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# PREDICTING THE BIODEGRADATION PRODUCTS OF PERFLUORINATED CHEMICALS USING CATABOL

S. DIMITROV[a], V. KAMENSKA[a], J.D. WALKER[b], W. WINDLE[C], R. PURDY[d], M. LEWIS[e] and O. MEKENYAN[a,]*

[a]*Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", Yakimov street 1, 8010 Bourgas. Bulgaria;* [b]*TSCA Interagency Testing Committee, U.S. EPA (7401), Washington. DC, USA* [c]*Chemical Evaluation Division, Existing Substances Branch, Environment Canada, Gatineau Quebec, Canada K1A OH3;* [d]*Independent Toxicologist. River Falls, MN, USA* [e]*New Chemicals Evaluation Division, New Substances Branch, Environment Canada, Gatineau Quebec, Canada K1A OH3*

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Perfluorinated chemicals (PFCs) form a special category of organofluorine compounds with particularly useful and unique properties. Their large use over the past decades increased the interest in the study of their environmental fate. Fluorocarbons may have direct or indirect environmental impact through the products of their decomposition in the environment. It is a common knowledge that biodegradation is restricted within non-perfluorinated part of molecules; however, a number of studies showed that defluorination can readily occur during biotransformation. To evaluate the fate of PFCs in the environment a set of principal transformations was developed and implemented in the simulator of microbial degradation using the catabolite software engine (CATABOL). The simulator was used to generate metabolic pathways for 171 perfluorinated substances on Canada's domestic substances list. It was found that although the extent of biodegradation of parent compounds could reach 60%, persistent metabolites could be formed in significant quantities. During the microbial degradation a trend was observed where PFCs are transformed to more bioaccumulative and more toxic products. Perfluorooctanoic acid and perfluorooctanesulfonate were predicted to be the persistent biodegradation products of 17 and 27% of the perfluorinated sulphonic acid and carboxylic acid containing compounds, respectively.

*Keywords:* Perfluorinated chemicals; Organofluorine compounds; PFOS; PFOA; Biodegradation

## INTRODUCTION

Perfluorinated chemicals (PFCs) have been extensively used for decades in a wide range of commercial products such as wetting agents, lubricants, corrosion inhibitors, insecticides and surfactants. Field monitoring studies indicate that PFCs are globally distributed, environmentally persistent and bioaccumulative [1-3]. Of special interest is the biodegradation potential of PFCs containing a chain of 7 or 8 perfluorinated carbons. Such chemicals are potential precursors of perfluorooctanesulfonamide, perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate and perfluorooctanoic acid (PFOA). PFCs have

*Corresponding author. Tel.: +359-56-880343. Fax: +359-56-880230. E-mail: omekenya@btu.bg

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been reported to occur in blood sera of humans [1], minks, otters, marine mammals, birds, fish and mussels [4-8]. The absence of sex or weight associated variations of perfluorochemical concentrations in marine mammals and birds suggests a different mechanism of bioaccumulation from those observed for lipophilic compounds such as polychlorinated biphenyls. Lower concentrations of eight-carbon PFCs were found in rat tissues high in lipid content such as brain and fat compared to liver and lung concentrations [9,10]. These facts suggested that perfluorochemicals accumulate in biota similar to tributyltin via binding to proteins [8]. The *in vitro* data presented in the study of Leubker *et al.* [11] supported the hypothesis that PFCs containing a chain of 7 or 8 perfluorinated carbons may interfere with the binding of fatty acids or other endogenous ligands to liver-fatty acid binding protein. PFOA and perfluorodecanoic acid are known to induce biogenesis of liver peroxisomes and significantly affect liver phospholipid metabolism [12]. Furthermore, it was found that perfluorinated fatty acids with a chain of 6-10 carbons reversibly inhibited gap junctional intercellular communication [13]. The down-regulation of intercellular communication by peroxisome proliferators results in abnormal cell growth and increased tumorogenicity [14].

The replacement of hydrogen atom for fluorine in organic molecules alters their reactivity and stability of neighboring functional groups. The carbon—fluorine bond is one of the strongest known in organic chemistry. It has also a strong dipole and can interact with other dipoles. In addition to the covalent bond fluorine atom can also form reversible, electrostatic bonds with certain functional groups. The replacement of a methylene function with a difluoromethylene function ($CF_2$ for $CH_2$) can have significant effect on conformation, physical properties and potential for metabolic transformations [15]. This functional group has found extensive use in the design of inhibitors of hydrolyzing enzymes [16]. Several studies have revealed that microbial degradation is limited to the non-fluorinated part of PFCs [17,18]. The observed partial defluorination of some PFCs suggests that metabolic transformations can affect the difluoromethylene group attached to the non-fluorinated part of the molecule. In general, the commercially used PFCs can be converted in the environment into recalcitrant metabolites that have highly hydrophobic and rigid perfluorinated carbon chains attached to strongly polar groups, such as sulfonyl or carboxyl groups.

The challenge of evaluating biodegradation potential and metabolic pathways of a large number of structurally diverse chemicals resulted in the development of the computer software CATABOL [19]. Previous experience has shown that the CATABOL can very accurately predict ready biodegradability [20,21]. The CATABOL rulebase is constantly refined to expand the structural domain of its applicability. In this respect, the aim of this paper is to present results of its performance for predicting microbial degradation of PFCs using newly implemented rules. The priority of these rules in the metabolic simulator was defined based on both expert knowledge and documented metabolic maps. The extended set of transformations was used to predict the most plausible biodegradation pathways and ultimate biodegradation potential for 171 PFCs selected by Environment Canada from their domestic substances list (DSL). More than one Simplified Molecular Input Line Entry System (SMILES) notation has been used to represent some of these substances being polymers or mixtures. For example, the chemical with CAS 178535-23-4, named as fatty acids, linseed oil, g-w-perfluoro-C8-14-alkyl esters was represented with 20 different SMILES depending on the number of carbon atoms in the perfluorinated chain and the type of fatty acid. As a result, a total of 462 individual structures with SMILES notations were studied. The quantitative distribution of the generated metabolites was also predicted. It was determined that practically all of the PFCs for which predictions were made would be transformed into extremely persistent perfluorinated sulphonic or carboxylic acids. It was predicted that most of the PFCs would biodegrade to PFOS or PFOA in the environment.

The evolution of hydrophobicity, bioaccumulation potential and fish acute toxicity of PFCs during their microbial biodegradation were also analyzed.

## MATERIALS AND METHODS

### CATABOL Methodology

The CATABOL system is a probabilistic scheme for simulating microbial degradation based on a hierarchically ordered set of principal metabolic transformations [19]. Currently the set of transformations includes 141 abiotic and biologically mediated reactions, which occur very rapidly, compared to the duration of the biodegradation tests. These rapid transformations were predicted to occur with the following highly reactive groups and intermediates: oxiranes, ketenes, acyl halides, thiocarboxylic acids, hydroperoxides, nitrenes and geminal diols. Various chemical equilibrium processes like carboxylic acids hydrolysis, keto-enol tautomerism, thiol-thion tautomerism and cyanuric acid isomerization were also included in this class of transformations. Many of the other 465 metabolic transformations such as oxidation, hydrolysis, decarboxylation and dehalogenation were grouped into subsets of reactions depending on the similarity of their target fragment and transformation products. The probabilities of 324 rate-determining reactions grouped in 50 subsets were estimated on the basis of experimental biodegradation data. Due the lack of sufficient data the probabilities of the remaining 141 reactions were determined on the basis of expert knowledge.

CATABOL was created to predict the most probable biodegradation pathway, distribution of stable metabolites and extent of biological oxygen demand or $CO_2$ production compared to theoretical limits. CATABOL matches the parent molecule with the source fragment associated with each transformation starting with the transformation having highest probability of occurrence. When a match is identified, the molecule is metabolized and transformation products are treated as parent molecule. The procedure is repeated for the newly-formed metabolite until the product of probabilities of consecutively performed transformations reaches a user-defined threshold (by default set 0.0001). The sequence of transformations that is obtained represents the most plausible catabolic pathway for the biodegradation of the parent chemical. Biological oxygen demand or CO2 production is calculated on the basis of the generated most plausible catabolic pathway for the parent chemical.

The generated metabolic trees can be used also to evaluate the quantitative distribution of the produced metabolites. The latter can be submitted for predicting their endpoints of interest, such as octanol—water partition coefficient (logKow). bioconcentration factor (log BCF), fish acute toxicity (log $1/LC_{50}$), estrogen receptor binding affinity, mutagenicity and other endpoints [22-28].

### Databases

The CATABOL system is trained to predict biodegradation within 28 days on the basis of 743 chemicals from MITI database [29] and another training set of 109 proprietary chemicals from Procter & Gamble Company obtained by OECD 301 C [30,31] and OECD 301 B [32] tests, respectively. In the first database biodegradation is expressed as the oxygen uptake relative to the theoretical uptake, while in the P&G database biodegradation is measured by CO2 production. Only nine fluorine-containing chemicals are included in MITI database. Four of them contained trifluoromethyl group and other two were perfluorinated. Biodegradation of these chemicals was in the range of 0-5%.

Documented microbial catabolic pathways were used to train CATABOL to reproduce the biodegradation pathways. Currently, we have collected a training set of observed catabolic pathways for more than 150 organic compounds from monographs [33-35] and the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD, http://umbbd.ahc.umn.edu/) [36-38]. Articles devoted to microbial degradation of specific classes of chemicals such as halogenacetic acids, terpenes, linear alkylbenzene sulfonate surfactants, bisphenols, etc. were also used [39-43]. The collection includes catabolism of Ci-compounds (10 pathways), aliphatic hydrocarbons (10 pathways), alicyclic rings (14 pathways), furans (4 pathways), halogenated hydrocarbons (12 pathways), aromatic hydrocarbons (30 pathways), haloaromatics (25 pathways). The rest of the collected microbial degradation pathways describe catabolism of amines, sulfonates, nitrates, nitro-derivates, nitriles, etc. and complex compounds containing more than one functional group. The biodegradation route for only one fluorinated chemical (methyl fluoride) was included in this database. The catabolic pathways database was used to train the simulator to reproduce the experimentally documented aerobic metabolism in prokaryotes. The produced metabolism simulator was suitable to predict biodegradation of non-perfluorinated part of the molecules. However, the microbial degradation of not-fluorinate fragment is quite different when they are directly attached to the perfluorinated carbon chains. Subsequently, additional information was necessary to model adequately the metabolism of such functional groups.

Little information was found about the biodegradation pathways for PFCs. To develop the transformation rules associated with these chemicals we collected available pieces of information for their observed modifications, which were combined, into metabolic maps. Key *et al.* [18] demonstrated that *Pseudomonas* sp., strain D2, is able to utilize and defluorinated compounds containing hydrogen such as difluoromethane sulfonate, 2,2,2-trifluoroethane sulfonate and 1H,1H,2H,2H-perfluoroctane sulfonate. However, chemicals without hydrogen atom at a-carbon, such as trifluorosulfonate and PFOS, were not degraded. In contrast, Visscher *et al.* [44] showed that trifluoroacetic acid could be microbially metabolized to fluoroform and consecutively defluorinated to acetate under aerobic and anaerobic conditions, respectively. In a recent field and laboratory study, no degradation of trifluoroacetic acid was observed [43]. PFOS was found also to be terminal metabolite of N-ethylperfluorooctanesulfonamido ethanol [45]. Four metabolic products were detected by Hagen *et al.* [46] resulting from a single oral dose of 1H,1H,2H, 2H-perfluorodecanol in adult rats. One of these metabolites was shown to be PFOA. In the degradation of 1H,lH,2H,2H-perfluorodecanol to PFOA two fluorines were lost.

1H,lH,2H,2H-perfluordecanol and similar substances also degrade without loss of fluorine to odd chain perfluorinated fatty acids [46-48]. These odd chain products have also been observed in wildlife [49] and water [50]. Such degradation is consistent with alpha oxidation common in mammals [51-53] and microbes [54,55]. Because of paucity information on microbial metabolism of fluorinated chemicals the metabolic fate of other fluorine-containing chemicals in rats and humans were also taken in to consideration [56-60].

## RESULTS AND DISCUSSION

### Generalized Metabolic Transformations for PFCs

Collected data for documented metabolic reactions of fluorine-containing and perfluorinated organic chemicals as well as expert knowledge for reactivity of these chemicals were used to predict 24 principal transformations listed in Table I. Because experimental biodegradability was available for two PFCs in MITI database only, experts defined the probabilities

TABLE I List of the principal transformations of PFCs

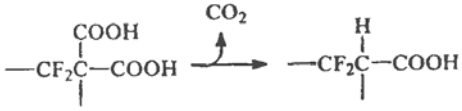
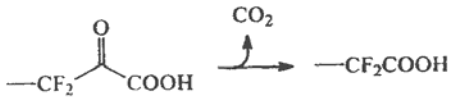
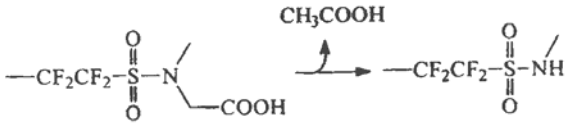
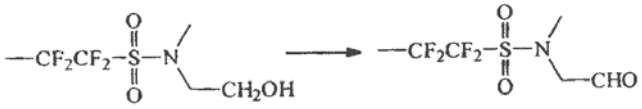
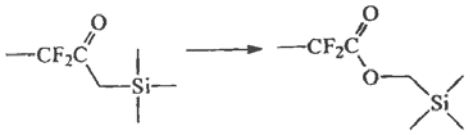
| *No.* | *Principal transformations* | *Probability* |
|---|---|---|
| *1* | Perfluoroacetone transformation  | 1.00 |
| 2 | Subterminal oxidation  | 1.00 |
| 3 | Defluorination of fluoroamines  | 0.90 |
| 4 | Decarboxilation  | 0.76 |
| 5 | Decarboxilation  | 0.76 |
| 6 | Sulfonamide hydrolysis  | 0.25 |
| 7 | Hydroxyl group oxidation  | 0.25 |
| 8 | Epoxidation  | 0.25 |
| 9 | Baeyer–Villiger oxidation  | 0.25 |

TABLE I – continued

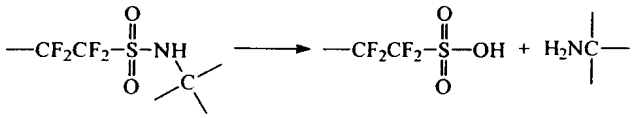
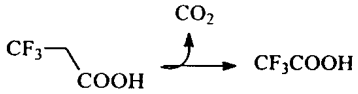
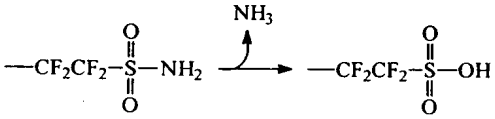
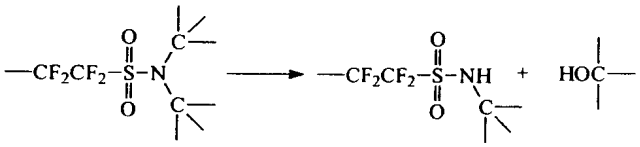
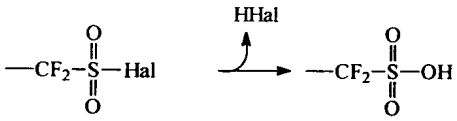
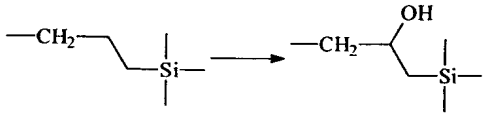
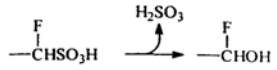
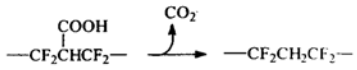
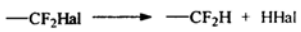
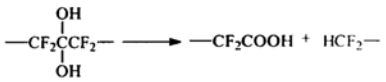
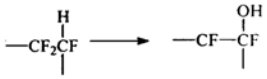
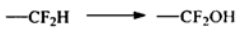
| *No.* | *Principal transformations* | *Probability* |
|---|---|---|
| 10 | Epoxidation  | 0.25 |
| 11 | Sulfonamide hydrolysis  | 0.25 |
| 12 | Decarboxilation  | 0.25 |
| 13 | Defluoration of $CF_2$ group adjacent to $CH_2$ group  | 0.25 |
| 14 | Sulfonamide hydrolysis  | 0.20 |
| 15 | Sulfonamide hydrolysis  | 0.20 |
| 16 | Dehalogenation of sulfonylhalogen derivatives  Hal = F, Cl, Br, I | 0.20 |
| 17 | Subterminal oxidation  | 0.01 |

TABLE I – continued

| *No.* | *Principal transformations* | *Probability* |
|---|---|---|
| 18 | Desulfonation  | 0.01 |
| 19 | Desulfonation  | 0.01 |
| 20 | Decarboxilation  | 0.01 |
| 21 | Dehagenation  | 0.01 |
| 22 | Perfluoroketone degradation  | 0.001 |
| 23 | Oxidation  | 0.001 |
| 24 | Oxidation  | 0.001 |

and hierarchy of most of these transformations. Most of the transformations from Table I are related to modification of the functional groups directly attached to the perfluorinated part of molecule. This is not unexpected because difluoromethylene functions can significantly alter the electronic properties of the neighboring groups. For example, geminal diols are usually unstable compounds and lose water spontaneously to give carbonyl compounds. In contrast, hexafluoroacetone forms stable hydrates (geminal diols) that are solid, crystalline compounds, due to the strong electron-withdrawing effect of trifluoromethyl groups. The same specific alteration of reactivity holds for defluorination, decarboxylation, desulfonation, etc. There is no evidence that the last four transformations presented in Table I are performed under enzyme control. They were included in the set of transformations to provide tentative information about the fate of PFCs under specific conditions such as combustion processes or oxidation in troposphere.

The reliability of the extended set of principal transformations to generate the most plausible catabolic pathways for PFCs is shown in Fig. 1. On the figure, the experimentally

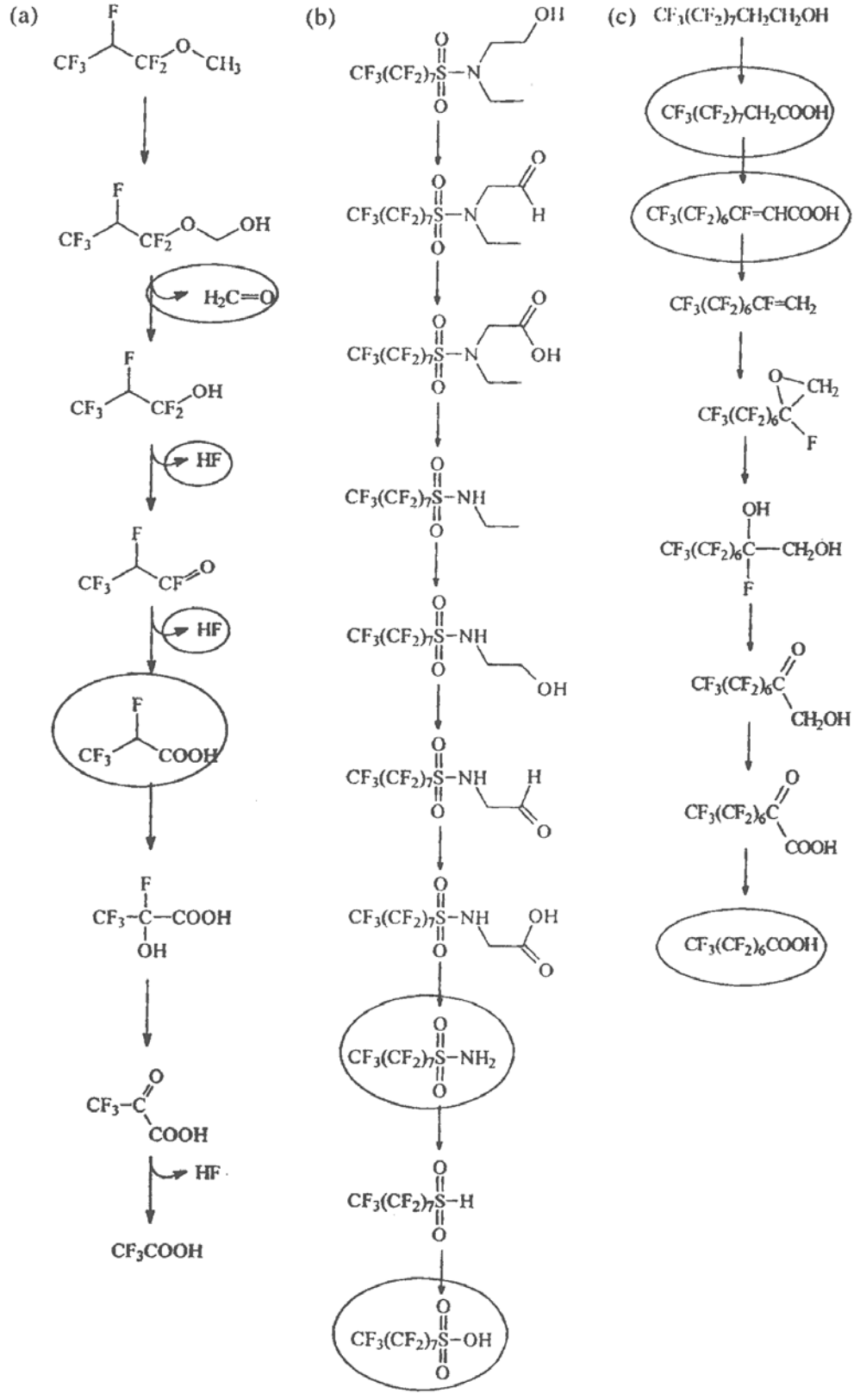



Figure 1. CATABOL biodegradation pathways and experimentally documented biodegradation products. (a) 1,1,2,3,3,3-Hexafluoropropyl methyl ether; (b) N-ethylperfluorooctanesulfonamido ethanol; (c) 1H,1H,2H,2H-pefluorodecanol;

○ Experimentally documented biodegradation products.

determined metabolites are located in circles [45,46,58]. It can be seen that CATABOL was able to generate metabolic pathways that are consistent with the experimental results.

## Evaluation of the Fate of PFCs on Canada's DSL

The most plausible biodegradation pathways were predicted for 462 PFCs with discrete SMILES notations on Canada's DSL by making use of the CATABOL software and extended set of principal transformations. Predicted biodegradability ranged from 0 to 73%. These results are in agreement with the experimentally obtained biodegradation of perfluorinated surfactants [17]. The distribution of PFCs according to the predicted biodegradability is shown in Fig. 2. It shows that about 60% of PFCs in ready biodegradability tests can have biodegradability greater than 20%. It should be mentioned that according to an expert scheme relating ready biodegradability test outcomes and half-lives [61] chemicals with biodegradability less than 20% are classified as recalcitrant. In this respect, on the basis of the predicted ready biodegradability 40% of 462 PFCs will be identified as recalcitrant chemicals. The remaining 60% of the studied PFCs were predicted to be ready biodegradable. Such a classification is misleading because both experimental and simulation studies emphasize that biodegradation is restricted to non-fluorinated part of molecules and persistent metabolites will be released in the environment. It is evident that identification of persistent profile of chemicals should be accompanied with studies of their potential to be biodegraded to recalcitrant chemicals.

CATABOL affords the opportunity to calculate variety of physical, chemical and environmental properties both for parent chemicals and their metabolites. Reliability of these predictions for PFCs can be assessed on the basis of experimental data for PFOS. Following an accidental release of fire fighting foam into Etobicoke Creek, Moody *et al.* [62] estimated the fish log BCF for PFOS was in the range of 3.0-5.12 based on PFOS concentration in liver and surface water. The authors pointed out that the calculated BCF for PFOS could be overestimated due to the metabolism of accumulated precursors of PFOS. Based on data for PFOS water concentration and whole fish burdens in catfish and large mouth bass, Purdy [63] estimated that the log BCF should be in the range of 2.9-4.4. The CATABOL predicted log BCF of 3.74 is in a good agreement with field data. Experimentally observed fish toxicity *(Pimephales promelas,* 96 h exposure) log $1/LC_{50}$ for PFOS was about 4.72, where $LC_{50}$ is 50% lethal concentration in mol/1 [64]. Predicted fish acute toxicity for PFOS using CATABOL was 6.99. The toxicity overestimation of about two log units is due to the fact that the prediction was based on a model derived for narcotic mode of action with lipophilicity as a main driving force. It was mentioned previously that the mechanism of bioaccumulation and especially tissue distribution and mode of toxic action of PFC may

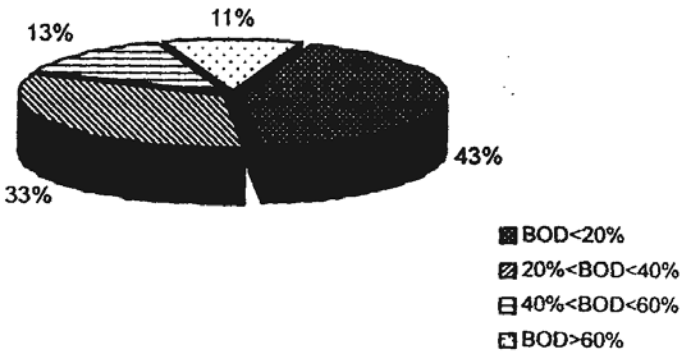



FIGURE 2 Distribution of PFCs based on predicted biochemical oxygen demand (BOD).

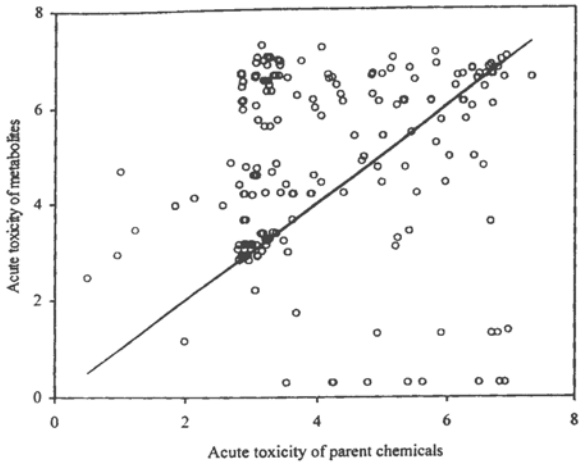



FIGURE 3 Change in fish acute toxicity of PFCs during biodegradation.

differ from those of usual narcotics. Unfortunately, sufficient data to build an adequate model for PFCs acute toxicity are still not available. In the following analysis, we assumed that toxicity overestimation is consistent for all PFCs and could not affect significantly the conclusions based on comparison between toxicity of parents and their metabolites.

The calculated hydrophobicity, bioconcentration and fish acute toxicity for the parent chemicals were compared with those of their stable biodegradation products that exceeded 30% molar amount related to the parent quantity. The search for first biodegradation products was performed analyzing the metabolic tree starting from the root of the metabolic map (parent chemical) and moving towards the end leaves of the tree. Of the studied 462 chemicals 323 were found to produce stable biodegradation products exceeding the 30% molar amount threshold. Hence, the next analysis for toxicity of biodegradation products was confined within these 323 parent chemicals and their stable biodegradation products. The evolution of acute fish toxicity from parents to biodegradation products is shown in Fig. 3. The comparison between toxicities of parent and biodegradation product shows that there is very well defined elevation of toxicity during biodegradation, which is greater than two log units for about 30% of the studied chemicals. Such an increase of toxicity is comparable with commonly used magnitude of excess toxicity distinguishing chemicals possessing narcotic and reactive mode of acute toxicity. It should be emphasized that the observed elevation of toxicity is not due to biodegradation of non-reactive chemicals into reactive ones. Most of chemicals are large molecules with extremely high lipophilicity that exceeds the log $_{AQW}$ value associated with the maximum of logBCF [65,66] resulting in a very low bioaccumulation potential. As a result of biodegradation, these molecules are reduced into smaller persistent biodegradation products that consist of highly hydrophobic perfluorinated carbon chains attached to polar groups.

As it was mentioned previously, concern about PFCs with 6-10 perfluorinated tails is increasing because of their wide use in commercial products. Perfluorochemicals have excellent chemical and thermal stability and thus have found many uses in products. CATABOL system affords the opportunity to identify which parent chemicals have the potential to be biodegraded to chemicals with selected perfluorinated tails. The potential of the studied 171 substances to be transformed in the environment to persistent perfluorinated

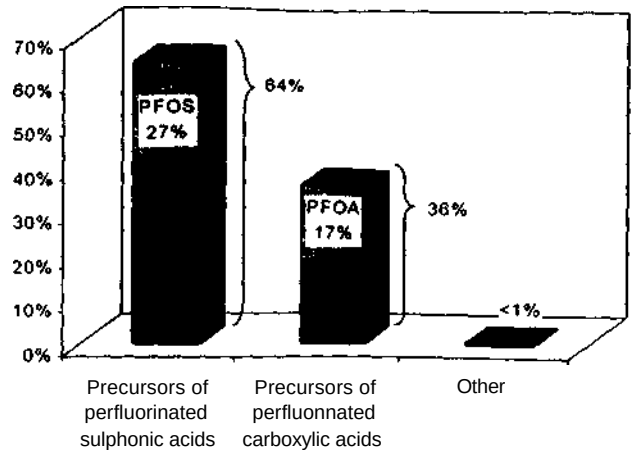



FIGURE 4 Ability of perfluorinated substances on Canada's DSL to degrade to perfluorinated acids.

acids is shown in Fig. 4. CATABOL predicted that of 171 substances, 46 (27%) could be biodegraded to PFOS. CATABOL also predicted that another 29 (17%) could be biodegraded to PFOA. At this time perfluorooctanyl chemicals and products containing PFOS are being phased out of the production. Chemicals used for their replacement are precursors of other perfluorinated sulphonic and carboxylic acids. The latter have properties similar to PFOS and PFOA. They are extremely persistent, bioconcentrate by the same mechanism and have similar mode of toxic action. From the studied 171 perfluorinated substances CATABOL predicted that 109 could be biodegraded to perfluorinated sulphonic acids. Additionally, CATABOL predicted that 61 could be biodegraded to perfluorinated carboxylic acids. Only one of the 171 perfluorinated substances was predicted to not biodegrade to the acids. Hence, one can summarize that more than 99% of perfluorinated substances could be transformed in the environment into extremely persistent perfluorinated acids.

## CONCLUSIONS

CATABOL's principal transformations for simulating catabolism and predicting biodegradation products in ready biodegradability tests were expanded in this study to cover the structural domain of PFCs. The new transformations were derived on the basis of literature data and expert knowledge. Most of the reactions were related to the functional groups directly attached to the perfluorinated part of molecule. Using the extended set of transformations, CATABOL was able to reproduce experimentally documented transformations of PFCs.

CATABOL system was used to screen 171 perfluorinated substances on Canada's DSL represented by 462 PFCs with discrete SMILES. Of the studied 462 chemicals 323 were found to produce stable biodegradation products exceeding the 30% molar amount threshold. Most of the stable biodegradation products had an enhanced bioaccumulation and acute toxicity potential compared to that of their parent chemicals. The enhanced bioaccumulation and acute toxicity potential of PFCs can be explained by their bioaccumulation through enterohepatic circulation and toxicity through their uncoupling mode of toxic action.

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