Structure-Activity Relationship Review Document

for

Sulfolane
[CAS No. 126-33-0]

Supporting Nomination for Toxicological Evaluation by the National Toxicology Program

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Abstract

There are numerous free and pay quantitative structure-activity relationship (QSAR) programs that are available to assess the biological and toxicological activity of a compound. These programs use a variety of databases and models to evaluate varied endpoints including neurotoxicity, carcinogenicity, and skin sensitization. It is noted that while several programs may evaluate the same endpoint (e.g., neurotoxicity), the programs may model widely different specific endpoints (e.g., pup behavior vs. *in vitro* neuronal cell death). The following document used several different QSAR programs to evaluate potential toxicity activity of sulfolane. The programs that were used were: GeneGo, Leadscope, Toxtree, the OECD Toolbox, Lhasa Derek, and MultiCASE. Descriptions of the models and results obtained are provided.

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1.0 GeneGo

The GeneGo summary provides an overview of the MetaDrug™ analysis method and the results of the quantitative structure-activity relationship (QSAR) analysis conducted on sulfolane. The background information provided in the GeneGo summary was obtained from the GeneGo Online Help Section (GeneGo, 2011a), unless otherwise noted.

1.1 Background and Overview of MetaDrug Analysis Methodology

MetaDrug, from GeneGo, Inc., combines chemical structural analysis tools (metabolite prediction, QSAR, structural similarity searching), a structure-activity database, and a systems biology database of molecular interactions (protein-protein, compound-protein, protein-enzymatic reaction, compound-enzymatic reaction), canonical signaling and metabolic pathways, and gene-biological property associations.

The MetaDrug analysis starts with uploading a chemical structure. Potential metabolites for the query compound are predicted and separated into major and minor phase 1 and phase 2 metabolites. A suite of pre-defined QSAR models is used to predict chemical and biological properties of the molecule (and, optionally, its metabolites). These include models for substrate affinity, inhibition of metabolic enzymes and transporters, water solubility, blood-brain barrier penetration, and plasma protein binding.

MetaDrug uses three methods with which to associate compounds to protein targets, which are subsequently subjected to functional analysis. The first method uses the MetaBase database, which contains compound-protein interactions. This database directly allows compounds with known biological activities to be incorporated into networks and their pharmacological properties further investigated. The second method uses QSAR predictions of protein target affinity from the included models that define a limited number of potential targets for novel molecules and/or their metabolites submitted for analysis. The third method performs a similarity search for the structure and its major metabolites against the database of existing structures and their targets. Potential targets for novel molecules are inferred through structurally similar compounds in the database (GeneGo, personal communication).

Having defined a list of known and predicted targets using the above approach, MetaDrug uses enrichment analysis (hypergeometric distribution) of the list across nine pre-defined biological ontologies to identify biological pathways, biological, metabolic, or toxicological processes, or diseases that may be affected by interaction of the query compound and its metabolites with biological systems. These are reported as enrichment scores (-log of the hypergeometric p-value) for the top 11 enriched categories in each ontology and, for canonical pathway maps, images of the top three enriched pathway maps with predicted targets of the query compound flagged (GeneGo, personal communication).

1.2 Metabolites

MetaDrug predicts first-pass and second-pass metabolites. Reactions are classified as Phase 1 and Phase 2, respectively. Phase 1 metabolic reactions typically include non-synthetic reactions (e.g., oxidation, reduction, and hydrolysis). These reactions are typically catalyzed by cytochrome P450 (CYP450) enzymes to increase chemical solubility. Phase 2 reactions include conjugation reactions with glucuronic acid, sulfate, glutathione, and amino acids. These

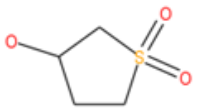
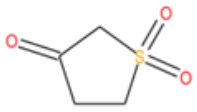
reactions are proposed to target the chemical for excretion. Seventy-four metabolic pathways (49 Phase 1 and 25 Phase 2) are used to predict metabolites. [Note: The help section notes that there are 81 metabolic rules; however, the total number of rules noted in the help section is 74.] The metabolic pathways describe "most likely metabolic reactions categorized according to the particular type of chemical transformation (e.g., aromatic hydroxylation or ester hydrolysis)." Phase 1 pathways include: C-oxidation, quinone formation, N-oxidation, S-oxidation, P-oxidation, spontaneous (e.g., ketone tautomerization, vicdiol to aldehyde), reduction, and hydrolysis. Phase 2 pathways include: glucuronide transfer, sulfate transfer, glutathione transfer, methyl transfer, cysteine transfer, other conjugation reactions (e.g., O-phosphate transfer), conjugation of amino acids, and N-acetyl transfer.

The metabolic pathways were derived from the analysis of a manually annotated human drug metabolism database that includes xenobiotic reactions, enzyme substrates, and enzyme inhibitors with kinetic data. MetaDrug also includes rules to predict and identify likely reactive metabolites (e.g., quinines and phenols).

In addition to classification as first-pass or second-pass metabolites, metabolites are further classified as predicted major or minor metabolites. The classification of major and minor metabolites is based on a score identified as the occurrence rate (OC). The OC is the "ratio of the occurrence of a particular metabolic reaction to the total number of metabolic reactions in the MetaCore™/MetaDrug™ database." The occurrence frequency is assigned to a metabolite as the negative log value. The greater the score, the higher the frequency the predicted metabolic reaction is present in the database. Major predicted metabolites have the highest OC values. Predicted metabolites are also identified as major metabolites "if they are produced by specific metabolic reactions, or when unique or highly reactive substructures undergo a transformation."

A single first-pass and a single second-pass metabolite were predicted to occur with sulfolane. The structures of these predicted metabolites are provided below.

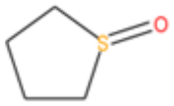
Metabolites

Pass	Name	Structure	SMILES	Formula	MW
First pass Major metabolites	1_Aliphatic_hydroxylation1		OC1CS(CC1)(=O)=O	C4H8O3S	136.02
Second pass Major metabolites	1_Aliphatic_hydroxylation1_Alcohol_dehydrogenation1		O=C1CS(CC1)(=O)=O	C4H6O3S	134

1.3 Structurally Similar Chemicals in Database

Based on the hypothesis that structurally similar compounds produce similar biological effects, similarity searches are conducted by searching the MetaCore™/MetaDrug™ database and results are ranked based on similarity (%). Two-dimensional fingerprints are developed for each chemical using the Accelrys Accord Cartridge. "Fingerprints are arrays generated for each molecule and containing as its elements binary hashes representing particular substructures (patterns) within that molecule." Similarity is quantified with the Tanimoto coefficient. The Tanimoto coefficient ranges from 0 to 1 and represents the ratio of the number of common fragments to the total number of fragments for two molecules. The greater the value, the greater degree of similarity noted.

Similar compounds for input molecule

#	Compound in database	Structure	Drug	Input molecule	Similarity, %	Network
1	thiolane 1-oxide			1	91.3	Yes
2	3-Methyl-tetrahydro-thiophene 1-oxide			1	80.77	Yes

1.4 Possible Targets for Sulfolane

Compound-target associations are based on the premise that structurally similar compounds have similar biological function. Reported are the predicted target, the input compound (MD object), Tanimoto similarity score (%), MetaDrug database compound to which the input compound is similar, effect of MetaDrug database compound on the target, and references to the literature used to make the compound-target associations.

Possible targets for input molecule

#	Target	Type	MD object	Similarity, %	Metadrug compound	Effect	Pubmed / Patent ID
1	ALDX		thiolane 1-oxide	91.3	1	Inhibition	3155552,6343601
2	ALDX		3-Methyl-tetrahydro-thiophene 1-oxide	80.77	1	Inhibition	3155552

Based on the available structurally similar chemicals with targets, one possible target was identified. The enzyme, identified as alcohol dehydrogenase [synonym: aldehyde reductase], catalyzes reduction of aromatic and aliphatic aldehydes to the alcohols.

1.5 QSAR

MetaDrug uses the ChemTree™ (Golden Helix) software with recursive partitioning algorithm to calculate QSAR models. A suite of pre-defined QSAR models is used to predict chemical and biological properties of the molecule (and, optionally, its metabolites) such as absorption, metabolism, distribution, excretion, and toxicology. Each model is developed based on literature and/or manually annotated training sets from MetaCore™/MetaDrug™ database.

The recursive partitioning method used in the ChemTree software separates data based on relationships between independent (e.g., atom connectivity) and dependent (e.g., activity) variables. Data separation continues (into nodes) until no further partitions can be made based on pre-defined stopping rules. Parameters that may be adjusted include path length (minimum number of compounds that must be present for a descriptor to be included), maximum segments (maximum number of nodes for any data separation), p-value threshold (disallows any split where the p-value is greater than the threshold), and number of random trees (maximum number of trees that can be generated).

Predicted activity is classified as active or non-active based on calculated values. For non-binary QSAR algorithms, values must comply with two QSAR thresholds to be classified as active. One threshold corresponds to the negative logarithm of activity value of the most active compound of the training set, which defines the predictability limit of the model. The second threshold is the negative logarithm of 50 μM (-1.7), which is considered the lower limit for active chemicals. If the QSAR value falls within these two thresholds, the compound is considered active. For binary QSAR models, values range from 0 to 1.

For non-binary QSAR models, the ideal training set would contain data as similar as possible (e.g., from the same origin, cell line, and experiment type). For the best results in developing binary QSAR models, the training sets used contained approximately equal numbers of positives and negatives. Examples of positives for therapeutic effects included marketed drugs, drug candidates in clinical trials, and preclinical compounds with *in vivo* activity. Chemicals that produce specific adverse effects were defined as producing toxic effects. Chemicals present in the database that produced a particular effect were assigned an arbitrary value of 1, while those that did not produce those effects were assigned a value of 0.

A percentage, representing the Tanimoto (structural) similarity to the most similar structure in the model's training set, is displayed in parentheses below the model. Results are color coded green or red. For pharmacological models, green color indicates an activity passing the cutoff threshold (thresholds are user adjustable; this report uses the default values given in the model description). For binary models, a probability ≥ 0.5 is colored green for target-based or therapeutic models, whereas toxicity models are colored red at ≥ 0.5 probability.

CYP450 QSAR models

#	Property	Model description	Value/(TP)
1	CYP1A2-inh, prob	Potential to inhibit CYP1A2 at 10 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential inhibitors. Data taken from PubChem Bioassay 1851. Model description: Training set N=9336, Test set N=3113, Sensitivity=0.81, Specificity=0.85, Accuracy=0.87, MCC=0.65.	0.09 (50.00)
2	CYP1A2-sub, prob	Potential to be metabolized by CYP1A2, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate that the compound is a substrate for CYP1A2. Reference: GeneGo data. Model description: Training set N=309, Test set N=62, Sensitivity=0.71, Specificity=0.91, Accuracy=0.78, MCC=0.60.	0.37 (22.22)
3	CYP2B6-sub, prob	Potential to be metabolized by CYP2B6, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate that the compound is a substrate for CYP2B6. Reference: GeneGo data. Model description: Training set N=228, Test set N=36, Sensitivity=0.90, Specificity=0.81, Accuracy=0.86, MCC=0.72.	0.59 (22.22)
4	CYP2C19-inh, prob	Potential to inhibit CYP2C19 at 10 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential inhibitors. Data taken from PubChem Bioassay 1851. Model description: Training set N=9392, Test set N=3149, Sensitivity= 0.80, Specificity=0.79, Accuracy=0.79, MCC=0.59.	0.08 (50.00)
5	CYP2C9-inh, prob	Potential to inhibit CYP2C9 at 10 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential inhibitors. Data taken from PubChem Bioassay 1851. Model description: Training set N=8977, Test set N=2993, Sensitivity=0.64, Specificity=0.88, Accuracy=0.80, MCC=0.54.	0.04 (50.00)
6	CYP2D6-inh, prob	Potential to inhibit CYP2D6 at 10 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential inhibitors. Data taken from PubChem Bioassay 1851. Model description: Training set N=9759, Test set N=3254, Sensitivity=0.62, Specificity=0.92, Accuracy=0.82, MCC=0.59.	0.06 (46.00)
7	CYP2D6-sub, prob	Potential to be metabolized by CYP2D6, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate that the compound is a substrate for CYP2D6. Reference: GeneGo data. Model description: Training set N=375, Test set N=69, Sensitivity=0.77, Specificity=0.84, Accuracy=0.81, MCC=0.62.	0.18 (30.43)
8	CYP3A4-inh, prob	Potential to inhibit CYP3A4 at 10 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential inhibitors. Data taken from PubChem Bioassay 1851. Model description: Training set N=9145, Test set N=3049, Sensitivity=0.74, Specificity=0.83, Accuracy=0.79, MCC=0.57.	0.04 (50.00)
9	CYP3A4-sub, prob	Potential to be metabolized by CYP3A4, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate that the compound is a substrate for CYP3A4. Reference: GeneGo data. Model description: Training set N=636, Test set N=116, Sensitivity=0.76, Specificity=0.85, Accuracy=0.80, MCC=0.61.	0.26 (26.67)
10	sEH-inh, pIC50	Human soluble epoxide hydrolase inhibition, pIC50 (uM). Cutoff is -1.7. The higher the value, the higher the inhibition activity. Reference: GeneGo data. Model description: N=675, R2=0.92, RMSE=0.51.	-2.58 (47.37)

Protein binding QSAR models

#	Property	Model description	Value/(TP)
1	5HT2B-act, prob	Potential to activate 5-hydroxytryptamine (serotonin) receptor 2B at 1 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate active compounds. Training set consists of chemicals and drugs that activate 5HT2B receptor resulting in valvular heart disease. Reference: Chekmarev et al., Chem Res Tox, 2008 (PMID: 18415049). Model description: Training set N=194, Test set N=38, Sensitivity=1.0, Specificity=0.96, Accuracy=0.97, MCC=0.94.	0.02 (15.94)
2	ADR-lig, prob	Potential to bind to Androgen receptor, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential Androgen receptor ligands. Reference: Fang H, Tong W, et al Chem Res Tox 2003 (PMID: 14565775). Model description: Training set N=165, Test set N=32, Sensitivity=1.0, Specificity=1.0, Accuracy=1.0, MCC=1.0.	0.65 (16.85)
3	ESR-lig, prob	Potential to bind to Estrogen receptor at 100 uM or less, range from 0 to 1. Cutoff is 0.5. Values higher than 0.5 indicate potential Estrogen receptor ligands. Training set is based on DSSTox KIERBL data (EPA). Model description: Training set N=164, Test set N=55, Sensitivity=1.0, Specificity=0.87, Accuracy=0.93, MCC=0.86.	0.04 (21.82)
4	PXR-act, prob	Pregnane X receptor activation binary model, range from 0 to 1. Values higher than 0.5 indicate potential PXR activators, values lower than 0.5 are preferable. Reference: GeneGo data. Model description: N=95, R2=0.64, RMSE=0.29.	0.59 (16.85)
5	Pgp-inh, pIC50	Human P-glycoprotein transporter inhibition, pIC50 (uM). Cutoff is -1.7. The higher the value, the higher the inhibition activity. GeneGo data. Model description: N=274, R2=0.85, RMSE=0.4.	-1.06 (14.29)
6	Pgp-sub, prob	Potential to be a substrate of human P-glycoprotein transporter, range from 0 to 1. Cutoff is 0.5. Values closer to 1 indicate potential ligands. Reference: Penzotti, Lamb, et al., 2002 (PMID: 11960484). Model description: N=192, R2=0.65, RMSE=0.3.	0.29 (30.00)
7	SERT-inh, pKi	Human serotonin transporter inhibition, pKi (uM). Cutoff is -1.7. The higher the value, the higher the inhibition activity of the metabolite. GeneGo data. Model description: N=256, R2=0.91, RMSE=0.36.	-0.07 (13.25)
8	hERG-inh, pKi	Human hERG (human ether a-go-go-related gene) channel inhibition , pKi (uM). Cutoff is -1.7. The higher the value, the higher the inhibition activity. Lower values are preferable. Reference: GeneGo data. Model description: N=196, R2=0.93, RMSE=0.23.	-0.49 (17.89)

ADME QSAR models

#	Property	Model description	Value/(TP)
1	BBB, log ratio	Blood brain barrier penetration model. The data is expressed as log values of the ratio of the metabolite concentrations in brain and plasma. Cutoff is -0.3. Larger values indicate that the metabolite is more likely to enter the brain. Reference: GeneGo data. Model description: N=107, R2=0.89, RMSE=0.26.	0.15 (18.52)
2	G-LogP	Lipophilicity, log of compound octanol-water distribution. Cutoffs are -0.4 to 5.6. Values greater than 5.6 correspond to overly hydrophobic compounds. Reference: Syracuse Research, PHYSPROP database. Model description: N=13474, R2=0.95, RMSE=0.21.	-0.16
3	Prot-bind, %	Human serum protein binding, %. Cutoff is 50%. A value of more than 95% is highly bound, less than 50% is a low binding metabolite. Reference: Thummel and Shen, 2001 in Goodman & Gilman's The Pharmacological Basis of Therapeutics. Model description: N=265, R2=0.909, RMSE=10.11.	34.89 (22.22)
4	Prot-bind, log t	Affinity to human serum albumin, log value of the retention time. Cutoff is 0. Positive values correspond to higher protein binding, negative values to lower protein binding. An acceptable level of binding is project dependent. The model is based on retention times of compounds assayed by HPLC using an immobilized HSA column. Values are expressed as log values of the retention time. Reference: Colmenarejo, Alvarez-Pedraglio, et al., 2001 (PMID: 11728183). Model description: N=95, R2=0.904, RMSE=0.2.	-0.38 (18.52)
5	WSol, log mg/L	Water solubility at 25?C, log mg/L. Cutoffs are from 2 to 4. An acceptable level of solubility is project dependent. Reference: Syracuse Research, PHYSPROP database. Model description: N=2871, 2=0.91, RMSE=0.54.	3.60

Prediction of therapeutic activity

#	Property	Model description	Value/(TP)
1	Allergy	Potential antiallergic activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=258, Test set N=47, Sensitivity= 0.87, Specificity=0.88, Accuracy=0.87, MCC=0.74. Click here for details.	0.78 (18.42)
2	Alzheimer	Potential activity against Alzheimer's disease. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=261, Test set N=44, Sensitivity= 0.91, Specificity=0.82, Accuracy=0.86, MCC=0.73. Click here for details.	0.89 (40.54)
3	Angina	Potential antianginal activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=546, Test set N=95, Sensitivity= 0.90, Specificity=0.93, Accuracy=0.92, MCC=0.83. Click here for details.	0.53 (25.40)
4	Arthritis	Potential activity against arthritis. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGO data. Model description: Training set N=460, Test set N=77, Sensitivity= 0.98, Specificity=0.94, Accuracy=0.96, MCC=0.92. Click here for details.	0.04 (26.67)
5	Asthma	Potential activity against asthma. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGO data. Model description: Training set N=366, Test set N=63, Sensitivity= 0.92, Specificity=0.86, Accuracy=0.89, MCC=0.78. Click here for details.	0.49 (22.22)
6	Bacterial	Potential antibacterial activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=530, Test set N=97, Sensitivity= 0.87, Specificity=0.90, Accuracy=0.89, MCC=0.77. Click here for details.	0.82 (20.37)
7	Cancer	Potential activity against cancer. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=886, Test set N=167, Sensitivity= 0.89, Specificity=0.83, Accuracy=0.86, MCC=0.72. Click here for details.	0.88 (35.71)
8	Depression	Potential activity against depression. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=335, Test set N=62, Sensitivity= 0.93, Specificity=0.82, Accuracy=0.87, MCC=0.75. Click here for details.	0.90 (20.83)
9	Diabetes	Potential antidiabetic activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=195, Test set N=34, Sensitivity= 0.85, Specificity=0.93, Accuracy=0.88, MCC=0.77. Click here for details.	0.41 (17.39)
10	HIV	Potential activity against HIV. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description:Training set N=491, Test set N=80, Sensitivity= 0.80, Specificity=0.86, Accuracy=0.84, MCC=0.67. Click here for details.	0.40 (22.22)
11	Heart Failure	Potential activity against heart failure. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=204, Test set N=33, Sensitivity= 0.78, Specificity=0.87, Accuracy=0.82, MCC=0.64. Click here for details.	0.29 (16.90)
12	Hyperlipidemia	Potential antihyperlipidemic activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=185, Test set N=24, Sensitivity= 0.75, Specificity=0.92, Accuracy=0.83, MCC=0.68. Click here for details.	0.43 (41.67)
13	Hypertension	Potential antihypertensive activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=554, Test set N=111, Sensitivity= 0.89, Specificity=0.81, Accuracy=0.85, MCC=0.70. Click here for details.	0.82 (18.84)
14	Inflammation	Potential anti-inflammatory activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=598, Test set N=93, Sensitivity= 0.86, Specificity=0.84, Accuracy=0.85, MCC=0.69. Click here for details.	0.39 (21.13)
15	Migraine	Potential activity against migraine. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=515, Test set N=98, Sensitivity= 0.81, Specificity=0.84, Accuracy=0.83, MCC=0.65. Click here for details.	0.44 (22.22)
16	Mycosis	Potential antifungal activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=172, Test set N=47, Sensitivity= 0.90, Specificity=0.88, Accuracy=0.89, MCC=0.79. Click here for details.	0.66 (19.57)
17	Obesity	Potential activity against obesity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGO data. Model description: Training set N=472, Test set N=75, Sensitivity= 0.89, Specificity=0.97, Accuracy=0.93, MCC=0.87. Click here for details.	0.01 (54.05)
18	Osteoporosis	Potential anti-osteoporosis activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=595, Test set N=86, Sensitivity= 0.84, Specificity=0.85, Accuracy=0.85, MCC=0.70. Click here for details.	0.41 (22.78)
19	Pain	Potential analgetic activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=525, Test set N=84, Sensitivity= 0.92, Specificity=0.67, Accuracy=0.79, MCC=0.60. Click here for details.	0.74 (19.61)
20	Parkinson	Potential activity against Parkinson's disease. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=298, Test set N=49, Sensitivity= 0.96, Specificity=0.96, Accuracy=0.96, MCC=0.92. Click here for details.	0.41 (20.83)
21	Psoriasis	Potential activity against psoriasis. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=199, Test set N=32, Sensitivity= 0.93, Specificity=0.82, Accuracy=0.89, MCC=0.74. Click here for details.	0.34 (16.33)
22	Schizophrenia	Potential activity against schizophrenia. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=616, Test set N=93, Sensitivity= 0.89, Specificity=0.91, Accuracy=0.90, MCC=0.80. Click here for details.	0.37 (22.58)
23	Skin Diseases	Potential activity against skin diseases. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=255, Test set N=36, Sensitivity= 1.00, Specificity=0.76, Accuracy=0.86, MCC=0.76. Click here for details.	0.83 (17.80)
24	Thrombosis	Potential antithrombotic activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs, drug candidates in clinical trials and preclinical compounds with in vivo activity. Reference: GeneGo data. Model description: Training set N=453, Test set N=80, Sensitivity= 0.98, Specificity=0.95, Accuracy=0.97, MCC=0.93. Click here for details.	0.37 (22.22)
25	Viral	Potential antiviral activity. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Reference: GeneGo data. Model description: Training set N=206, Test set N=35, Sensitivity= 0.92, Specificity=0.95, Accuracy=0.94, MCC=0.88. Click here for details.	0.54 (22.22)

Prediction of toxic effects

#	Property	Model description	Value/(TP)
1	AMES	Potential to be mutagenic (AMES positive), range from 0 to 1. A value of 1 is AMES positive (mutagenic), and a value of 0 is AMES negative (non-mutagenic). Cutoff is 0.5. Values close to zero are preferable. The AMES assay is based upon the reversion of mutations in the histidine operon in the bacterium Salmonella enterica sv Typhimurium. Reference: Young, Gombar, et al., 2002 (DOI: 10.1016/S0169-7439(01) 00181-2). Model description: N=1780, R2=0.69, RMSE=0.29.	0.24 (35.29)
2	Anemia	Potential for causing anemia. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing anemia in vivo. Model organisms: human. Reference: GeneGo data. Model description: Training set N=324, Test set N=51, Sensitivity= 0.82, Specificity=0.90, Accuracy=0.86, MCC=0.72. Click here for details.	0.94 (26.67)
3	Carcinogenicity	Potential for inducing carcinogenicity in rats and mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: mouse, rat. Reference: ISSCAN data. Model description: Training set N=1210, Test set N=185, Sensitivity= 0.96, Specificity=0.90, Accuracy=0.93, MCC=0.86. Click here for details.	1.00 (34.09)
4	Carcinogenicity Mouse Female	Potential for inducing carcinogenicity in female mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: female mice. Reference: ISSCAN data. Model description: Training set N=640, Test set N=94, Sensitivity= 0.90, Specificity=0.93, Accuracy=0.92, MCC=0.83. Click here for details.	0.99 (29.17)
5	Carcinogenicity Mouse Male	Potential for inducing carcinogenicity in male mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: mouse male. Reference: ISSCAN data. Model description: Training set N=584, Test set N=93, Sensitivity= 0.91, Specificity=0.88, Accuracy=0.89, MCC=0.78. Click here for details.	0.94 (29.17)
6	Carcinogenicity Rat Female	Potential for inducing carcinogenicity in female rats. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: female rat. Reference: ISSCAN data. Model description: Training set N=667, Test set N=120, Sensitivity= 0.90, Specificity=0.96, Accuracy=0.93, MCC=0.86. Click here for details.	0.99 (34.09)
7	Carcinogenicity Rat Male	Potential for inducing carcinogenicity in male rats. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: male rat. Reference: ISSCAN data. Model description: Training set N=715, Test set N=117, Sensitivity= 0.92, Specificity=0.88, Accuracy=0.90, MCC=0.79. Click here for details.	1.00 (29.17)
8	Cardiotoxicity	Potential for inducing cardiotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing cardiotoxicity in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=143, Test set N=30, Sensitivity= 0.80, Specificity=1.00, Accuracy=0.90, MCC=0.82. Click here for details.	0.71 (27.27)
9	Cytotoxicity model, -log GI50 (M)	Growth inhibition of MCF7 cell line (human caucasian breast adenocarcinoma), pGI50. Cutoff is 6. Values from 6 to 8 correspond to a toxic metabolite, values less than 6 are preferable, values less than 3 are more preferable and less toxic. Reference: DTP/NCI. Model description: N=1474, R2=0.9, RMSE=0.05.	4.65 (50.00)
10	Genotoxicity	Potential for inducing genotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing genotoxicity in vivo. Model organisms: mouse, rat. Reference: NTP data (Li et al. Chem Res Tox 2005). Model description: Training set N=372, Test set N=86, Sensitivity= 0.75, Specificity=0.84, Accuracy=0.79, MCC=0.59. Click here for details.	0.90 (20.37)
11	Hepatotoxicity	Potential for inducing hepatotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing hepatotoxicity in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=1380, Test set N=231, Sensitivity= 0.73, Specificity=0.88, Accuracy=0.81, MCC=0.62. Click here for details.	0.89 (29.69)
12	Kidney Necrosis	Potential for inducing kidney necrosis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing renal necrosis in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=221, Test set N=42, Sensitivity= 0.96, Specificity=1.00, Accuracy=0.98, MCC=0.95. Click here for details.	0.97 (26.67)
13	Kidney Weight Gain	Potential for inducing kidney weight gain. Cutoff is 0.5. The values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing kidney weight gain in vivo. Model organisms: mouse, rat. Reference: GeneGo data. Model description: Training set N=240, Test set N=49, Sensitivity= 0.95, Specificity=1.00, Accuracy=0.98, MCC=0.96. Click here for details.	0.98 (29.69)
14	Liver Cholestasis	Potential for inducing cholestasis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing cholestasis in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=218, Test set N=35, Sensitivity= 0.79, Specificity=0.67, Accuracy=0.74, MCC=0.46. Click here for details.	0.86 (22.92)
15	Liver Lipid Accumulation	Potential for inducing liver lipid accumulation. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing lipid accumulation in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=172, Test set N=28, Sensitivity= 0.80, Specificity=0.85, Accuracy=0.82, MCC=0.64. Click here for details.	0.93 (29.17)
16	Liver Necrosis	Potential for inducing liver necrosis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing hepatic necrosis in vivo. Model organisms: mouse, rat, human. Model description: Training set N=300, Test set N=57, Sensitivity= 0.91, Specificity=0.91, Accuracy=0.91, MCC=0.82. Reference: GeneGo annotation, click here to view training set.	0.88 (29.17)
17	Liver Weight Gain	Potential for inducing liver weight gain . Cutoff is 0.5. Values higher than 0.5 indicate potential liver weight-changing compounds. Training set consists of chemicals and drugs causing liver weight gain in vivo. Model organisms: mouse, rat. Reference: GeneGo data. Model description: Training set N=292, Test set N=52, Sensitivity= 1.00, Specificity=1.00, Accuracy=1.00, MCC=1.00. Click here for details.	1.00 (29.69)
18	MRTD	Maximum Recommended Therapeutic Dose, log mg/kg-bm/day, range is from -5 to 3. Cutoff is 0.5. Chemicals with high log MRTDs can be classified as midly toxic compounds, chemicals with low log MRTDs as highly toxic compounds. Reference: Matthews, Kruhlak, et al., 2004 (PMID: 16472220). Model description: N=1209, R2= 0.86, RMSE=0.42.	0.69 (37.50)
19	Nephron Injury	Potential for inducing nephron injury. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing nephron injury in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=598, Test set N=109, Sensitivity= 0.91, Specificity=1.00, Accuracy=0.96, MCC=0.93. Click here for details.	0.97 (27.50)
20	Nephrotoxicity	Potential for inducing nephrotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing nephrotoxicity in vivo. Model organisms: mouse, rat, human. Reference: GeneGo data. Model description: Training set N=847, Test set N=154, Sensitivity= 0.90, Specificity=0.84, Accuracy=0.87, MCC=0.74. Click here for details.	0.96 (29.69)
21	Neurotoxicity	Potential for inducing neurotoxicity. Training set consists of chemicals and drugs causing neurotoxicity in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Reference: GeneGo data. Model description: Training set N=175, Test set N=34, Sensitivity= 0.94, Specificity=0.94, Accuracy=0.94, MCC=0.88. Click here for details.	0.98 (22.22)
22	SkinSens, EC3	Skin sensitization potential expressed as effective concentration 3, EC3 %. Values higher than 10 indicate weak and moderate sensitizers. Reference: Ren et al. (PMID: 17723489). Model description: N=89, R2=0.67, RMSE=22.56.	1.31 (34.78)

QSAR modeling results indicate the following predicted properties of sulfolane (Tanimoto Percentage [TP] values ≥50%):

- Not an inhibitor of cytochrome (CYP) 1A2 (0.09, TP = 50.00)
- Not an inhibitor of CYP2C19 (0.08, TP = 50.00)
- Not an inhibitor of CYP2C9 (0.04, TP = 50.00)
- Not an inhibitor of CYP3A4 (0.04, TP = 50.00)
- Does not have potential activity against obesity (0.01, TP = 54.05)
- Does not inhibit growth of MCF7 cells (4.65 [values from 6-8 suggest toxic metabolite, value less than 6 are preferred], TP = 50.00)

1.6 GeneGo Functional Ontologies

Enrichment analysis of the identified target list is shown across seven functional biology ontologies; two ontologies (process networks and disease biomarker networks) were not provided since there were no targets provided. The enrichment calculation uses the Fisher's exact test or hypergeometric distribution to calculate the probability that the degree of overlap between the list of possible protein targets generated from the query compound analysis and the proteins represented in the functional ontology category can happen by chance given an identical number of proteins selected at random from the universe of proteins annotated within the ontology. The p-value generated is used to rank order the categories within each ontology by their significance to the list of targets, thereby identifying maps or biological processes likely to be affected by compound exposure (GeneGo, personal communication). Those entries with a p-value ≤0.01000 are highlighted in yellow.

GeneGo maps

Name	Map	pValue
1	Pyruvate metabolism	5.556e-03
1	Serotonin - melatonin biosynthesis and metabolism	5.783e-03
1	Triacylglycerol metabolism p.1	6.803e-03
1	Naphthalene metabolism	6.917e-03
1	Pyruvate metabolism/ Rodent version	7.484e-03
1	Prostaglandin 2 biosynthesis and metabolism FM	1.145e-02

GeneGo drug target networks

Name	Network	pValue
1	Metabolism_Glucuronid metabolism via BGLR and ALDR	2.439e-02

GeneGo toxicity networks

Name	Network	pValue
1	Metabolism_Alcohol metabolism	1.246e-02

GeneGo metabolic networks		
Name	**Network**	**pValue**
1	Carbohydrate metabolism_Pyruvate metabolism and transport_new	1.190e-02
1	D-glucuronic acid pathway	1.241e-02
1	Lipid metabolism_Triacylglycerol metabolism	1.717e-02
1	Glucose pathway	1.818e-02
1	Carbohydrate metabolism_Glycolisys, Glucogenesis and glucose transport	2.345e-02
1	Lipid metabolism_Prostaglandin metabolism	2.685e-02

GO processes		
Name	**Process**	**pValue**
1	D-glucuronate catabolic process	4.575e-05
1	D-glucuronate metabolic process	4.575e-05
1	glucuronate catabolic process	4.575e-05
1	aldehyde catabolic process	1.373e-04
1	L-ascorbic acid biosynthetic process	1.830e-04
1	L-ascorbic acid metabolic process	2.745e-04
1	uronic acid metabolic process	3.203e-04
1	glucuronate metabolic process	3.203e-04
1	water-soluble vitamin biosynthetic process	1.190e-03
1	vitamin biosynthetic process	1.647e-03
1	cellular aldehyde metabolic process	1.967e-03
1	water-soluble vitamin metabolic process	3.569e-03
1	vitamin metabolic process	6.909e-03
1	carbohydrate catabolic process	6.955e-03
1	carboxylic acid catabolic process	8.144e-03
1	organic acid catabolic process	8.144e-03
1	glucose metabolic process	9.014e-03
1	hexose metabolic process	1.126e-02
1	organic acid biosynthetic process	1.281e-02
1	carboxylic acid biosynthetic process	1.281e-02
1	monosaccharide metabolic process	1.336e-02
1	monocarboxylic acid metabolic process	1.999e-02
1	cellular carbohydrate metabolic process	2.544e-02
1	alcohol metabolic process	2.782e-02
1	small molecule biosynthetic process	2.974e-02
1	carbohydrate metabolic process	3.459e-02
1	carboxylic acid metabolic process	3.935e-02
1	oxoacid metabolic process	3.935e-02
1	cellular ketone metabolic process	4.017e-02
1	organic acid metabolic process	4.040e-02
1	small molecule catabolic process	4.566e-02
1	oxidation-reduction process	5.207e-02
1	cellular catabolic process	7.824e-02
1	catabolic process	9.251e-02
1	small molecule metabolic process	1.176e-01
1	cellular biosynthetic process	1.853e-01
1	biosynthetic process	1.907e-01
1	cellular metabolic process	3.845e-01
1	primary metabolic process	3.856e-01
1	metabolic process	4.576e-01
1	cellular process	6.777e-01

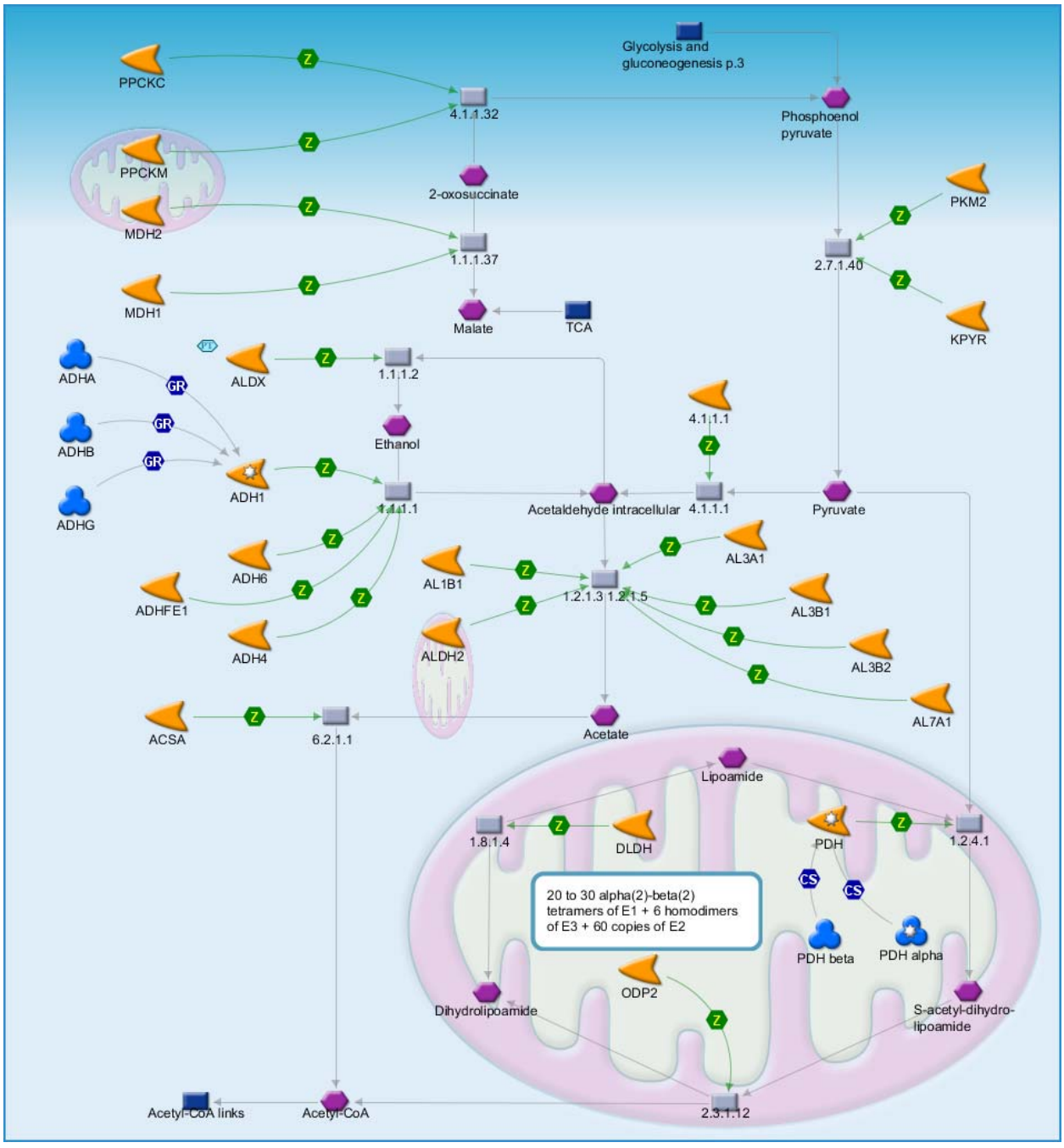
GO molecular functions		
Name	**Function**	**pValue**
1	L-glucuronate reductase activity	4.426e-05
1	alcohol dehydrogenase (NADP+) activity	2.656e-04
1	aldehyde reductase activity	3.098e-04
1	aldo-keto reductase activity	1.062e-03
1	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	5.621e-03
1	oxidoreductase activity, acting on CH-OH group of donors	6.241e-03
1	electron carrier activity	1.071e-02
1	oxidoreductase activity	3.696e-02
1	catalytic activity	2.637e-01
1	protein binding	3.812e-01
1	binding	6.059e-01

GO localizations		
Name	**Localization**	**pValue**
1	apical plasma membrane	1.201e-02
1	apical part of cell	1.530e-02
1	cytosol	1.099e-01
1	plasma membrane part	1.271e-01
1	plasma membrane	2.368e-01
1	cell periphery	2.409e-01
1	cytoplasmic part	3.355e-01
1	membrane part	3.638e-01
1	membrane	4.457e-01
1	cytoplasm	5.245e-01
1	intracellular part	7.003e-01
1	intracellular	7.173e-01
1	cell part	9.371e-01
1	cell	9.372e-01

1.7 Top GeneGo Pathway Maps

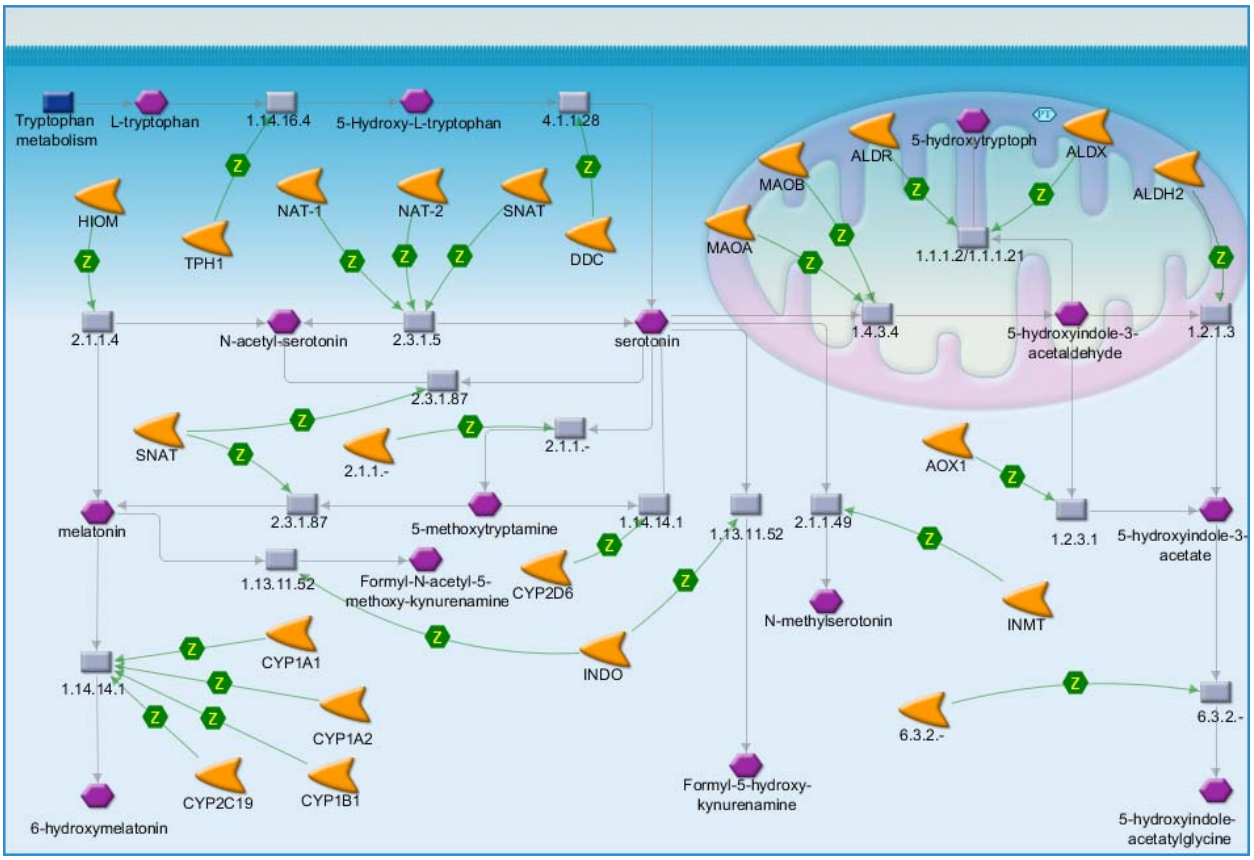
GeneGo pathway maps comprise pictorial representations of human and rodent signaling and metabolic pathways. The three most significant maps are shown below. Compounds are represented by purple hexagons, proteins by colored shapes representing different classes of compound, and enzymatic reactions by gray rectangles. Protein-protein, compound-protein, and compound-reaction interactions are shown as unidirectional arrows, and a mechanism of interaction is represented by letters in hexagonal boxes over the arrows.

1.7.1 Pyruvate Metabolism



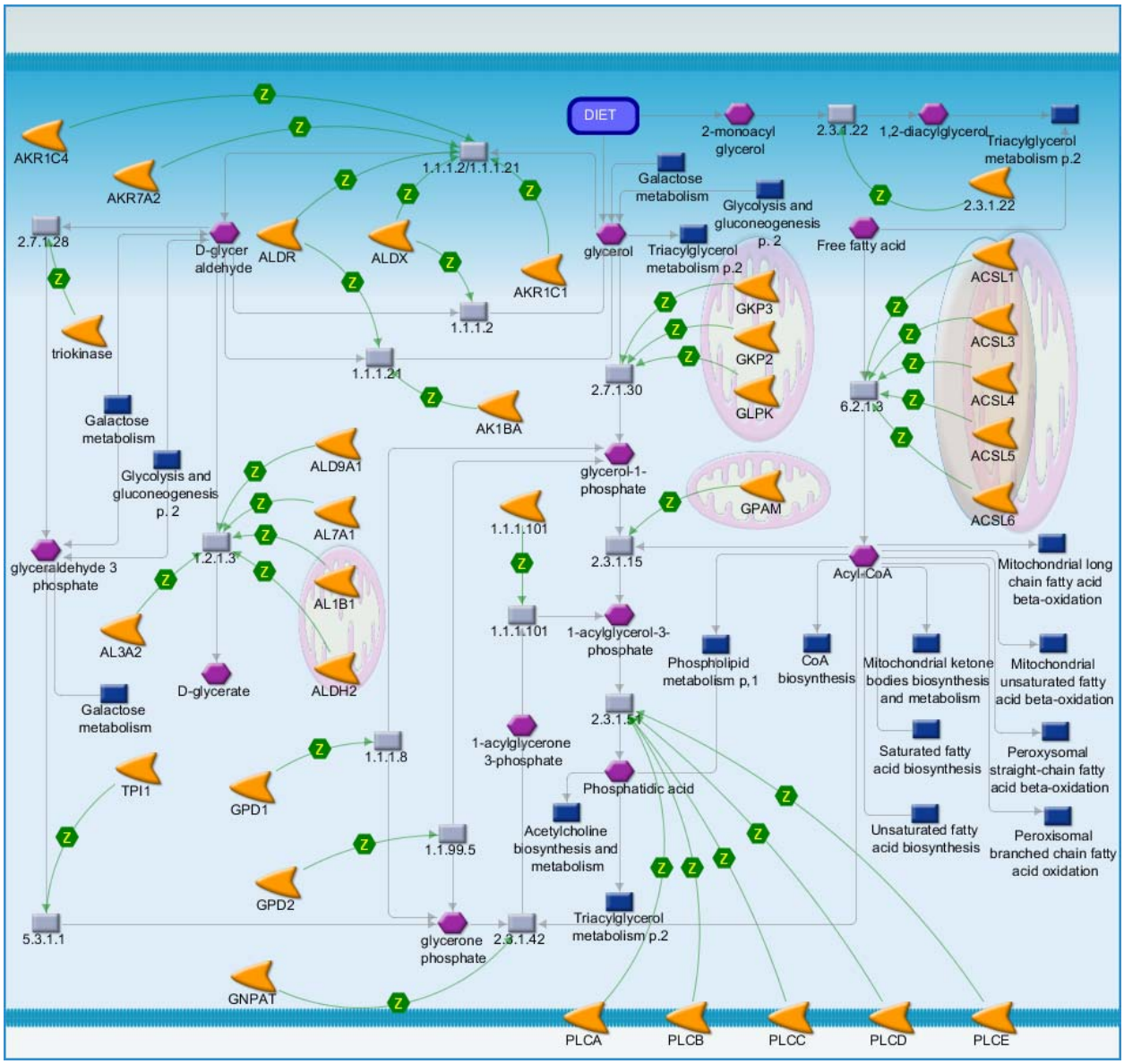
Pyruvate is a product of glycolysis. In one of the available metabolism pathways, pyruvate may be converted to acetaldehyde through the action of pyruvate decarboxylase. The carbonyl on acetaldehyde then may be reduced to produce ethanol. Based on the structural similarity of sulfolane to thiolane 1-oxide and 3-methyl-tetrahydro-thiophene 1-oxide, it was predicted that the chemical would inhibit conversion of acetaldehyde to ethanol (GeneGo, 2011b).

1.7.2 Serotonin - Melatonin Biosynthesis and Metabolism



Serotonin is a neurotransmitter that produces effects through receptor mediated mechanisms. Serotonin may be metabolized through a variety of pathways. One pathway involves conversion of serotonin to 5-hydroxyindole-3-acetaldhyde through action of monoamine oxidases. 5-Hydroxyindole-3-acetaldehyde then may be converted to 5-hydroxyindole-3-acetate through action of an aldehyde dehydrogenase or may be converted to 5-hydroxytryptophol through the actions of alcohol dehydrogenase [synonym: aldehyde reductase] and/or aldose reductase. Based on the structural similarity of sulfolane to thiolane 1-oxide and 3-methyl-tetrahydro-thiophene 1-oxide, it was predicted that the chemical would inhibit conversion of 5-hydroxyindole-3-acetaldhyde to 5-hydroxytryptophol through inhibition of alcohol dehydrogenase (GeneGo, 2011c).

1.7.3 Triacyl Glycerol Metabolism (p. 1)



Glycerol may be obtained from a variety of sources including diet, galactose metabolism, and glycolysis. Glycerol is used in the formation of fatty acids. One metabolic pathway associated with glycerol is conversion to D-glyceraldehyde through the action of aldo-keto reductase enzymes. Based on the structural similarity of sulfolane to thiolane 1-oxide and 3-methyl-tetrahydro-thiophene 1-oxide, it was predicted that the chemical would inhibit conversion of glycerol to D-glyceraldhyde through inhibition of alcohol dehydrogenase (GeneGo, 2011d).

2.0 Leadscope

This summary provides an overview of the Leadscope method and the results of the QSAR analysis conducted on sulfolane. The background information provided in this summary was obtained from the *Leadscope Model Applier Documentation* (Leadscope Inc., 2009), unless otherwise noted.

2.1 Background and Overview of Leadscope Analysis Methodology

The QSAR model suites are divided into (1) human clinical endpoints and (2) non-human toxicity endpoints. The human clinical endpoint suites model potential adverse cardiac effects, adverse hepatobiliary effects, and adverse urinary tract effects. The non-human toxicity endpoints are comprised of rodent carcinogenicity, genetic toxicity, reproductive toxicity, developmental toxicity, and neurotoxicity.

Most of the QSAR models used in this analysis were based on public information, which included structures of the chemicals present in the training set and the biological/toxicological result for the particular endpoint being modeled. The exceptions are the rodent, rat, and mouse carcinogenicity models, which were developed using confidential data. The QSAR models were constructed by the Informatics and Computational Safety Analysis Staff at the U.S. Food and Drug Administration (FDA) within the Leadscope Prediction Data Miner software. In designing the models, all default settings were used.

The modeling strategy was described in six steps by Yang and colleagues (2004):

(1) diagnose the data set – data set is analyzed for structural diversity, similarity, and distribution
(2) assembly of macrostructures - macrostructures associated with activity are identified
(3) preselection of features – selection of a subset of features based on statistical analyses
(4) develop model – model is developed based on selected model building algorithms
(5) evaluate the model with chemical inference – evaluate results of known chemicals and evaluate why model worked or failed for particular chemicals
(6) refine model – based on evaluation, refine model with new features

Structural features and calculated properties are used to develop the models. "The structural features include Leadscope® default hierarchy features plus the predictive scaffolds generated with default settings." In addition to the structural features, calculated properties are used. These are: parent molecular weight, LogP, polar surface area, hydrogen bond acceptors, hydrogen bond donors, number of rotational bonds, and Lipinski score (rule violation). [ILS Note: The *Leadscope Model Applier Documentation* notes that there were eight calculated properties used, but seven are listed. In reviewing an article discussing the prediction modeling methodology used, it was noted that in addition to the seven calculated properties that the calculated property of parent atom count was also noted (Yang et al., 2004).]

Predictive performance of a model is dependent on the ratio of active to inactive compounds present in the training set. Sub-models were developed for some of the models to improve predictive performance. The active/inactive compound ratios were between 0.30 and 0.35 for these sub-models. Overall prediction results were based on averaging the probabilities for the sub-models.

Output from the models includes a prediction status and a prediction probability. The prediction status of a test compound was defined as "positive," "negative," or "not-in-domain." Test compounds are defined as "not-in-domain" when they are not within the parameters of the specified model. "The model domain is defined within the Leadscope application for two factors: 1) containing structural model features in addition to property descriptors; 2) being within a similar structure group with at least 30 % similarity." The prediction probability is given as a value between 0 and 1. The greater the number, the greater the likelihood that the test compound is toxic for the evaluated model. Within the FDA, a probability ≥0.5 is defined as active.

In addition to the prediction status and prediction probability, the structural features and calculated properties associated with the predicted activity are provided for review. For the models that were developed using confidential data, the Leadscope default hierarchy is provided, but the scaffold structures are not revealed. Additionally, the structures of the compounds in the training data set for models developed using confidential data are encrypted and randomly generated numbers are presented as the compound names.

2.2 Suite Results

2.2.1 Rodent Carcinogenicity

This suite is composed of a total of 11 models, seven *in vivo* and four *in vitro*. The *in vivo* models are based on results from the 2-year rodent bioassay; training sets were based on confidential data. The *in vitro* models are based on cell transformation studies. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Carcinogenicity Mouse	Negative	0.368	1132-1260	37.7-40.8	91.6-92.9
Carcinogenicity Male Mouse	Negative	0.282	1106-1235	37.1-38.1	90.2-91.7
Carcinogenicity Female Mouse	Negative	0.3775	1110-1246	35.7-38.9	90.3-92.0
Carcinogenicity Rat	Not in domain		1206-1415	33.7-40.5	93.8-95.1
Carcinogenicity Male Rat	Negative	0.271	1155-1361	35.4-39.7	93.0-94.2
Carcinogenicity Female Rat	Not in domain		1164-1356	37.9-40.1	93.2-94.1
Carcinogenicity Rodent	Negative	0.402	1153-1569	32.5-37.9	91.6-94.2
In Vitro Cell Transformation	Not in domain		640	87.8	50.8
SHE	Not in domain		425	88.8	55.8
BALB/c-3T3	Positive	0.763	316	87.8	54.7
C3H10T1/2	Not in domain		138	93.9	22.5

*Ranges are provided for those models where sub-models were developed.

Sulfolane was classified as positive in one model, negative in five models, and not in domain in five models. Sulfolane was classified as positive in the BALB/c-3T3 model; prediction probability was 0.763. Within the model, a single structural feature was identified: sulfonyl group. Within the training set used to develop the model, a single chemical was identified as structurally similar: 3-sulfolene.

2.2.2 Genetic Toxicity

This suite is composed of 29 models. There are 12 *in vitro* mammalian and microbial mutagenicity models evaluated. Additionally, there is a mouse lymphoma mutagenicity model. Three *in vitro* unscheduled DNA synthesis models are used to assess DNA damage. Clastogenicity models are based on *in vivo* micronucleus and chromosomal aberration studies. Finally, three sister chromatid exchange models and five chromosomal aberration models are described using results from a variety of cell types. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Mutagenicity models					
In vitro microbial	Not in domain		3683	64.3	87.5
In vitro Salmonella	Not in domain		3575	62.0	89.5
In vitro E. coli	Not in domain		524	76.3	76.7
E. coli w strains	Not in domain		277	62.6	90.1
In vitro yeast	Not in domain		435-603	59.5-63.5	89.6-91.1
In vitro S. cerevisiae	Not in domain		356-473	65.5-66.5	89.6-90.8
In vivo Drosophila	Not in domain		595	73.0	81.9
In vivo Drosophila sex linked recessive lethal	Not in domain		588	71.6	82.8
In vivo Drosophila heritable translocations	Not in domain		118	77.4	84.6
In vivo mammalian	Not in domain		213	62.7	88.5
In vivo mammalian dominant lethal	Not in domain		182	61.5	90.6
In vitro CHO V79 hgprt	Not in domain		472-643	42.1-46.5	91.4-92.7
Mouse lymphoma mutagenicity model					
Mouse lymphoma 5178Y-tk	Negative	0.428	565-809	48.8-68.0	72.6-87.2
DNA damage models					
UDS *in vitro*	Not in domain		374	61.5	90.0
UDS *in vitro* rat hepatocytes	Not in domain		143	63.6	90.9
UDS *in vitro* human lymphocytes	Not in domain		194	66.7	89.4
Clastogenicity models					
Micronucleus *in vivo*	Not in domain		824	41.3	95.4
Micronucleus *in vivo* mouse	Not in domain		624	45.7	90.7
Chromosome aberrations *in vivo*	Not in domain		285	48.0	91.4
Chromosome aberrations *in vivo* rat	Not in domain		110	6.67	96.8
Chromosome aberrations *in vivo* other rodent	Not in domain		153	48.1	86.9
Chromosomal aberrations models					
In vitro chrom. ab.	Negative	0.385	1182-1596	43.5-44.1	89.2-90.6
In vitro chrom. ab. CHO	Negative	0.3	591-688	42.8-46.9	91.0-91.5
In vitro chrom. ab. CHL	Not in domain		535-734	44.8-52.4	91.9-94.8
In vitro chrom. ab. HL	Not in domain		186	75.3	81.9
In vitro chrom. ab. Other cells	Negative	0.396	281	54.9	81.9
Sister chromatid exchange models					
SCE *in vitro*	Not in domain		410-758	70.1-72.7	66.5-74.0
SCE *in vitro* CHO	Not in domain		624	87.7	42.4
SCE *in vitro* other cells	Not in domain		204	96.0	38.7

*Ranges are provided for those models where sub-models were developed.

Sulfolane was classified as negative in four models and not in domain in 25 models.

2.2.3 Reproductive Toxicity

A total of nine models are used to predict reproductive toxicity; six male and three female. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Repro Rodent Male	Not in domain		786	36.3	93.8
Repro Rat Male	Not in domain		717	41.7	92.0
Repro Mouse Male	Not in domain		146	63.8	83.9
Repro Rodent Female	Not in domain		476-965	46.1-53.3	91.4-92.9
Repro Rat Female	Not in domain		435-900	35.4-50.4	90.6-96.5
Repro Mouse Female	Not in domain		150	62.5	90.2
Sperm Rodent	Not in domain		684-910	44.0-50.4	88.1-89.8
Sperm Rat	Not in domain		542-726	52.3-57.5	89.7-90.2
Sperm Mouse	Not in domain		260	50.0	87.1

*Ranges are provided for those models where sub-models have been developed.

Sulfolane was classified as not in domain for all the models evaluated.

2.2.4 Developmental Toxicity

A total of 27 developmental toxicity models are included in this suite. The models can be classified as structural dysmorphogenesis (four models), visceral dysmorphogenesis (three models), fetal survival (12 models), and fetal growth (eight models). The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Structural dysmorphogenesis					
Structural dysmorphogenesis rodent	Not in domain		2019	28.6	94.4
Structural dysmorphogenesis rat	Not in domain		1330-1759	40.7-43.4	88.7-89.8
Structural dysmorphogenesis mouse	Not in domain		979	34.6	90.5
Structural dysmorphogenesis rabbit	Not in domain		432-1014	50.4-55.3	87.3-90.0
Visceral dysmorphogenesis					
Visceral dysmorphogenesis rodent	Not in domain		1004-2019	35.6-38.0	89.4-92.3
Visceral dysmorphogenesis rat	Not in domain		743-1654	42.3-42.7	88.9-92.9
Visceral dysmorphogenesis mouse	Not in domain		321-978	30.8-51.9	85.7-93.2
Fetal growth					
Fetal growth retardation rodent	Not in domain		2019	22.1	92.6
Fetal growth retardation rat	Not in domain		1317-1759	33.3-34.9	89.4-89.8
Fetal growth retardation mouse	Not in domain		727-978	39.1-40.4	89.8-90.3
Fetal growth retardation rabbit	Not in domain		269-1013	29.4-52.9	87.2-89.7
Fetal weight decrease rodent	Not in domain		2019	30.8	91.8
Fetal weight decrease rat	Not in domain		1325-1759	35.4-36.7	89.0-89.9
Fetal weight decrease mouse	Not in domain		732-978	39.3-43.9	89.8-91.4
Fetal weight decrease rabbit	Not in domain		420-1013	26.6-48.4	87.2-95.3
Fetal survival					
Fetal death rodent	Not in domain		1538-2019	27.7-29.8	89.8-92.1
Fetal death rat	Not in domain		1519-1759	27.9-28.9	91.1-91.8

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Fetal death mouse	Not in domain		842-978	34.4-36.9	90.4-90.9
Fetal death rabbit	Not in domain		760-1013	40.9-42.9	89.5-89.9
Post implantation loss rodent	Not in domain		2019	30.9	92.5
Post implantation loss rat	Not in domain		1321-1759	30.0-32.3	89.5-91.3
Post implantation loss mouse	Not in domain		978	28.3	92.6
Post implantation loss rabbit	Not in domain		432-1013	43.4-49.0	84.4-89.0
Pre implantation loss rodent	Not in domain		1516-2019	31.3-32.3	90.2-90.6
Pre implantation loss rat	Not in domain		1059-1759	35.4-38.7	89.0-89.1
Pre implantation loss mouse	Not in domain		589-978	43.3-51.2	89.7-90.2
Pre implantation loss rabbit	Not in domain		323-1013	38.3-57.4	87.0-90.0

*Ranges are provided for those models where sub-models have been developed.

Sulfolane was classified as not in domain for all the models evaluated.

2.2.5 Neurotoxicity

Neurotoxicity models were developed based on alterations in newborn rodent, rat, and mouse. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Behavioral toxicity newborn rodent	Not in domain		502-671	55.8-60.7	86.4-89.7
Behavioral toxicity newborn rat	Not in domain		466-628	52.5-58.2	90.2-91.4
Behavioral toxicity newborn mouse	Not in domain		127-172	43.2-78.4	86.7-90.0

*Ranges are provided for those models where sub-models have been developed.

Sulfolane was classified as not in domain for all the models evaluated.

2.2.6 Human Adverse Cardiological Effects

A total of 13 models are used to assess potential human adverse cardiac effects of tested chemicals. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Conduction disorders	Not in domain		370-1628	54.2-64.2	88.4-93.6
Coronary artery disorders	Not in domain		700-1628	50.0-52.9	88.3-89.5
Electrocardiogram disorders	Not in domain		535-1628	47.7-52.3	87.1-88.2
Heart failure disorders	Not in domain		679-1628	41.0-48.8	90.7-91.6
Arrhythmia disorders	Not in domain		682-1509	43.8-54.3*	91.1-92.0
Bradycardia disorders	Not in domain		324-1628	47.2-65.7	86.2-90.4
QT prolongation	Not in domain		444-1628	52.0-61.3	88.5-88.9
Tachycardia disorders	Not in domain		554-1628	48.7-60.3	86.4-89.1
Torsades	Not in domain		374-1628	53.6-61.0	86.9-88.8
Myocardial infarct disorders	Not in domain		366-1628	53.0-64.3	87.6-90.5
Myocardial disorders	Not in domain		314-1629	38.1-57.7	85.8-93.2
Palpitations	Not in domain		548-1628	54.0-58.2	86.4-88.6

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Rate Rhythm Disorders	Not in domain		813-1628	32.1-40.2	87.7-90.8

*Ranges are provided for those models where sub-models have been developed.

Sulfolane was classified as not in domain for all the models evaluated.

2.2.7 Human Adverse Hepatobiliary Effects

Five models are used to assess the potential for adverse human hepatobiliary effects produced by test compounds. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Bile duct disorders	Not in domain		567-1043	23.9-27.2	97.9
Gall bladder disorders	Not in domain		607-1055	41.3-42.5	92.9-93.7
Liver jaundice disorders	Not in domain		692-1604	49.6-51.7	91.4-92.7
Liver acute damage disorders	Not in domain		646-1603	47.3-51.5	92.7-93.5
Liver enzyme release disorders	Not in domain		624-1602	40.4-48.5	94.3-95.7

*Ranges are provided for those models where sub-models have been developed.

2.2.8 Human Adverse Urinary Tract Effects

Six models are used to assess the potential for adverse urinary tract effects produced by test compounds. The table below provides the results for sulfolane including the prediction call and prediction probability. The number of training compounds used to develop the models and the sensitivity and specificity of each model are also provided.

Endpoint	Prediction Call	Prediction Probability	Number of Training Compounds*	Sensitivity*	Specificity*
Bladder disorders	Not in domain		689-1591	43.9-51.5	89.2-90.2
Blood in urine disorders	Not in domain		638-1591	43.6-53.3	93.7-95.2
Kidney disorders	Not in domain		625-1590	35.4-38.9	94.8-96.1
Kidney function tests	Not in domain		687-1589	45.6-50.6	89.8-90.0
Nephropathy disorders	Not in domain		667-1590	44.2-55.8	90.2-91.6
Urolithiasis disorders	Not in domain		626-1591	34.5-48.3	94.2-95.5

*Ranges are provided for those models where sub-models have been developed.

3.0 ToxTree

3.1 Background

This summary provides an overview of the ToxTree method and the results of the QSAR analysis conducted on sulfolane. ToxTree is an open source software that was commissioned by the European Commission Joint Research Centre (JRC). The program estimates toxicity hazards by using a decision tree approach for each endpoint evaluated (JRC, 2011a). [ILS Note: Only those modules related to mammalian toxicity were included in this evaluation.]

3.2 Results

3.2.1 Cramer Classification Scheme

The Threshold of Toxicological Concern (TTC) is a principle which attempts to develop a minimal exposure level for a chemical, below which there would be negligible human health

risk. The Cramer classification scheme uses chemical structures and total human intake estimates to estimate TTC. In addition to chemical structure, the scheme uses metabolic pathways, toxicity data, and the presence of the chemical in foods or as an endogenous metabolite in developing a TTC. The chemical is then classified into one of three classes:

> Class I contains substances of simple chemical structure with known metabolic pathways and innocuous end products which suggest a low order of oral toxicity.
>
> Class II contains substances that are intermediate. They possess structures that are less innocuous than those in Class 1 but they do not contain structural features that are suggestive of toxicity like those in Class 3.
>
> Class III contains substances with chemical structures that permit no strong initial impression of safety and may even suggest a significant toxicity (JRC, 2011b).

Sulfolane was classified as a Class III chemical. This classification was based on the presence of (1) a non-divalent sulfur atom and (2) all elements present in sulfolane do not occur as (a) a sodium, potassium, calcium, magnesium, or ammonium salt of a carboxylic acid, (b) a sulfate or hydrochloride of an amine, or (c) a sodium, potassium or calcium sulfonate, sulfamate, or sulfate (Cramer et al., 1978).

3.2.2 Kroes TTC

The Kroes TTC principle is based on the principle that below the human exposure level for a chemical, there is a probability of human health risk. The TTC uses this principle to evaluate chemicals that lack a full toxicological database based on comparison to structurally similar chemicals that have similar structural characteristics. Chemicals are initially evaluated the presence of genotoxic or high potency carcinogenic structural alerts. Non-genotoxic compounds are evaluated separately to evaluate concerns associated with increased intake of the compound (Kroes et al., 2004). The Kroes TTC Decision Tree, based on the assumption that daily intake of the chemical would be ≤1.5 μg/day, predicted that the substance would not be of safety concern.

3.2.3 Benigni/Bossa Rules for Carcinogenicity and Mutagenicity

Chemicals are evaluated for the presence of structural alerts associated with carcinogenic and/or mutagenic activity. Structural alerts for non-genotoxic and genotoxic compounds are evaluated. Structural alerts that are evaluated include acyl halides, hydrazine, nitro aromatics, thiocarbonyls, and halogenated benzene (Benigni et al., 2008). Based on the lack structural alerts, sulfolane was predicted to be negative for genotoxic or non-genotoxic carcinogenic activity.

[ILS Note: Three QSAR models were included in the rules for this evaluation. The models focused on evaluating (1) mutagenic activity of aromatic amines in *Salmonella typhimurium* strain TA100, (2) mutagenic activity of α,β-unsaturated aldehydes in *S. typhimurium* strain TA100, and (3) carcinogenic activity of the aromatic amines in rodents. The applicability domains of the three QSAR models were (1) compounds containing (a) homocyclic amines (excluding aromatic amines containing aromatic nitro groups) and (b) diazo, isocyanate, and imine groups, (2) linear aldehydes, and (3) compounds containing (a) homocyclic amines (including aromatic amines containing aromatic nitro groups) and (b) diazo, isocyanate, and imine groups, respectively.]

3.2.4 Structural Alerts for the *In Vivo* Micronucleus Assay in Rodents

Chemicals are evaluated for the presence of structural alerts associated with micronucleus formation in rodents. Structural alerts that are evaluated include acyl halides, hydrazine, quinones, isocyanate and isothiocyanate groups, and nitro aromatic groups (Benigni et al., 2009). A review of the structure of sulfolane indicates that there were no structural alerts which may predict *in vivo* micronucleus formation. [ILS Note: Much of the data used in the ToxTree analysis were obtained from the "FDA SAR Genetox Database" developed by Leadscope.]

3.2.5 Structural Alerts for Eye Irritation and/or Corrosion

Based on general chemical class, chemicals are evaluated for physicochemical properties and the presence of structural alerts associated with eye irritation and/or corrosion. For the current evaluation, physicochemical properties were not included in the evaluation, and sulfolane was only evaluated for the presence of structural alerts. [Note: The user manual notes that exclusion of physicochemical properties may lead to a low quality prediction (Ideaconsult Ltd., 2009). Physicochemical properties were not included because data for all the necessary properties were not available.] Structural alerts that are evaluated included presence of aliphatic monoalcohol, pyrrolidine, and aliphatic carboxylic acid (Ideaconsult Ltd., 2009). Sulfolane was classified as unknown.

3.2.6 Structural Alerts for Skin Irritation and/or Corrosion

This model estimates skin irritation and/or corrosion potential based on physicochemical properties and the presence of structural alerts. For the current evaluation, physicochemical properties were not included in the evaluation, and sulfolane was only evaluated for the presence of structural alerts. [Note: The user manual notes that exclusion of physicochemical properties may lead to a low quality prediction (Ideaconsult Ltd., 2009). Physicochemical properties were not included because data for all the necessary properties were not available.] Sulfolane was classified as unknown.

3.2.7 Skin Sensitization

This model evaluates chemicals for the presence of structural alerts associated with skin sensitization. There were no structural alerts for skin sensitization identified in sulfolane.

3.2.8 START Biodegradation and Persistence

Chemicals are evaluated for the presence of structural alerts associated with biodegradation and/or environmental persistence. Chemicals are then classified into one of three categories: Class 1 (easily biodegradable), Class 2 (persistent chemical), or Class 3 (unknown biodegradability) (Molecular Networks, 2008). Structural alerts that are evaluated include epoxides, two or more rings, and a tertiary amine. Sulfolane was classified as Class 3.

3.2.9 Michael Acceptor

This model evaluates whether the chemical may be a Michael acceptor based on the presence of structural alerts. The model indicated that sulfolane is not reactive by Michael addition.

3.2.10 Cytochrome P450-Mediated Drug Metabolism

This model evaluates chemicals for sites that may be metabolized by cytochrome P450 isoform 3A4. The model evaluates sites of metabolism but not the proposed metabolite. Based on the

chemical structure, it was predicted that the carbons at the 2- and 5-positions on the ring would be the primary sites of metabolism. The carbons at the 3- and 4-positions were identified as the secondary metabolic sites, while sulfur was identified as the tertiary metabolic site. No additional sites of predicted metabolism were noted. [Note: A web-based application of the SMARTCyp model indicated that the predicted sulfur metabolic site was not considered a possible site of metabolism since there was no matching energy rule. Some limitations of the model noted on the website include: (a) sites with low accessibility in three dimensions are ranked too high, (b) metabolites produced due to entropy are ranked too low, and (c) for reactive sites for large compounds (e.g., >40 non-hydrogen atoms), the proposed reactive sites are not usually found experimentally.]

4.0 Organisation for Economic Co-operation and Development (OECD) Tool Box

4.1 Background

The OECD ToolBox is a program developed to incorporate (Q)SAR techniques to fill in data gaps in (eco)toxicity data needed to assess hazards of chemicals. Similar to other QSAR programs, the program identifies structural characteristics and potential mechanisms or modes of action for various toxicity endpoints. Other chemicals with similar structural characteristics or proposed mechanisms or modes of action are then identified, based on the user input. The experimental data for the structurally or mechanistically similar chemicals are then used to predict activity of the target chemical (OECD, undated).

More specifically, the program initially "profiles" the chemical using a variety of databases to retrieve information regarding the chemical. The profilers are grouped into four categories: predefined (e.g., database affiliation), general mechanistic (e.g., estrogen receptor binding), endpoint specific (e.g., micronucleus alerts by Benigni/Bossa), and empiric (e.g., organic function groups (US EPA)) (OECD, 2010).

Following the profiling step, the chemical is then evaluated for the available data for a variety of endpoints. Endpoints that may be evaluated include aquatic toxicity, eye irritation, genotoxicity, micronucleus formation, skin irritation, skin sensitization, and repeated dose toxicity. Data are obtained from a variety of databases and sources including the European Center of Ecotoxicology and Toxicology (OECD, 2010).

The results of the profiling and endpoint portions of the program relate to the focus of the QSAR analyses to be conducted. QSAR analyses may be evaluated based on potential mechanism/mode of action related to an endpoint of interest (e.g., protein binding as a mode of action for skin sensitization). Alternatively, if information regarding a mode of action is not available, then a structural analog approach may be used to identify chemicals that are structurally similar with potentially similar effects (OECD, 2010).

Once chemicals are identified to fill in a data gap, three different tools may be used: read-across analysis, trend analysis, and (Q)SAR models. The read-across and trend analyses use the available data to estimate the missing data. The (Q)SAR models option allows the user to access the library of models available for use. The read-across analysis was identified as appropriate for "qualitative" endpoints (e.g., skin sensitization) where there are limited result options (e.g., positive, negative, or equivocal) or for "quantitative endpoints" (e.g., 96-hour LC_{50} for fish)

where there are limited number of analogs identified. The trend analysis was identified as appropriate for those situations where a high number of analogs with experimental data were identified. The (Q)SAR model option was proposed to be used when no adequate analogs were identified (OECD, 2010).

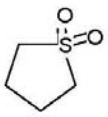
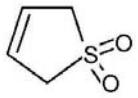
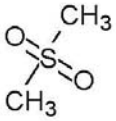
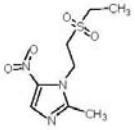
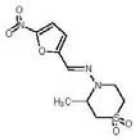
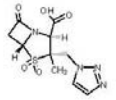
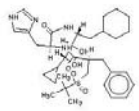
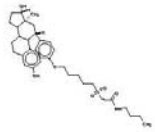
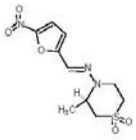
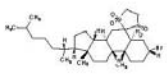
4.2 Results

A search of the available data for sulfolane indicated that there was limited mechanism and mode of action information available. For most of the general mechanistic and endpoint specific modules evaluated (e.g., protein binding and presence of micronucleus alerts), the results were either identified as "no binding" or "no alerts."

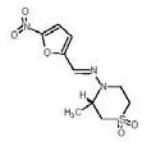
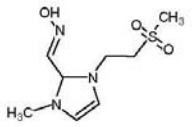
Based on the limited mechanistic information available, a search was conducted for chemicals that were structurally similar to sulfolane. Two different searches for identifying structurally similar chemicals were used within the program. Searches were conducted based on the organic functional groups, as identified by the U.S. Environmental Protection Agency (EPA), and on chemicals that were at least 70% structurally similar to sulfolane as calculated by the Tanimoto method.

The organic functional groups, identified by the EPA, that were searched were "Aliphatic Carbon [CH] and Aliphatic Carbon [-CH2-], and Miscellaneous sulfide (=S) or oxide (=O) and Suflur {v+4} or {v+6} and Sulfone, aliphatic attach [-SO2-] and Sulfoxide, aliphatic attach [-S(=O)-] and Sulfur, aliphatic attach [-S-]." This led to the identification of 10 compounds that were identified as having at least those functional groups within their structure definition. (See table below for identified structures.) [ILS Note: While the program notes that 11 chemicals were identified as structurally similar, nifurtimox was identified twice.]

QSAR Toolbox 2.2.1.1120; Aliphatic Carbon [CH]<AND>Aliphatic Carbon [-CH2-]<AND>Miscellaneous sulfide (=S) or o:

#1 C1CCCS1(=O)=O 126-33-0 2,3,4,5-tetrahydrothiophene-1 ,1-dioxide;tetrahydrothiophene-1,1-dioxide;tetrahydrothiophene 1,1-dioxide;thiophene, tetrahydro-, 1,1-dioxide 	#2 C1=CCS(=O)(=O)C1 77-79-2 2,5-dihydrothiophene 1,1-di oxide;beta-sulfolene;3-sulfolene;thiophene, 2,5-dihydro-, 1,1-dioxide 
#3 CS(C)(=O)=O 67-71-0 sulfonyldimethane;dimethyl sulpho ne;methane, sulfonylbis-;methyl sulfone;dimethyl sulfone 	#4 C1(N(=O)=O)=CN=C(C)N1CCS(=O)(=O)CC 19387-91-8 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1h-imidazole;tinidazole;1h -imidazole, 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro- 
#5 C1(C=NN2C(C)CS(=O)(=O)CC2)=CC=C(N(=O)=O)O1 23256 -30-6 nifurtimox 	#6 C(=O)(O)C{P-}1C{P+}(C)(CN2C=CN=N2)S(=O)(=O)C{P+}2CC(=O)N12 89786-04-9 4-thia-1-azabicyclo[3.2.0]heptane-2-car boxylic acid, 3-methyl-7-oxo-3-(1h-1,2,3-triazol-1-ylmethyl)-, 4,4-dio xide, (2s,3s,5r)- 
#7 C(=O)(C{P-}(Cc1ccccc1)CS(=O)(=O)C(C)(C)C)NC{P+}(C(=O)NC {P-}(C{P+}(O)C{P+}(O)C1CC1)CC1CCCCC1)CC1=CNC=N1 1262 22-34-2 remikiren 	#8 c12c(C3C{P-}(c4ccc(OCCCCCS(=O)(=O)CC(=O)NCCCC)cc4)CC{P -}4(C)C(C3CC1)CCC{P+}4O)ccc(O)c2 N/A - 
#9 C1(C=\{t\}NN2C{P+}(C)CS(=O)(=O)CC2)=CC=C(N(=O)=O)O1 23256-30-6 nifurtimox 	#10 C12(C{P+}3C{P-}(C)(C{P-}4C{P-}(C{P+}5C{P-}(C)C{P+}(C{P +}(C)CCCC(C)C)CC5)CC4)C1)CCC{P-}(Br)C3)S(=O)(=O)CCS2(=O)= O 133331-34-7 - 

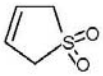
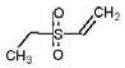
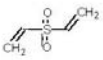
QSAR Toolbox 2.2.1.1120; Aliphatic Carbon [CH]<AND>Aliphatic Carbon [-CH2-]<AND>Miscellaneous sulfide (=S) or o:

#11 C1(C={t}NN2C{P-}(C)CS(=O)(=O)CC2)=CC=C(N(=O)=O)O1 *0-23-8 - 	#12 C(C1N(C)C=CN1CCS(C)(=O)=O)={t}NO 131431-60-2 - 

A review of the available data indicated that there were limited data available for any of the endpoints where sulfolane lacked data (i.e., only one chemical of those identified had experimental data). The lack of available data did not allow for a read-across or trend analysis to be conducted.

The second analysis method used structural similarity, as defined by Tanimoto, to identify potential analogs to allow for prediction of activity. Using a minimal Tanimoto percentage of 70%, three chemicals were identified (see table below).

QSAR Toolbox 2.2.1.1120; Similarity: Threshold=70%; Tanimoto(Atom pairs) (Structure similarity), page 1/1

#1 C1CCCS1(=O)=O 126-33-0 2,3,4,5-tetrahydrothiophene-1 ,1-dioxide;tetrahydrothiophene-1,1-dioxide;tetrahydrothiophene 1,1-dioxide;thiophene, tetrahydro-, 1,1-dioxide 	#2 C1=CCS(=O)(=O)C1 77-79-2 2,5-dihydrothiophene 1,1-di oxide;beta-sulfolene;3-sulfolene;thiophene, 2,5-dihydro-, 1,1-dioxide 
#3 C(=C)S(=O)(=O)CC 1889-59-4 (ethylsulfonyl)ethene;(eth ylsulphonyl)ethylene;ethyl vinyl sulfone 	#4 C(=C)S(=O)(=O)C=C 77-77-0 1,1'-sulfonyldiethene;divin yl sulfone;divinyl sulphone;1,1'-sulfonylbisethene;ethene, 1,1'-sulfonylbis- 

As with the previous analysis, there were limited data available for the analogs to allow for prediction of activity for sulfolane.

5.0 Lhasa

The Lhasa Derek Nexus program is a program that uses expert-based toxicology rules to predict chemical toxicity. Using the structure of the chemical, the program applies structure-activity relationship rules as well as expert knowledge rules to make predictions as to the potential

toxicity of the chemical. Once the results are provided, support for the predictions (e.g., literature references, examples, and comments) are provided to allow the user to review the evidence and develop conclusions. The expert knowledge rules are updated based on testing by collaborators who compare the predictions with known results (Lhasa Limited, 2011; Matthews et al., 2008).

The Derek Nexus program was accessed using the National Institute of Environmental Health Sciences (NIEHS) Integrated Predictive System (ISP). The endpoints evaluated included thyroid toxicity, miscellaneous endpoints, carcinogenicity, irritation, genotoxicity, respiratory sensitization, skin sensitization, HERG channel inhibition, hepatotoxicity, chromosome damage, mutagenicity, reproductive toxicity, ocular toxicity, bradycardia, nephrotoxicity, hepatotoxicity, and "All Endpoints." [ILS Note: There is no information currently available on the NIEHS ISP to provide background on these endpoints. ILS is currently contacting Lhasa to obtain additional information regarding the endpoints displayed.]

The results for all endpoints were identified as "No result." [ILS Note: In communications with Lhasa, the outcome of "No result" could indicate that there were no toxic structural alerts identified in the chemical. However, there is no current functionality in the program to allow further analysis of the results to allow for individual assessment of the results. Additional information is forthcoming from Lhasa.]

6.0 MultiCASE

The MultiCASE program, accessed through the NIEHS ISP, evaluates the input chemical for the presence of biophores. The chemical is also evaluated for the presence of molecular fragments and molecular descriptors that may modulate the effect of the identified biophore in producing the proposed toxic effect. The combination of these data is used to produce a quantitative estimate of toxicity for the tested chemical (Matthews et al., 2008; Teasdale, 2011).

The endpoints evaluated from MultiCASE were carcinogenicity, genotoxicity, reproductive toxicity, kidney and bladder toxicity, behavioral toxicity, cardiac toxicity, liver toxicity, skin irritation, sensory irritation, and eye irritation. Of the models evaluated, sulfolane was identified as active in three models: Fertility, male sperm rats (Sub B), Kidney toxicity, FDA Blood Urine (6x6), and Kidney toxicity, FDA Urolithiasis (6x5). [ILS Note: As of the development of this text, the description of the models was not available. Additionally, the results output for the fertility endpoint was not available. Therefore, only the results for the kidney toxicity endpoints are discussed below. Leadscope has been contacted regarding these issues, and they are currently in the process of being updated and corrected.]

For the FDA Blood Urine (6x6) model, it was noted that four of four chemicals with the identified biophore (SO2-CH2-) had been identified as kidney toxicants. The average activity of the four compounds was 46 CASE units. The QSAR contribution, combined with identified modulators, led to a total predicted QSAR activity of 68.00 CASE units, which was classified as extremely active. The probability that sulfolane is a kidney toxicant was 83%.

For the FDA Urolithiasis (6x5) model, it was noted that three of four chemicals with the identified biophore (SO2-CH2-) had been identified as kidney toxicants. The average activity of

the three compounds was 39 CASE units. The program noted that the confidence level in the biophore was not very good. Furthermore, it was noted that the biophore in the sulfolane existed in a significantly different environment than present in the database and may not be relevant. The QSAR contribution, combined with identified modulators, led to a total predicted QSAR activity of 62.24 CASE units, which was classified as extremely active. The probability that sulfolane is a kidney toxicant was 66%.

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Appendix: Units and Abbreviations

CYP450 = cytochrome P450
EPA = Environmental Protection Agency
FDA = Food and Drug Administration
ILS = Integrated Laboratory Systems, Inc.
IPS = Integrated Predictive System
JRC = Joint Research Centre, part of the European Commission
NIEHS = National Institute of Environmental Health Sciences
NTP = National Toxicology Program
OC = occurrence rate; the "ratio of the occurrence of a particular metabolic reaction to the total number of metabolic reactions in the MetaCore™/MetaDrug™ database"
QSAR = quantitative structure-activity relationship
TP = Tanimoto similarity percentage
TTC = threshold of toxicological concern