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Research Article

MICROBIAL VOLATILE ORGANIC COMPOUNDS AS INDOOR AIR POLLUTANTS: PREDICTION OF ACUTE ORAL TOXICITY, HEPATOTOXICITY, IMMUNOTOXICITY, GENETIC TOXICITY ENDPOINTS, NUCLEAR RECEPTOR SIGNALLING AND STRESS RESPONSE PATHWAYS BY USING PROTOX–II WEBSERVER

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ABSTRACT

Microbial volatile organic compounds (MVOCs) are well-known indoor air pollutants that generate from bacteria and fungi through metabolism during growth. The present objective was a predictive assessment to detect acute toxicity, hepatotoxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling (NRS), and stress response (SR) pathways of MVOCs by using ProTox-II web server.The MVOCs of 15 nos. were selected as per available literature. The ProTox-II web server was used for predictive toxicity studies. The predictive results for the toxicity of MVOCs, Acetic acid obtained highly toxic compound among 15 compounds as class I and others observed class III, IV and V. None of these were showed hepatotoxic, immunotoxic, cytotoxic and mutagenic active but Isoprene, 2-methyl-2-propanol, Ethyl acetate, Styrene and 3-Methylfuran were obtained carcinogenic active. In case of NRS pathways, all MVOCs were inactive except 1 compound namely Geosmin was AR active, 3 compounds viz. Geosmin, 3-methyl-2-Pentanone and 3-Hexanone were obtained ER active and only Geosmin was obtained ER-LBD active. For SR pathways, all the parameters were obtained inactive for all 15 MVOCs. In conclusion, the predictive results revealed easily the acute toxicity and genotoxic profiles of MVOCs. The present study detects a narrow range of toxic or less toxic compounds, which can be validated in experimental study by further research. This web server helps faster screening of large numbers of compounds within short duration and without animal testing. It is suggested further chronic toxicity test with these highly toxic MVOCs to detect health hazards.

Keywords: Indoor air pollutants, Microbial volatile organic compounds, Predictive toxicology, Molecular mechanism of toxicity, Nuclear receptor signaling, stress response pathways

1. INTRODUCTION

Air pollution in the indoor environment is a matter of great concern because human spent major time in closed room. The indoor air is contaminated by several volatile organic compounds (VOCs) from different household and personal care products, which volatilize within the living place and cause health disorders due to toxic effect [1-5].

Among several established VOCs, microbial volatile organic compounds (MVOCs) are well-known, which are emitted from microorganisms especially bacteria and fungi during growth and multiplication [6-7]. Many researchers have been studied the mechanism of formation of MVOCs during both the primary and the secondary metabolism as side-products, mainly in the metabolic oxidation of glucose, from various precursors, such as acetate, amino acids, fatty acids, and keto acids [8-9].

Moreover, details acute toxicity study along with organ toxicity, cytotoxicity, mutagenicity, carcinogenicity and other nuclear signalling as well as stress response pathways of each MVOC is required long duration, huge cost and *in vitro* and *in vivo* functional assay through animal testing. In this context, researchers have been developed an alteration of experimental toxicity study, the predictive toxicity study through computational simulation to prevent cost, less duration and no animal harming. On the other hand, several compounds can be screened within an hour to obtain short range of chemicals. Major research works have been done to detect the microbes in indoor air and their metabolites as volatile organic compounds along with health disorders

[8, 11-20]. But the data are lacking on overall toxicity profiling to know molecular mechanisms of MVOCs while genotoxicity study was carried out for few MVOCs by Nakajima et al. [20].

Till date, the study of toxicity profiles with special reference to oral acute toxicity, hepatotoxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways for many MVOCs are required in new research arena. The ProTox-II is a web server (http://tox.charite.de/protox_II/) to predict toxicity and multiple toxicological endpoints for several chemical compounds developed by Drwal et al. [21] and Banerjee et al. [22]. Ghosh et al. [23] reported an easy and faster screening to know the toxicity profiles of synthetic pyrethroids by using this online server.

Present predictive study was to determine oral acute toxicity, hepatotoxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of MVOCs by using ProTox-II web server.

2. MATERIALS AND METHODS

2.1. Selection of MVOCs

The microbial volatile organic compounds (MVOCs) were selected as per available literature [8, 11-19]. The MVOCs such as Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone, Dimethyldisulfide, Styrene and 3-Methylfuran were selected for computational prediction.

2.2. Predictive study of MVOCs

According to Banerjee et al. [22], the ProTox-II platform is classified into a five different steps such as (1) oral acute toxicity prediction model as per six different toxicity classes; (2) organ toxicity model especially liver toxicity prediction;(3) toxicological (immunotoxicity model) and genotoxicological (cytotoxicity, mutagenicity and carcinogenicity model) endpoints; (4) toxicological pathways such as nuclear receptor signalling pathways is classified seven target-pathway based models viz. aryl hydrogen receptor (AhR), androgen receptor (AR), androgen receptor ligand binding domain (AR-LBD), aromatase, estrogen receptor alpha (ER), estrogen receptor ligand binding domain (ER-LBD), and peroxisome proliferator activated receptor gamma (PPARGamma) as well as (5) stress response pathways is classified five target-pathway based models such as nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (ARE), heat shock factor response element (HSE), mitochondrial membrane potential (MMP), phosphoprotein tumor suppressor (p53), and ATPase family AAA domain-containing protein 5 (ATAD5) and toxicity targets model of 14 nos. All the predictive models for toxicology pathways have been implemented as toxicology in the 21st Century (Tox21), which is a federal collaboration among United States Environmental Protection Agency (EPA), National Institute of Health (NIH), including National Center for Advancing Translational Sciences, and the National Toxicology Program at the National Institute of Environmental Health Sciences, and the Food and Drug Administration [24].

3. RESULTS

Table 1 describes molecular weight, number of hydrogen bond donor and acceptor, number of atoms, bonds and rings for each microbial volatile organic compounds (MVOCs). The MVOCs such as Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone, Dimethyldisulfide, Styrene and 3-Methylfuran were selected. In the present predictive results, among 15 MVOCs, the value of molecular weight obtained in this manner as manner as Acetic acid> Isoprene> 2-Butanone> 2-methyl-2-propanol> 3-Methylfuran> Ethyl acetate> Cyclopentanone> Dimethyl disulphide> 3-methyl-2-Pentanone, 2-Hexanone and 3-Hexanone> Styrene> 1-Octen-3 ol> Limonene> Geosmin.

Table 2 indicates the rat oral acute toxicity (LD_{50}) as mg/Kg, predicted different toxicity classes (I–VI) and prediction accuracy in % for different volatile organic compounds (VOCs). Among 15 compounds, Acetic acid obtained the highest toxicity as LD_{50} of 4.0 mg/Kg as class I, i.e. prescribed as death after swallowing ($5<LD50\leq50$) with 100% prediction accuracy. Other 2 VOCs such as 3-Methylfuran and Dimethyl disulphide showed LD_{50} value of 190 and 360 as class III i.e. prescribed toxic after swallowing ($50<LD_{50}\leq300$) with prediction accuracy of 67.4% and 100% respectively. In case of toxicity class IV i.e. prescribed harmful after swallowing ($2000<LD_{50}\leq5000$), 8 VOCs viz. Styrene, 1-Octen-3-ol, 2-Butanone, Geosmin, Isoprene, Cyclopentanone, 3-methyl-2-Pentanone and 2-methyl-2-Propanolfor LD50 values as 316,340, 610, 940, 1800, 1820, 1600 and 2733 mg/Kg with 100% prediction

accuracy were obtained. The LD_{50} values 2430, 2430, 4100 and 4400 mg/Kg for 4 VOCs such as 2-Hexanone, 3-Hexanone, Ethyl acetate and Limonene were found as class V i.e. prescribed may be harmful if swallowing ($2000<LD_{50} \leq 5000$) with prediction accuracy 100% for 3 compounds except 71% for 3-Hexanone.

Table 1: Prediction of physico-chemical properties of MVOCs

Sl. No.	Compounds name	MolWt (gm/mol)	HBD (nos.)	HBA (nos.)	A (nos.)	B (nos.)	R (nos.)
1.	Isoprene	68.12	0	0	5	4	0
2.	Limonene	136.23	0	0	10	10	1
3.	Geosmin	182.30	0	1	14	15	2
4.	1-Octen-3-ol	128.21	0	1	9	8	0
5.	2-Methyl-2-propanol	74.12	0	1	5	4	0
6.	Acetic acid	60.05	0	2	4	3	0
7.	Ethyl acetate	88.11	0	2	6	5	0
8.	2-Butanone	72.11	0	1	5	4	0
9.	3-Methyl-2-Pentanone	100.16	0	1	7	6	0
10.	2-Hexanone	100.16	0	1	7	6	0
11.	3-Hexanone	100.16	0	1	7	6	0
12.	Cyclopentanone	84.12	0	1	6	6	1
13.	Dimethyl disulfide	94.2	0	0	4	3	0
14.	Styrene	104.15	0	0	8	8	1
15.	3-Methylfuran	82.1	0	1	6	6	1

MolWt = Molecular weight; HBD = Hydrogen bond donor; HBA = Hydrogen bond acceptor; A = Atoms; B = Bonds and R = Rings

Table 2: Prediction of oral acute toxicity, class and accuracy of MVOCs

Sl. No.	Compounds name	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1.	Isoprene	1800	IV	100
2.	Limonene	4400	V	100
3.	Geosmin	940	IV	100
4.	1-Octen-3-ol	340	IV	100
5.	2-Methyl-2-propanol	2733	IV	100
6.	Acetic acid	4	I	100
7.	Ethyl acetate	4100	V	100
8.	2-Butanone	610	IV	100
9.	3-Methyl-2-Pentanone	1600	IV	100
10.	2-Hexanone	2430	V	100
11.	3-Hexanone	2430	V	71
12.	Cyclopentanone	1820	V	100
13.	Dimethyldisulfide	190	III	100
14.	Styrene	316	IV	100
15.	3-Methylfuran	160	III	67.4

Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class VI: non-toxic ($LD_{50} > 5000$)

In Table 3, the prediction of organ toxicity especially liver toxicity or hepatotoxicity was observed. All 15 compounds such as Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone,

Dimethyldisulfide, Styrene and 3-Methylfuran were found non-hepatotoxic or hepatotoxic inactive with probability scores of 0.86, 0.76, 0.67, 0.74, 0.93, 0.80, 0.84, 0.76, 0.82, 0.73, 0.72, 0.73, 0.86, 0.85 and 0.84 respectively. The immunotoxicity end points of studied 15compoundssuch as Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone, Dimethyldisulfide, Styrene and 3-Methylfuran were also obtained immunotoxic inactive with probability scores of 0.99, 0.95, 0.98, 0.94, 0.99, 0.99, 0.99, 0.99, 0.99, 0.99, 0.99, 0.99, 0.99, 0.99 and 0.99 respectively (Table 3).

Table 3: Prediction of organ toxicity and immunotoxicity end points of MVOCs

Sl. No.	Compounds name	Hep	P	Imm	P
1.	Isoprene	I	0.86	I	0.99
2.	Limonene	I	0.76	I	0.95
3.	Geosmin	I	0.67	I	0.98
4.	1-Octen-3-ol	I	0.74	I	0.94
5.	2-methyl-2-propanol	I	0.93	I	0.99
6.	Acetic acid	I	0.80	I	0.99
7.	Ethyl acetate	I	0.84	I	0.99
8.	2-Butanone	I	0.76	I	0.99
9.	3-methyl-2-Pentanone	I	0.82	I	0.99
10.	2-Hexanone	I	0.73	I	0.99
11.	3-Hexanone	I	0.72	I	0.99
12.	Cyclopentanone	I	0.73	I	0.99
13.	Dimethyldisulfide	I	0.86	I	0.99
14.	Styrene	I	0.85	I	0.99
15.	3-Methylfuran	I	0.84	I	0.99

Hep = Hepatotoxicity; Imm = Immunotoxicity; I = Inactive; A = Active and P = Probability

Table 4: Prediction of genetic toxicity end points of MVOCs

Sl. No.	Compounds name	Cytt	P	Mutg	P	Carci	P
1.	Isoprene	I	0.69	I	0.58	A	0.73
2.	Limonene	I	0.82	I	0.97	I	0.65
3.	Geosmin	I	0.88	I	0.92	I	0.84
4.	1-Octen-3-ol	I	0.79	I	0.95	I	0.68
5.	2-Methyl-2-propanol	I	0.80	I	0.92	A	0.79
6.	Acetic acid	I	0.77	I	0.98	I	0.72
7.	Ethyl acetate	I	0.82	I	0.93	A	0.62
8.	2-Butanone	I	0.79	I	0.97	I	0.51
9.	3-Methyl-2-Pentanone	I	0.80	I	0.91	I	0.50
10.	2-Hexanone	I	0.77	I	0.95	I	0.66
11.	3-Hexanone	I	0.74	I	0.97	I	0.64
12.	Cyclopentanone	I	0.72	I	0.82	I	0.68
13.	Dimethyldisulfide	I	0.80	I	0.78	I	0.56
14.	Styrene	I	0.90	I	0.90	A	0.75
15.	3-Methylfuran	I	0.81	I	0.98	A	0.91

Cytt = Cytotoxicity; Mutg = Mutagenicity; Carci = Carcinogenicity; I = Inactive; A = Active and P = Probability

In Table 4, the prediction of genotoxicity especially cytotoxicity, mutagenicity and carcinogenicity were studied. Among 15 compounds, all compounds such as Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone, Dimethyldisulfide, Styrene and 3-Methylfuran were found non-cytotoxic or cytotoxic inactive with probability scores of 0.69, 0.82, 0.88, 0.79, 0.80, 0.77, 0.82, 0.79, 0.80, 0.77, 0.74, 0.72, 0.80, 0.90 and 0.81 respectively.

Table 5: Prediction of Tox21-nuclear receptor signalling pathways of MVOCs

Sl. No.	Compounds name	Tox21-Nuclear receptor signalling pathways							
		Ahr	**P**	**AR**	**P**	**AR-LBD**	**P**	**Aro**	**P**
1.	Isoprene	I	1.00	I	1.00	I	1.00	I	0.99
2.	Limonene	I	1.00	I	0.99	I	1.00	I	0.99
3.	Geosmin	I	0.99	A	0.53	I	0.63	I	0.98
4.	1-Octen-3-ol	I	1.00	I	1.00	I	1.00	I	1.00
5.	2-Methyl-2-propanol	I	1.00	I	1.00	I	1.00	I	1.00
6.	Acetic acid	I	1.00	I	1.00	I	1.00	I	0.98
7.	Ethyl acetate	I	1.00	I	1.00	I	1.00	I	1.00
8.	2-Butanone	I	1.00	I	1.00	I	0.99	I	1.00
9.	3-Methyl-2-Pentanone	I	0.99	I	1.00	I	0.99	I	0.99
10.	2-Hexanone	I	1.00	I	1.00	I	0.99	I	1.00
11.	3-Hexanone	I	1.00	I	1.00	I	1.00	I	1.00
12.	Cyclopentanone	I	0.99	I	0.95	I	0.99	I	0.99
13.	Dimethyldisulfide	I	0.99	I	1.00	I	1.00	I	0.99
14.	Styrene	I	0.99	I	1.00	I	1.00	I	1.00
15.	3-Methylfuran	I	1.00	I	0.99	I	0.99	I	0.97
		ER	**P**	**ER-LBD**	**P**	**PPAR-Gamma**	**P**		
1.	Isoprene	I	0.99	I	1.00	I	1.00		
2.	Limonene	I	0.84	I	1.00	I	1.00		
3.	Geosmin	A	0.75	A	0.75	I	0.99		
4.	1-Octen-3-ol	I	0.99	I	1.00	I	0.99		
5.	2-Methyl-2-propanol	I	0.99	I	1.00	I	0.99		
6.	Acetic acid	I	0.99	I	0.99	I	1.00		
7.	Ethyl acetate	I	0.99	I	1.00	I	1.00		
8.	2-Butanone	I	0.94	I	0.99	I	0.99		
9.	3-Methyl-2-Pentanone	A	1.00	I	0.99	I	0.99		
10.	2-Hexanone	I	0.64	I	1.00	I	0.99		
11.	3-Hexanone	A	0.88	I	1.00	I	0.99		
12.	Cyclopentanone	I	0.99	I	0.99	I	0.95		
13.	Dimethyldisulfide	I	0.99	I	1.00	I	0.99		
14.	Styrene	I	0.99	I	1.00	I	1.00		
15.	3-Methylfuran	I	0.98	I	1.00	I	1.00		

AhR = Aryl hydrocarbon Receptor; AR = Androgen receptor; AR-LBD = Androgen Receptor Ligand Binding Domain; Aro = Aromatase; ER = Estrogen Receptor Alpha; ER-LBD = Estrogen Receptor Ligand Binding Domain; PPAR-Gamma = Peroxisome Proliferator Activated Receptor Gamma; I = Inactive; A = Active and P = Probability

In case of mutagenicity endpoints, all 15 compounds Isoprene, Limonene, Geosmin, 1-Octen-3-ol, 2-methyl-2-propanol, Acetic acid, Ethyl acetate, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone,

Cyclopentanone, Dimethyldisulfide, Styrene and 3-Methylfuran were found non-mutagenic or mutagenic inactive with probability scores of 0.58, 0.97, 0.92, 0.95, 0.92, 0.98, 0.93, 0.97, 0.91, 0.95, 0.97, 0.82, 0.78, 0.90 and 0.98 respectively. All the studied 15 compounds, 5 VOCs such as Isoprene, 2-methyl-2-propanol, Ethyl acetate, Styrene and 3-Methylfuranwere obtained carcinogenic active with probability scores of 0.73, 0.79, 0.62, 0.75 and 0.91respectively. Rest 10 VOCs viz. Limonene, Geosmin, 1-Octen-3-ol, Acetic acid, 2-Butanone, 3-methyl-2-Pentanone, 2-Hexanone, 3-Hexanone, Cyclopentanone, and Dimethyldisulfide were obtained carcinogenic inactive with probability scores 0.65, 0.84, 0.68, 0.72, 0.51, 0.50, 0.66, 0.64, 0.68 and 0.56 respectively. For Tox21-nuclear receptor signalling pathways, several parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD and PPAR-Gamma were predicted for 15 VOCs (Table 5).

All the studied 15 compounds were observed AhR inactive with probability scores 1.00, 1.00, 0.99, 1.00, 1.00, 1.00, 1.00, 1.00, 0.99, 1.00, 1.00, 0.99, 0.99, 0.99 and 1.00 respectively. Except 1 compound namely Geosmin found AR active with probability score of 0.53 while rest 14 compounds were obtained AR inactive with probability scores 1.00, 0.99, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 0.95, 1.00, 1.00 and 0.99 respectively. For AR-LBD parameter, all 15 compounds were observed inactive and the probability scores 1.00, 1.00, 0.63, 1.00, 1.00, 1.00, 1.00, 0.99, 0.99, 0.99, 1.00, 0.991.00, 1.00 and 0.99 respectively.

Table 6: Prediction of Tox21-stress response pathwaysof MVOCs

Sl. No.	Compounds name	Tox21-Stress response pathways									
		nrf2/ARE	P	HSE	P	MMP	P	p53	P	ATAD5	P
1.	Isoprene	I	0.99	I	0.99	I	0.99	I	0.99	I	1.00
2.	Limonene	I	0.98	I	0.98	I	1.00	I	1.00	I	1.00
3.	Geosmin	I	0.96	I	0.96	I	0.64	I	0.99	I	0.99
4.	1-Octen-3-ol	I	0.96	I	0.96	I	0.97	I	1.00	I	0.99
5.	2-Methyl-2-propanol	I	1.00	I	1.00	I	0.99	I	1.00	I	1.00
6.	Acetic acid	I	1.00	I	1.00	I	0.98	I	0.99	I	1.00
7.	Ethyl acetate	I	1.00	I	1.00	I	0.97	I	1.00	I	0.99
8.	2-Butanone	I	1.00	I	1.00	I	1.00	I	1.00	I	1.00
9.	3-Methyl-2-Pentanone	I	0.99	I	0.99	I	0.99	I	0.99	I	0.99
10.	2-Hexanone	I	1.00	I	1.00	I	1.00	I	1.00	I	1.00
11.	3-Hexanone	I	1.00	I	1.00	I	0.99	I	1.00	I	1.00
12.	Cyclopentanone	I	0.99	I	0.99	I	0.79	I	0.99	I	0.98
13.	Dimethyldisulfide	I	0.97	I	0.97	I	0.97	I	0.96	I	0.97
14.	Styrene	I	1.00	I	1.00	I	1.00	I	1.00	I	1.00
15.	3-Methylfuran	I	0.99	I	0.99	I	0.98	I	1.00	I	1.00

nrf2/ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour supressor); ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active and P = Probability

In case of the parameter Aromatase or Aro, all the studied 15 compounds were observed inactive with probability scores 0.99, 0.99, 0.98, 1.00, 1.00, 0.98, 1.00, 1.00, 0.99, 1.00, 1.00, 0.99, 0.99, 1.00 and 0.97 respectively. For the parameter ER, 3 compounds viz. Geosmin, 3-methyl-2-Pentanone and 3-Hexanone observed ER active with probability scores of 0.75, 1.00 and 0.88 while rest 12 compounds were obtained ER inactive with probability scores 0.99, 0.84, 0.99, 0.99, 0.99, 0.99, 0.94, 0.64, 0.99, 0.99, 0.99 and 0.98 respectively. For the parameter ER-LBD, 1 compound namely Geosmin found AR active with probability score

of 0.75 while rest 14 compounds were obtained ER-LBD inactive with probability scores 1.00, 1.00, 1.00, 1.00, 0.99, 1.00, 0.99, 0.99, 1.00, 1.00, 0.99, 1.00, 1.00 and 1.00 respectively. For the parameter as PPAR-Gamma, it was also found inactivity of 15 compounds and probability scores were recorded 1.00, 1.00, 0.99, 0.99, 0.99, 1.00, 1.00, 0.99, 0.99, 0.99, 0.99, 0.95, 0.99, 1.00 and 1.00 respectively.

In Table 6, Tox21-stress response pathways parameters such as nrf2/ARE, HSE, MMP, p53 and ATAD5 for studied compounds were predicted. All studied 15 compounds were showed inactivity for the parameters of nrf2/ARE, HSE, MMP, p53 andATAD5. For nrf2/ARE, the probability scores 0.99, 0.98, 0.96, 0.96, 1.00, 1.00, 1.00, 1.00, 0.99, 0.97, 0.97, 1.00 and 0.99 respectively were obtained. For HSE, the probability scores 0.99, 0.98, 0.96, 0.96, 1.00, 1.00, 1.00, 1.00, 0.99, 1.00, 1.00, 0.99, 0.97, 1.00 and 0.99 respectively were observed. For MMP, the probability scores 0.99, 1.00, 0.64, 0.97, 0.99, 0.98, 0.97, 1.00, 0.99, 1.00, 0.99, 0.79, 0.97, 1.00 and 0.98 respectively were found. For ATAD5, the probability scores 1.00, 1.00, 0.99, 0.99, 1.00, 1.00, 0.99, 1.00, 0.99, 1.00, 1.00, 0.98, 0.97, 1.00 and 1.00 respectively were obtained.

4. DISCUSSION

In case of VOCs, volatility depends on several physico-chemical parameters such as boiling point, vapour pressure, molecular weight, and size. According to the report of WHO [25], the volatility of the compounds is depending on the boiling point (300°C) of indoor air pollutants. Herrmann [26] reported VOCs are a large class of low-molecular-weight and carbon-containing compounds. In the present study, among 15 compounds, Acetic acid observed low molecular weight compared to other compounds (Table 1). These toxicity classes have been prescribed by Drwal et al. [21] in ProTox-II web server.

Earlier research work reported by Korpi et al.[10] that dimethyl disulphide showed slightly toxic with LC_{50} in the range 1000-10000 ppm in rats and this was reported in CHEMINFO database [27]. The concentrations of MVOCs need to be thousands of mg/m^3 in order to produce lethal effects in animals, whereas the concentrations of individual MVOCs indoors in general are in the range of 100ng to<1mg/m3. In the present prediction, Acetic acid showed highest acute toxicity in rat, but earlier study reported the value oral LD_{50} in rats is 3530mg/Kg [28] and as per AIHA [29] the rodent oral LD_{50}3310 mg/Kg while rest MVOCs are toxicity class of III, IV and V that means may cause health hazards (Table 2). According to Schleibinger et al. [30], fungal metabolites as mycotoxins cause toxicity in lower dose.

The present prediction obtained no hepatotoxicity and immunotoxicity of studied MVOCs (Table 3). As such no earlier reports are available about the studied compounds regarding hepatotoxic and immunotoxic potential. Interestingly, these compounds uptake through respiratory tract and less responsive to gastrointestinal tract and associated organs. In other research it has been emphasized that MVOCs concentration showed normally 2–3 decimal powers lower compared to VOCs, these low concentrations should not be expected to have any effects in liver or immune system [31]. In other words, mycotoxins are toxic, hepatotoxic, immunotoxic and carcinogenic when treated as indoor air pollutants [30].

Among 15 MVOCs, none of these were observed cytotoxic and mutagenic while Isoprene, 2-methyl-2-propanol, Ethyl acetate, Styrene and 3-Methylfuran were obtained carcinogenic (Table 4). Isoprene has already been reported carcinogenic due to chronic inhalation toxicity in rat and mice models [32]. The other experiment with 2-Methyl-2-propanol showed carcinoma or renal tubule adenoma in male rats but not occurred in female rat while follicular cell adenoma of the thyroid gland found in both male and female mice [33]. The Styrene has potent carcinogenic effect especially lymphatic/hematopoietic cancers in humans [34]. As per researchers Furan showed toxicity and carcinogenicity [35] while 3-Methylfuran is the derivative of Furan and Furan induced cholangio carcinomas in rat [36-37]. These 3 MVOCs were showed similarities with previous research works on carcinogenicity. The carcinogenicity of Ethyl acetate has not been found any positive result in experimental study, but this compound has tendency to hydrolyse and form Ethyl alcohol and Acetic acid that may lead to carcinogenic effect. According to Khan et al. [38], Ethyl acetate vapour is suitable as complimentary medicine for breast cancer. The present study is found some contradiction for Ethyl acetate as carcinogenic active.

The present predictive results indicated inactivity for all the parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD and PPAR-Gamma under nuclear receptor (NR) signalling pathways for all MVOCs (Table 5) except 1 compound namely Geosmin AR active, 3 compounds viz. Geosmin, 3-methyl-2-Pentanone and 3-Hexanone were obtained ER active and only Geosmin was obtained ER-LBD active. The results revealed that 1 compound

observed androgenic active while few compounds exhibited estrogenic active, but some were also observed antiandrogenic and antiestrogenicvia AR and ER test, which is supported the present prediction [39]. According to Kolodkin et al. [40], NR signalling occurs to maintain development, cellular growth, inflammation and metabolism and ligand distribution appeared dynamic with few NRs found predominantly in the nucleus (pregnane X receptor and peroxisome proliferator-activated receptor gamma), while some are located either in both compartments (vitamin D receptor and mineralocorticoid receptor) or mostly in the cytoplasm (glucocorticoid receptor and androgen receptor).

Different types of cellular stress in response pathways have been investigated individually through *in vitro* studies, and the major signalling components and molecular mechanisms have been identified by researchers. Adaptive stress response pathways are signal transduction pathways that ultimately resulted in the transcriptional activation of cytoprotective genes [41]. All the compounds were obtained nrf2/ARE, HSE, MMP and p53 inactive that may not cause reactive oxygen species (ROS) [41-43] while in the second case, another stress response pathway i.e. heat shock factor response element (HSE), which caused transcriptional upregulation of a family of genes called as heat shock proteins and occurred protein denaturation because chemical insult [41, 44-46]. In the present predictive results (Table 6), all the MVOCs were obtained not harmful for cellular stress but may alter the molecular mechanisms after chronic exposure. Another stress response pathway i.e. mitochondrial membrane potential (MMP), all compounds were obtained inactive. It is well-known that mitochondria consist double membrane, which provides the energy to the cell through oxidative phosphorylation and prevent apoptosis [47]. According to Parikh et al. [48] yeast mitochondria have adapted a mitochondria-to-nucleus signal transduction pathway which induce the transcription of nuclear-encoded mitochondrial genes, and alleviate mitochondrial stress. Moreover, mitochondrial stress by toxins may lead to several diseases [49]. Recently, Richter et al. [50] emphasized that toxins inhibit the mitochondrial protein synthesis and block with the stress response. Other two parameters such as p53 or Phosphoprotein (tumour suppressor) and ATPase family AAA domain-containing protein 5 (ATAD5) observed inactive for all the studied compounds. The p53 gene controls the cell cycle arrest, carcinogenesis, DNA damage, apoptosis, etc. and inactivity in the present prediction showed no incidence of carcinogenesis obtained for all compounds except 3 MVOCs in Table 4. On the other hand, ATAD5 is involved in DNA damage response. This is also involved in a RAD9A-related damage checkpoint, a pathway which is important in determining whether DNA damage is compatible with cell survival or whether it requires cell elimination by apoptosis [51]. The inactivity of studied compounds revealed that DNA damage may repair due to no stress response of ATAD5.

5. CONCLUSIONS

It is concluded from the above predictive results that indoor air pollutants created by microbial metabolites as MVOCs cause health hazards because these were found somehow toxic and few of these carcinogenic, androgen and oestrogen disruptor. The present *in silico* results are suitable for further experimental research in which toxic MVOCs were obtained in a narrow range. This online tool helps faster screening of large numbers of compounds within short duration as well as without animal testing. This study is suggested future experimental assay to validate the present prediction of studied MVOCs.

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Conflict of interest

No conflict of interest.

7. REFERENCES

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