contaminants include polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), the dioxins (PCDD), dibenzo furans, tributyltin, nitroaromatics, organochlorines, radionuclides, and metals [1]. In view of the array of pollutants encountered, a variety of approaches have been taken both to monitor their entry into the aquatic environment and to measure levels of persistence and turnover under natural conditions [2,3]. Traditional methods of monitoring, apart from the direct measurement of health and survival in aquatic organisms, have focused on the chemical analysis of pollutant levels in water, sediment or the biota. However, measurements of pollutant levels give little indication of their effect on the flora and fauna, nor indeed do they take account of the ability of living organisms to adapt to chemical challenge. For this reason, the use of molecular biomarkers is now being actively investigated. These biomarkers attempt to associate traditionally measured changes due to pollution, such as of health, growth and reproduction, with more specific and rapid analyses of underlying cellular change or molecular damage.

Molecular biomarkers

A variety of biological parameters have been examined in different species to quantify aquatic pollutant-induced change and damage at the molecular level [3,4]. However, many of these changes can be induced by a variety of stressors, pollutants or toxins and are not sufficiently selective for use as specific biomarkers of exposure to defined groups of chemicals or individual compounds. Some biomarkers, however, do show compound specificity in their response to exposure. Included here are the formation of bulky