DEVELOPMENT OF A COMPUTER MODEL FOR PREDICTION OF PCB DEGRADATION ENDPOINTS

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DEVELOPMENT OF A COMPUTER MODEL FOR PREDICTION OF PCB DEGRADATION ENDPOINTS

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ABSTRACT
Several researchers have demonstrated the transformation of polychlorinated biphenyls (PCBs) by both aerobic and anaerobic bacteria. This transformation, or conversion, is characteristic and often dependent on PCB congener structure and, in addition, dictates the products or endpoints. Since transformation is linked to microbial activities, bioremediation has been hailed as a possible solution for PCB-contaminated soils and sediments, and several demonstration activities have verified laboratory results. This paper presents results from mathematical modeling of PCB transformation as a means of predicting possible endpoints of bioremediation. Since transformation can be influenced by both starting composition of the PCBs and microbial activity, this paper systematically evaluates several of the most common transformation patterns. The predicted data are also compared with experimental results. For example, the correlation between laboratory-observed and predicted endpoint data was, in some cases, as good as 0.98 (perfect correlation = 1.0). In addition to predicting chemical endpoints, the possible human effects of the PCBs are discussed through the use of documented dioxin-like toxicity and accumulation in humans before and after transformation.

INTRODUCTION
Polychlorinated biphenyls are a family of compounds regulated by the Toxic Substances Control Act due to their suspected carcinogenic effect in humans. These compounds, which were produced and marketed worldwide prior to the mid-1970s, were used in transformers, capacitors, printing inks, paints, antidusting agents, pesticides, etc. Today, they are well known as a widely distributed environmental pollutants [1].

Although PCBs are relatively inert, biological degradation by anaerobic dechlorination and aerobic oxidation is possible [2]. The enzymes involved in the aerobic ring cleavage have been studied and reviewed extensively [2, 3]; however, details are not known about the enzymes responsible for anaerobic dechlorination, primarily due to the difficulty in isolating the microorganisms responsible [4]. The anaerobic dechlorination follows distinct dechlorination patterns, which refer to the type of chlorines removed, and is dependent on environmental conditions. As the knowledge of microbial dechlorination and degradation increases, it may soon be possible to control these bioremediation processes for optimal results.

Safe [5] and Brown [6] have published the most recent and complete studies on the possible effects of PCBs on human health. Safe noted that certain PCBs were similar to dioxin in their biochemical and toxic reactions in humans and animals. Relationships were developed on the basis of PCB structure, and toxic equivalence factors (TEQs) were postulated for the most dioxin-like PCB congeners. Brown discussed the metabolism and natural attenuation of PCB compounds within humans and animals; it was concluded that the results could be used to evaluate bioremediation processes.

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This work presents the development of a predictive modeling tool to aid in evaluation of PCB degradation outcomes. This tool is a computer model based on the susceptibility of individual PCB compounds (congeners) to undergo bacterial transformation [4, 7, 8]. The model has been tested and compared with experimental data taken from the dechlorination of complex PCB mixtures (Aroclors). The computer model also incorporates literature data from Safe [5] and Brown [6] to estimate the effects on humans.

**METHODOLOGY**

The model was developed for use on a personal computer using Microsoft Excel (Microsoft Corporation, Seattle, WA); and in order to utilize the various built-in functions offered in Excel, the construction of a new nomenclature for PCBs was incorporated (Table 1). The nomenclature name was 11 characters wide, and both the position and the numeric value of a character in the name mark the chlorine substitution type. A proxy name was also constructed, as indicated in Table 1. The compounds listed in Table 1 are shown as examples of PCB chemical structure (Figure 1).

<table>
<thead>
<tr>
<th>IUPAC no.</th>
<th>common nomenclature name</th>
<th>computer model nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-monochlorobiphenyl or 2</td>
<td>20000-00000 or 00000-20000</td>
</tr>
<tr>
<td>16</td>
<td>2,2',3-trichlorobiphenyl or 23-2</td>
<td>23000-20000 or 20000-23000</td>
</tr>
<tr>
<td>164</td>
<td>2,3,3',4',5',6-hexachlorobiphenyl or 236-345</td>
<td>23006-03450 or 03450-23006</td>
</tr>
</tbody>
</table>

![Image of PCB congeners]

Figure 1. PCB congener structure corresponding to listing in Table 1.

The susceptibility of individual congeners to undergo anaerobic transformation has been postulated and demonstrated by Williams [8], who found that the chlorine position on the PCB carbon backbone determined sensitivity to attack. These dechlorination systems were adapted, modified, and used in the computer model. Examples of the most common dechlorination systems are displayed in Table 2. These anaerobic, reductive transformations result in the replacement of a chlorine atom with a hydrogen atom (from water) and produce a new PCB congener.

As is noted from the examples in Table 2, the susceptibility for attack is based on simple rules related to the structure of the PCB congeners. The example of para chlorine (UFP) removal in the attack on 2,2',4,5',6-pentachlorobiphenyl (20406-20050) demonstrates the need for the proxy nomenclature name since the outcome of this attack results in a structure that is "reversed" from the traditional nomenclature name.
Table 2. Common Dechlorination Systems with the Reactive Position Underlined

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubly flanked meta chlorine (DFM)</td>
<td>23450-20450</td>
<td>180</td>
<td>20450-20450</td>
<td>153</td>
</tr>
<tr>
<td>Doubly flanked para chlorine (DFP)</td>
<td>23450-20450</td>
<td>180</td>
<td>23050-20450</td>
<td>146</td>
</tr>
<tr>
<td>Singly flanked para chlorine (SFP)</td>
<td>23450-20450</td>
<td>180</td>
<td>23450-20505</td>
<td>141</td>
</tr>
<tr>
<td>Singly flanked meta chlorine (SFM)</td>
<td>23450-20450</td>
<td>180</td>
<td>23400-20400</td>
<td>85</td>
</tr>
<tr>
<td>Unflanked meta chlorine on di- or tri-substituted ring (UFP)</td>
<td>20406-20050</td>
<td>103</td>
<td>20406-20000</td>
<td>50</td>
</tr>
<tr>
<td>Unflanked para chlorine on di- or tri-substituted ring (UFP)</td>
<td>20406-20050</td>
<td>103</td>
<td>20006-20050 or 53</td>
<td>50</td>
</tr>
<tr>
<td>Lone para chlorine on ring opposite substituted ring (LP)</td>
<td>20400-00400</td>
<td>28</td>
<td>20400-00000</td>
<td>7</td>
</tr>
</tbody>
</table>

*Data obtained from Williams (8). Williams’ list is slightly more extensive and includes rare dechlorination of the ortho-position chlorines.

Rules for the seven dechlorination systems are easily incorporated into computer algorithms and may be expressed as the Excel formulas shown in Figure 2. The structure of the congener under attack is found in column A and the end-product structure is found in cells in column C. In this manner, any PCB congener can be evaluated for susceptibility, and the resulting endpoint product (also a PCB congener) can be determined. Results from the formulas in Figure 2 are shown in Figure 3.

![Figure 2. Excel spreadsheet with underlying functions corresponding to dechlorination systems described in Table 1. Column A contains examples of congeners.](image)

![Figure 3. Excel spreadsheet with congener structures following dechlorination systems described in Table 1 and Figure 3. Column A contains starting structure and column C contains the final dechlorinated structure.](image)

The dechlorination systems described above and by Williams [8] occur both in nature and in laboratory experiments in the form of combinations — not as isolated individual systems. The more complex of
these systems are often referred to as dechlorination activities or processes, which are denoted with letters such as C, H, H', M, N, P, Q, etc. [4]. For example, activity M is described as the removal of flanked and unflanked meta chlorines, and activity Q is described as the removal of flanked and unflanked para chlorines. Activity C can be described as a combination of activities M and Q. The significance is that dechlorination activities characterize the type of PCB structure susceptible for attack, and thus the products can be predicted via individual dechlorination systems. If knowledge exists about the starting concentration of each congener in a contaminated material, as well as about the type of dechlorination activity expected in a bioremediation effort (active or intrinsic), there is opportunity to predict the end-points (products) of microbial transformation.

As previously mentioned, dechlorination processes do not result in destruction of PCBs — only alteration. The most common pathway of biological destruction is through aerobic cometabolism with biphenyl. It has been shown that the most common aerobic processes are carried out by enzymes that attack C-C bonds in the biphenyl structure when these carbons are not linked to chlorines [7]. In general, this means that heavily chlorinated PCB congeners are less likely to be degraded, suggesting that a sequential anaerobic-aerobic bioremediation scheme is likely to yield the most complete destruction of PCBs [9]. There are two known enzymatic oxidative processes, one that initially oxygenates the C-C bond in the 2,3-position [3], and another that oxygenates the bond in the 3,4-position [7].

Figure 4 displays the computer algorithms for aerobic degradation of PCB congeners, assuming that the only requirement is unchlorinated adjacent carbons on either ring. The initial structure is in column A, and the resulting structure is in column C. The 2,3-dioxygenase enzyme is effective in attacking the C-C bond in either the 2,3 or the 5,6 position due to symmetry. This is the case with 3,4-dioxygenase as well, which is also effective for the 4,5 position.

![Figure 4. Excel spreadsheet with underlying functions corresponding to two degradation systems. The 2,3-dioxygenase attack is listed in row 1, and the 3,4-dioxygenase attack is listed in row 2. If the congener is susceptible to attack, the resulting structure is simply an empty cell in the computer spreadsheet.](image)

### RESULTS

Figure 5 shows the predicted dechlorination of Aroclor 1242 through process C [4, 10]. The individual charts were constructed by entering published values for Aroclor 1242 congener composition [11] and evaluating each congener’s susceptibility to dechlorination in a sequence of dechlorination systems:

1. doubly flanked meta (DFM), followed by
2. singly flanked meta (SFM), followed by
3. unflanked meta on di- or tri- substituted ring (UFM), followed by
4. unflanked para on di- or tri- substituted ring (UFP), and followed by
5. lone para on ring opposite substituted ring (LP).

After each sequence step, the concentration of each of the 209 possible PCB congeners was recalculated to account for the products created, and the individual congeners were combined for comparison with experimental data [10]. To elaborate; analysis of Aroclor PCBs using gas chromatography (GC) yields chromatograms with approximately 40–90 peaks. Each chromatographic peak corresponds to the elution
of one congener or, sometimes, many congeners. When experimental results are reported, individual congener concentrations are seldom shown; instead "peak concentrations" are used. Thus, congener concentrations calculated by the model were combined into peak concentrations for easy comparison with literature data.

![Figure 5. Dechlorination of Aroclor 1242 was modeled via DFM+SFM+UFM+UFP+LP. The correlation coefficient of the predicted "peaks" and the experimental endpoint results were 0.11, 0.18, 0.44, 0.56, and 0.98 for DFM, DFM+SFM, DFM+SFM+UFM, DFM+SFM+UFM+UFP, and DFM+SFM+UFM+UFP+LP. Values on the Y-axis refer to mole percent. The congeners in the three largest peaks have been identified.](image)

The construction of the charts (displaying chromatograms) in Figure 5 was made by generating these congener-containing peaks. After each sequential step, the constructed chromatogram was compared with Quensen et al. [10] and a correlation coefficient \( \left[ \frac{\sum (X_i - \bar{X})(Y_i - \bar{Y})}{\sum (X_i - \bar{X})^2 \sum (Y_i - \bar{Y})^2} \right]^{0.5} \) [12] was calculated comparing predicted results with experimental values. Quensen et al. obtained the laboratory data by combining Aroclor 1242 with Hudson River (NY) sediment organisms, inducing dechlorination.
activity. The correlation between starting values in the computer model and the sterile control in the experimental case was 0.95. It should be noted that the initial congener composition in the predictive case was based on data by Schulz et al. [11], and the starting composition in the experimental case was based on the actual analyses calibrated with known congener mixtures. The correlation of the final values in the predictive case and the experimental case was 0.98.

Many dechlorination activities are unique to the microbial consortium utilized; for example, the combination of Aroclor 1242 and Silver Lake (Pittsfield, PA) sediment organisms does not usually result in the same end-product composition as that of the combination of Aroclor 1242 and Hudson River microorganisms [10]. A computer model prediction via DFM+SFM+UFM dechlorination of Aroclor 1242 compares well with experimental data for dechlorination of Aroclor 1242 with Silver Lake sediment organisms. The model accurately predicts major endpoint products and the correlation between end values for the predictive case and the experimental case was 0.92.

Dechlorination simulations of Aroclors 1254 and 1260 via process C and H by Hudson River sediment organisms and the conversion of Aroclor 1260 via process N by Silver Lake organisms accurately predicted the end products measured in the laboratory [10] (data not shown). The computer simulation generally projected more complete dechlorination than laboratory results; however, qualitative agreement was good. The authors [10] noted that there was a very long lag phase in some of the experiments for Aroclor 1260 and Hudson River sediment organisms, which may have affected the outcome.

Other experimental results conducted with various sources of bacteria and different PCB Aroclors have been reviewed by Bedard and Quensen [4], who summarized published dechlorination activities (processes). The preferential order of the systems was also listed for each of these activities and matches well with that proposed by Williams [8]. Algorithms proposed for the individual systems (Figure 2), when combined appropriately, accurately predict the same end products as those listed by Bedard and Quensen [4]. One notable exception is the dechlorination of 2345-2346 octachlorobiphenyl via process N; Bedard and Quensen list 246-25 pentachlorobiphenyl, while the computer model predicts 246-24, as the end product of meta chlorine removal activity.

Several research groups have studied sequential anaerobic-aerobic treatment in the laboratory [13, 14, 15]. These studies have generally shown that overall degradation is not as complete as the model would predict; certain congeners remain even after extensive treatment times. Bedard and coworkers [9] investigated the aerobic degradation of environmentally altered (dechlorinated via process C) Aroclor 1242 and found that 81% of the dechlorinated PCBs were aerobically degraded by Alcaligenes eutrophus H850 in 48 h. They also concluded that a longer incubation period may have resulted in more complete degradation and that it was possible that some Aroclor 1260 and its dechlorination products were present in the environmental sample and masking the results. The computer simulation predicts that essentially all congeners remaining after a process C dechlorination of Aroclor 1242 would be biodegradable (Table 3), which perhaps overpredicts the effectiveness of a sequential anaerobic-aerobic bioremediation scheme. It is difficult to determine whether the model is too optimistic or whether the experimental conditions have not been ideal during reported experiments. There is clear evidence that prolonged incubation results in more extensive aerobic degradation [9]. It should also be noted that congeners found to be partially degraded in experimental studies are likely to be completely degraded if more favorable conditions are employed (e.g., continuous addition of nutrients and removal of products).

Since individual congener concentrations are calculated in the computer model throughout the progression, estimations of potential health-related issues of PCBs are possible. The dioxin TEQs of various PCB congeners have been reported by Safe [5] and the accumulation extents of PCB congeners in organisms (including humans) have been reported by Brown [6]. To assess health-related issues, the endpoints for a potential sequential anaerobic-aerobic PCB degradation strategy were generated using the
algorithms described earlier. Prediction show that aerobic degradation of the unaltered and altered Aroclors should be quite effective in reducing TEQs except in the case of unaltered Aroclor 1260 (Table 3).

As is shown in Table 3, Aroclors 1242, 1254, and 1260 (at 100 ppm) have TEQ values of approximately 10, 5.1, and 3 ppb of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), respectively. All of the main dechlorination activities result in a substantial reduction of TEQ. The activity of the 2,3-dioxygenase is predicted to be sufficient in reaching the lowest TEQ levels and does not have to be complemented with 3,4-dioxygenase activity. The most efficient anaerobic dechlorination process preceding aerobic degradation are the ones that include DFM and SFM systems (activities M, C, H', and N). It is encouraging to see that PCB Aroclors 1242, 1254, and 1260 may potentially be almost completely biodegradable if the correct combination of anaerobic and aerobic treatments is applied under optimal conditions.

Table 3. Summary Results of Predicted Anaerobic Dechlorination Followed by Aerobic Degradation (via Ring Cleavage) by 2,3-Dioxygenase, 3,4-Dioxygenase, and a Combination of Both Enzyme Activities. Activity M was modeled as DFM+SFM+UFM, activity Q was modeled as DFP+SFM+SFP+UF+LP, activity C was modeled as DFM+SFM+UFM+UF+LP, activity H' was modeled as DFP+DFM+SFP+SF, activity H was modeled as DFP+DFM+SFP, activity P was modeled as DFP+SFP, and activity N was modeled as DFM+SFM.

As an example, this indicate that congeners amounting to 51.5 ppm of 100 ppm Aroclor 1242 have a half-life of 0.1 to 1.0 year.

As an example, this indicate that congeners amounting to 45.4 ppm after dechlorination of 100 ppm Aroclor 1242 via process H have a half-life of 0.1 to 1.0 year.

As an example, this indicate that congeners amounting to 9.6 ppm after dechlorination of 100 ppm Aroclor 1242 via process H followed by an aerobic 2,3-dioxygenate attack have a half-life of 0.1 to 1.0 year.

As an example, this indicate that congeners amounting to 0 ppm after dechlorination of 100 ppm Aroclor 1242 via process H followed by an aerobic 2,3- and 3,4-dioxygenate attacks have a half-life of 0.1 to 1.0 year.

The most effective activity in reducing the accumulation tendency in humans is dechlorination process C. This activity has not been observed with Aroclor 1260 [4], but if it was or could be induced, the computer model predicts that all end products would have half-lives in humans of less than 0.1 year (data not shown). It is worth noting that the total amounts (in ppm) listed for the half lives do not add up to 100.
ppm; this is because dechlorination decreases the average molecular weight and, thus, the total ppm values. On a molar basis, total concentrations before and after predicted dechlorination are the same.

CONCLUSIONS
Simple algorithms have been developed to assess the implications of sequential anaerobic-aerobic bioremediation of PCBs, based on literature data. The method appears to be accurate in predicting anaerobic dechlorination activities, although it tends to overpredict microbial efficiencies. However, due to their simple structure, the algorithms may easily be adjusted, as more reliable data become available. The model predicts that most PCBs could be completely biodegraded if the ideal conditions could be employed. The incorporation of human health risks allows for additional evaluation of potential remediation strategies and the potential impact.

REFERENCES