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Prediction of the Biodegradation and Toxicity of Naphthenic Acids

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1. Introduction

Crude oil is a complex mixture of hydrocarbons, basically composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds. The constituent hydrocarbon compounds are present in varied proportion resulting in great variability in crude oils from different sources (Speight, 1999). There are several reports indicating the recalcitrance and potential health hazards of the different constituents of crude oil (Kanaly&Harayana, 2000). These compounds have been reported to be carcinogenic, mutagenic and have immunomodulatory effects on humans, animals and plant life (van Gestel et al., 2001; Miller& Miller, 1981). The sites contaminated with hydrocarbons are ecologically important locations as one may encounter microbial flora of diverse nature, which may be potential candidates for important industrial processes (Jain et al., 2005).

The microorganisms possess the greatest enzymatic diversity found on earth and metabolize millions of organic compounds to capture chemical energy for growth. This metabolism, called catabolism or biodegradation, is the principal driving force in the degradative half of the earth's carbon cycle (Dagley, 1987). Microorganisms are increasingly used in engineered systems to biodegrade hazardous, xenobiotic compounds, an application commonly known as bioremediation (Alexander, 1994).

There have been numerous efforts to predict both the biodegradability or the pathway(s) of biodegradation for a given compound under a given set of conditions, typically either aerobic or anaerobic (Boethling et al., 1989; Parsons& Govers, 1990; Howard et al., 1991; Klopmann et al., 1995; Damborsky, 1996; Punch et al., 1996). Most of the efforts have been rule based, drawing general conclusions about what structures would or would not be readily biodegraded. Some only address whether a compound will be biodegraded and, if so, will biodegradation proceed slowly or quickly. The expert system projects, META (Klopmann et al., 1995) and BESS (Punch et al., 1996), also seek to determine at least one plausible biodegradation pathway.

Biodegradation pathway prediction requires the use of biochemical knowledge sometimes called metabolic logic. This requires knowledge of:

- organic functional groups to match a new chemical structure to one whose metabolism is already known;

- intermediary metabolism pathways to deduce how a new biodegradation can funnel a metabolite into a common pathway most efficiently;
- microbial enzymatic reactions to match a given reaction with a known enzyme;
- organic chemistry reactions to deduce what new reactions are chemically plausible to decompose a compound when precedents are not available.

Scientists studying biodegradation acquire this knowledge and these skills through many years of study and experimentation. This requires a means of organizing biodegradation reactions in some systematic fashion (Wackett& Ellis, 1999).

The University of Minnesota Biocatalysis/ Biodegradation Database (UM-BBD) began in 1995 and now contains information on almost 1200 compounds, over 800 enzymes, almost 1300 reactions and almost 500 microorganism entries. Besides these data, it includes a Biochemical Periodic Table (UM-BPT) and a rule-based Pathway Prediction System (UM-PPS) that predicts plausible pathways for microbial degradation of organic compounds (Gao et al., 2010). The inherent biodegradability of these individual components is a reflection of their chemical structure, but is also strongly influenced by the physical state and toxicity of the compounds. Therefore, the physical state is that strongly influences their biodegradation (Bartha& Atlas, 1977).

Naphthenic acid are most significant environmental contaminants. They are comprised of a large collection of saturated aliphatic and alicyclic carboxylic acids found in hydrocarbon deposits (petroleum, oil sands bitumen, and crude oils). Moreover, they are toxic components in refinery wastewaters and in oil sands extraction waters. In addition, there are many industrial uses for naphthenic acids, so there is a potential for their release to the environment from a variety of activities. Studies have shown that naphthenic acids are susceptible to biodegradation, which decreases their concentration and reduces toxicity.

They are described by the general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number and Z is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation. Naphthenic acids have dissociation constants, which is typical of most carboxylic acids. Naphthenic acids are non-volatile, chemically stable, and act as surfactants (Seifert, 1975).

The presence of naphthenic acids in the environment is seldom studied and little is known about their fate. The investigations that used actual naphthenic acids focused on the biodegradation of these compounds as a group, because current analytical methods do not allow the study of individual compounds in the complex mixture. The aim of this study was to predict the biodegradation of the individual naphthenic acids and the possible toxicity of the parent structure and their metabolites. The software used for prediction of the microbial metabolism (biodegradation) of the naphthenic acids is the OECD (Q)SAR Application Toolbox (OECD (Q)SAR Project). The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco) toxicity data needed for assessing the hazards of chemicals. Degradation pathways used by microorganism to obtain carbon and energy from 200 chemicals are stored in a special file format that allows easy computer access to catabolic information. Most of pathways are related to aerobic conditions. Single pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of

the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the source fragment associated with the transformation having the highest hierarchy.

2. Biodegradation of naphthenic acids

Naphthenic acids are highly toxic, recalcitrant compounds that persist in the environment for many years, and it is important to develop efficient bioremediation strategies to decrease both their abundance and toxicity in the environment. However, the diversity of microbial communities involved in naphthenic acid-degradation, and the mechanisms by which naphthenic acids are biodegraded, are poorly understood. This lack of knowledge is mainly due to the difficulties in identifying and purifying individual carboxylic acid compounds from complex naphthenic acid mixtures found in the environment, for microbial biodegradation studies.

2.1 Microbial degradation of naphthenic acids

Due to the high degree of complexity of the natural naphthenic acid mixtures and a lack of sources of individual naphthenic acid compounds, surrogate naphthenic acids were used in early microbial degradation studies (Herman et al., 1993; Herman et al., 1994, Lai et al., 1996). Naphthenic acids are acutely toxic to a range of organisms (Clemente& Fedorak, 2005; Headley& McMartin, 2004). MacKinnon and Boerger (MacKinnon& Boerger, 1986) demonstrated that with chemical and microbiological treatment approaches, the toxicity of tailings water could be reduced, presumably by removal or biodegradation of Naphtehic acids, although this was not shown directly. Herman et al. (Herman et al., 1994) followed biodegradation of naphthenic acids extracted from Mildred Lake Settling Basin (Syncrude) in laboratory cultures and also observed detoxification, as determined by the Microtox method. Clemente et al. (Clemente, MacKinnon& Fedorak, 2004) used enrichments of naphthenic acid-degrading microorganisms to biodegrade commercially available naphthenic acids (Kodak Salts and Merichem). Microtox analyses of culture supernatants revealed a reduction in toxicity after less than 4 weeks of incubation (Clemente, MacKinnon& Fedorak, 2004).

2.2 Microbial degradation prediction of naphthenic acids

The software used for prediction of the microbial metabolism (biodegradation) of petroleum thiophene is the OECD (Q)SAR Application Toolbox. The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco) toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical workflow (OECD (Q)SAR Project).

Degradation pathways used by microorganism to obtain carbon and energy from 200 chemicals are stored in a special file format that allows easy computer access to catabolic information. The collection includes the catabolism of C1-compounds, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulfonates, nitrates, nitro-derivatives, nitriles, and compounds containing more than one functional group. Most of pathways are related to aerobic

conditions. Different sources including monographs, scientific articles and public web sites such as the UM-BBD (Ellis, Roe & Wackett, 2006) were used to compile the database.

The original CATABOL simulator of microbial metabolism is implemented in the OECD (Q)SAR Application Toolbox (Jaworska et al., 2002; Dimitrov et al., 2002; Dimitrov et al., 2004). Single pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the source fragment associated with the transformation having the highest hierarchy. If the match is not found search is performed with the next transformation, etc. When the match is identified, the transformation products are generated. The procedure is repeated for the newly formed products. Predictability (probability that the metabolite is observed, given that the metabolite is predicted) evaluated on the bases of documented catabolism for 200 chemicals stored in the database of "Observed microbial catabolism" is 83%.

In this work will be researched the possible metabolites (observed and predicted) for some naphthenic acids. For this aim we will use the OECD (Q)SAR Application Toolbox system. Predictions are based on biotransformation rules that, in turn, are derived from reactions found in the UM-BBD and the scientific literature. The UM-PPS most accurately predicts compounds that are similar to compounds with known biodegradation mechanisms, for microbes under aerobic conditions and when the compounds are the sole source of energy, carbon, nitrogen or other essential elements for these microbes. Results in the OECD (Q)SAR Application Toolbox system are presented in Table 1.

3. Toxicity of naphthenic acids

Napthenic acids likely behave as surfactants as they consist of a hydrophilic head and a hydrophobic tail giving them unique solubility properties (Ivanković & Hrenović, 2010). These compounds are commonly found in detergents or cleaning products used in mining, oil, food and textile industries (Sandbacka, Christianson & Isomaa, 2000). Untreated industrial effluents often contain surfactants or surfactant-like compounds in concentrations sufficient to elicit acute toxicity in aquatic organisms (Ankley& Burkhard, 1992). Investigation into the influence that molecular structure exerts on the toxicity of naphthenic acid revealed that the observed acute toxicities for naphthenic acid-like surrogates to aquatic organisms rise with increasing Molecular weight and decreased with greater carboxylic acid content (Frank et al., 2009). These results suggested that the acute toxicity of naphthenic acid was influenced by hydrophobicity, thereby supporting narcosis as the probable mode of action (Kőnemann, 1981). Chemicals acting by a nonpolar narcotic mode of action are biologically unreactive, and their toxicity acts as a function of their concentration at the site of action, typically the cellular membrane (Cronin & Schultz, 1997).

Persistent Organic Pollutants (POPs) and Persistent, Bioaccumulative and Toxic (PBT) substances are carbon-based chemicals that resist degradation in the environment and accumulate in the tissues of living organisms, where they can produce undesirable effects on human health or the environment at certain exposure levels (Pavan & Worth, 2006).

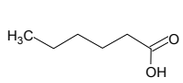
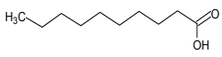
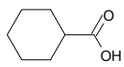
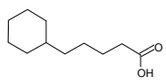
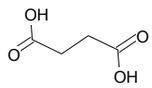
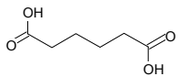
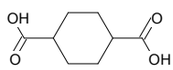
№	CAS number	Name of compound	Observed Microbial metabolism	Predicted Microbial metabolism
1	142-62-1	Hexanoic acid 	No metabolite	8 metabolites
2	334-48-5	Decanoic acid 	No metabolite	16 metabolites
3	98-89-5	Cyclohexane carboxylic acid 	4 metabolites	13 metabolites
4	5962-88-9	Cyclohexane pentanoic acid 	No metabolite	21 metabolites
5	110-15-6	Succinic acid 	No metabolite	1 metabolite
6	124-04-9	Adipic acid 	No metabolite	6 metabolites
7	1076-97-7	1,4-Cyclohexane dicarboxylic acid 	No metabolite	13 metabolites

Table 1. Observed and predicted microbial metabolism of some selected naphthenic acids.

3.1 Toxic prediction of naphthenic acids

Naphthenic acids have been reported to be acutely toxic to various organisms. However, the critical mechanism of toxicity remains largely unknown. Naphthenic acids are persistent in aquatic environments and are acutely toxic to aquatic bacteria, invertebrates, fish, and plants (Clemente & Fedorak, 2005]. Narcosis has been suggested to be the probable mode of acute toxicity by naphthenic acids, particularly for lower molecular weight of naphthenic acids. Higher molecular weight of naphthenic acids are less acutely toxic than lower molecular weight of naphthenic acids. Toxicity of naphthenic acids is inversely proportional to carboxylic acid content within naphthenic acid structures of higher molecular weight of naphthenic acids (Frank et al., 2009). Toxicity of naphthenic acids is also related to the amount of naphthenic acid that can be accumulated into the organisms as well as their inherent toxic potency.

In general, the complex and changing nature of mixtures of naphthenic acids make it difficult to predict toxicity. By determining the critical mechanism of toxicity of naphthenic acids, it might be possible to develop more effective predictive relationships to account for the toxic effects observed in living organisms exposed to naphthenic acids.

3.1.1 Use methods for toxic prediction of naphthenic acids

The PBT Profiler is a screening-level tool that provides estimates of the persistence, bioaccumulation, and chronic fish toxicity potential of chemical compounds. It is designed to be used when data are not available. In order to help interested parties make informed decision on a chemical's PBT characteristics, the PBT profiler automatically identifies chemicals that may persistent in the environment and bioaccumulate in the food chain. These chemicals are identified using thresholds published by the EPA (PBT Profiler).

The PBT Profiler combines the persistence criteria for water, soil, and sediment and highlights chemicals with an estimated half-life ≥ 2 months and < 6 months as persistent and those with an estimated half-life ≥ 6 months as very persistent. The half-life in air is not used in the PBT Profiler's Persistence summary (chemicals with an estimated half-life > 2 days are considered as persistent). The PBT Profiler uses 30 days in a month for its comparisons.

The PBT Profiler combines the bioaccumulation criteria and highlights chemicals with a BCF ≥ 1000 and < 5000 as bioaccumulative and those with a BCF ≥ 5000 as very bioaccumulative.

To highlight a chemical that may be chronically toxic to fish, the PBT profiler uses the following criteria: Fish ChV (Chronic Value) > 10 mg/l (low concern), Fish ChV = 0.1 - 10 mg/l (moderate concern) and Fish ChV < 0.1 mg/l (high concern).

Toxicity values of some naphthenic acids to Tetrahymena pyriformis were obtained from the literature (Schultz, 1997) and reported in Table 3. Population growth impairment was assessed after 40h with the common ciliate *Tetrahymena pyriformis*. The experimental data for rat (oral LD50 values) were collected from the literature (ChemIDplus).

Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software (US EPA, KOWWIN). Where possible measured log P values were verified and used in preference to calculated values.

In this study several models were used for non-polar compounds to aquatic and terrestrial species to determine the acute toxicity of selected naphthenic acids (Tables 3).

Baseline model (saturated alcohols and ketones) of *Tetrahymena pyriformis* (Ellison et al., 2008):

$$\log(1/IGC50) = 0.78*logP - 2.01 \quad (1)$$

$$n = 87 \quad R2 = 0.96 \quad s = 0.20 \quad F = 2131$$

Baseline model (saturated alcohols and ketones) of Rat (oral) (Lipnick, 1991):

$$\log(1/LD50) = 0.805*logP - 0.971*\log(0.0807*10\log P+1) + 0.984 \quad (2)$$

$$n = 54 \quad R2 = 0.824 \quad s = 0.208 \quad F = 35.3$$

The property - excess toxicity - was used to define the toxicity of chemicals (reactive or nonrective) (Lipnick, 1991). The extent of excess toxicity was determined as the toxic ratio (TR), which was calculated by the following equations 3-4 (Lipnick, 1991, Nendza & Müller, 2007):

$$TR = \log(1/C)exp - \log(1/C)calc \quad (3)$$

or

$$TR = (\text{predicted baseline toxicity}) / (\text{observed toxicity}) \quad (4)$$

3.1.2 Results and discussion

The results of estimation of naphthenic acids for persistence, bioaccumulation and toxicity are presented in Table 2. The components of naphthenic acids are commonly classified by their structures and the number of carbon atoms in the molecule.

Naphthenic acids are a family of carboxylic acid surfactants, primarily consisting of cyclic terpenoids used in source and geochemical characterisation of petroleum reserves (Brient, Wessner & Doyle, 1995). The compound group is composed predominately of alkyl-substituted cycloaliphatic carboxylic acids with smaller amounts of acyclic aliphatic (paraffinic or fatty) acids. Aromatic olefinic, hydroxyl and dibasic acids are also present as minor components of naphthenic acids. The cycloaliphatic acids include single rings and fused multiple rings.

The PBT profiler uses a well-defined set of procedures to predict the persistence, bioaccumulation, and toxicity of chemical compounds when experimental data are not available. The persistence, bioaccumulation, and fish chronic toxicity values estimated by the PBT profiler are automatically compared to criteria published by the EPA.

Analysis of data in Table 2 reveals that as all naphthenic acids are not persistence, bioacculumative and toxic (Fish ChV), but some compounds are exception about their chronic toxic. The compounds with moderate toxic (0.1-10 mg/l) are decanoic and cyclohexanepentanoic acids.

№	Name of compound	Persistence		Bio-accumu-lation	Toxicity
		Media (water, soil, sediment, air) Half-life (days)	Percent in Each Medium	BCF	Fish ChV (mg/l)
1	Hexanoic acid	8.7; 17; 78; 2.9	30%; 65%; 0%; 5%	3.2	84
2	Decanoic acid	8.7; 17; 78; 1.4	25%; 69%; 3%; 3%	3.2	2.7
3	Cyclohexanecarboxylic acid	8.7; 17; 78; 1.6	31%; 65%; 0%; 4%	3.2	51
4	Cyclohexanepentanoic acid	15; 30; 140; 1	22%; 70%; 6%; 2%	3.2	1.6
5	Succinic acid	8.7; 17; 78; 5.8	34%; 66%; 0%; 0%	3.2	21,000
6	Adipic acid	8.7; 17; 78; 2.9	34%; 66%; 0%; 0%	3.2	3,800
7	1,4-Cyclohexanedicarboxylic acid	8.7; 17; 78; 1.5	31%; 69%; 0%; 0%	3.2	1,100

Table 2. PBT Profiler estimate of the naphthenic acids.

All organic chemicals have the potential to cause narcosis. Their ability to do so is mainly governed by their concentration and their ability to cause more serious toxic effects, which would mask any narcotic effect the chemical may cause (van Wezel & Opperhuizen, 1995). The toxicity is not observed to be related to hydrophobicity and is in excess of baseline toxicity for the more compounds (Fig. 1 and Table 3).

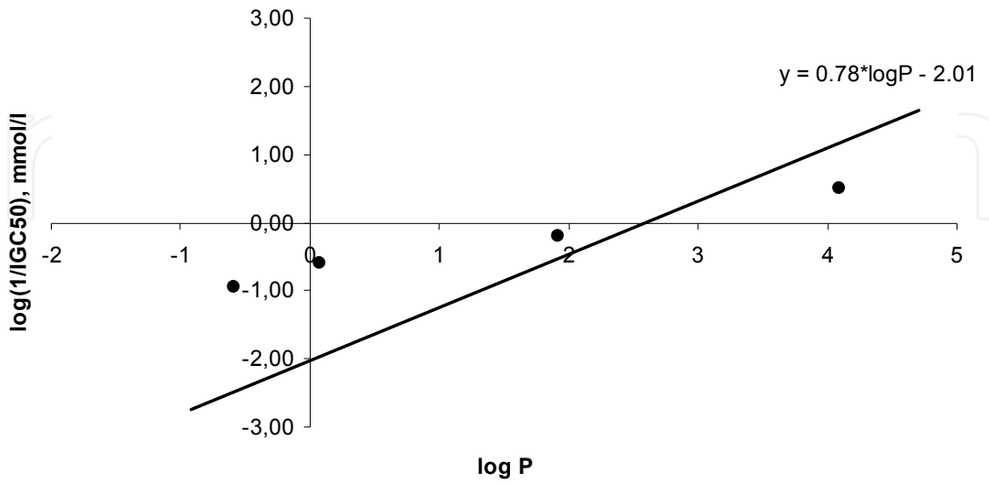


Fig. 1. Plot of toxicity to Tetrahymena pyriformis vs log P for naphthenic acids showing baseline toxicity.

CAS number	Name of compound	EcoSAR classification	log P	Exp. *T. pyriformis* log(1/IGC$_{50}$), mmol/l	Pred. *T. pyriformis* log(1/IGC$_{50}$), mmol/l /TR	Exp. oral Rat LD$_{50}$ mmol/kg	Pred. oral Rat LD$_{50}$ mmol/kg / TR
142-62-1	Hexanoic acid	Neutral organic-acid	1.92[a]	-0.208	-0.512/ 0.30	17.65	46.57/ 2.64
334-48-5	Decanoic acid	Surfactant -anionic-acid	4.09[a]	0.506	1.180/ -0.67	58.05	23.23/ 0.40
98-89-5	Cyclohexane carboxylic acid	Neutral organic-acid	1.96[a]			25.47	46.38/ 1.82
5962-88-9	Cyclohexane pentanoic acid	Neutral organic-acid	4.32[b]				
110-15-6	Succinic acid	Neutral organic-acid	-0.59[a]	-0.94	-2.470/ 1.53	19.14	3.16/ 0.16
124-04-9	Adipic acid	Neutral organic-acid	0.08[a]	-0.606	-1.948/ 1.34	75.27	10.22/ 0.14
1076-97-7	1,4-Cyclohexane dicarboxylic acid	Neutral organic-acid	0.95[b]				

[a]Experimental value of log P; [b]Calculated value of log P.

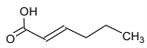
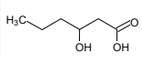
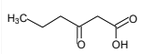
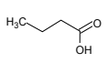
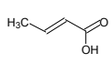
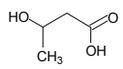
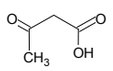
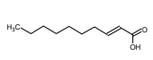
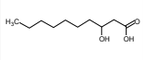
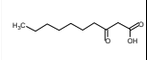
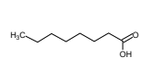
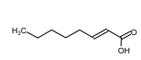
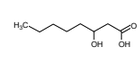
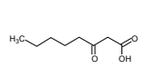
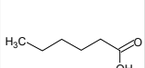
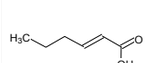
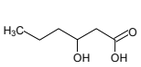
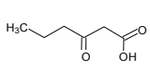
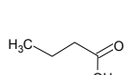
Table 3. Experimental and predicted values of acute toxicity for some naphthenic acids.

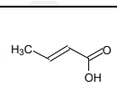
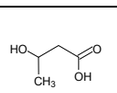
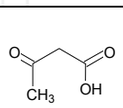
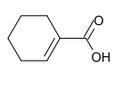
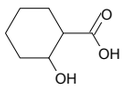
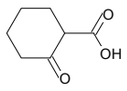
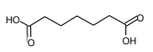
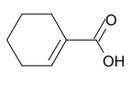
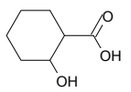
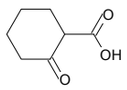
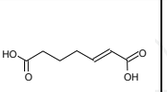
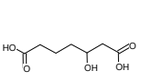
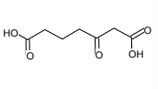
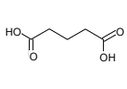
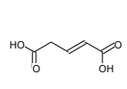
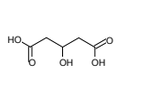
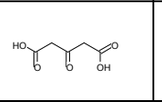
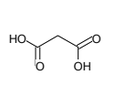
On the basis of calculated and experimental values for acute toxicity, the toxicity ratio (TR) as the ratio of the calculated baseline toxicity over the experimentally determined value was calculated (Table 3). A TR-value less than one could indicate rapid hydrolysis and/or biotransformation of the parent compound by the organism to non-toxic metabolites (Aptula & Roberts, 2006).

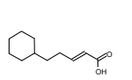
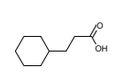
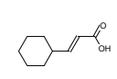
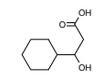
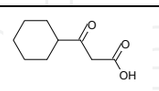
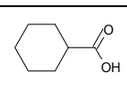
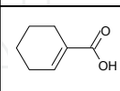
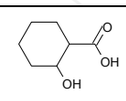
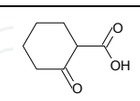
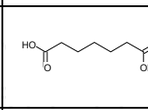
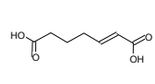
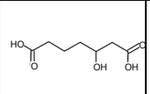
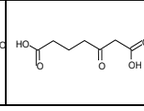
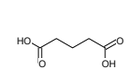
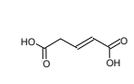
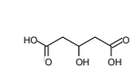
3.2 Toxic prediction of their metabolites

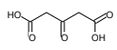
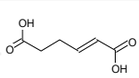
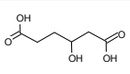
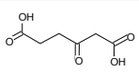
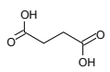
The reactions should be considered as an approximation of the real catabolism by mixed cultures of bacteria. Each chemical transformation includes source fragment (transformation target) and its bidegradation products. Results of possible metabolism (observed and predicted) for some selected naphthenic acids, the EcoSAR classification of their metabolites

and protein binding in the OECD (Q)SAR Application Toolbox system are presented in Table 4.

Hexanoic acid (142-62-1)						
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophilic addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	7	8				
EcoSAR classification	Neutral organics-acid	Neutral organics-acid				
Possible mechanism of action	Nucleophilic addition to ketone					
Decanoic acid (334-48-5)						
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophilic addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	7	8	9	10	11	12

EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action	Nucleophilic addition to ketone				Nucleophilic addition to ketone	
Predicted Microbial metabolism						
Number of metabolite	13	14	15	16		
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid		
Possible mechanism of action			Nucleophilic addition to ketone			
Cyclohexane carboxylic acid (98-89-5)						
Observed Microbial metabolism						
Number of metabolite	1	2	3	4		
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid		
Possible mechanism of action			Nucleophilic addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophilic addition to ketone			
Predicted Microbial metabolism						

Number of metabolite	7	8	9	10	11	12
EcoSAR classifi cation	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action	Nucleophilic addition to ketone				Nucleophilic addition to ketone	
Predicted Microbial metabolism						
Number of metabolite	13					
EcoSAR classifi cation	Neutral organics-acid					
Possible mechanism of action						
Cyclohexane pentanoic acid (5962-88-9)						
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classifi cation	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophili c addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	7	8	9	10	11	12
EcoSAR classifi cation	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action	Nucleophilic addition to ketone				Nucleophilic addition to ketone	
Predicted Microbial metabolism						

Number of metabolite	13	14	15	16	17	18
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophilic addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	19	20	21			
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid			
Possible mechanism of action	Nucleophilic addition to ketone					
Succinic acid (110-97-7)						
Predicted Microbial metabolism						
Number of metabolite	1					
EcoSAR classification	Neutral organics-acid					
Possible mechanism of action						
Adipic acid (124-04-9)						
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classification	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophilic addition to ketone			

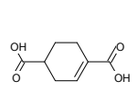
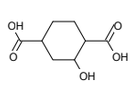
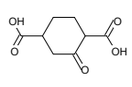
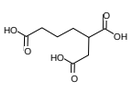
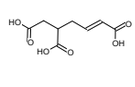
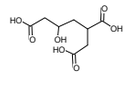
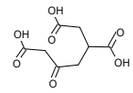
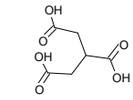
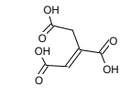
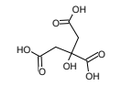
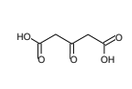
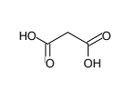
1,4-Cyclohexane dicarboxylic acid (1076-97-7)						
Predicted Microbial metabolism						
Number of metabolite	1	2	3	4	5	6
EcoSAR classifi cation	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action			Nucleophili c addition to ketone			
Predicted Microbial metabolism						
Number of metabolite	7	8	9	10	11	12
EcoSAR classifi cation	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid	Neutral organics-acid
Possible mechanism of action	Nucleophilic addition to ketone				Nucleophilic addition to ketone	
Predicted Microbial metabolism						
Number of metabolite	13					
EcoSAR classifi cation	Neutral organics-acid					
Possible mechanism of action						

Table 4. Predicted microbial metabolites, EcoSAR classification and possible protein binding (mechanism of action).

4. Conclusion

Naphthenic acids can enter the environment from both natural and anthropogenic processes. Naphthenic acids are highly toxic, recalcitrant compounds that persist in the environment for many years, and it is important to develop efficient bioremediation strategies to decrease both their abundance and toxicity in the environment. However, the diversity of microbial communities involved in naphthenic acid-degradation, and the mechanisms by which naphthenic acids are biodegraded, are poorly understood. This lack

of knowledge is mainly due to the difficulties in identifying and purifying individual carboxylic acid compounds from complex naphthenic acid mixtures found in the environment, for microbial biodegradation studies. Further, the persistence and fate of naphthenic acids in the environment is not well documented due to a lack of adequate analytical methods to determine the concentration, composition and extent of these crude-oil based compounds in environmental samples. This lack of knowledge constitutes a critical gap in scientific understanding. Acute toxicity is one of endpoints used in environmental risk assessment to determine the safe use and disposal of organic chemicals.

Degradation by the action of microorganisms is one of the major processes that determines the fate of organic chemicals in the environment. Quantitative Structure-Activity Relationships (QSAR) methods can be applied to biodegradation. Such relationships, often referred to as Quantitative Structure-Biodegradability Relationships (QSBRs), relate the molecular structure of an organic chemical to its biodegradability and consequently aid in the prediction of environmental fate.

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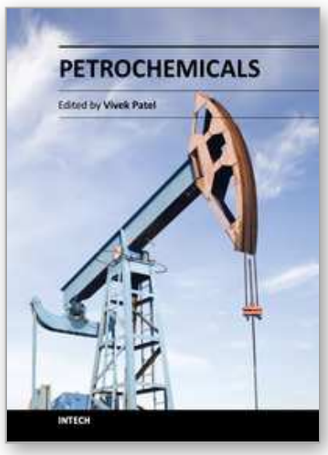

Petrochemicals

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The petrochemical industry is an important constituent in our pursuit of economic growth, employment generation and basic needs. It is a huge field that encompasses many commercial chemicals and polymers. This book is designed to help the reader, particularly students and researchers of petroleum science and engineering, understand the mechanics and techniques. The selection of topics addressed and the examples, tables and graphs used to illustrate them are governed, to a large extent, by the fact that this book is aimed primarily at the petroleum science and engineering technologist. This book is must-read material for students, engineers, and researchers working in the petrochemical and petroleum area. It gives a valuable and cost-effective insight into the relevant mechanisms and chemical reactions. The book aims to be concise, self-explanatory and informative.

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