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Characterization of polychlorinated alkane mixtures—a Monte Carlo modeling approach

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Abstract A Monte Carlo model was developed to characterize the molecular composition of polychlorinated alkane mixtures. The model is based upon a simulation of the free-radical chlorination process by which polychlorinated alkane mixtures are produced industrially from *n*-alkanes. In the model, the free-radical chlorination reaction was simulated by randomly selecting a position on a partially converted alkane molecule for target by chlorine free-radical attack. The relative reactivities of the hydrogen atoms on the alkane chain towards chlorine free-radical substitution were either determined experimentally or extrapolated from experimental results and incorporated into the model. The result of the simulation is the prediction of the detailed molecular composition of any PCA mixture. Good agreement was found when comparing the distribution of molecules predicted by the model to analytically determined distributions of real PCA mixtures. Results from the model were then coupled with rules describing the action of biological enzymes to estimate the upper limit possible for the aerobic biodegradation of PCA mixtures.

Keywords Biodegradation · Composition · Modeling · Monte Carlo · PCA, polychlorinated alkanes

Abbreviations

1,10-DCD	1,10-dichlorodecane
1,2-DCD	1,2-dichlorodecane
1-CD	1-chlorodecane
3-CD	3-chlorodecane
5,6-DCD	5,6-dichlorodecane
$A_{1\text{-CD}}$	The GC chromatogram peak areas associated with 1-CD
$A_{3\text{-CD}}$	The GC chromatogram peak areas associated with 3-CD
EPA	United States Environmental Protection Agency
GC	Gas Chromatograph
N	Number of carbon atoms in an unbranched carbon chain
NMR	Nuclear Magnetic Resonance spectroscopy
PCA	Polychlorinated Alkane
$R_{P/S}$	The relative reactivity of a hydrogen atom located on a primary carbon to a hydrogen atom located on a secondary carbon atom
UV	Ultraviolet
$h\ v$	Light source (photon)

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β	Number of chlorine atoms bound to a PCA molecule
(π_P/π_S)	The probability of encountering a hydrogen atom on a secondary carbon relative to the probability of encountering a hydrogen atom on a primary carbon

Introduction

Polychlorinated alkanes (PCAs), also known as chlorinated paraffins, are produced industrially by the free-radical chlorination of *n*-alkanes. PCAs are classified according to their chlorine content (30–70% by mass) and carbon chain length, as being short (C_{10}–C_{13}), medium (C_{14}–C_{17}) or long ($>C_{17}$). Most of the PCAs produced are used as high-temperature lubricants in the metal working industry, but the compounds also find utility as flame-retardants, plasticizers and additives in paints and rubbers. In 1992, the estimated worldwide production of PCA products was 300,000 tons per year, making them the largest class of chlorinated organic compounds produced in Western Europe and North America in that year (Tomy et al. 1998, 2000a, b; Allpress and Gowland 1999).

The release of PCAs into the environment during production and use in industry is a cause for concern. The short chain PCAs are toxic to marine life, bioaccumulate and have been implicated as carcinogens and endocrine disrupters (Tomy et al. 2000b; Wyatt et al. 1993). Although the highest levels of PCAs are found near production facilities, the compounds are susceptible to long range transport. Detectable levels of PCAs are found in water, soil and biota worldwide, including human tissue and arctic mammals (Madeley and Robin 1980; Environment-Canada 1993). Because of these attributes, the US Environmental Protection Agency (EPA) has placed short chain PCAs on the Toxic Release Inventory. In Canada, these mixtures are classified as Track 1 Priority Toxic Substances in the Canadian Environmental Protection act (Tomy et al. 2000a, b; Wyatt et al. 1993; Madeley and Robin 1980; Environment-Canada, 1993, 1997; Vogel et al. 1987).

Commercial PCA products are comprised of thousands of isomers and congeners. The large number of isomers results from the free radical mechanism by which PCAs are produced. In the synthesis process ultraviolet light is used to generate two radicals from molecular chlorine. The free radicals then interact with the target alkane, resulting in the displacement of hydrogen atoms by chlorine. To date, no analytical technique has been developed that is capable of resolving these mixtures into its various constituent elements (Tomy et al. 1998; Shojania 1999; Muller and Schmid 1984).

Work aimed at the understanding the underlying processes associated with biological dechlorination has focused for the most part on pure compounds. These efforts have identified chain length, degree of chlorination, and stereochemistry as key determinants in assessing the degradability of pure chloroalkanes. A suite of enzyme systems and their substrate specificity have also been identified (Wyatt et al. 1993; Environment-Canada 1997).

Extrapolating towards more relevant cases, the complexity and logistics increase dramatically when mixtures of chlorinated alkanes are considered. It is recognized that some isomers or congeners within a mixture of chlorinated alkanes may be readily biodegradable, while others may only be partially degradable or altogether recalcitrant (Damborsky et al. 2001). Furthermore, some isomers or congeners are likely to be toxic or carcinogenic whereas others are not. As a result the toxicity of PCA mixtures found in the environment will change as the biodegradable molecules are completely or partially mineralized by microorganisms (Tomy et al. 1998).

For ill-defined mixtures in which the composition cannot even be resolved, pre-assessment of the biodegradability of the oil becomes impossible. Since the chemical disposition is unknown, researchers are relegated to a “trial and error” approach, and the ultimate degree of biological removal achievable remains an unknown. For instance it has been established that short chain PCA mixtures have higher toxicity than medium and long chain PCAs and that more extensively chlorinated mixtures are more recalcitrant to biodegradation than less chlorinated mixtures (Tomy et al. 1998; Madeley and Robin 1980; Environment-Canada 1993, 1997; Vogel et al. 1987). However, for a given PCA formulation the ultimate degradation remains an open question, with claims between 0% through 100% being claimed by different researchers for some mixtures (Tomy et al. 1998; Wyatt et al. 1993).

Knowledge of the chemical composition of the oil under consideration is a key consideration when assessing the applicability of biological treatment. As a simple example, it has been well established that pure hexadecane can be completely mineralized under aerobic conditions by a number of organisms. If this result is not realized in practice then it follows that factors other than the genetic capabilities of the organism must be responsible. These may include the absence of trace minerals, availability and transport issues, or others. Without adequate definition of the disposition of the substrate it is impossible to ascertain whether the ultimate level of degradation has been achieved as determined by the genetic potential, or whether higher levels can be reached through manipulation of various environmental conditions. The literature concerned with the biological degradation of PCA mixtures has been hindered by the lack of definition of the oils under study. It has been impossible to critically assess the levels of degradation realized experimentally since an estimate of the maximum degree of removal attainable remains an unknown as the oils are chemically ill-defined.

From this discussion it is apparent that an estimate of the compositions of PCA mixtures is required as a compliment to biological studies involving defined substrates. Results from the studies involving pure compounds can then be applied to the characterization, providing a first approximation as to the action of the microbes on PCA mixtures. The result would allow for an assessment of the feasibility of biodegradation as it applies to a particular PCA mixture, and will also provide an estimate of the maximum amount of degradation achievable.

The work presented here represents a first attempt at addressing the shortcomings in the collective ability of current analytical techniques to adequately characterize PCA mixtures. These deficiencies are addressed through a novel modeling approach that captures the mechanism by which PCAs are produced. The model incorporates experimentally determined reactivity data and information found in the chemistry literature to arrive at a complete, detailed estimate of the distribution of the molecules contained in a PCA mixture of any carbon chain length and any degree of chlorination.

The chemical distribution generated by the model was then coupled with published biological degradation involving pure compounds, to arrive at an estimate of the maximum degree of degradation attainable for the given mixture. To assess the validity of this approach this result was then compared with published studies concerned with the biodegradation of the corresponding PCA mixtures.

Materials and methods

Chemicals

Decane, 1-chlorodecane (1-CD), 1-decene, 5-decene, 1,10-dichlorodecane (1,10-DCD) and chlorine gas were purchased from Sigma-Aldrich (Mississauga, Ontario, Canada). All chemicals had purities ranging from 95% to 99.5%. Dichloromethane (CH_2Cl_2, spectrophotometric grade) was purchased from J.T. Baker Chemical Co. (Phillipsburg, N.J., USA).

Analysis of chlorinated alkanes

Analyses of the chlorinated alkanes were carried out with a gas chromatograph (Varian CP-3800) equipped with a Supelco SPB-5 column (30 m × 0.32 mm ID, 0.25 μm film thickness) and a flame ionization detector. The carrier gas (UHP grade helium) was set at a column flow of 4 ml/min and split ratio of 40:1. The temperature program for all hydrocarbon analyses was as follows: initial temperature of 70°C; ramp to

300°C at 15°C/min; and held for 5 min. The injector temperature was constant at 300°C and the detector temperature was fixed at 320°C. Chlorinated decanes were dissolved in dichloromethane before injection.

Measurements of ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were performed using a 300 MHz Varian Mercury spectrometer.

Syntheses of di-chlorinated alkanes

Pure chlorinated alkanes were synthesized from alkenes as described elsewhere (Heath et al. 2006). In summary, chlorine gas was bubbled through a volume of 2 ml decene dissolved in 10 ml dichloromethane, while stirring. The extent of the reaction was tracked by gas chromatography (see above). The chemicals 1-decene and 5-decene were used as reactants to obtain 1,2-dichlorodecane (1,2-DCD) and 5,6-dichlorodecane (5,6-DCD) respectively. The final products were confirmed using ^1H and ^{13}C-NMR spectroscopy.

Free-radical chlorination of decane and chlorinated decanes

Glass vials (4 ml) were charged with approximately 2 ml of neat decane or chlorinated decane (1-CD, 1,2-DCD or 5,6-DCD). Chlorine gas was bubbled through the alkane using a Pasteur pipette, which was connected to the chlorine gas supply by teflon tubing and a vacuum trap. The chlorine gas was supplied at a rate of 3–6 bubbles per second. The free-radical chlorination reaction was carried out in the presence of ultraviolet light supplied by a UV-lamp (UVGL-25, MINERALIGHT® Lamp, UVP Inc.) placed in close proximity to the glass vial. The following reactants were used for these experiments: *n*-decane, 1-CD, 1,2-DCD, 5,6-DCD and 1,10-DCD. The extent of the reaction was tracked by gas chromatography. Retention times of individual chlorinated decane products were determined either by using pure standards of chlorinated decanes or by comparison with published values. The overall chlorine content of each chlorinated alkane mixtures was determined by ^1H-NMR.

Model description

Background

The industrial production of PCAs is typically carried out in a batch process where mixtures of pure *n*-alkanes are contacted with chlorine gas in the presence of ultraviolet light (Tomy et al. 1998; McMurry 1994). In general, chlorine atoms are exchanged for hydrogen atoms according to the equation:

$$C_nH_{2n+2-\beta}Cl_\beta + Cl_2 \xrightarrow{h\nu} C_nH_{2n+1-\beta}Cl_{\beta+1} + HCl \tag{1}$$

Any of the hydrogen atoms on the alkane carbon chain can potentially be replaced by a chlorine atom upon encountering a chlorine free-radical. The reactivity of a hydrogen atom to chlorine free-radical attack depends upon the stability of the carbon free-radical intermediate formed in the chlorination reaction. This stability is primarily affected by the intra-molecular location of the carbon atom on which the hydrogen resides (primary or secondary) and the number and intra-molecular distribution of chlorine atoms already incorporated in the molecule (Huyser 1969; McMurry 1994).

Mathematically, the process can be viewed as being comprised of a series of discrete events, with each step having only two possible outcomes. Any given chlorine radical either encounters a hydrogen atom or it does not. If an interaction occurs, then the chlorine free radical either displaces the hydrogen atom or it does not. By defining appropriate probabilities describing the outcome of these events, the chlorination process can be modeled, and the molecular makeup of the resulting distribution can be estimated.

From a modeling perspective, this description lends itself to a Monte Carlo approach. In the Monte Carlo framework a pool of alkane molecules is established of a magnitude large enough so as to give reproducible results, typically 4,000–20,000. An alkane molecule is selected randomly from the pool. A specific atom on the molecule, selected at random, is then targeted for possible chlorine free-radical attack through the mechanism described in equation (1) (Tomy et al. 1998). Whether the encounter is successful in substituting a chlorine atom for a hydrogen atom depends upon the reactivity of the hydrogen atom selected.

Each hydrogen atom is assigned a reactivity that represents the probability that the selected hydrogen atom will be replaced by a chlorine free radical. From the literature, the reactivity of a given hydrogen atom is related to the stability of the resulting carbon free radical. Stability is affected by such factors as the position of the carbon atom in the chain, and the intra-molecular distribution of chlorine atoms (Huyser 1969; McMurry 1994). The complete set of reactivity values represents the parameters in the Monte Carlo model.

For convenience, reactivities of the various hydrogen atoms were considered relative to that associated with a hydrogen atom residing on a secondary carbon atom on the pure alkane. From the literature, hydrogen atoms located on secondary carbon atoms on unchlorinated alkane chains had the highest reactivity (Colburne and Stern 1965). The relative reactivities associated with all other hydrogen atoms were a fraction of this value. Relative reactivities defined in this way are bounded between 0 and 1, coupling easily with the Monte Carlo approach.

The Monte Carlo model was written in Visual Basic for Applications in the Microsoft Excel environment. Incorporating the spreadsheet interface enables the user to easily specify the desired carbon chain length, number of molecules used for the simulation and the final overall degree of chlorination. Each chlorinated alkane molecule is represented by a text string. The characters in each string represent the carbon–hydrogen and carbon–chlorine σ-bonds. Hence an alkane chain with n carbon atoms is represented by a text string with $2n + 2$ characters. In the text string, a "0" character represents a carbon–hydrogen bond, while the character "1" represents a carbon–chlorine bonds. With these definitions, a collection of non-chlorinated alkane molecules is represented on the worksheet by a set of strings consisting only of the "0" character. The number of text strings generated for the simulations is limited only by the available computing power. Typically, 20,000 molecules were considered.

When a hydrogen atom is substituted by a chlorine atom during an iteration of the model the zero character that corresponds to the position of the hydrogen atom under consideration is replaced by a "1". The characters on the text strings, shown on the worksheet, are changed in real time, which allows the user to visually follow the progress of the virtual chlorination process. The result of the simulation is a complete representation of the set of molecules in the PCA mixture.

Model assumptions and limitations

A number of assumptions were required in formulating the model. Most importantly, the reactants were assumed to be well mixed. As a consequence of this assumption, the chlorine free-radicals are distributed uniformly in the mixture and each molecule has an equal probability of encountering a chlorine free-radical.

The relative reactivity of a hydrogen atom was assumed to be unaffected by the alkane chain length. Studies on the relative reactivities of hydrogen atoms on octane and decane have shown that the effect of chain lengths is negligible (Huyser 1969; Colburne and Stern 1965). The reactivities of all hydrogen atoms bonded to secondary carbon atoms were assumed to be equal for unchlorinated alkane molecules. In reality, the relative reactivities for hydrogen bound to secondary carbon adjacent to primary carbon have slightly lower reactivity than those associated with more centrally located secondary carbons. However, this difference in reactivity is small compared to the effect of the presence of proximal chlorine atoms on the carbon chain and was therefore assumed to be negligible (Huyser, 1969). In all cases, the relative reactivities used were averages of available values.

Finally, the effect of temperature is implicitly considered by virtue of the approach taken. The literature has shown that while the absolute reactivity of a hydrogen atom in a particular intra-molecular environment increases significantly with increasing temperature, hydrogen atoms in all environments are affected to a similar magnitude. Therefore, on a relative basis, the effect is minor. Since the extent of the reaction is considered in this approach instead of reaction time the temperature has no impact on the final results (McMurry 1994; Colburne and Stern 1965).

Results and discussion

Parameter estimation

The reactivity of a hydrogen atom depends upon its intra-molecular environment. In the most general case each hydrogen atom must be considered as unique, with all possible chlorinated isomers considered. This approach leads to an unmanageable number of model parameters (see Appendix). Fortunately, there is no justification for this level of complexity based on the prevailing literature. The relative reactivities of hydrogen atoms on an *n*-alkane chain are higher for those on secondary carbon atoms than for those on a primary carbon atom (Tables 1 and 2). This well-known effect is attributed to the resonance stability of the intermediate carbon radical (Huyser 1969; Colburne and Stern 1965; McMurry 1994). It is not necessary to consider tertiary carbon atoms because they are absent in the starting materials (*n*-alkanes). The effect of chlorine atoms located in close proximity to the reacting hydrogen is determined by two opposing phenomena—polar deactivation and resonance activation. Polar deactivation is caused by the electron-withdrawing nature of the chlorine atoms making the carbon–hydrogen bonds in close proximity electron-poor and, thus, less reactive. Resonance stabilization is a result of an increase in the degree of bonding between the carbon and chlorine atoms and by charge transfer between the two during the formation of reaction intermediates. The net result of the two effects is a reduction in reactivity of the hydrogen atoms residing close to a chlorine atom (Huyser 1969). Literature data have shown that the deactivating effect of chlorine substitutes acts only on the carbon–hydrogen bonds on both the α and β carbon atoms (see Fig. 1 for naming conventions) (Huyser 1969). Considering all possible arrangements of chlorine atoms located near a hydrogen atom of interest, there are a total of 77 model parameters, or possible values, for relative reactivities of the hydrogen atoms that must be specified.

Table 1 Relative reactivity for hydrogen atoms located on terminal carbon atoms

# chlorine atoms on β-carbon[a] atom	# chlorine atoms on terminal carbon atom bound to the hydrogen atom of interest	# chlorine atoms on α-carbon[a] atom		
		0	1	2
0	0	**0.370** (0.37–0.40)	**0.067**	0.021
	1	**0.074**	0.022	0.011
	2	0.037	0.020	0.011
1	0	**0.310** (0.30)	**0.067**	0.021
	1	0.062	0.022	0.011
	2	0.033	0.020	0.011
2	0	0.279	0.067	0.021
	1	0.062	0.022	0.011
	2	0.033	0.020	0.011

Values shown in bold font are experimentally determined values; values shown in regular font are interpolated values; values shown in parenthesis are from the literature [14]

[a] See naming convention on Fig. 8

Of the 77 parameters required by the Monte Carlo model, only 12 could be derived from experimental data. These values are shown in parentheses in Tables 1 and 2. For moderately chlorinated PCAs (<55% w/w chlorine), this subset was sufficient for describing the majority (77%) of chlorination events. This is due to the fact that, for mixtures of this degree of chlorination, most hydrogen atoms are in close proximity to only one or, more commonly, no chlorine atoms. As the chlorine content of the alkane chain increases, so does the probability that a hydrogen atom of interests is located adjacent to two or more chlorine atoms. For these cases more accurate estimates of the unknown model parameters are required.

To supplement the available data free-radical chlorination experiments were carried out with selected pure and chlorinated alkanes. The intent was to determine the effect of the intra-molecular chlorine distribution on the reactivity

Table 2 Relative reactivity for hydrogen atoms for hydrogen atoms located on secondary carbon atoms

Total # chlorine atoms on β- and β'-carbon[a] atom	# chlorine atoms on secondary carbon atom bound to the hydrogen atom of interest	Total # chlorine atoms on α- and α'-carbon[a] atoms				
		0	1	2	3	4
0	0	**1.000** (0.93–1.00)	**0.500**	**0.111**	0.025	0.025
	1	**0.095**	**0.067**	0.030	0.007	0.007
1	0	**0.720**	**0.410**	0.090	0.025	0.025
	1	0.080	0.052	0.028	0.007	0.007
2	0	0.600	0.350	0.081	0.025	0.025
	1	0.070	0.042	0.026	0.007	0.007
3	0	0.540	0.320	0.077	0.025	0.025
	1	0.065	0.037	0.025	0.007	0.007
≥ 4	0	0.540	0.320	0.077	0.025	0.025
	1	0.065	0.037	0.025	0.007	0.007

Values shown in bold font are experimentally determined values; values shown in regular font are interpolated values; values shown in parenthesis are from the literature [14]

[a] See naming convention on Fig. 8

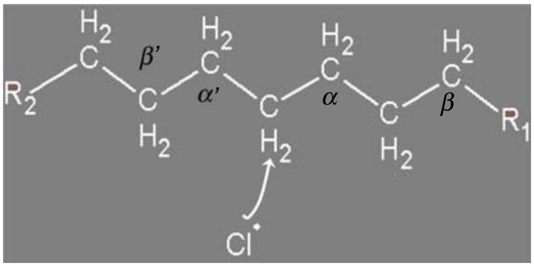

Fig. 1 Naming convention: Carbon atoms adjacent to the carbon atom bound to the hydrogen atom of interest are referred to as α or α' carbon atoms. Carbon atoms adjacent to α or α' carbon atoms are referred to as β or β' carbon atoms

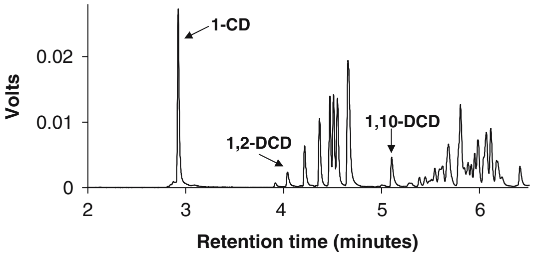

Fig. 2 Gas chromatogram of products of 1-chlorodecane free-radical chlorination

of a hydrogen atom on chlorine free-radical substitution. These data were then used to derive values for relative reactivities of hydrogen atoms for incorporation in the Monte Carlo model. The relative reactivities of the hydrogen atoms were determined by quantifying the formation of some selected chlorinated products of the free-radical chlorination reaction.

Figure 2 shows a typical GC-chromatogram associated with the free-radical chlorination of 1-chlorodecane. The peaks associated with 1-chlorodecane and the dichlorinated reaction products are well resolved, making it possible to quantify the amount of individual chlorination products. Each chlorination experiment was performed in at least triplicate and average values for areas under GC-chromatogram peaks were used to determine the quantity of each chlorinated product species. The calibration curves associated with various pure mono-, di-, tri-, and tetra-chlorinated alkanes were shown to be equivalent on a molar basis, within statistical error (data not shown). Therefore, peak areas were assumed to be proportional to the molar concentration of the particular species, independent of its degree of chlorination. On this basis, the relative reactivity of the individual hydrogen atoms was obtained directly from the relative quantity of the chlorinated products formed.

For example, two distinguishable chlorinated products from chlorination of decane are 1-chlorodecane (1-CD) and 3-chlorodecane (3-CD). The selectivity of the chlorination reactions for 1-CD relative to 3-CD follows from a simple analysis of competing parallel reactions, and is given by:

$$S_{P/S} = \frac{6}{4}\left(\frac{A_{1-\mathrm{CD}}}{A_{3-\mathrm{CD}}}\right) \tag{2}$$

where $A_{1\text{-CD}}$ and $A_{3\text{-CD}}$ are the chromatograph peak areas associated with 1-CD and 3-CD respectively. Since the selectivity is unbounded, it is more convenient to considering the rate of formation of the species of interest relative to all possible outcomes. This quantity was termed the 'relative reactivity'. With regards to the current example, all hydrogen atoms on secondary carbons on the pure alkane have been shown to have approximately the same reactivity (Huyser 1969; Colburne and Stern 1965). The same has been demonstrated for hydrogen atoms residing on primary carbons. Therefore the relative reactivity of a primary carbon is given by:

$$R_{P/S} = \frac{6}{4}\left(\frac{A_{1-\mathrm{CD}}}{6A_{1-\mathrm{CD}} + 4A_{3-\mathrm{CD}}}\right) = \frac{3}{2}X_{1-\mathrm{CD}} \qquad (3)$$

Here $X_{1\text{-CD}}$ is the mole fraction of 1-CD in the organic phase, considering only chlorinated species. Hydrogen atoms for which relative reactivities could be determined using Eq. (3) are indicated by the bold entries in Tables 1 and 2. In these tables, it can be seen that, where available, reactivity data obtained from the literature compares favorably with estimates determined experimentally, validating the approach.

Figure 3 shows the dependence of the model output on experimentally determined parameters as a function of the overall degree of chlorination. It is apparent that the possible impact of more highly chlorinated species becomes more important as higher degrees of chlorination targeted. Ideally, additional free-radical chlorination experiments should be carried out using appropriate starting compounds. However, such starting materials are decidedly difficult to synthesize and are not commercially available.

Fortunately, it is possible to obtain reasonable estimates of the parameters that could not be determined experimentally by extrapolating the trends associated with the experimental values. From the literature, it is known that the hydrogen reactivity decrease as the number of chlorine atoms near the hydrogen of interest increases. For an unchlorinated alkane chain, the decrease in hydrogen reactivity is most pronounced upon addition of the first chlorine atom, with additional chlorination having less of an impact, suggesting a "saturation" effect. Figure 4 shows a plot of data from the literature showing the effect of the relative reactivities of hydrogen atoms on ethane molecules with different numbers of chlorine atoms (Huyser 1969; Colburne and Stern 1965). The asymptotic convergence of the relative reaction values for hydrogen atoms with increasing numbers of adjacent chlorinated carbon atoms supports the notion of a saturating effect. Figure 5 shows some of the relative hydrogen reactivities determined experimentally in this study and the parameters that were estimated by extrapolating those data. The "saturation" effect discussed above is again illustrated by the asymptotic convergence of the relative reaction

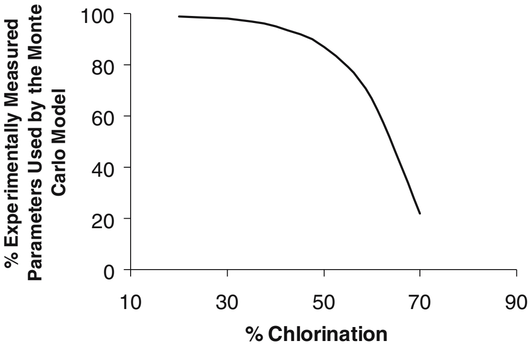

Fig. 3 The percent reliance by the Monte Carlo Model on experimentally measured model parameters (as opposed to estimated parameters) versus overall degree of chlorination

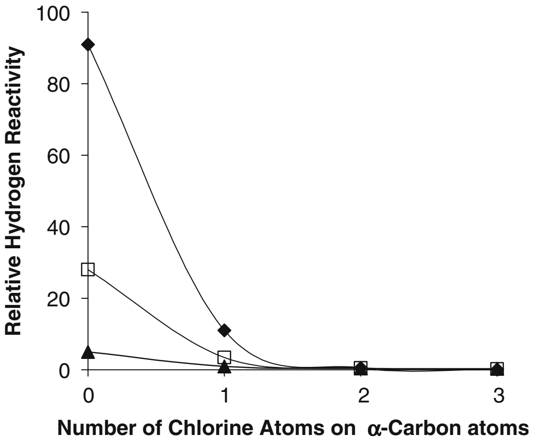

Fig. 4 Relative reactivity of hydrogen atoms on an ethane molecule towards chlorine free-radical substitution. (—◆—) No chlorine atoms, (—□—) one chlorine atom, and (—▲—) two chlorine atoms bound to the carbon atom with the hydrogen of interest [50]

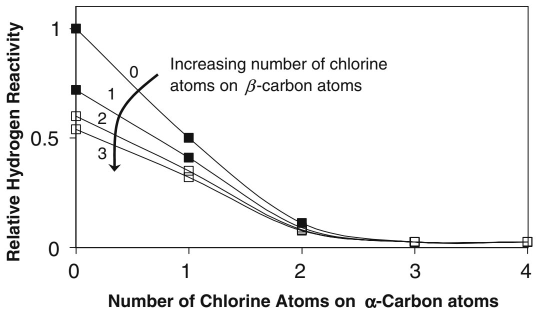

Fig. 5 Graphical representation of selected model parameters from Table 1. (—■—) experimentally measured parameters and (—□—) estimated parameters

values for hydrogen atoms with increasing numbers of adjacent chlorinated carbon atoms. The model parameters that could not be calculated from experimental results were estimated using a non-linear extrapolation procedure similar to that shown in Fig. 5. These estimated model parameters are shown by the entries in regular font in Tables 1 and 2.

Model validation

The basic model structure was validated against a simplified version of the model. The purpose of this validation was to ensure that the programmed model algorithm was free or errors. In the simplified version of the model it was assumed that all hydrogen atoms on the alkane chain had equal reactivity. This assumption made it possible to derive an analytical solution describing the change in molecular weight distribution of any PCA mixture as a function of reaction extent (see Appendix). The analytical solution was compared to a molecular weight profile generated by the Monte Carlo model where all relative reactivities were assigned a value of unity. Both models predicted identical molecular weight distributions, as expected (data not shown). This result validates the program algorithm. Upon validation, the actual relative reactivities shown in Tables 1 and 2 were incorporated into the model.

The lack of analytical techniques for PCA characterization made it difficult to validate the Monte Carlo model with the actual reactivities against experimental data. As stated above, the result of the simulation is a complete virtual representation of all molecules in any given PCA mixture. Although it is easy to determine any number of physical characteristics from the set of virtual molecules, it is difficult to obtain experimental data that allow for comparison with the model results. Tomy et al. (1998, 2000a) performed a gross chemical analysis on sPCA-60, a short chain PCA product with 60% w/w chlorine content. In this study, the researchers were able to quantify the distribution of isomers, grouped by the number of chlorine atoms present on the alkane molecules. Figure 6 shows the distribution of isomers grouped by the number of chlorine atoms from this study and the distribution predicted by the Monte Carlo model presented here. For comparison, the results from the analytical model were also plotted, in which the impact of chlorination on reactivity was not considered. The Monte Carlo model did a reasonable job at

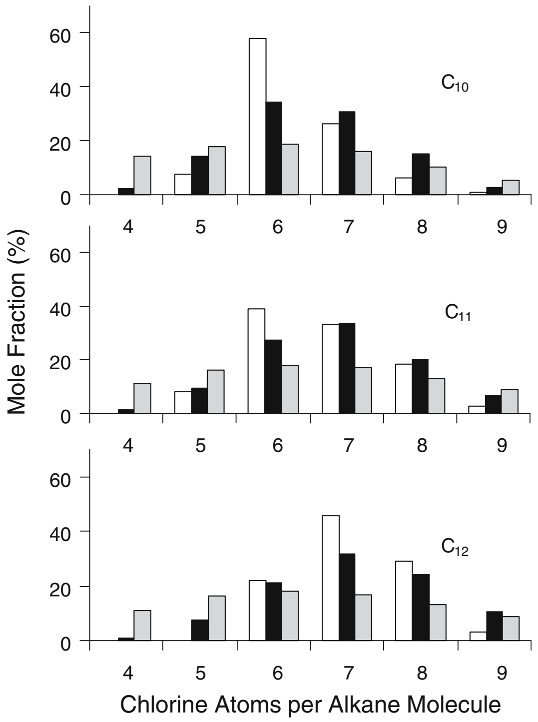

Fig. 6 Comparison of molecular distribution results from experimental results (□) [3], the Monte Carlo model (■), and the model assuming equal relative reactivity of all hydrogen atoms (Eq. 6) (▨) for a mixture of short chain PCAs with 60% (w/w) chlorine content. The graphs show the distribution for decane (C_{10}), undecane (C_{11}), and dodecane (C_{12})

capturing the median and general character of the expected distribution. Clearly, results from the model with the actual reactivities incorporated presented here resembled the experimental results much more closely than the results from the model distribution given by the analytical model in which all hydrogen atoms were assigned the same reactivity. The distribution of molecules derived from chlorinated undecane and dodecane are in better agreement with the experimental results than the distribution of molecules from chlorinated decane. Unfortunately, uncertainties in the experimental results were not reported by Tomy et al. (1998, 2000a, b), making any statistical comparisons impossible.

Data obtained from ^{1}H-NMR spectra of PCA mixtures provide another means of validating the model. The ^{1}H-NMR spectra show distinct double peaks at shifts below 1.1 ppm which correspond to hydrogen atoms located on primary carbon atoms that have no chlorine atoms substituted on associated α-carbon atoms. ^{1}H-NMR spectra of pure chlorinated decanes showed that the peak areas are quantitative to within ±2% (data not shown). Figure 7 shows the percentage of hydrogen atoms with no chlorine atoms substituted on associated α-carbon atoms as determined by ^{1}H-NMR versus the Monte Carlo model prediction for eight PCA preparations generated in the laboratory by free-radical chlorination. Five mixtures were synthesized using *n*-decane as the starting material, while 1-CD, 1,2-CD, and 5,6-DCD were used for the remaining three.

Model predictions were generated by running the Monte Carlo model with a set of 20,000 virtual molecules. This number of molecules was adequate for properly representing the PCA mixture since the model results of consecutive runs were identical (data not shown). In fact, the number of molecules could be reduced to less than 4,000 with no apparent impact on the model predictions.

Figure 7 shows excellent agreement between the modeled and experimentally measured results. These results indicate that the model has captured the overall intra-molecular distribution of chlorine atoms of the molecules in the chlorinated decane mixtures. The specific arrangement of chlorine atoms quantified by ^{1}H-NMR represented the combined interaction of all model parameters. The fact that this very specific pattern was accurately reproduced by the model over a wide range of chlorination degrees indicates that the model has successfully captured the true reaction mechanisms. Furthermore, the result validates the assumption that the reactivity of the carbon atoms adjacent to the terminal carbon atoms on the alkane chains has the same reactivity as other secondary carbon atoms. Future studies using ^{13}C-NMR could provide further validation of the Monte Carlo model.

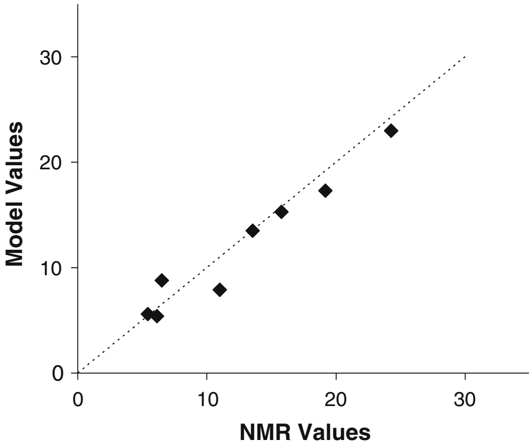

Fig. 7 The percentage of hydrogen atoms bound to terminal carbon atoms with no chlorine atoms on the associated α-carbon: Model prediction versus values determined experimentally by NMR

Application of the model

The Monte Carlo model is particularly well suited to studies of how physical and environmental properties of the PCA molecules relate to specific chlorine atom arrangements on the alkane chain. Possible applications include the quantification of toxic components of PCA mixtures based on knowledge of the toxic effects of specific chlorine atom arrangements within the PCA molecules, or estimation of the aqueous solubility of PCA mixtures based on knowledge of how specific chlorine atom arrangements within the PCA molecules contribute to solubility. The example application considered here is the investigation of the theoretical upper limit of biodegradation of PCAs in an aerobic environment.

Many factors influence the rate at which the degradation of recalcitrant compounds occurs in nature. These include the toxicity of the compounds, mass transfer limitations, nutrient limitations, temperatures, pH or the absence of microorganisms capable of metabolizing the compounds of interests. However, given appropriate growth conditions, the biological degradation process is ultimately limited by the presence and specificity of the catabolic enzymes (Baker and Herson 1994).

In general the first step in the degradation of chlorinated alkanes with chain lengths greater than C_9 is the hydroxylation of one of the two primary carbon atoms on the alkane chain (Allpress and Gowland 1999). This process is carried out through the action of either an oxygenolytic dehalogenase or the enzymes associated with beta oxidation. For unchlorinated alkane molecules the hydroxylation is carried out by alkane hydroxylase or similar enzymes (Marín et al. 2001). If a chlorine atom is present on the primary carbon atom, or on the α-carbon atom, the primary carbon atom is no longer accessible for the hydroxylases due to steric hindrance (Allpress and Gowland 1999). In these circumstances, the oxygenolytic dehalogenases are sometimes able to remove the chlorine, and replace it with a hydroxyl group (Tomy et al. 1998), with the outcome dependant upon the intra-molecular chlorine distribution. For instance, the initial oxidation of α, ω-dichlorinated *n*-alkanes requires an oxygenolytic dehalogenase (Allpress and Gowland 1999; Armfield et al. 1995; Wischnak et al. 1998, 2000). Further studies have been carried out by Heath et al. (2002, 2006), which suggest that if two chlorine atoms are present on the primary carbon atom, or if both the primary carbon atom and the adjacent α-carbon atom have chlorine atoms associated with them then the oxygenolytic dehalogenation process does not proceed. Other studies have shown that the presence of more than one chlorine atom on a carbon atom in a polychlorinated carbon chain prevents the action of aerobic dehalogenase enzymes (Vogel et al. 1987). From these results it appears that only chlorine atoms that are located in a position on the alkane chain where there are no chlorine atoms on the surrounding carbon atoms can be removed by oxygenolytic dehalogenation.

A simple set of rules capturing the major features of catabolism of pure chlorinated alkanes was then formulated.

- Dechlorination does not occur when vicinal chlorine atoms are encountered or when a carbon atom with two chlorine atoms is encountered.
- Dehalogenation and subsequent degradation by beta oxidation of PCA molecules can be initiated from either end of a molecule (Mathews and Van Holde 1996). This rule allows degradation to continue on a partially consumed molecule that has become recalcitrant to degradation on one end. This situation can arise if biological activity yields a chlorination pattern that cannot be handled by the enzyme systems present.

The theoretical limit to aerobic degradation was then estimated by applying these rules to the distribution of molecules as estimated by the Monte Carlo model. This was accomplished by searching the set of virtual molecules for arrangements of chlorine atoms that, in theory, are accessible for removal by the enzymes. The molecule was then modified to account for the activity of the enzyme systems present.

In the literature, a number of studies have been carried out that have attempted to determine the ultimate degree of biological conversion possible for a PCA mixture. Varied methodologies were employed, utilizing either pure bacterial strains containing oxygenolytic dehalogenases or undefined consortia of microorganisms (Tomy et al. 1998; Allpress and Gowland 1999; Madeley and Robin 1980; Heath et al. 2002). For a PCA mixture of given chlorination extent, the degree of biodegradation varied dramatically. In some cases no biodegradation was observed while in others over 80% of chlorine in the mixture was released. However, a maximum degree of conversion should exist, based on the potential contained in the enzyme systems.

The upper limit to aerobic biodegradation of PCAs with a carbon chain length of C_{11} was estimated using the modeling approach described above (Fig. 8). Also included on this figure are the published dechlorination results from all studies that could be extracted from the public domain. Given the simplicity of the biodegradation rules applied the curve for theoretical maximum enzymatic chloride removal encompasses a good deal of the experimental data reported. The fact that results have been reported that exceed the maximum predicted by the model suggests that either the oxygenolytic enzymes are able to dehalogenate internally located chlorine atoms on the carbon chain or that some abiotic, or other mechanism facilitates the degradation process. Curragh et al. (1994) has suggested that lactonization is a likely mechanism by which abiotic dehalogenation can occur during aerobic biodegradation. In this process, the carboxyl groups that have been formed by beta-oxidation at the terminal end of a chlorinated *n*-alkane can spontaneously form a lactone if a chlorine atom is bound to an appropriate carbon atom displacing the chlorine atom in the process (Heath et al. 2006; Curragh et al. 1994). Mechanisms such as this may explain why some of the data reported appear to exceed the predictions.

The modeling approach outlined in this study could be applied to any study involving PCA mixtures. The model offers a complete characterization of any PCA mixture, which can be analyzed for any specific intra-molecular arrangement of chlorine atoms. The model code could, with little effort, be modified to include other halides or functional groups such as bromium, hydroxy or carboxy group. Existing knowledge about the metabolic pathways responsible for biodegradation as well as rate data could be introduced implemented in the model for detailed environmental fate studies. For example, estimates of the identity and quantity of chlorinated carboxylic acids that are generated as metabolic byproducts during the incomplete degradation of PCAs could be generated. Heath et al. (2006) showed that no chloride release was detected during the degradation of 5,6-DCD although all of the chlorinated decane that was initially added had been degraded. It was concluded that the biodegradation of 5,6-DCD was a result of the action of the regular alkane catabolic pathway where hydroxyl groups are added to the terminal carbon atoms on the alkane chains, which is followed by beta oxidation (van Beilen et al. 1994). Recalcitrant di-chlorinated carboxylic acids would accumulated in the culture broth as by-products this degradation process (Heath et al. 2006). A prediction of the identity, toxicity and stability of such chlorinated acids should be considered when the toxicity and environmental impact of PCAs are evaluated. To date this has not occurred since neither data nor studies have been published concerning the properties or potential toxicity of the metabolic by-products of PCA degradation.

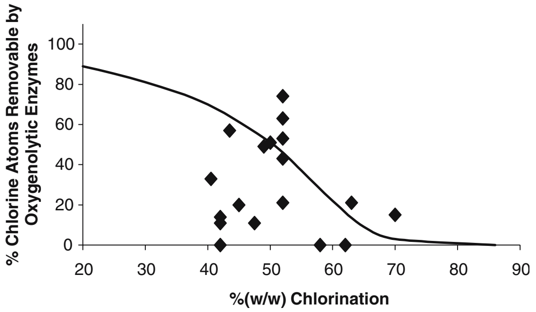

Fig. 8 Model prediction of the upper limit for the percentage of chlorine atoms that can be removed by oxygenolytic enzymatic action. PCA biodegradation results from the literature (◆) [1, 2, 5, 23]

Conclusion

The current study has demonstrated that the molecular composition of PCA mixtures can be characterized by modeling of the free-radical chlorination reaction of *n*-alkanes. The Monte Carlo model relied on experimentally determined parameters to estimate the outcome of chlorination events for low to moderate degrees of chlorination. The model was particularly useful for studies that sought to correlate the presence and quantity of specific arrangements of chlorine atoms on the alkane chains to the overall properties of the PCA mixtures. The model results are in good agreement with available analytical results. Although further model validation is desirable, the model provides a good first estimate of the compositions of PCA mixtures where no alternative exists today.

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Appendix: derivation of analytical solution

For the chlorination process governed by equation (Tomy et al. 1998), each isomer i containing β chlorine atoms can be identified by the set of β elements $\mathbf{S}_i^\beta$, as:

$$\mathbf{S}_i^\beta = \{p_1, p_2, p_3, \ldots p_\beta\} \qquad (4)$$

If the hydrogen atoms are numbered in sequence from the end of the molecule, $1 \le p_j \le 2n + 2$ represents the position of the hydrogen atom in the alkane chain that has been replaced by a chlorine atom. It follows that each element of the set is unique, repeats are not permitted, and the total number of elements in the set cannot exceed $2n + 2$. The molar concentration of an isomer containing n carbon and β chlorine atoms distributed according to the set $\mathbf{S}_i^\beta$ is given by $C_{n,\beta}^{\mathbf{S}_i^\beta}$. The total concentration of all unique isomers containing β chlorine atoms, $C_{n,\beta}$, is given by:

$$C_{n,\beta} = \sum_{p_1=1}^{2n+3-\beta} \sum_{p_2=p_1+1}^{2n+4-\beta} \sum_{p_3=p_2+1}^{2n+5-\beta} \cdots \sum_{p_{\beta-1}=p_{\beta-2}+1}^{2n+1} \sum_{p_\beta=p_{\beta-1}+1}^{2n+2} C_{n,\beta}^{\mathbf{S}_i^\beta}$$

The molar ratio of chloride atoms to alkane molecules in the hydrocarbon phase is given by:

$$X = \frac{C_{\mathrm{Cl}}}{\sum_{\beta=0}^{2n+2} C_{n,\beta}} \qquad (6)$$

Choosing a basis of one mole of alkane chains, $\sum_{\beta=0}^{2n+2} C_{n,\beta} = 1$ and the total amount of chlorine contained in the hydrocarbon phase is equal to:

$$X = (0)C_{n,0} + (1)C_{n,1} + (2)C_{n,2} + \cdots + (2n+2)C_{n,2n+2} \qquad (7)$$

In a closed batch process, the change in the chlorine content in the hydrocarbon phase as the extent of the reaction proceeds is given by:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (1)\frac{\mathrm{d}C_{n,1}}{\mathrm{d}t} + (2)\frac{\mathrm{d}C_{n,2}}{\mathrm{d}t} + \cdots + (2n+2)\frac{\mathrm{d}C_{n,2n+2}}{\mathrm{d}t} \qquad (8)$$

The rate of formation of an isomer $\mathbf{S}_i^\beta$ by the replacement of a hydrogen atom at any position p_j in a reactant $\mathbf{S}_i^{\beta-1} = \{p_1, p_2, p_3, \ldots p_{\beta-1}\}$, where $\mathbf{S}_i^{\beta-1} \subset \mathbf{S}_i^\beta$ and $p_j \notin \{p_1, p_2, p_3, \ldots, p_{\beta-1}\}$ is defined in the usual way in terms of the reacting species $\mathbf{S}_i^{\beta-1}$, represented as $r_{n,\beta}^{\mathbf{S}_i^{\beta-1} \to \mathbf{S}_i^\beta}$.The total number of independent reactants that can form the isomer $\mathbf{S}_i^\beta$ is given by the binomial coefficient formula $\begin{pmatrix} \beta \\ \beta-1 \end{pmatrix}$, which yields β as a result. The set of β reactants includes all independent subsets of the set $\mathbf{S}_i^\beta$ having β –1 elements. In the general case where $\beta < 2n + 2$, the isomer $\mathbf{S}_i^\beta$ can be lost from the system if one of the hydrogen atoms is displaced by a chlorine atom to yield an isomer with $\beta + 1$ chlorine atoms. For each isomer $\mathbf{S}_i^\beta$, there are $2n + 2–\beta$ possible products. The net rate of formation of the isomer $\mathbf{S}_i^\beta$ can be determined by considering the sum of all β reactions involving the complete set of isomers containing β –1 chlorine atoms, less the $2n + 2–\beta$ possible rates of destruction. Finally, the net rate of removal considering all isomers containing β chlorine atoms can be derived by summing the expressions associated with all isomers containing this degree of chlorination, and is equal to $\mathrm{d}C_{n,\beta}/\mathrm{d}t$ in Eq. (8). The complete expression is cumbersome, but is omitted here for clarity.

If chlorine is in excess, the reaction is first order, described by a rate equation of the form:

$$r_{n,\beta}^{\mathbf{S}_i^{\beta-1} \to \mathbf{S}_i^\beta} = k_{n,\beta}^{\mathbf{S}_i^{\beta-1} \to \mathbf{S}_i^\beta} C_{n,\beta}^{\mathbf{S}_i^{\beta-1}} \qquad (9)$$

For reactants consisting of pure *n*-alkane, the initial conditions are given by:

$$C_n = \sum_{i=1}^{2n+2} C_{n,i} = C_{n,0}$$

It is more convenient to work in the chlorination domain. Applying the chain rule to (8) provides the necessary conversion from the time domain.

$$\frac{\mathrm{d}C_{n,\beta}}{\mathrm{d}X} = \frac{\mathrm{d}C_{n,\beta}}{\mathrm{d}t} \Big/ \frac{\mathrm{d}X}{\mathrm{d}t} \tag{11}$$

In the simplest case it can be assumed that all hydrogen atoms are replaced at equal rates once encountered by a chlorine free-radical. This is equivalent to assuming that the rate constant of any isomer is proportional to the number of hydrogen atoms on the reacting alkane. From this result, all molecules with the same number of chlorine atoms will have the same reactivity. Thus, from Eq. (9):

$$r_{n,\beta} = k_{n,\beta} C_{n,\beta} \tag{12}$$

At any instant during the chlorination process:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{\mathrm{d}C_{n,1}}{\mathrm{d}t} + \frac{2\mathrm{d}C_{n,2}}{\mathrm{d}t} + \frac{3\mathrm{d}C_{n,3}}{\mathrm{d}t} + \frac{(2n+1)\mathrm{d}C_{n,(2n+1)}}{\mathrm{d}t} + \frac{(2n+2)\mathrm{d}C_{n,(2n+2)}}{\mathrm{d}t} \tag{13}$$

Under the simplifying assumption, it follows from a mass balance that:

$$\frac{\mathrm{d}C_{n,\beta}}{\mathrm{d}t} = r_{n,\beta-1} - r_{n,\beta} \tag{14}$$

Combining:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = k_{n,(2n+1)} C_{(2n+1)} + \cdots + k_{n,1} C_{n,1} + k_{n,0} C_{n,0} \tag{15}$$

The values of rate constants are proportional to the number of hydrogen in the carbon chain. Selecting 1 as a basis for the rate constant $k_{n,(2n+1)}$ it follows:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (1)C_{n,2n+1} + (2)C_{n,2n} + \cdots + (2n+1)C_{n,1} + (2n+2)C_{n,0} \tag{16}$$

By inspection, the above expression is equal to the total moles of hydrogen, which can also be written as:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (2n+2) - \mathrm{X} \tag{17}$$

These equations can be integrated subject to the condition $C_{n,\beta} = 0$ when $X = 0$ to yield the solution:

$$C_{n,\beta} = \frac{X^\beta}{(2n+2)^\beta} \left(\frac{(2n+2) - X}{(2n+2)} \right)^{(2n+2-\beta)} \times \frac{(2n+2)!}{(2n+2-\beta)!(\beta)!} \tag{18}$$

An alternative solution to this problem, previously published was used by Colburne and Stern (1965) for comparing experimental chlorination data. This model is of the form:

$$\frac{C_{n,0}}{C_n} = e^{-X} \quad \text{for } \beta = 0 \tag{19}$$

$$\frac{C_{n,\beta}}{C_n} = \frac{X^\beta}{\beta!} \cdot e^{-X} \quad \text{for } \beta = 1, \ldots, n-1 \tag{20}$$

The assumption that allows for this solution is that all alkane molecules react with chlorine radicals at equal rates regardless of the degree of chlorination. However, the model violates the mass balance, or the condition that $\lim_{\beta \to 2n+2} C_{n,2n+2}/C_n = 1$ and hence does not represent a correct solution.

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