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Radioactive Imagine Agents: 99mTc-Toxicological Study

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Abstract

Radioactive pharmaceutical materials (radiopharmaceuticals) that contain radioactive atom or/ and ion (emits nuclear (α, β, or / and γ) ray according to its energy and half-life duration) companied with organic or inorganic molecule are important materials in nuclear imaging or therapeutically medicine. Many biomolecules labeled with Technetium-99 in relationship to toxicity issues can be transported, distributed, and detected with the computerized algorithm detector depending upon chemical, biological, metabolic, and functional properties of tissue or organ (bone, liver, heart, kidney, and others). According to literatures' review about this subject, this is the first try in Iraq and other countries to study Technetium – radiomolecules with *in Silico* depending on two approachs. LD_{50}, Class, Polar Surface Area PSA, logP, Hepatotoxicity, Carcinogenity, Immunotoxicity, Mutagenicity, Cytotoxicity, AMES test, Max. tolerated dose (human), hERG I and II inhibitor, Oral Rat Acute (LD_{50}) and Chronic (LOAEL) Toxicity, Hepatotoxicity besides Skin Sensitisation showed that Tc-biomolecules under study are structurally unsafe having toxic response to liver, immune system, cellular components, DNA, and/or cardiac repolarization through hERG inhibition of action. These mainly conclusion notes depended upon Technetium oxidation state, heteroatoms presence, surface properties besides bio-target specifications, concentration, exposure time, genetic factors of human, and health problems.

1. Introduction

Radioactive pharmaceutical materials (radiopharmaceuticals) are important materials in nuclear imaging or therapeutically medicine. These materials contain radioactive atom or/ and ion companied with organic or inorganic molecule. The important component in these molecules is the radioactive element (radionuclide) that emits nuclear (α, β, or / and γ) ray according to its energy and half-life duration like Rhenium Re-186 or Re-188, iodine-131 or Iodine -129, Gallium-67, and Technetium- 99 that used in nuclear medicinal therapy as shown in Figure (1) where radioactive Rhenium emits beta and gamma rays while others emit gamma as shown in Table (1) and Figure (2). Clinical situation of human, radionuclide energy and half – life time, target organ or tissue penetration, kinetic-dynamic behavior of these pharmaceutics specify the choosing of the nuclear tracker or monitor such as gamma camera [1].

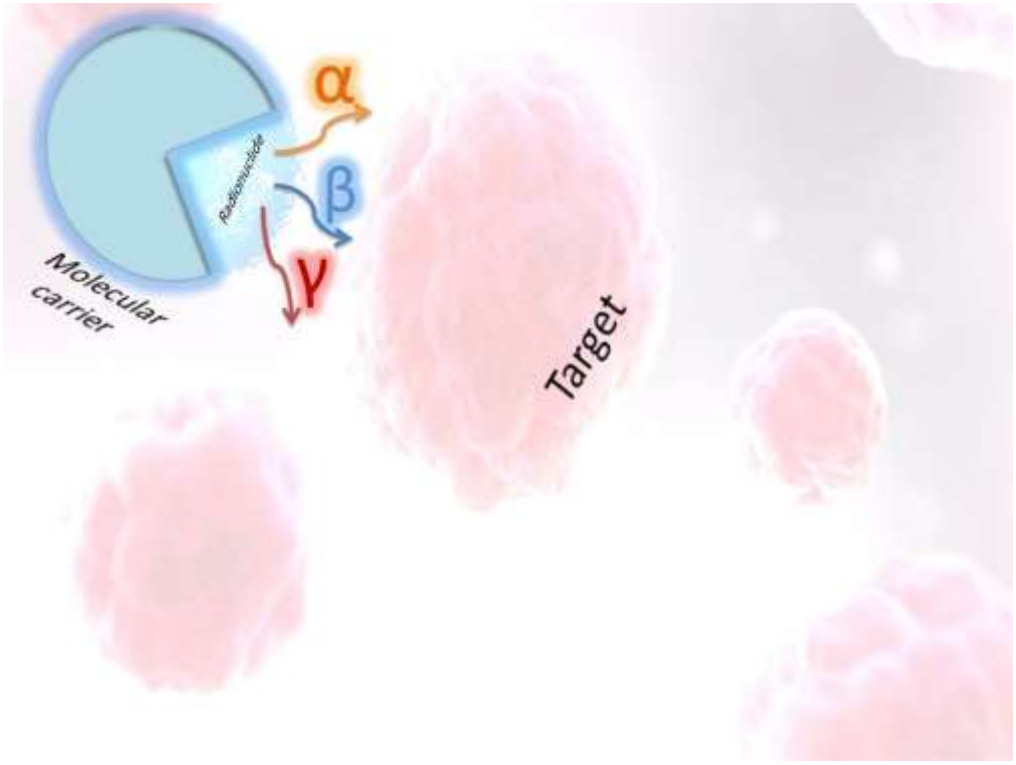

Figure (1). Image of radiopharmaceutical molecule emits (α, β, or / and γ) ray towards bio-target.

Table (1). Examples of various radionuclides.

Isotope	Emitting radiation	Example	Function(s)
Chromium-51	Gamma	Cr-51-EDTA	Non-imaging
Cobalt-57	Gamma	Co-57 cyanocobalamine	Non-imaging
Cobalt-58	Gamma	Co-58 cyanocobalamine	Non-imaging
Fluorine-18	Positron	F-18 Fluorocholine	Imaging
Gallium-67	Positron	Ga-68 Dotatoc	Imaging
Indium-111	Gamma	In-111 DTPA	Imaging
Iodine-123	Gamma	I-123 MIBG	Imaging
Iodine-131	Beta Gamma	I-131 MIBG	Imaging
Selenium-75	Gamma	Se-75 Selenocholesterol	imaging

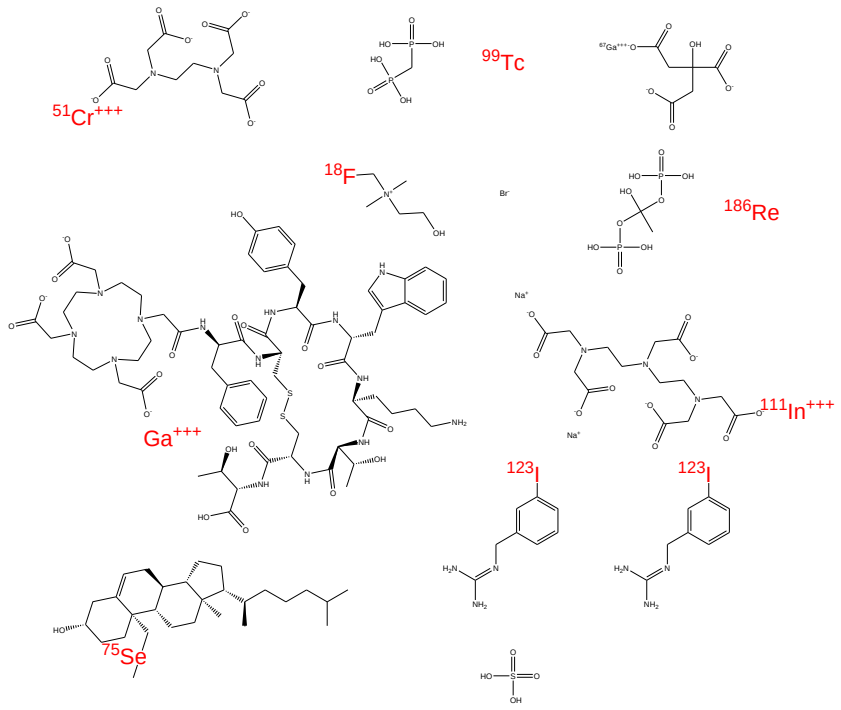

Figure (2). Various radiopharmaceutical structures.

To get best results of molecular nuclear imaging, radioactive material must be transported, distributed, and detected with the computerized algorithm detector. So, intravenously administration of any radiopharmaceuticals mainly depends upon chemical, biological, metabolic, and functional properties of tissue or organ (bone, hepatic, cardiac, renal, and others) [2].

Man-made Tc-99 isotope can be found in nuclear waste having very long half-life ($2.1x10^5$ years) that extends its presence in environment and concentrates in some plants or animals. Human exposure to this radioactive represents a high chance of cancer where it can be concentrated in thyroid and gastrointestinal tract [3].

Technetium-99m is a radioactive imaging transition metal supplied by Molybdenum -99/ Technetium -99 generators and considered as an expanded radioisotope having half- life time of 6 hours - 140 KeV produced from Molybdenum-99 where Molybdenum -99 (^{99}Mo, (1-20)Ci where 1Ci=37 GBq) that produced from highly or low enriched Uranium -235 reactors localized in Canada, Belgium, South Africa, France, Australia, and Argentina [4]. Production of Molybdenum-99(M0-99) routes are starting from U-235 or natural Mo-98 that targets with neutrons, U-238 or Mo-100 that targets with gamma, Zr-96 target with alpha ray and Mo-100 targets with proton [5]. From health points, ^{99m}Tc may deteriorate DNA and minimize cell remaining in existence [6,7].

In contrast to Carbon -11, iodine-123, or Fluorine -18, Technetium -99m does not substituted any carbon or hydrogen atom in any pharmaceutical molecule to form Technetium -99m imaging agent or any labeling molecule [8].

Technetium-99m bioactive molecule consists of inorganic Tc-99m (the core) and coordinated biomolecule in a specific molecular geometry according to Tc-99m oxidation state and coordination site. Various examples of Tc-99m cores as presented in Figure (3) are [2, 9, 10]:

- 6-hydrazinonicotinamide (HYNIC) forms ^{99m}Tc (5+) hydrazido complex used in neuroendocrine tumor imaging.
- ^{99m}Tc (5+) having π bonding with trans-oxo with penta-coordination towards square pyramidal, example of this coordination is ^{99m}Tc-TRODAT-1 as Parkinson' disease detector.
- ^{99m}Tc (1+) with carbonyl group as in histidine complex

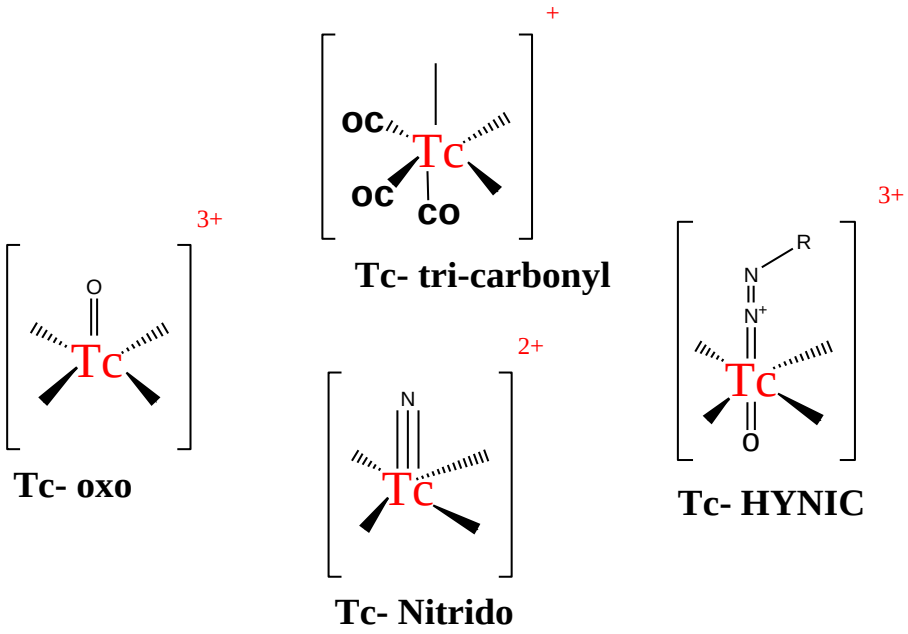

Figure (3). Examples of Tc-99m cores [11].

Different superior Tc-99m agents were prepared and used in diagnosis stage combined with antibiotics (rifampicin, cefuroxime, delafloxacin, cefepime, …), protein or peptide (ghrelin, insulin, vasopressin, …) and other bio-organic molecules. According to our acknowledgment, all Technetium radio-biomolecules were not subjected to online *in Silico* - Toxicity investigation. With this point of interest, two free online predication websites [https://tox-new.charite.de/protox_II/ and http://biosig.unimelb.edu.au/pkcsm/] were chosen for thirty Technetium complexes having various oxidation states through their isomeric SMILES obtained from https://pubchem.ncbi.nlm.nih.gov.

2. Experimental Procedure

Thirty tested Technetium radio-biomolecules as tabulated in Table (2) were checked where their isomeric Simplified Molecular –Input Line – Entry System (SMILES) were obtained from online https://pubchem.ncbi.nlm.nih.gov. website. They are Technetium 99mTc Pentetate, Technetium 99mTc Exametazime, 99mTc MIBI or Technetium 99mTc Sestamibi, 99mTc-CCMSH, Technetium-99 Tin(4+,2+) complex, 99mTc –A-MSH, 99mTc- (V) DMSA, 99mTc TRODAT, 99mTc –DG, 99mTc-EDDA/HYNIC_C(RGDyK), 99mTc-Hypericin, Technetium 99mTc Bicisate, Technetium 99mTc- Apcitide, 99mTc- DTPA-TOR, 99mTc-PrDP, 99mTc-MDP, EC-DG-99mTc, Technetium 99mTc glucoheptonate, 99mTc-DO3A-Folate, 99mTc-HYNIC-EGF, 99mTc-MIP-1404, 99mTc-Hl91, EMIDP99mTc, MAG3-HBP99mTc, EC2099mTc, 99mTc -Rp128, Technetium(99mTc) Etrarfolatide, Technetium 99mTc Tetrofosmin, Technetium Tc-99m TMPDA, and Technetium 99mTc Disofenin (Figure (3)).

Two free online https://tox-new.charite.de/protox_II/ and http://biosig.unimelb.edu.au/pkcsm/ were selected for in Silico prediction. The prediction toxicity items are LD50, Class, Polar Surface Area PSA, logP, Hepatotoxicity, Carcinogenity, Immunotoxicity, Mutagenicity, Cytotoxicity, AMES test, Max. tolerated dose (human), hERG I inhibitor, hERG II inhibitor, Oral Rat Acute Toxicity (LD50), Oral Rat Chronic Toxicity (LOAEL), Hepatotoxicity, and Skin Sensitisation as seen in Table (3).

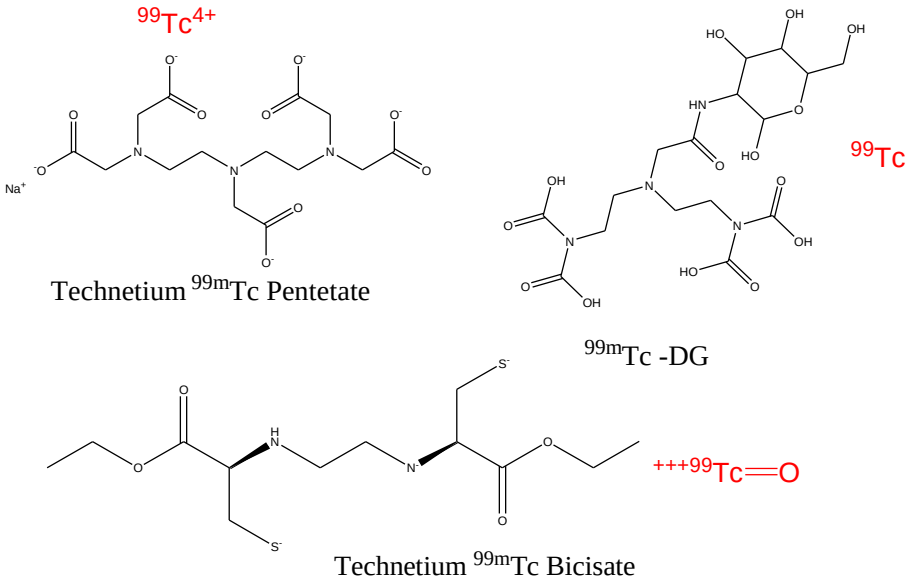

Figure (3). Some of Tc- molecules under study.

3. Results and Discussion

Tc-pharmaceuticals as a chelating complex composed of Tc- radionuclide (essential core) in a certain oxidation state and tagged molecule. 99mTc –biomolecules target particular organ such as liver, heart, lung, brain, thyroid, bone, and kidney to detect cancer occurrence basing on cellular blood flowing, or ionic transportation, molecular charge, lipophilic- hydrophilic balance, blood-brain barrier transportation [12].

According to [13], toxicity definition is the "mount or degree of a substance needed to be poisonous depending on its concentration, frequency of use, personal interaction(s) that determine reversibility and acute-chronic states. When it is at cell level causing organ failure with possible death, this terms systemic toxicity, while local type is reflection of reversibility effects." In lab, single pure material assessment is concerned as actual toxicity testing that may be supported by computerized models [14] known as in Silico, QSAR, ADMET models including online website prediction. This prediction or testing may include natural or synthesized materials.

With above abbreviated statements, two free online websites were selected to predict toxicity of thirty Technetium radioactive materials that may use in imaging of suspected human cancer or other diseases. As a new prediction try of these radiopharmaceuticals, both online (https://tox-new.charite.de/protox_II/ and http://biosig.unimelb.edu.au/pkcsm/) websites were easily to use with the help of isomeric SMILES (Table (2)) obtained from https://pubchem.ncbi.nlm.nih.gov website in numeric and (Yes/No) response (Figures (4 and 5)) that arranged in a tabular form (Table (3)).

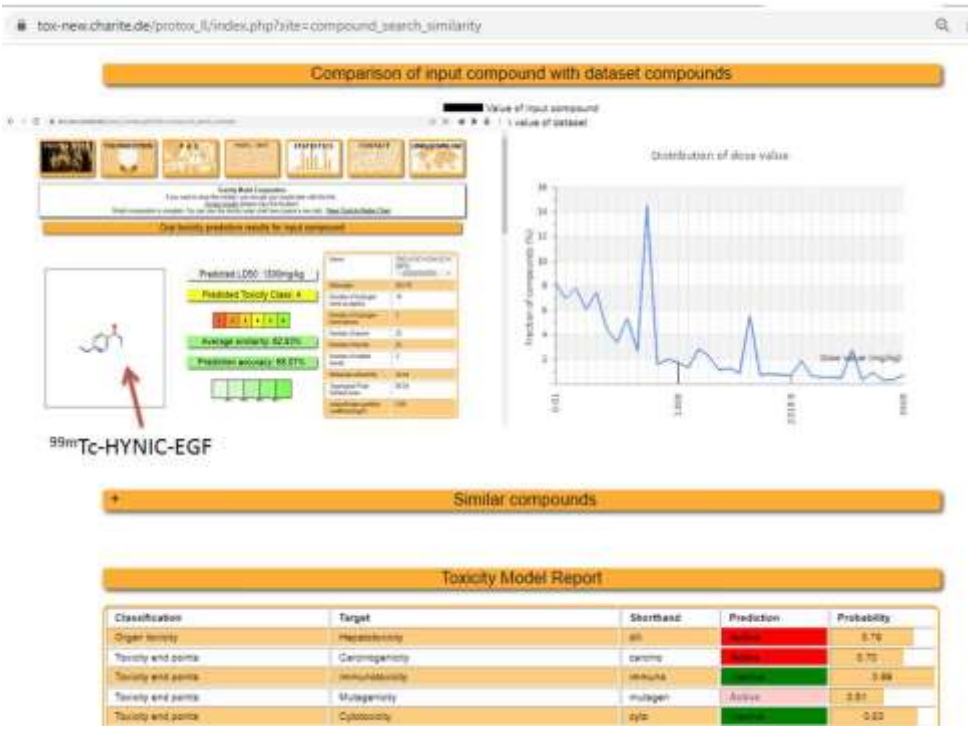

Figure (4). Results of online Protox-II website of ^{99m}Tc-HYNIC-EGF as an example.

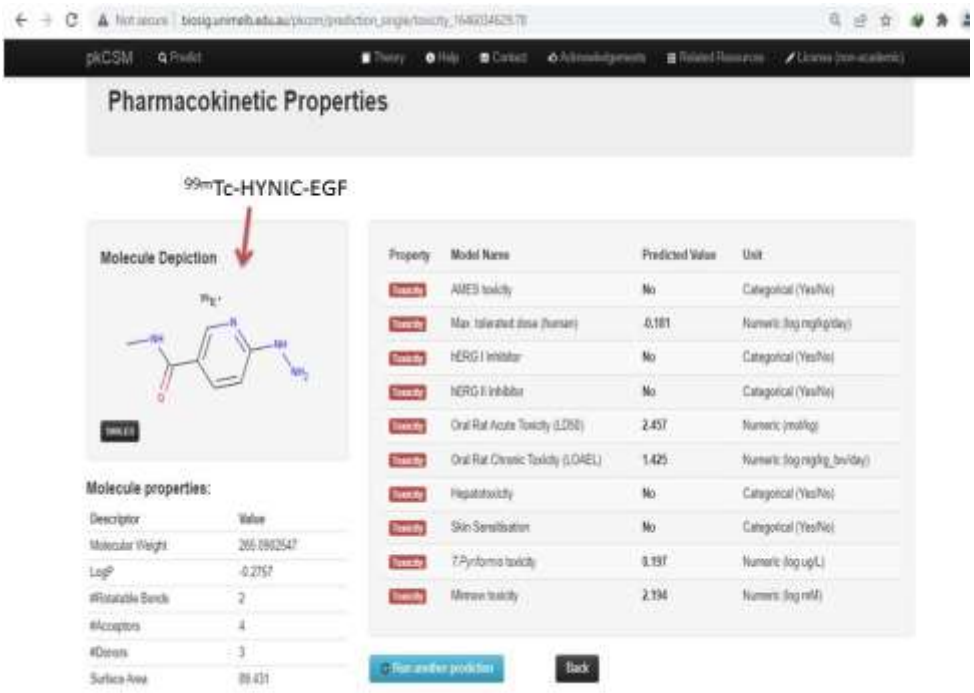

Figure (5). Results of pKcsm -toxicity prediction website of ^{99m}Tc-HYNIC-EGF toxicity as an example.

Table (2). Technetium -99m complexes as recorded in https://pubchem.ncbi.nlm.nih.gov.

Symbol	Agent name	IUPAC name	Isomeric SMILES
Pentetate	Technetium ^{99m}Tc Pentetate Or Technetium ^{99m}Tc DTPA	sodium; 2-[bis[2-[bis(carboxylatomethyl) amino]ethyl]amino]acetate; technetium99(4+) or 2-[bis[2-[bis(carboxylatomethyl) amino]ethyl]amino] acetate; technetium-99(4+)	C(CN(CC(=O)[O-])CC(=O)[O-])N(CCN(CC(=O)[O-])CC(=O)[O-])CC(=O)[O-].[Na+].[99Tc+4] Or C(CN(CC(=O)[O-])CC(=O)[O-])N(CCN(CC(=O)[O-])CC(=O)[O-])CC(=O)[O-].[99Tc+4]
EXM	Technetium ^{99m}Tc Exametazime	(NZ)-N-[(3S)-3-[[3-[[(2S,3Z)-3-hydroxyiminobutan-2-yl]amino]-2,2-dimethylpropyl]amino]butan-2-ylidene]hydroxylamine;(NZ)-N-[(3R)-3-[[3-[[(2R,3Z)-3-hydroxyiminobutan-2-yl]amino]-2,2-dimethylpropyl]amino]butan-2-ylidene] hydroxylamine; technetium-99	C[C@@H](NCC(CN[C@@H](/C(=N/O)/C)C)(C)C)/C(=N/O)/C.C[C@H](NCC(CN[C@H](/C(=N/O)/C)C)(C)C)/C(=N/O)/C.[99Tc].[99Tc]
MIBI	^{99m}Tc MIBI Or Technetium ^{99m}Tc Sestamibi	1-isocyano-2-methoxy-2-methylpropane;technetium-99(7+)	CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.[99Tc+7] Or CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.CC(C)(C[N+]#[C-])OC.[Tc]
CCMSH	^{99m}Tc-CCMSH	(2R)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[(2S)-2-acetamido-3-sulfidopropanoyl]azanidyl-3-sulfidopropanoyl]amino]-4-carboxybutanoyl]amino]-3-(1H-imidazol-5-yl)propanoyl]amino]-2-phenylacetyl]amino]-5-(diaminomethylamino)pentanoyl]amino]-3-(1H-indol-3-yl)propanoyl]amino]-3-[[(2S)-6-amino-1-[2-[[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]carbamoyl]pyrrolidin-1-yl]-1-oxohexan-2-yl]amino]-3-oxopropane-1-thiolate;oxo(99Tc)technetium-99(4+)	CC(C)[C@@H](C(=O)N)NC(=O)C1CCCN1C(=O)[C@H](CCCCN)NC(=O)[C@H](C[S-])NC(=O)[C@H](CC2=CNC3=CC=CC=C32)NC(=O)[C@H](CCCNC(N)N)NC(=O)[C@H](C4=CC=CC=C4)NC(=O)[C@H](CC5=CN=CN5)NC(=O)[C@H](CCC(=O)O)NC(=O)[C@@H](C[S-])[N-]C(=O)[C@@H](C[S-])NC(=O)C.O=[99Tc+4]
TcSn Complex	Technetium-99 Tin(4+,2+) complex	2-[2-[[2-[[4-[2-[2-[2-amino-2-(3,4-dihydroxyphenyl)acetyl] oxyethoxy]ethoxy]-3,5-bis[2-[2-[2-[(2-methylpropan-2-yl)oxy]ethoxy]ethoxy]ethoxy]phenyl]methoxy]-2-oxoethyl]-(carboxymethyl)amino] ethyl-[2-[bis(carboxymethyl)amino]ethyl] amino] acetic	[H+].[H+].[H+].[H+].[H+].[H+].[H+].[H+].CC(C)(C)OCCOCCOCCOC1=CC(=CC(=C1OCCOCCOC(=O)C(C2=CC(=C(C=C2)O)O)N)OCCOCCOCCOC(C)(C)C)COC(=O)CN(CCN(CCN(CC(=O)O)CC(=O)O)CC(=O)O)CC(=O)O.O.O.O.O.[O-][99Tc](=O)(=O)=O.[O-][99Tc](=O)(=O)=O.O=[99Tc]=O.O=[

Symbol	Agent name	IUPAC name	Isomeric SMILES
		acid;dioxo(99Tc)technetium-99; hydron;oxido(trioxo)(99Tc) technetium-99;tin(2+);tin(4+); tetrahydrate	99Tc]=O.[Sn+2].[Sn+2].[Sn+2].[Sn+4].[Sn+4].[Sn+4]
MSH	^{99m}Tc –A-MSH	(4S)-4-[[(2R)-2-[[(2R)-2-acetamido-3-sulfanylpropanoyl]amino]-3-sulfanylpropanoyl]amino]-5-[[(2S)-1-(1H-imidazol-5-yl)-3-oxobutan-2-yl]amino]-5-oxopentanoic acid;(2S)-1-[(2S)-2-acetylpyrrolidin-1-yl]-2-hydrazinyl-3-methylbutan-1-one;(2S)-2-amino-5-(diaminomethylideneamino)-N-[(2R)-1-[[(2S)-1-(1H-indol-3-yl)-3-oxobutan-2-yl]amino]-1-oxo-3-sulfanylpropan-2-yl]pentanamide;ethane;technetium-99;hydrate	CC.CC(C)[C@@H](C(=O)N1CCC[C@H]1C(=O)C)NN.CC(=O)[C@H](CC1=CNC2=CC=CC=C21)NC(=O)[C@H](CS)NC(=O)[C@H](CCCN=C(N)N)N.CC(=O)[C@H](CC1=CN=CN1)NC(=O)[C@H](CCC(=O)O)NC(=O)[C@H](CS)NC(=O)[C@H](CS)NC(=O)C.O.[99Tc]
DMSA	^{99m}Tc-(V) DMSA	carbanide;1,4-dihydroxy-1,4-dioxobutane-2,3-dithiolate;oxo(99Tc)technetium-99(2+)	[CH3-].C(C(C(=O)O)[S-])(C(=O)O)[S-].C(C(C(=O)O)[S-])(C(=O)O)[S-].O=[99Tc+2]
TRODAT	^{99m}Tc TRODAT	2-[2-[[(2R,3S)-3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octan-2-yl]methyl-(2-sulfidoethyl)amino]acetyl]azanidylethanethiolate;oxo(99Tc) technetium-99	CN1C2CCC1[C@H]([C@H](C2)C3=CC=C(C=C3)Cl)CN(CC[S-])CC(=O)[N-]CC[S-].O=[99Tc]
DG	^{99m}Tc -DG	carboxy-[2-[2-(dicarboxyamino)ethyl-[2-oxo-2-[[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]amino]ethyl]amino]ethyl]carbamic acid;technetium-99	C(CN(C(=O)O)C(=O)O)N(CCN(C(=O)O)C(=O)O)CC(=O)NC1C(C(C(OC1O)CO)O)O.[99Tc]
EDDA	^{99m}Tc-EDDA/HYNIC_C(RGDyK)	carboxymethyl-[2-(carboxymethylazanidyl)ethyl]azanide;carboxymethyl-[2-[carboxymethyl(methanidyl) amino]ethyl]azanide;[5-[2-[4-[(2S,5R,8S,14S)-8-(carboxymethyl)-14-[3-(diaminomethylideneamino)propyl]-5-[(4-hydroxyphenyl)methyl]-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentazacyclopentadec-2-yl] butylamino]-2-oxoethyl]pyridin-2-yl]iminoazanide; technetium-99(5+)	[CH2-]N(CC[N-]CC(=O)O)CC(=O)O.C1C(=O)N[C@H](C(=O)N[C@H](C(=O)N[C@@H](C(=O)N[C@H](C(=O)N1)CC(=O)O)CC2=CC=C(C=C2)O)CCCCNC(=O)CC3=CN=C(C=C3)N=[N-])CCCN=C(N)N.C(C[N-]CC(=O)O)[N-]CC(=O)O.[99Tc+5]
HYP	^{99m}Tc-Hypericin	2-[2-[2-[5-[(7,11,13,16,18,22-hexahydroxy-12-(123I)iodanyl-24-methyl-9,20-dioxooctacyclo[13.11.1.12,10.03,8.04,25.019,27.021,26.014,28]octacosa-1,5,7,11,13,15(27),17,21,23-nonaene-5-carbonyl)amino]pentylazanidyl]-2-oxoethyl]azanidyl-2-oxoethyl]azanidyl-2-oxoethanethiolate;technetium-99(4+)	CC1=CC(=C2C3C1C4C5C6=C3C7=C(C(C=C(C7C2=O)O)O)C8=C(C(=C(C(C68)C(=O)C5=C(C=C4C(=O)NCCCCC[N-]C(=O)C[N-]C(=O)C[N-]C(=O)C[S-])O)O)[123I])O)O.[99Tc+4]

Symbol	Agent name	IUPAC name	Isomeric SMILES
BIC	Technetium ^{99m}Tc Bicisate	(2R)-3-ethoxy-2-[2-[(2R)-1-ethoxy-1-oxo-3-sulfidopropan-2-yl]azanidylethylamino]-3-oxopropane-1-thiolate;oxo(99Tc)technetium-99(3+)	CCOC(=O)[C@H](C[S-])NCC[N-][C@@H](C[S-])C(=O)OCC.O=[99Tc+3]
APC	Technetium ^{99m}Tc-Apcitide	sodium;2-[(3R,6S,12R,15R)-3-[[2-[[2-[[(2R)-3-(acetamidomethylsulfanyl)-1-[[2-[[(2R)-3-(acetamidomethylsulfanyl)-1-[2-[2-[(2R)-1-amino-1-oxo-3-sulfidopropan-2-yl]imino-2-oxidoethyl]imino-2-oxidoethyl]imino-1-oxidopropan-2-yl]amino]-2-oxoethyl]amino]-1-oxopropan-2-yl]amino]-2-oxoethyl]amino]-2-oxoethyl]carbamoyl]-12-(3-aminopropylsulfanylmethyl)-15-[(4-hydroxyphenyl)methyl]-5,8,11,14,17-pentaoxo-1-thia-4,7,10,13,16-pentazacyclooctadec-6-yl]acetate;oxygen(2-);technetium-99(5+)	CC(=O)NCSC[C@@H](C(=O)NCC(=O)N[C@@H](CSCNC(=O)C)C(=NCC(=NCC(=N[C@@H](C[S-])C(=O)N)[O-])[O-])[O-])NC(=O)CNC(=O)CNC(=O)[C@@H]1CSCC(=O)N[C@@H](C(=O)N[C@H](C(=O)NCC(=O)N[C@H](C(=O)N1)CC(=O)[O-])CSCCCN)CC2=CC=C(C=C2)O.[O-2].[Na+].[99Tc+5]
DTPA-TOR	^{99m}Tc-DTPA-TOR	[(Z)-4-[4-[2-(dimethylamino)ethoxy]phenyl]-3,4-diphenylbut-3-enyl] 2-[bis[2-(2,6-dioxomorpholin-4-yl)ethyl]amino]acetate;technetium-99	CN(C)CCOC1=CC=C(C=C1)/C(=C(/CCOC(=O)CN(CCN2CC(=O)OC(=O)C2)CCN3CC(=O)OC(=O)C3)\C4=CC=CC=C4)/C5=CC=CC=C5.[99Tc]
PrDP	^{99m}Tc-PrDP	(1-hydroxy-3-imidazol-1-yl-1-phosphonopropyl) phosphonic acid; technetium; dihydrate	C1=CN(C=N1)CCC(O)(P(=O)(O)O)P(=O)(O)O.C1=CN(C=N1)CCC(O)(P(=O)(O)O)P(=O)(O)O.O.O.[Tc]
MDP	^{99m}Tc-MDP	Phosphonomethyl phosphonic acid; technetium; dihydrate	C(P(=O)(O)O)P(=O)(O)O.C(P(=O)(O)O)P(=O)(O)O.O.O.[Tc]
EC-DG	EC-DG-^{99m}Tc	3-oxo-2-[2-[1-oxo-3-sulfido-1-[[(2R,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]amino]propan-2-yl]azanidylethylazanidyl]-3-[[(2R,4R)-2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]amino]propane-1-thiolate;technetium-99(4+)	C(C[N-]C(C[S-])C(=O)NC1[C@H](C(C(O[C@H]1O)CO)O)O)[N-]C(C[S-])C(=O)NC2[C@@H]([C@@H](C(O[C@H]2O)CO)O)O.[99Tc+4]
GHate	Technetium ^{99m}Tc glucoheptonate	(2R,3S,4S,5S,6R)-2,3,4,5,6,7-hexahydroxyheptanoic acid; oxo technetium	C([C@H]([C@@H]([C@@H]([C@@H]([C@H](C(=O)O)O)O)O)O)O)O.C([C@H]([C@@H]([C@@H]([C@@H]([C@H](C(=O)O)O)O)O)O)O)O.O=[Tc]
DO3A-Folate	^{99m}Tc-DO3A-Folate	2-[2-[4-[(7-amino-5-hydroxyquinoxalin-2-yl)methylamino]phenyl]acetyl]-5-oxo-5-[[4,7,10-tris(carboxymethyl)-1,4,7,10-tetrazacyclododec-1-yl]methylamino]pentanoic acid;technetium-99(4+)	C1CN(CCN(CCN(CCN1CC(=O)O)CC(=O)O)CNC(=O)CCC(C(=O)CC2=CC=C(C=C2)NCC3=CN=C4C(=CC(=CC4=N3)N)O)C(=O)O)CC(=O)O.[99Tc+4]
HYNIC-EGF	^{99m}Tc-HYNIC-EGF	6-hydrazinyl-N-methylpyridine-3-carboxamide;technetium-99	CNC(=O)C1=CN=C(C=C1)NN.[99Tc]
MIP-1404	^{99m}Tc-MIP-1404	(2S)-2-[[(1S)-4-[[(1S)-5-[bis[[1-[2-[bis(carboxymethyl)amino]-2-oxoethyl]imidazol-2-yl]methyl]amino]-1-	[CH-]=O.[CH-]=O.[CH-]=O.C1=CN(C(=N1)CN(CCCC[C@@H](C(=O)O)NC(=O)CC[C@@H](C(=

Symbol	Agent name	IUPAC name	Isomeric SMILES
		carboxypentyl]amino]-1-carboxy-4-oxobutyl]carbamoylamino]pentanedioic acid;methanone;technetium-99(4+);chloride	O)O)NC(=O)N[C@@H](CCC(=O)O)C(=O)O)CC2=NC=CN2CC(=O)N(CC(=O)O)CC(=O)O)CC(=O)N(CC(=O)O)CC(=O)O.[Cl-].[99Tc+4]
Hl91	^{99m}Tc-Hl91	(NE)-N-[3-[4-[[(3Z)-3-hydroxyimino-2-methylbutan-2-yl]amino]butylamino]-3-methylbutan-2-ylidene]hydroxylamine; technetium	C/C(=N\O)/C(C)(C)NCCCCNC(C)(C)/C(=N\O)/C.[Tc]
EMIDP	EMIDP^{99m}Tc	[2-(2-ethyl-4-methylimidazol-1-yl)-1-hydroxy-1-phosphonoethyl]phosphonic acid;technetium-99;dihydrate	CCC1=NC(=CN1CC(O)(P(=O)(O)O)P(=O)(O)O)C.CCC1=NC(=CN1CC(O)(P(=O)(O)O)P(=O)(O)O)C.O.O.[99Tc]
MAG3-HBP	MAG3-HBP^{99m}Tc	2-[2-[2-[2-[(4-hydroxy-4,4-diphosphonobutyl)amino]-2-oxoethyl]azanidyl-2-oxoethyl]azanidyl-2-oxoethyl]azanidyl-2-oxoethanethiolate; oxotechnetium(2+)	C(CC(O)(P(=O)(O)O)P(=O)(O)O)CNC(=O)C[N-]C(=O)C[N-]C(=O)C[N-]C(=O)C[S-].O=[Tc+2]
EC20	EC20^{99m}Tc	2R)-2-[(2S)-2-[(2S)-3-[[(4R)-4-[[4-[(2-amino-4-oxo-3H-pteridin-6-yl)methylamino]benzoyl]amino]-4-carboxybutanoyl]amino]-2-azanidylpropanoyl]azanidyl-3-carboxypropanoyl]azanidyl-3-hydroxy-3-oxopropane-1-thiolate;oxo(99Tc)technetium-99(2+)	C1=CC(=CC=C1C(=O)N[C@H](CCC(=O)NC[C@@H](C(=O)[N-][C@@H](CC(=O)O)C(=O)[N-][C@@H](C[S-])C(=O)O)[NH-])C(=O)O)NCC2=CN=C3C(=N2)C(=O)NC(=N3)N.O=[99Tc+2]
Rp128	^{99m}Tc - Rp128	: (2R)-3-[[2-[[(2S,3R)-1-[[(2S)-6-amino-1-[(3R)-3-[(3R)-3-[[(1S)-1-carboxy-4-(diaminomethylideneamino)butyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-oxohexan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-2-oxoethyl]amino]-2-[(2S)-2-[2-(dimethylamino)acetyl]azanidyl-3-hydroxypropanoyl]azanidyl-3-oxopropane-1-thiolate;oxotechnetium(3+)	C[C@H]([C@@H](C(=O)N[C@@H](CCCCN)C(=O)N1CC[C@H](C1)C(=O)N2CC[C@H](C2)C(=O)N[C@@H](CCCN=C(N)N)C(=O)O)NC(=O)CNC(=O)[C@H](C[S-])[N-]C(=O)[C@H](CO)[N-]C(=O)CN(C)C)O.O=[Tc+3]
Etar	Technetium (^{99m}Tc) Etrarfolatide	(2S)-2-[[(2S)-3-[[(4R)-4-[[4-[(2-amino-4-oxo-3H-pteridin-6-yl)methylamino]benzoyl]amino]-4-carboxybutanoyl]amino]-2-azanidyl-1-oxidopropylidene]amino]-N-[(1R)-1-carboxy-2-sulfidoethyl]-4-hydroxy-4-oxobutanimidate;oxotechnetium(4+)	C1=CC(=CC=C1C(=O)N[C@H](CCC(=O)NC[C@@H](C(=N[C@@H](CC(=O)O)C(=N[C@@H](C[S-])C(=O)O)[O-])[O-])[NH-])C(=O)O)NCC2=CN=C3C(=N2)C(=O)NC(=N3)N.O=[Tc+4]
Myview	Technetium ^{99m}Tc Tetrofosmin	: 2-[bis(2-ethoxyethyl)phosphaniumyl]ethyl-bis(2-ethoxyethyl)phosphanium;dioxo(99Tc)technetium-99	CCOCC[PH+](CCOCC)CC[PH+](CCOCC)CCOCC.CCOCC[PH+](CCOCC)CC[PH+](CCOCC)CCOCC.O=[99Tc]=O
DISIDA	Technetium ^{99m}Tc Disofenin	2-[carboxymethyl-[2-[2,6-di(propan-2-yl)anilino]-2-oxoethyl]amino]acetic acid;technetium-99	CC(C)C1=C(C(=CC=C1)C(C)C)NC(=O)CN(CC(=O)O)CC(=O)O.[99Tc]
TMPDA	Technetium Tc-	1-[3-[3-(dimethylamino)propyl-methylamino]-2-(2-methyl-2-sulfidopropyl)azanidylpropyl]azanidyl-2-	[H+].CC(C)(C[N-]CC(CN(C)CCCN(C)C)[N-]CC(C)(C)[S-])[S-].O=[99Tc+3]

Symbol	Agent name	IUPAC name	Isomeric SMILES
	99m TMPDA	methylpropane-2-thiolate;hydron;oxo(99Tc)technetium-99(3+)	

Table (3). Toxicological prediction of Technetium complexes. (Continued).

	Pentetate	EXM	MIBI	CCMSH	TcSn Complex	MSH	DMSA	TRODAT	DG	EDDA	HYP	BIC	APC	DTPA-TOR	PrDP
PSA*	210.37	178.48	55.38	531.3	634.04	581.99	166.27	40.62	278.17	474.22	235.83	81.70	686.13	135.23	363.92
logP*	-9.36	4.9	3.37	2.57	-3.41	4.29	-1.7	3.02	-3.09	1.07	4.38	0.18	-3.52	2.47	-1.58
Class*	2	4	5	5	4	5	5	4	5	6	3	5	6	4	4
LD_{50}*	5000	435	2450	2400	1589	2400	5011	874	5000	6000	100	3470	10000	1700	1400
Hepatotox.*	0.96	0.90	0.90	0.89	0.85	0.68	0.91	0.78	0.85	0.68	0.79	0.76	0.84	0.87	0.88
Carcinogen.*	0.61	0.50	0.63	0.66	0.71	0.54	0.85	0.68	0.69	0.51	0.72	0.72	0.72	0.54	0.68
Immunotox.*	0.98	0.99	0.99	0.69	0.99	0.99	0.99	0.90	0.97	0.98	0.99	0.99	0.87	0.99	0.98
Mutagen.*	0.87	0.58	0.78	0.70	0.67	0.62	0.83	0.64	0.65	0.54	0.63	0.66	0.72	0.76	0.61
Cytotox.*	0.75	0.65	0.75	0.76	0.62	0.64	0.75	0.60	0.68	0.60	0.67	0.68	0.63	0.75	0.67
Max. tolerated dose (human)**	0.435	1.028	0.049	0.438	0.438	0.438	0.508	-0.351	0.437	0.442	-1.26	0.126	0.487	-0.009	0.335
Oral Rat Acute Toxicity (LD50)**	2.482	2.282	1.953	2.482	2.482	2.482	2.178	2.883	2.482	2.482	2.909	3.117	2.586	2.521	2.477
Oral Rat Chronic Toxicity (LOAEL)**	2.204	0.201	0.962	8.884	3.657	3.409	3.097	0.733	4.079	4.046	1.57	1.219	3.506	3.223	7.482
AMES **	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No
hERG I inhibitor**	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor **	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No	No	Yes	No
Hepatotox.**	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Skin Sensitisation **	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Table (4). Toxicological prediction of Technetium complexes.

	MDP	EC-DG	GHate	DO3A-Folate	HYNIC-EGF	MIP-1404	Hl91	EMIDP	MAG3-HBP	EC20	Rp128	Etar	Myview	DISIDA	TMPDA
PSA*	287.82	238.5	334.43	292.39	80.04	499.34	89.24	363.92	252.29	342.92	379.65	379.62	162.34	106.94	23.55
logP*	-1.53	-5.82	-8.39	0.47	0.89	-5.98	2.98	-0.62	-1.6	0.37	-2.04	0.22	6.88	2.41	2.52
Class*	4	5	6	4	4	4	4	4	4	3	4	3	4	3	4
LD_{50}*	1125	5000	9800	1700	1300	1100	810	608	552	135	2000	135	750	234	1250
Hepatotox.*	0.97	0.82	0.96	0.78	0.79	0.76	0.83	0.76	0.91	0.86	0.94	0.87	0.88	0.88	0.87
Carcinogen.*	0.71	0.75	0.83	0.62	0.70	0.76	0.50	0.68	0.73	0.60	0.57	0.55	0.68	0.71	٠.82
Immunotox.*	0.99	0.99	0.99	0.95	0.99	0.99	0.99	0.99	0.97	0.69	0.99	0.89	0.99	0.98	0.81
Mutagen.*	0.77	0.66	0.78	0.63	0.51	0.67	0.56	0.60	0.61	0.60	0.64	0.58	0.77	0.80	0.76
Cytotox.*	0.76	0.74	0.80	0.69	0.83	0.73	0.66	0.66	0.65	0.69	0.71	0.68	0.80	0.52	0.75
Max. tolerated dose (human)**	0.521	0.992	0.559	0.405	-0.181	0.438	0.579	0.417	0.79	0.215	0.642	0.174	1.099	0.454	0.187
Oral Rat Acute Toxicity (LD50)**	1.928	1.726	2.51	2.482	2.457	2.482	2.169	2.482	2.289	2.5	2.501	2.528	0.92	2.346	2.776
Oral Rat Chronic Toxicity (LOAEL)**	5.377	4.181	7.653	5.256	1.425	3.949	0.734	7.133	5.104	3.283	2.489	3.353	0.411	2.661	0.636
AMES **	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG I inhibitor**	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

	MDP	EC-DG	GHate	DO3A-Folate	HYNIC-EGF	MIP-1404	Hl91	EMIDP	MAG3-HBP	EC20	Rp128	Etar	Myview	DISIDA	TMPDA
hERG II inhibitor **	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	Yes
Hepatotox. **	No	No	No	Yes	No	No	No	No	No	Yes	Yes	Yes	No	Yes	No
Skin Sensitisation **	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No

Figure (5). Numeric toxicity prediction data from both websites.

Topological Polar Surface Area, PSA		**Octanol/ water partitioning coefficient , logP**		**Toxicity Class**		**LD_{50}, mg/Kg**	
Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
23.55	686.13	-9.36	6.88	2	6	100	10000
Hepatotoxicity		**Carcinogenicity**		**Immunotoxicity**		**Mutagenicity**	
Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
0.68	0.97	0.5	0.85	0.69	0.99	0.51	0.87
Cytotoxicity		**Max. tolerated dose (human), log mg/Kg/day**		**Oral Rat Acute Toxicity (LD_{50}), mol./Kg**		**Oral Rat Chronic Toxicity (LOAEL), log mg/Kg -bw/day**	
Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
0.52	0.83	-1.26	1.099	0.92	3.117	0.201	8.884

Polar surface area (PSA) is topological molecular descriptor depends upon polar atoms like oxygen, phosphor, or nitrogen attached to hydrogen (functional groups) as a basic in biological conformations in cooperation with QSAR prediction descriptors like octanol/ water partitioning coefficient (logP) in drug transport study. This virtual screener presents a primary image of molecular polar interaction affected barrier crossing in medicinal chemistry subject [15, 16]. Lipophilic-hydrophilic ratio (logP) measures partitioning target molecule in n-octanol/water phases in equilibrium state expressed in logarithm form ranged from -3 (low hydrophobic or high hydrophilic) to +10 (high hydrophobic or low hydrophilic). Usually, this character is measured by shake-flask method [17]. It is important in drug research and development, toxicity, chromatographic methods especially High Performance Liquid Chromatography (HPLC) [18]. From Table (3) and Figures (6 and 7), both PSA and logP were influenced by Technetium oxidation state, heteroatoms presence, molecular weight, stereo-structure, ring (aromatic, hetero, aliphatic), branching, length of chain, and other effective factors.

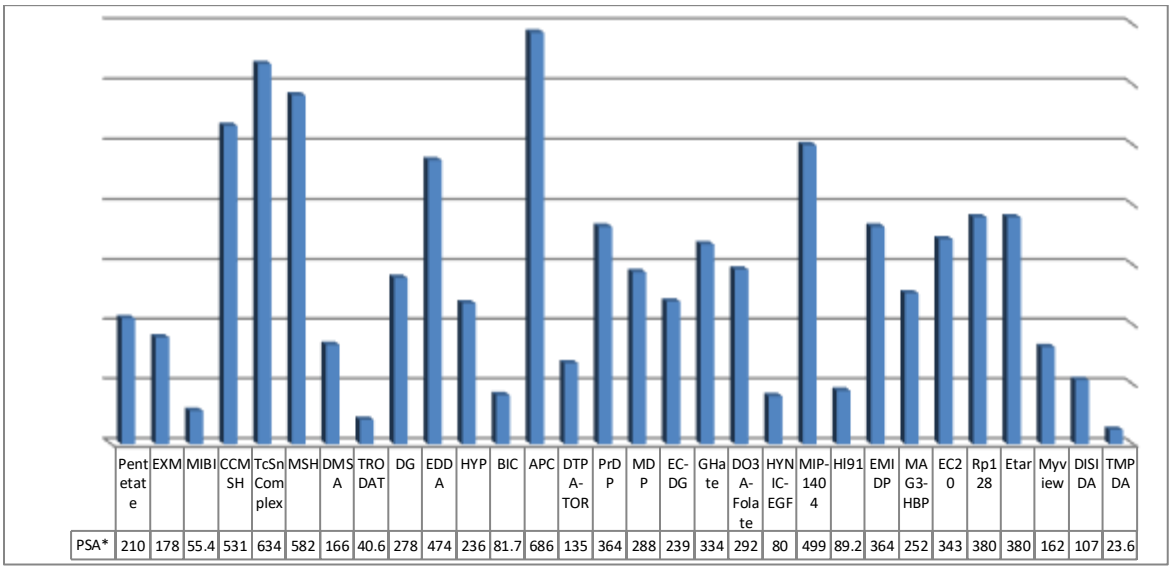

Figure (6). Topological Polar Surface Area (PSA) of the tested Tc-radiomolecules.

Among toxicity characters, medium lethal dose (LD_{50}) and toxicity class are important factors in characterization of chemicals which are classified according EPA (U.S Environmental Protection Agency) and GHS (Globally Harmonized System of Classification and Labelling of Chemicals). LD_{50} can be defined as the required dose of chemical that kills 50% (half) of the rats under test and ranges from toxic to nontoxic (safe) (in numbers, it ranges from less than 50 mg/Kg to more than 2000 mg/Kg). Table (3) and Figure (8) show medium lethal dose to rodent (LD_{50}) ranged from (100-10000)mg/Kg calculated by https://tox-new.charite.de/protox_II/ while http://biosig.unimelb.edu.au/pkcsm/ showed Oral Rat Acute Toxicity (LD_{50}) range (0.92 to 3.117) mol/Kg. LD_{50} values were influenced by PSA and logP and their determinants as mentioned above. Pentetate was predicted as the most toxic material (Class 2).

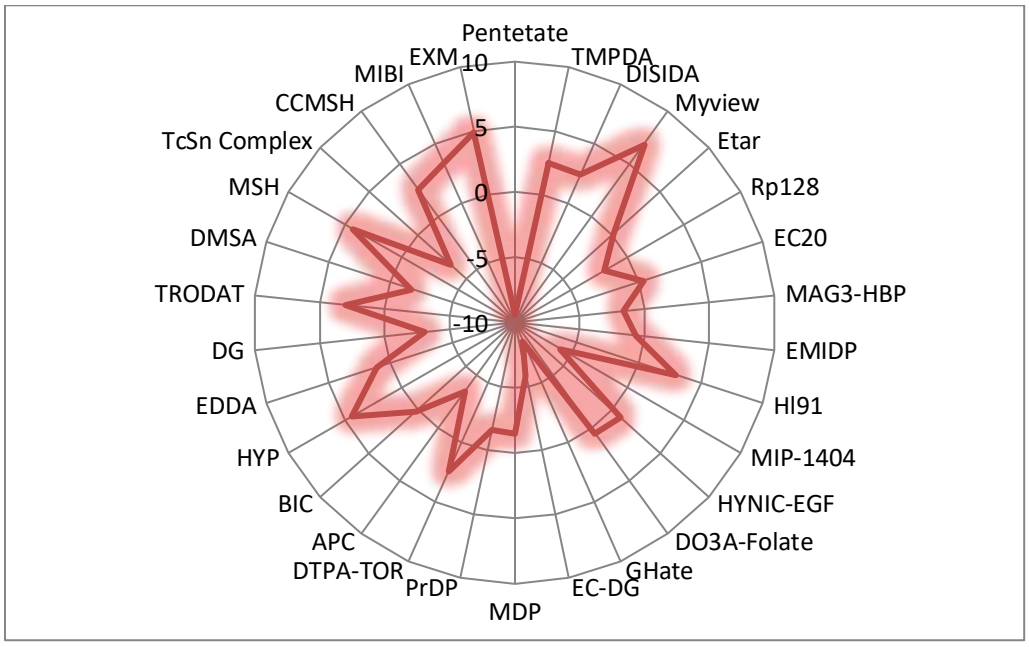

Figure (7). Octanol/ Water partitioning coefficient (logP) of the tested Technetium -99 molecules.

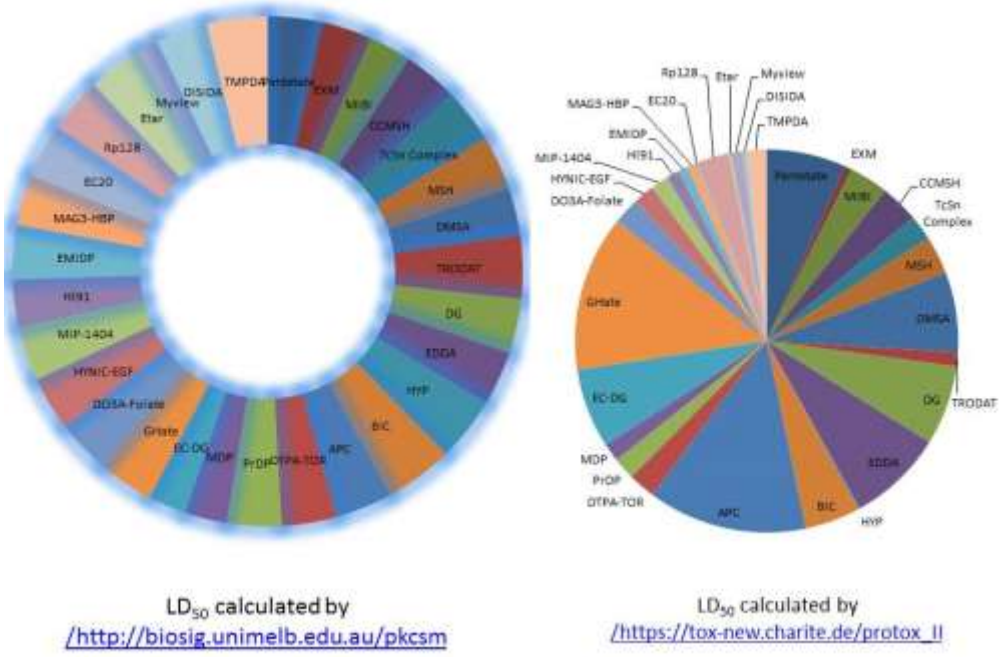

Figure (8). LD_{50} prediction by both online websites.

To detect acute liver failure resulted by chemical substance especially with radionuclide, hepatotoxicity character is the correct scientific classification. Age, gender, smoking, incorrect consumption of drug especially non-hydrophilic or alcohol, genetic, changing in β- oxidation of fatty acids, mitochondrial abnormality in its function, or other factors may increase liver toxicity in either self-compatibility or predictable form towards cell or organ death [19]. According to [20], more than 1000 drugs have been linked to this failure where hepatitis histological characterizations are acute, chronic fulminant, cholestatic, biliary plugs, macro- or micro- lipid droplet in the cytoplasm with non-particular symptoms. Also, High level of special liver enzymes may highly influence liver damage that may be cooperated with candidate toxic materials [19]. Highest Drug Induced liver injury (Hepatotoxicity) probability of these Tc- complexes in percentage term was with MDP(97%) then Pentetate (96%), and GHate (96%) as predicted by https://tox-new.charite.de/protox_II/. Other online website http://biosig.unimelb.edu.au/pkcsm/ prediction showed 50% of Tc-molecules were with (Yes) response including Pentetate but not MDP or GHate.

Encyclopedia of Toxicology [21] states that" potential occupational carcinogen means any substance or combination or mixture of substance, which causes an increased incidence of benign and / or malignant neoplasms or a substantial decrease in the latency period between exposure and onset of neoplasms in human or in one or more experimental mammalian species as the result of any oral, respiratory, or dermal exposure, or any other exposure which results in the induction of tumors at a site other than the site of administration". Even with its expensive and time consuming, carcinogenicity is essential requirement in drug and/or its metabolite(s) industry research and development for short – or long- term testing after mutagenicity evaluation to prevent tumor initiation as a result of using candidate medication [22]. Time and cost require primer, ultimate and post-final carcinogenicity testing. This post- validation testing is necessary to overcome any accidental health problems including new lab and *in Silico* trials. From this objective point, Table (3) and Figure (9) show the prediction probability of carcinogenicity where its percentage range was (50% - 85%) and DMSA (85%), GHate (83%), and TMPDA (82%) were with highest point in this study.

Every living body has a defense system against foreign material(s) and immune system in human is the protector against tumor, fungi, bacteria, virus, parasites, chemicals, etc. with makeable distinguishing roles (self- from non-self-component(s)). Immunotoxics may generate reactive oxygen species, and/ or induce severe damage in DNA or P53 gene expression [23]. In Silico study is among other evaluating methods depending on tissue, blood, and urine examinations that assess mechanism, response, then outcome of Tc- radiomaterials (and other toxins). It is noticeable results that most of Tc-materials under study were with high probability of immunotoxicity (Table (3), Figure (9)) where:

- Percentage range (69-99)%.
- More than 55% (seventeen Tc- molecules from thirty tested molecules) had 99% prediction probability of immunotoxicity.
- More than 12% (four Tc-molecules from thirty tested molecules) had 99% prediction probability of immunotoxicity. So, 70% of tested molecules had more than 98% prediction probability of immunotoxicity (twenty-one Tc- molecules from thirty tested molecules).

In mutagenicity issue, bacteria influenced by toxics represent induced mutation assay that is known as Ames Test. In consequence, exposure to chemical(s) dramatically changes DNA (nucleotide sequence) as mentioned by many in vivo studies [24, 25]. In this study, mutagenicity probability (percentage term) that tested by https://tox-new.charite.de/protox_II/ ranged from 51% to 87% where 90% of Tc-molecules under prediction were below 80% mutagenicity probability and only DISIDA (80%), DMSA (83%), and Pentetate (87%) were with highly mutagenicity probability (Table (3), Figure (9)).

By another online website http://biosig.unimelb.edu.au/pkcsm/, mutagenicity (Ames test) was with (No) response for all Tc- molecules expect MIBI (Yes) response. Reasons behind these data variations by the same website or in comparison of both online websites may be related to oxidation state of Technetium radionuclide, heteroatoms presence, concentration, surface properties, bio-target specifications, and mathematical base of each website.

The other toxicity term that deals with *in vitro* compatibility of chemical under experimental cellular conditions is cytotoxicity. In this term, primary cell structures and functions may be changed when it exposures to toxic substance and this changing may observed by computational prediction and lab methods [26, 27]. In lab, its mechanism assay mainly involves substance chemistry and response with using cell lines of corneal, lung, ovary, renal, three-dimensional tissue culture and various microorganisms [28]. In human body, inducing cell-lysis and apoptosis mechanisms are the main role in cellular cytotoxicity that can be measured *in vitro* with radioactive and non- radioactive reagent(s) release as measurable label target in short time with non-inflammatory response [29]. But these cost-time measuring methods can be in advanced replaced with computational models as approximated approach.

In this study, cytotoxicity probability (Table (3), Figure (9)) ranged from 52% to 83% where ^{99m}Tc-HYNIC-EGF had the highest prediction probability (83%). As initial conclusion about this predictor, high cytotoxicity range is an elementary indication of deficiency in cellular biological mechanisms.

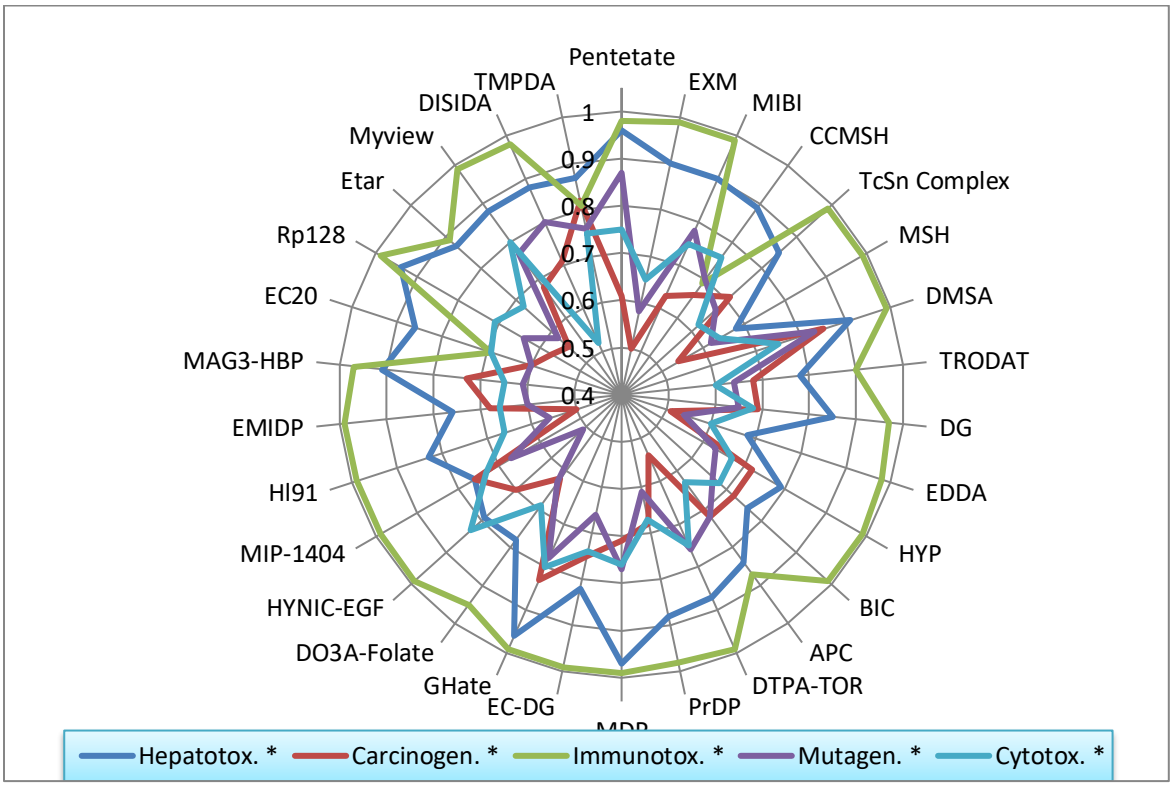

Figure (9). Probability prediction of Hepatotoxicity, Carcinogenity, Immunotoxicity, Mutagenicity, and Cytotoxicity of the molecules under test.

The second website (pkCSM) results of Maximum tolerated dose (human), Oral Rat Acute Toxicity (LD_{50}), and Oral Rat Chronic Toxicity (LOAEL) are presented in Table (3) and Figure (10). These results were ranged ((0.0549-0.079) mg/Kg/day), (0.92-3.117) mol./Kg, and (1.588-768423829.33) mg/Kg.BW/day respectively. Maximum tolerated dose data display an obvious form about Tc-molecules action dose threshold in human as a starting point in clinical trial, Phase I. LD_{50} of rat in its definition submit the amount leads to 50% death of test animals. While, Oral Rat Chronic Toxicity (LOAEL) outcomes offer lowest dose over long time of exposure in an observed adverse effect. All three toxicological predictors ensure that Tc-molecules under this evaluation were toxic even at low numerical data [30].

In cardiac repolarization, hERG blockage by chemical causes a lethal action in life-threatening. Here, all Tc-molecules showed (No) response to hERG I inhibition while 23% of them showed (Yes) to hERG II inhibition as tabulated in Table (3) that match with some antibiotic, antipsychotics, and others [31] as they exhibit in www.crediblemeds.org website.

Finally, it is remarkable notice that only H191 had skin sensation (Table (3)). So, more than 96% of tested Tc-molecules were with no allergic contact dermatitis induction in all cutaneous sensation points related to the median nerve [32].

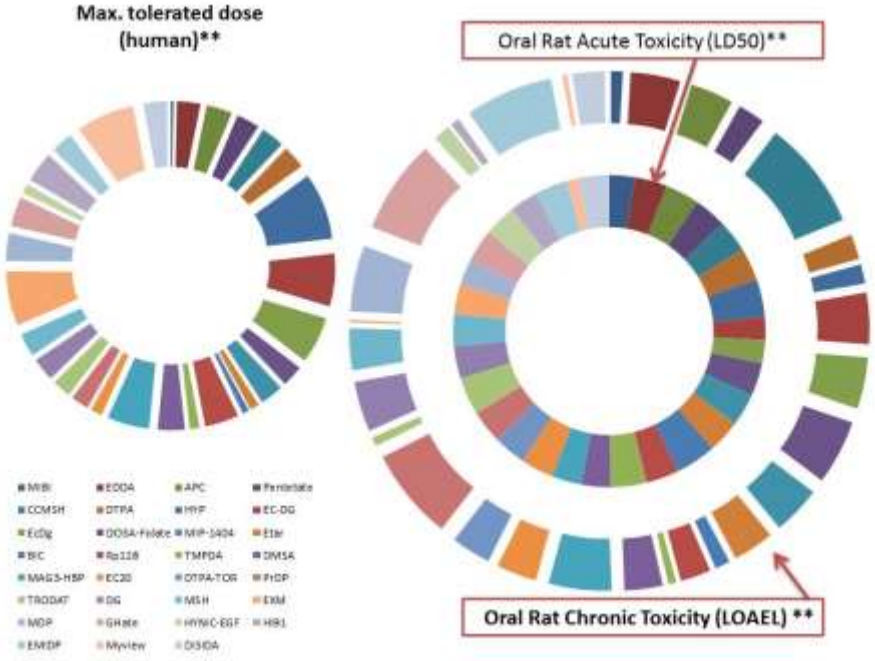

Figure (10). Results of Maximum tolerated dose (human), Oral Rat Acute Toxicity (LD_{50}), Oral Rat Chronic Toxicity (LOAEL).

To get an apparent vision about this study, Pentetate molecule is an example. This substance is a radionuclide imaging complex for renal, lung, and gastrointestinal tract, and it is stable to oxidation by ascorbic acid or other free radical scavengers. Also, it is bounded to protein and excreted by kidney where it used in blood flow in brain and heart, cerebrospinal fluid, and inhalation studies. It is produced by several trademarks including DRAXMAGE® with health notice about eye and skin irritations at high quantity. According to this prediction study, this substance belongs to class 2 (fatal if swelled, inhaled, in contact to eye) with very highly toxic probability to liver and immune system. It may have considered as mutagen- cytotoxic agent but not as an inhibitor of hERG Types I and II or skin sensation. It is structurally unsafe compound where it may be interacted with molecular target giving toxic response. Here, toxicity notes are mainly depending upon concentration, exposure time, genetic factors of human, health problems, and others.

4. Conclusions

According to literatures review about this subject, this is the first try in Iraq and other countries to study Technetium – radiomolecules with *in Silico* approach depending on two online websites. Thirty biomolecules labeled with Technetium-99 in relationship to toxicity issues were studied by applying two online websites [https://tox-new.charite.de/protox_II/ and http://biosig.unimelb.edu.au/pkcsm/]. The obtained data showed that these Tc-biomolecules are structurally unsafe having toxic response to liver, immune system, cellular components, DNA, and/or cardiac repolarization through hERG inhibition of action. These mainly conclusion notes depended upon Technetium oxidation state, heteroatoms presence, surface properties such as molecular weight, stereo-structure, ring (aromatic, hetero, aliphatic), branching, length of chain, and other effective factors besides bio-target specifications, concentration, exposure time, genetic factors of human, and health problems.

References

[1] J. Mukiza, E. Byamukama, J. Sezirahiga, K. Ngbolua, V. Ndebwanimana " A review on Technetium and Rhenum based radiopharaceuticals for diagnostic imaging and therapeutic nuclear medicine." *Rwanda Medical Journal* vol.75, no. 1, pp.14-22, 2018.

[2] A. Boschi, L. Uccelli, and P. Martini. "A picture of modern Tc-99 radiopharmaceuticals: Production,

Chemistry, and applications in molecular imaging." *Applied Sciences* vol. 9, article number 2526, 2019.
[3] https://www.epa.gov/radiation/radionuclide-basics-technetium-99.
[4] T. Ruth. "The shortage of Technetium -99m and possible solutions." *Annual Review of Nuclear and Particle Science* vol. 70, pp.77-94, 2020.
[5] National Research Council (US) Committee on " Medical Isotope Production without Highly Enriched Uranium. Medical Isotope Production without Highly Enriched Uranium", Washington (DC): National Academies Press (US). Molybdenum-99/ Technetium-99m production and use, Chapter 2. 2009, Available from https://www.ncbi.nlm.nih.gov/books/NBK215133/
[6] U. Mauchsch, R. Runge, G. Wunderlich, R. Freudenberg, A. Naumann, and J. Kotzerke. "Comparison of the radiotoxicity of the ^{99m}Tc – labeled compounds ^{99m}Tc-pertechnetate, ^{99m}Tc-HMPAO and ^{99m}Tc- MIBI." *International Journal of Radiation Biology (Proceeding of the 8th Auger symposium)*, vol. 92, no.11, pp. 698-706 2016.
[7] I. Costa, N. Siksek, A. Volpe, F. Man, K. Osytek, E. Verger, G. Schettino, G. Fruhwirth, and S. Terry. "Relationship of in Vitro toxicity of Technetium -99m to subcellular localisation and absorbed dose." *International Journal Of Molecular Sciences* vol. 22, article number 13466, 2021.
[8] T. Witney and P. Blower. " The chemical tool-kit for molecular imaging with radionuclides in the age of targeted and immune therapy." *Cancer Imaging* vol. 21, pp. 18, 2021.
[9] S. Lui. " Bifunctional coupling agents for radiolabeling of biomolecules and target –specific delivery of metallic radionuclides." *Advanced Drug Delivery Reviews* vol. 60, no.12, pp. 1347-1370. 2008.
[10] A. Duatti. " Review on 99mTc radiopharmaceuticals with emphasis on new advancements." *Nuclear Medicine and Biology* vol. 92, pp. 202-216, 2021.
[11] D. Papagiannopulou. " Technetium-99m radiochemistry for pharmaceutical applications." *Journal of Labeled Compounds and Radiopharmaceuticals* vol. 60, no. 11, pp. 502-520, 2017.
[12] M. James and S. Gambhir. " A molecular imaging primer: modalities, imaging agent, and applications." *Physiological Reviews* vol. 92, no. 2, pp. 897-965, 2012.
[13] J. Buckle. " Clinical Aromatherapy. Essential oils in Healthcare", 3rd edition, Elsevier Ltd, UK, 2016.
[14] R. Tisserand and R. Young. " Essential oil safety. A guide for health care professionals", 2nd edition, Elsevier Ltd, UK, 2014.
[15] S. Prasanna and R. Doerksen. "Topological polar surface area: a useful descriptor in 2D-QSAR." *Current Medicinal Chemistry* vol. 16, no. 1, pp. 21-41, 2009.
[16] J. Taylor and D. Triggle (editors)." Comprehensive Medicinal Chemistry", Volume II, Reference work, Elsevier Ltd, UK, 2007.
[17] H. Cumming and C. Rücker. " Octanol-water partition coefficient measurementby a simple ^{1}HNMR method." *ACS Omega* vol. 2, no. 9, pp. 6244-6249, 2017.
[18] K. Hason, D. Hoff, T. Lahren, D. Mount, A. Squillace, and L. Burkhard. " Estimating n-octanol-water partition coefficients for neutral highly hydrophobic chemicals using measured n-butanol-water partition coefficients." *Chemosphere* vol. 218, pp. 616-623, 2019.
[19] N. Osna, T. Donohue, and K. Kharbanda. " Alcoholic liver disease: pathogenesis and current management." *Alcohol Research, Current Review* vol. 38, no.2, pp. 147-161, 2017.
[20] A. Cano, L. Cifuentes, and P. Amariles. " Structural literature review of hepatic toxicity caused by medicine." *Revista Colombiana de Gastroenterologia* vol. 22, no.4, pp. 337-348, 2017.
[21] I. Kamrin." Encyclopedia of Toxicology, Carcinogen Classification Schemes," P. Wexler (editor), 3rd edition, reference module in biomedical sciences, Academic Press, Elsevier, USA, 2014.
[22] P. Ducheyne (editor). " Comprehensive Biomaterials II, Reference work, Biocompatibility, Surface engineering, and Delivery of drugs, gene, and other molecules," volume 4, Elsevier Ltd., USA, 2017.
[23] M. Bou Zerdan, S. Moussa, A. Atoui, and H. Assi. " Mechanisms of immunotoxicity: stressors and evaluators." *International Journal of Molecular Sciences* vol. 22, no. 15, pp. 8242, 2021.
[24] H. Du, B. Pan, and T. Chen, "Evaluation of chemical mutagenicity using next generation sequencing: A review," *Journal of Environmental Science and Health*, Part C, vol. 35, no. 3, pp. 140-158, 2017.
[25] S. Lamponi. " Preliminary in Vitro cytotoxicity, mutagenicity, and antitumoral activity evaluation of graphene flake and aqueous graphene paste." *Life* vol. 12,pp. 242 (21 pages), 2022.
[26] A. Schrey, J. Seeber, M. Drwal, P. Zwicker, N. Schultze, et al., "Computational predication of immune cell cytotoxicity," *Food and Chemical Toxicology*, vol. 107, Part A, pp. 150-166, 2017.
[27] B. David, M. Jane, and C. Marco, "The natural cytotoxicity receptors in health and disease," *Frontiers in*

Immunology, vol.10, pp. 909, 2019.
[28] Z. Li. " *In Vitro* micro tissue and organ models for toxicity testing", volume 5, in *Comprehensive Biotechnology*, 3rd edition, Elsevier, USA, 2019.
[29] M. Karimi, E. Lee, M. Bachmann, A. Salicioni, E. Behrens, T. Kambayashi, and C. Balwin. " Measuring cytotoxicity by bioluminescence imaging outperforms the standard Chromium-51 release assay." *PLOS ONE* vol. 9, no. 2, e89357, 2014.
[30] C. Araujo-Lima, R. Nunes, R. Carpes, C. Ajub, and I. Felzenszwab." Pharmacokinetic and toxicological evaluation of zinc gluconate- based chemical sterilant using *in vitro* and *in Silico* approaches." *BioMed Research International* vo. 2017, Article ID 5746768, 8 pages, 2017.
[31] S. Munaware, M. Windley, E. Tse, M. Todd, A. Hill, J. Vandenberg, and I. Jabeen. " Experimental validated pharmacoinformatics approach to predict hERG inhibition potential of new chemical entities." *Frontiers in Pharmacology* vol. 9, Article 1035, 20 pages, 2018.
[32] M. Aminoff and R. Daroff (editors). " Encyclopedia of the Neurological Sciences," reference work, 2nd edition, Elsevier Ltd., US, 2014.