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The probable reactivity of a petroleum component

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Oil spills threaten the global and local environment in both short and long term. Therefore, the fate and environmental impact of crude oil and its petroleum products requires serious study. Most of the studies at this stage have focused on the main hydrocarbons in crude oil while a minor fraction of hydrocarbons containing heteroatoms such as nitrogen (N), sulfur (S) and oxygen (O) have been neglected. However, these heterocyclic compounds may be disproportionately important to ecosystem health, necessitating their study. Toxicological data of organosulfur compounds in oil are limited. This necessitates the use of alternative methods to assess their toxicological properties. In the present work, the probable reactivity of the parent structure (2,3-dimethyl-1-benzothiophene) and its generated hepatic metabolites (for both conditions (rat *in vivo* and *in vitro*)) with respect to DNA and protein binding were studied by the QSAR Toolbox software. The reactive hepatic metabolites in both conditions (rat *in vivo* and *in vitro*) have different mechanisms of action (radical mechanism, A_N^2 and non-covalent interaction) with respect to DNA binding and the following mechanisms of action (Michael addition and Schiff base formation) with respect to protein binding.

Keywords*:* 2,3-dimethyl-1-benzothiophene, predict, metabolic activation, hepatic, QSAR Toolbox

INTRODUCTION

An oil spill is the release of a liquid petroleum hydrocarbon into the environment, especially the marine ecosystem, due to human activity, and is a form of pollution. The term is usually given to marine oil spills, where oil is released into the ocean or coastal waters. When oil is released into the sea, not only it increases pollution, but it is also difficult to clean. In fact, most of the methods for cleaning oil spills are ineffective, and often damage the marine life and environment. So these countermeasures should be applied depending on interrelated factors like ecological protection, socioeconomic effects and health risks. Crude oil is a mixture of hydrocarbons, but each kind has a different composition of molecular compounds, for example: sulfur, nitrogen, oxygen, metals, and other elements [1].

The petroleum industry has continually been troubled with various problems related to sulfur compounds in petroleum and its products, such as product odor and storage stability, catalyst poisoning, corrosion of processing equipment, and pollution emitted during usage. Sulfur is usually the most abundant hetero element in petroleum. Most of the sulfur present in crude oils is organically bound sulfur while elemental sulfur and hydrogen sulfide usually represent a very minor portion [2]. Furthermore, noxious sulfur dioxide is produced during combustion of sulfur-containing fuels. As such they are toxic and some of them are suspected mutagens and/or carcinogens. Better knowledge of the forms in which sulfur occurs in fossil fuels might aid the development of methods for its removal [3].

The sulfur content is in the range of 0.1—3.0% in most crudes [4] but can reach 8% in the vacuum residue of heavy crudes [5]. Organic sulfur compounds in crude oils are distributed over a wide range of molecular structures: aliphatic thiols, mono- and disulfides [6], as well as alkyl phenyl disulfides [7], but a large amount occurs in aromatic structures, especially as alkylated thiophene benzologues [8]. After distillation, mercaptanes, sulfides, and thiophenes are concentrated in the gasoline-range products [9] while benzothiophenes (BTs), dibenzothiophene (DBT), and alkylated dibenzothiophenes (DBTs) are concentrated in the middle distillate fractions. They may represent up to 70% of the sulfur present in diesel fuel.

A major part of the organic sulfur present in these materials occurs as thiophenic compounds, which makes this an important class of sulfur compounds to study. In petroleums the thiophene ring is mostly present as part of ring systems (primarily benzo- and dibenzothiophenes) [10-12] but alkylated thiophenes also occur [13] and are the most abundant thiophenic compounds present in shale oils [14].

The presence of organosulfur compounds in petroleum poses important production, environmental and health problems. In 1998, the

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European Union first mandated new sulfur specifications for drastically reduced levels that started to be phased in from the year 2000 [15].

However, the knowledge about the possible toxicities caused by this type of compounds is limited, necessitating the application of alternative methods (*in silico*) for their evaluation. Some theoretical studies show that the parent compounds (the basic structure) of organosulfur compounds are not reactive, but under certain conditions (e.g. in the liver) they can generate metabolites that are reactive, i.e. can cause health problems [16, 17].

The aim of the present work is to study the probable reactivity of the parent structure (2,3-dimethyl-1-benzothiophene) and its generated hepatic metabolites (for both conditions (rat *in vivo* and *in vitro*)) with respect to DNA and protein binding, using the QSAR Toolbox software.

MATERIAL AND METHODS

Compound. Heterocyclic sulfur compounds such as alkyl benzothiophenes (2,3-dimethyl-1-benzothiophene) are major sulfur components in the hydrodesulfurized oil fractions because they are highly recalcitrant to chemical catalysts [18]. The structural formula of 2,3-dimethyl-1-benzothiophene with CAS number 4923-91-5 is presented in Figure 1 [19].

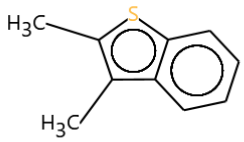

Figure 1. Structural formula of 2,3-dimethyl-1-benzothiophene

Organisation for Economic Co-operation and Development (*OECD*) (*Q)SAR Toolbox (version 4.3)*. (Quantitative) Structure - Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed [20].

METAPATH is a software platform to manage experimental data for the observed metabolism (*in vivo* and *in vitro*), providing very powerful and flexible search capabilities in identifying of metabolites, biotransformations and relative biotransformation rates observed in specific test environments, as well as specific enzymes responsible for a given biotransformation [20]. The metabolism databases assembled in METAPATH may be used as stand-alone datasets to increase efficiency of metabolism data evaluation and assessment by searching for specific compounds and identification of metabolism commonalities and, also, differences across chemical classes, species and dose-groups. These databases can be also employed for development and improvement of existing metabolic simulators that are used to perform metabolic predictions for chemical lists of concern [20].

Observed rat in vivo metabolism. The observed (documented) metabolic pathways for 647 chemicals, extracted from the scientific literature, and associated with the *in vivo* biotransformations of xenobiotic chemicals in rodents (mostly rats) are stored in a database format that allows easy computer access to the metabolism information. This database includes structurally different chemicals of various functionalities [20].

In vivo rat metabolism simulator. The current *in vivo* rat liver metabolic simulator (transformation table) represents an electronically designed set of 671 structurally generalized, hierarchically arranged abiotic and enzymatic transformation reactions which are characteristic for the metabolism for *in vivo* experimental systems such as rodent (mostly rat). The principal applicability of this simulator is associated with the reproduction, as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit *in vivo* genotoxicity effects [20].

Observed rat liver S9 metabolism. The documented metabolic pathways for 261 chemicals observed with the use of *in vitro* experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction are stored in a database format that allows easy computer access to the metabolism information. This database includes structurally different chemicals of various functionalities and fields of application [20].

Rat liver S9 metabolism simulator. The current *in vitro* rat liver metabolic simulator (transformation table) represents an electronically designed set of 551 structurally generalized, hierarchically arranged biotransformation reactions which are characteristic for the metabolism for *in vitro* experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction [20].

DNA binding by OASIS. The profiler is based on Ames Mutagenicity model part of OASIS TIMES system. The profiler consists of 85 structural alerts

responsible for interaction with DNA analyzed in Ames Mutagenicity model. The scope of the profiler is to investigate the presence of alerts within target molecules which may interact with DNA [20].

Protein binding by OASIS. The scope of the profiler is to investigate the presence of alerts within target molecules responsible for interaction with proteins. The list of 112 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than 2 mechanistic alerts. The profiling result outcome assigns a target to the corresponding structural alert, mechanistic alerts and domain [20].

RESULTS AND DISCUSSION

Toxicology is undergoing a paradigm shift, from predominantly observational science (based on animal testing), to predominantly predictive science focusing on target-specific, mechanism-based biological observations, contingent upon *in vitro* data and *in silico* predictions, often referred to as toxicology for the twenty-first century [21]. The development and application of modern tools can provide deeper insights into the molecular mechanisms underlying toxicity in a high throughput manner [22, 23]. Such developments are being driven by the need to improve the safety evaluation of chemicals in a more efficient, human-relevant context [24] to meet changing regulations and promote the use of non-animal models to predict toxicity [25].

Generally, toxicity studies require large numbers of animals, take several months to years to complete, are usually very costly, and can only test low numbers of compounds in a given time period. Current animal testing is primarily performed in rats and mice, and although these rodents exhibit many of the same responses to chemicals as humans, there are qualitative and particularly quantitative differences [26].

The software QSAR Toolbox (version 4.3) was applied to predict the possible metabolites of 2,3-dimethyl-1-benzothiophene in the liver (rat *in vivo* and *in vitro*) and their probable DNA and protein binding. The parent structure of 2,3-dimethyl-1-benzothiophene cannot bind to DNA and protein. The experimental pathways of metabolic activation were not observed in both conditions (rats *in vivo* and *in vitro*). The generated hepatic metabolites of 2,3-dimethyl-1-benzothiophene in the software QSAR Toolbox (rat *in vivo*) are presented in Table 1.

The possible DNA binding by OASIS (reaction mechanism) of the generated hepatic metabolites of 2,3-dimethyl-1-benzothiophene was predicted using the QSAR Toolbox software. The probable DNA binding of the generated hepatic metabolites of 2,3-dimethyl-1-benzothiophene is presented in Table 2.

Twenty metabolites are non-reactive and two are reactive, i.e. structural alerts are found for DNA binding. The structural alerts (quinones and trihydroxybenzenes) of the two metabolites were identified in the mechanistic domains (radical mechanism, A_N2 and non-covalent interaction) with mechanistic alerts (radical mechanism *via* ROS formation, Michael-type addition, quinoid structures and DNA intercalation). The probable protein binding of the generated hepatic metabolites (liver *in vivo*) of 2,3-dimethyl-1-benzothiophene is presented in Table 3. Eleven metabolites are not reactive and eleven are reactive, i.e. structural alerts are found for protein binding. The structural alerts (polarised alkenes – sulfinyl, di-substituted α,β-unsaturated aldehydes and aldehydes) of the eleven metabolites were identified in the mechanistic domains (Michael addition and Schiff base formation) with mechanistic alerts (Michael addition on polarized alkenes, direct acting Schiff base formers and Schiff base formation with carbonyl compounds).

The probable hepatic metabolites of 2,3-dimethyl-1-benzothiophene that were generated using the QSAR Toolbox (*in vitro* rat metabolism simulator) are fourteen. The generated hepatic metabolites of 2,3-dimethyl-1-benzothiophene in the software QSAR Toolbox (rat *in vitro*) are presented in Table 4.

The probable DNA binding of the generated hepatic metabolites (*in vitro*) of 2,3-dimethyl-1-benzothiophene is presented in Table 5. Twelve metabolites are not reactive and two are reactive, i.e. structural alerts are found for DNA binding. The structural alerts (quinones and trihydroxybenzenes) of the two metabolites were identified in the mechanistic domains (radical mechanism, A_N2 and non-covalent interaction) with mechanistic alerts (radical mechanism via ROS formation, Michael-type addition, quinoid structures and DNA intercalation).

The probable protein binding of the generated hepatic metabolites (*in vitro*) of 2,3-dimethyl-1-benzothiophene is presented in Table 6. Nine metabolites are not reactive and five are reactive, i.e. structural alerts are found for protein binding. The structural alerts (polarized alkenes – sulfinyl and aldehydes) of the five metabolites were identified in the mechanistic domains (Michael addition and Schiff base formation) with mechanistic alerts (Michael addition on polarized alkenes and Schiff base formation with carbonyl compounds).

Table 1. Number and structure of the generated hepatic metabolites (*in vivo*) of 2,3-dimethyl-1-benzothiophene

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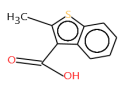	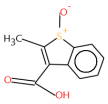	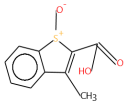	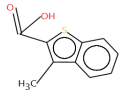	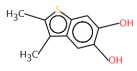
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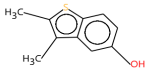	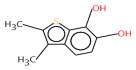	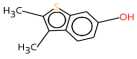	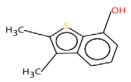	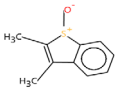
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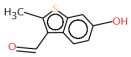	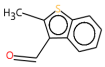	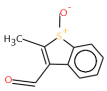	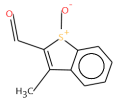	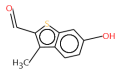
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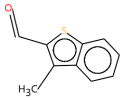	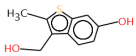		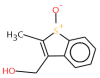	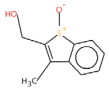
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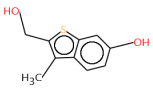	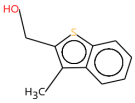			

Table 2. DNA binding of hepatic metabolites of 2,3-dimethyl-1-benzothiophene by QSAR Toolbox (liver *in vivo* metabolism simulator)

Number of metabolite	DNA binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1-4,6,8-22	No alert found		
5,7	Quinones and trihydroxybenzenes	Radical mechanism *via* ROS formation	Radical mechanism
5,7	Quinones and trihydroxybenzenes	Michael-type addition, quinoid structures	A_N^2
5,7	Quinones and trihydroxybenzenes	DNA intercalation	Non-covalent interaction

Table 3. Protein binding of hepatic metabolites of 2,3-dimethyl-1-benzothiophene by QSAR Toolbox (liver *in vivo* metabolism simulator)

Number of metabolite	Protein binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1,4-9,17,18,21,22	No alert found		
2,3,10,13,14,19,20	Polarised alkenes - sulfinyl	Michael addition on polarized alkenes	Michael addition
14	Di-substituted α,β-unsaturated aldehydes	Direct acting Schiff base formers	Schiff base formation
11-16	Aldehydes	Schiff base formation with carbonyl compounds	Schiff base formation

Table 4. Number and structure of the predicted hepatic metabolites (*in vitro*) of 2,3-dimethyl-1-benzothiophene

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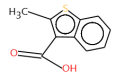	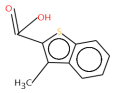	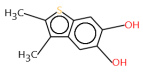	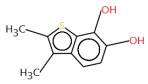	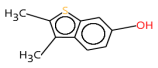
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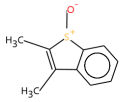
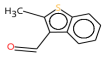
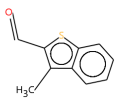
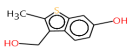
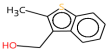
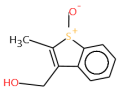
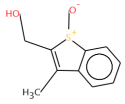
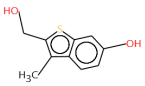
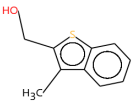
				
11	**12**	**13**	**14**	
				

Table 5. DNA binding of the hepatic metabolites of 2,3-dimethyl-1-benzothiophene by QSAR Toolbox (liver *in vitro* metabolism simulator)

Number of metabolite	DNA binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1,2,5-14	No alert found		
3,4	Quinones and trihydroxybenzenes	Radical mechanism *via* ROS formation	Radical mechanism
3,4	Quinones and trihydroxybenzenes	Michael-type addition, quinoid structures	A_N^2
3,4	Quinones and trihydroxybenzenes	DNA intercalation	Non-covalent interaction

Table 6. Protein binding of hepatic metabolites of 2,3-dimethyl-1-benzothiophene by QSAR Toolbox (liver *in vitro* metabolism simulator)

Number of metabolite	Protein binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1-5,9,10,13,14	No alert found		
6,11,12	Polarised alkenes - sulfinyl	Michael addition on polarized alkenes	Michael addition
7,8	Aldehydes	Schiff base formation with carbonyl compounds	Schiff base formation

CONCLUSIONS

The presence of sulfur organic compounds in oil can lead to various harmful effects on the environment and living organisms. There are no toxicological data of 2,3-dimethyl-1-benzothiophene, which requires the use of alternative methods (*in silico*) to study its reactivity. The probability of 2,3-dimethyl-1-benzothiophene to generate metabolites in the liver and their possible reactivity was investigated using the QSAR Toolbox software. Some of the generated hepatic metabolites of 2,3-dimethyl-1-benzothiophene are reactive to DNA and protein, i.e. have an electrophilic effect. Therefore, 2,3-dimethyl-1-benzothiophene may have toxic effects on living organisms.

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