

Human Health Hazard Assessment for the Sustainable Futures Program

Analog Analysis, Read Across, and the OncoLogic Tool

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Non-Cancer Health Hazard Screen

- Background
 - No computer models for assistance
 - Analysis based on available experimental data for the compound of interest and closely related analogs
 - Includes a broad range of acute, subchronic, and chronic endpoints

Non-Cancer Health Hazard Endpoints

Endpoints generally used to assign hazard concern levels for PBT score and potential regulation of PMNs

- **Systemic toxicity (e.g., liver, kidney, or generalized toxicity)**
 - Subchronic or chronic duration
 - Acute studies may offer evidence of potential health hazards if longer duration studies are not available
- **Neurotoxicity**
 - Behavioral evidence of neurotoxicity, brain pathology
- **Reproductive toxicity**
 - Effects on ability to reproduce (e.g., fertility)

Non-Cancer Health Hazard Endpoints

Endpoints generally used to assign hazard concern levels for PBT score and potential regulation of PMNs (Cont.)

- **Developmental toxicity**
 - **Effects on the developing fetus**
 - **Maternal toxicity may indicate greater sensitivity of pregnant animals with respect to systemic effects**
- **Immunotoxicity**
 - **Effects on immune system organs (spleen, thymus)**
 - **Immune suppression observed in immunotoxicity studies**

Non-Cancer Health Hazard Endpoints

- **Other hazard endpoints considered**
 - **Mutagenicity**
 - **Skin Sensitization**
 - **Irritation (eye, skin, respiratory)**
- **Not generally the sole basis for regulating a chemical or assigning the PBT score**
 - **These endpoints should be identified in the MSDS**

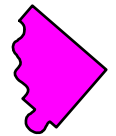
Five Steps in Conducting a Non-Cancer Hazard Screen

- **Step 1. Locate relevant information on substance (if available) and report information in SF summary assessment**
- **Step 2. Determine if chemical is a member of a category known to be associated with hazards**
- **Step 3. Identify appropriate analog(s) if available data on PMN chemical is not sufficient to allow for toxicity characterization**
- **Step 4. Locate measured toxicity data on analog(s)**
 - **Quality measured data on chemical substance supercedes analog data**
- **Step 5. Assign **HAZARD** concern level**

Factors in Health Hazard Assessment



Chemical toxicity data



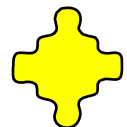
Analogue toxicity data



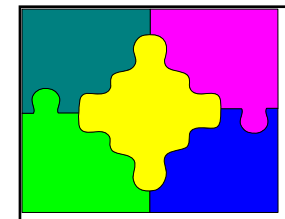
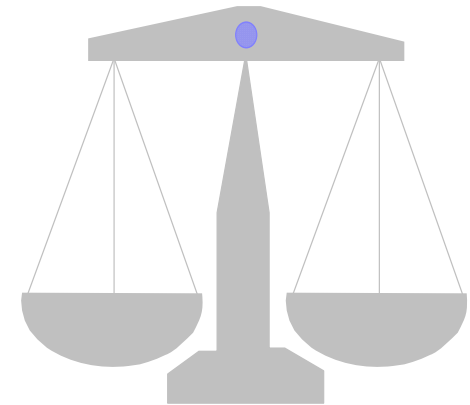
Chemical Class toxicity data



Mechanistic considerations



Professional judgement



Step 1. Search for toxicity data on chemical of interest

- **Where to search for chemical toxicity data**
 - In-house data
 - TOXNET (toxnet.nlm.nih.gov)
 - HSDB, TOXLINE, CCRIS, GENETOX, DART/ETIC
 - IARC, IPCS, NTP, U.S. EPA HPV program, SIDS data sets, RTECS, TSCATS, and others (URLs included in attachment)
- **Other sources are available**

Important Details to Record From Toxicity Studies

- **Hazard concern identified**
- **Type of study (e.g., 2-generation reproductive toxicity study, 28-day repeated-dose study)**
- **Study duration**
- **Animal species**

Important Details to Record From Toxicity Studies

- **Exposure route (oral gavage, diet, dermal, inhalation)**
- **Effect Levels**
 - **No adverse effect levels (NOAEL) for each hazard identified**
 - **Lowest adverse effect levels (LOAEL) for each hazard identified**
- **Reference**

Important Factors in Evaluating Available Data

- **No data is NOT equivalent to negative data**
 - Hazard concerns based on scientific judgment
- If conflicting data exist, a weight of evidence approach should be used to support conclusions

Other Factors to Consider

- **Absorption Differences**
 - Absorption is necessary for some chemicals to be toxic
 - May vary by exposure route
 - May be estimated using measured data (chemical or analog) or by chemical and physical properties
 - Molecular weight, Kow, water solubility, physical state
 - Often need to extrapolate across exposure routes
 - Not appropriate for portal of entry effects

Dermal Absorption

- **Assessed on a case-by-case basis**
- **The following generally absorb well through the skin:**
 - **Liquid chemicals with log P values of 2 to 4**
 - **Liquid chemicals with MW <500**
 - **Formulated products with surfactants and detergents**
 - **Small molecular weight amines and carboxylic acids**

Dermal Absorption (Cont.)

- The following generally absorb poorly through the skin:
 - Solids
 - Solids may absorb better if the melting point is at approximately skin temperature
 - Chemicals with MW >500
 - Charged chemicals and salts

Absorption (Other Exposure Routes)

- **Assessed on a case-by-case basis**
- **Important chemical factors often include molecular weight, physical state, K_{ow} , solubility, and ionization state**

Other Factors to Consider

- Biological activity, metabolism, bioactivation, pharmacokinetics, distribution
 - Toxicity study on appropriate analog mitigates the need to consider these factors separately

Step 2. Determining if a Chemical Belongs to a Category of Concern

- **U.S. EPA Category Statements**
 - www.epa.gov/oppt/newchemicals/cat02.htm
 - **55 categories based on PMN data that have consistently been shown to induce toxic effects**
 - **Concern may exist for chemicals with structures that are not consistent with an EPA category**

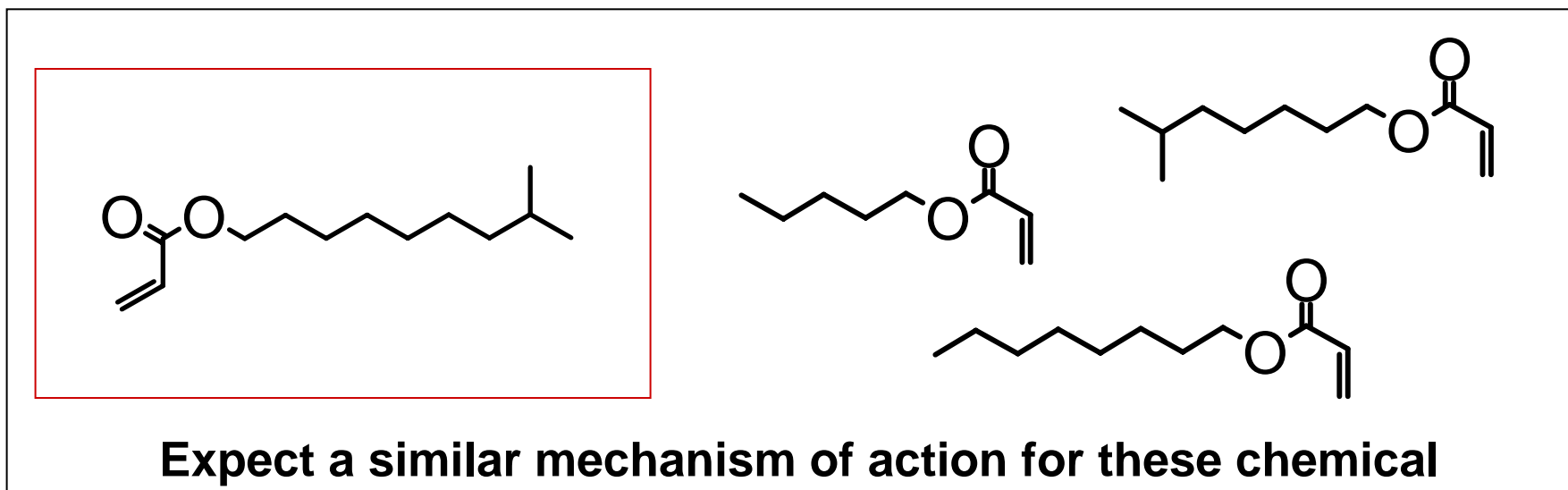
- **Review articles**

Step 3. Identifying Appropriate Analog(s)

- **Substructure or similarity searches**
 - **Publicly available databases that allow substructure searches**
 - **CHEMID.**
<http://chem.sis.nlm.nih.gov/chemidplus/cmplxqry.html>
 - **CHEMFINDER**
<http://chemfinder.cambridgesoft.com/>
 - **TSCATS**
<http://esc.syrres.com/efdb/tscats.htm>

Step 3. Identifying Appropriate Analog(s)

- Characteristics of an appropriate analog
 - Size and functional groups are representative of chemical of interest
 - Does not contain biologically active groups that are NOT represented on chemical of interest



Step 4. Collection of toxicity data for all identified analog(s)

- **Same Type of Information as Step 1 (data on chemical of interest)**
 - Hazard concern identified
 - Type of study (e.g., 2-generation reproductive toxicity study, 28-day repeated-dose study)
 - Study duration
 - Animal species

Step 5. Assigning a Hazard Concern Level

- Hazard endpoints used in assigning concern levels
 - Systemic Toxicity
 - Includes reproductive, developmental, neurotoxicity, target organ toxicity, etc.
 - Environmental vs. occupational exposures
 - Endpoints not generally used as sole basis for assigning concern levels for PBT scores
 - Skin sensitization and irritation
 - Mutagenicity

Step 5. Guidance in Assigning EPA Concern Levels

Concern Level	Criteria	Exposure and Risk Assessment Needed?
HIGH	<ul style="list-style-type: none">• Evidence of adverse effects in human populations• Conclusive evidence of severe effects in animal studies	Yes
MODERATE	<ul style="list-style-type: none">• Suggestive animal studies• Analog data• Class known to produce toxicity	Yes
LOW	<ul style="list-style-type: none">• No concern identified	No

Interpreting Hazard Concern Levels

- Risk assessment performed only if moderate or high concern is identified
- Specific distinction between a moderate and a high call is not critical since both indicate the need to complete an exposure analysis to make a determination of potential risk

Performing a Cancer Hazard Assessment Using the OncoLogic Tool



Why is Cancer a Separate Toxicity Endpoint (from Non-Cancer Effects)?

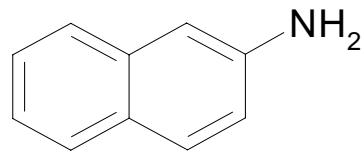
- Default assumption is that there is no threshold for carcinogens that act by genotoxic mechanisms
- Risk Assessment methods are different for cancer and non-cancer endpoints
 - Also differences in framework for risk determination between genotoxic ($q1^*$) and (well-defined) non-genotoxic (MOE) carcinogens

Why is OncoLogic different than EpiSuite and ECOSAR?

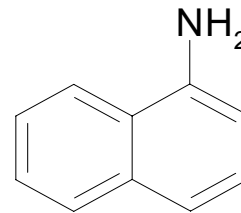
- Difficult to relate specific chemical/physical properties to carcinogenicity
 - Many properties have multiple possible effects on carcinogenicity
 - Multiple stages of carcinogenicity
 - Metabolism to carcinogenic intermediate
 - Isomers that have very similar properties may have dramatically different cancer concerns
- No all-encompassing descriptors have been identified for carcinogenicity even within many chemical classes

Challenges in Predicting Carcinogenicity (Cont.)

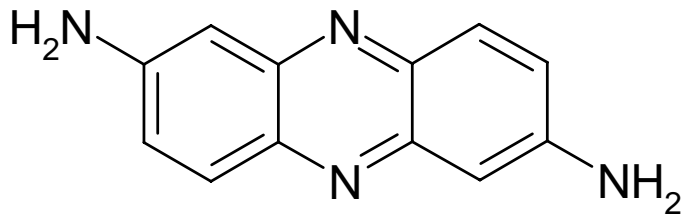
- Carcinogenicity of a chemical may be drastically different for chemicals with similar chemical/physical properties



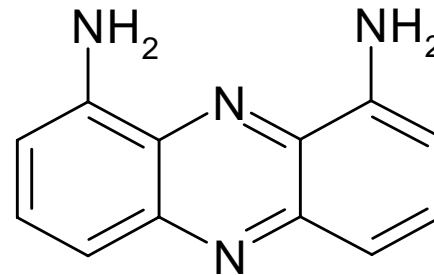
Potent human carcinogen



Marginal or inactive

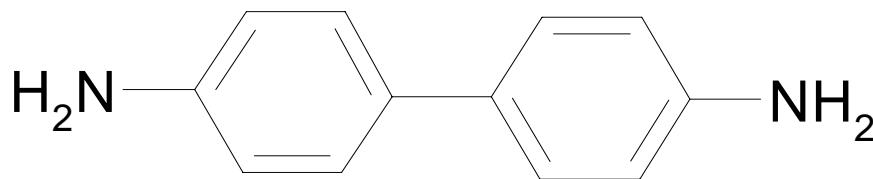


Strong Mutagen



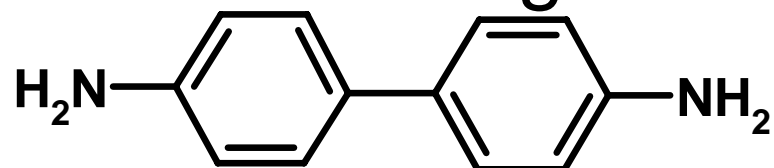
Approx. 10,000X less mutagenic

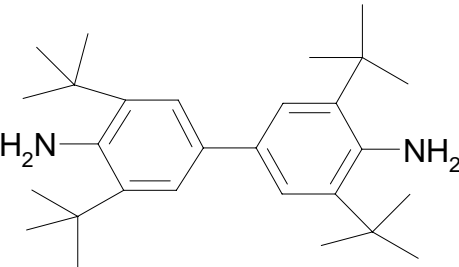
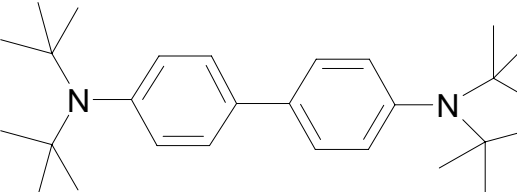
Examples of how “Knowledge Rules” can be used in chemical design



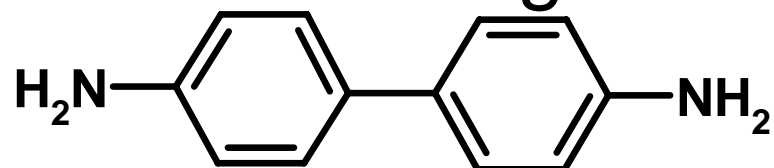
OncoLogic Cancer Concern = High

Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential



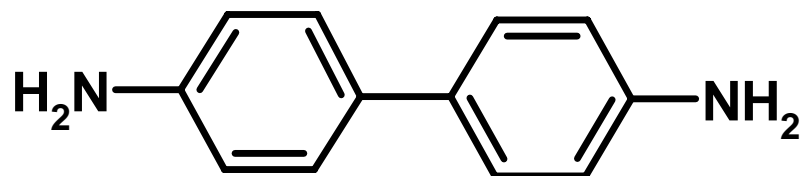
Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-bis(tert-butylamino)diphenylmethane, where the amino groups of the parent structure are substituted with tert-butyl groups.</p>	Introduce bulky substituent(s) <u>ortho</u> to amino / amine-generating group(s).	
 <p>Chemical structure of N,N'-bis(tert-butyl)-4,4'-diaminodiphenylmethane, where the amino groups of the parent structure are substituted with tert-butyl groups on the nitrogen atoms.</p>	Introduce bulky N-substituent(s) to amino / amine-generating group(s).	

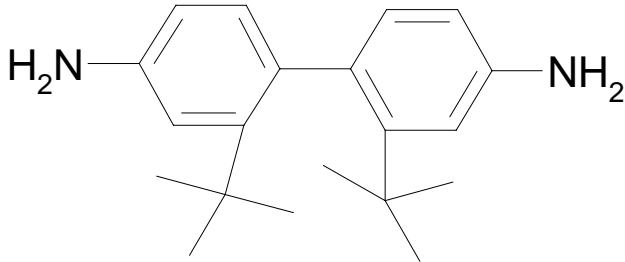
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential



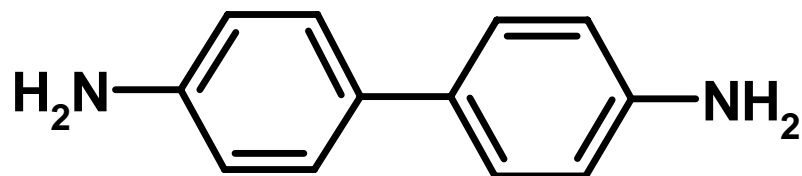
Example	Action	Effect on Cancer Concern/Justification
<chem>CC(C)(C)c1ccc(N)c(C(C)(C)C)c1Cc2ccc(N)c(C(C)(C)C)c2</chem>	Introduce bulky substituent(s) <u>ortho</u> to amino / amine-generating group(s).	Provide steric hindrance to inhibit bioactivation. Concern = Marginal
<chem>CC(C)(C)N(c1ccc(cc1)Cc2ccc(N)cc2)C(C)(C)C</chem>	Introduce bulky N-substituent(s) to amino / amine-generating group(s).	Make it a poor substrate for the bioactivation enzymes. Concern = Marginal

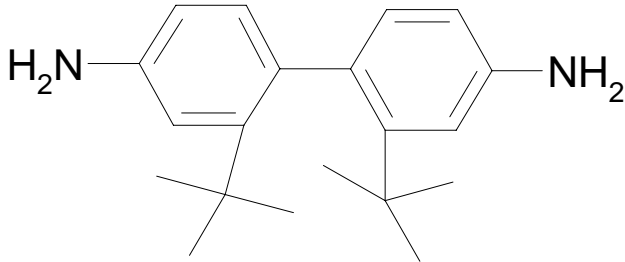
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



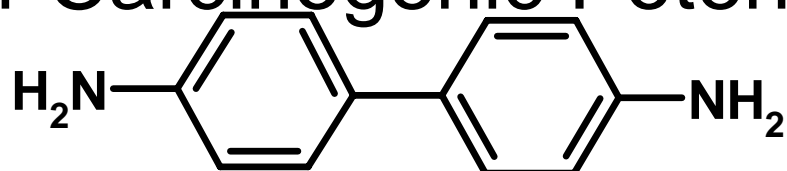
Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-diaminodiphenylmethane with tert-butyl groups attached to the ortho positions of both benzene rings.</p>	Introduce bulky groups <u>ortho</u> to intercylic linkages.	

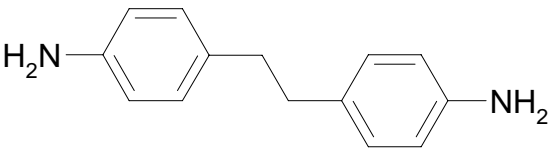
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



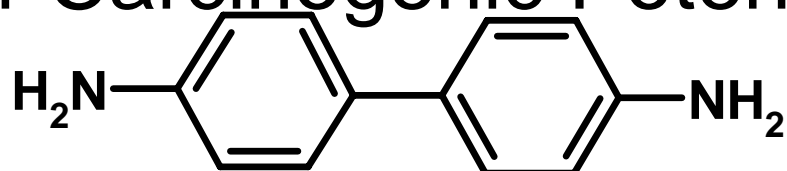
Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-diaminodiphenylmethane with two tert-butyl groups attached to the ortho positions of the benzene rings, illustrating the introduction of bulky groups.</p>	Introduce bulky groups <u>ortho</u> to intercylic linkages.	Distort the planarity of the molecule making it less accessible and a poorer substrate for the bioactivation enzymes. Concern = Marginal

Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



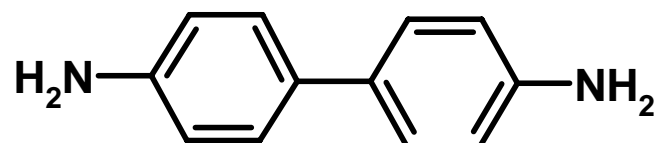
Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-diaminodiphenylpropane, showing two benzene rings connected by a three-carbon alkyl chain, with an amino group (H_2N) attached to each ring.</p>	Replace electron-conducting intercylic linkages by electron-insulating intercylic linkages.	

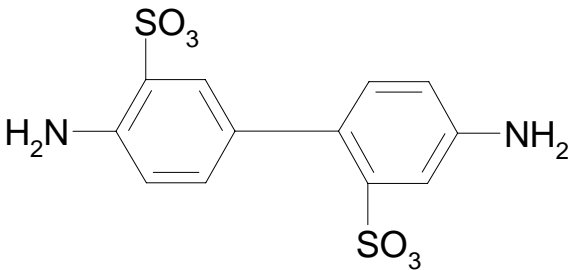
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



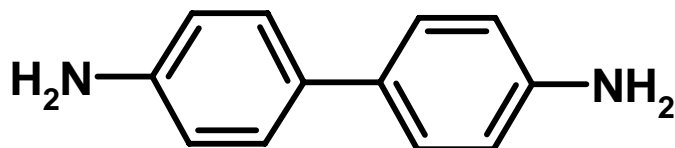
Example	Action	Effect on Cancer Concern/Justification
<chem>Nc1ccc(cc1)CCc2ccc(N)cc2</chem>	<p>Replace electron-conducting intercylic linkages by electron-insulating intercylic linkages.</p>	<ol style="list-style-type: none"> 1. Reduce length of conjugation path and thus the force of conjugation, which facilitates departure of acyloxy anion. 2. Less resonance stabilization of electrophilic nitrenium ion. <p>Concern = Marginal</p>

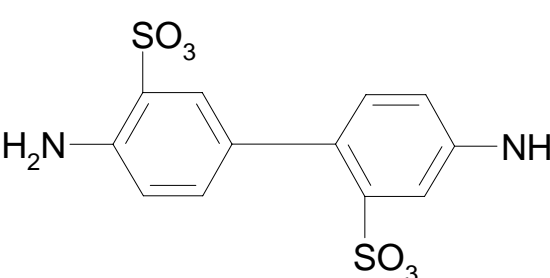
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-diaminodiphenylmethane with sulfonic acid groups (-SO₃H) attached to the para positions of both benzene rings, adjacent to the amino groups.</p>	Ring substitution with hydrophilic groups (e.g., sulfonic acid); especially at ring(s) bearing amino / amine-generating group(s).	

Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential (Cont.)



Example	Action	Effect on Cancer Concern/Justification
 <p>Chemical structure of 4,4'-diaminodiphenylmethane with sulfonic acid groups (-SO₃H) attached to the para positions of both benzene rings, adjacent to the amino groups.</p>	Ring substitution with hydrophilic groups (e.g., sulfonic acid); especially at ring(s) bearing amino / amine-generating group(s).	Render molecule more water-soluble thus reducing absorption and accelerating excretion. Concern Level = Low

OncoLogic® - Expert System

How it Works

- Mimic the thinking and reasoning of human experts using knowledge based rules for chemical classes to predict cancer concern
 - Assigns a baseline concern level ranging from low to high
 - Evaluates how substituents on the chemical may affect carcinogenicity
 - Concern level changes accordingly

Critical Factors for SAR Consideration

- Electronic and Steric Factors
 - Resonance stabilization
 - Steric hindrance
 - Molecular size and shape
- Metabolic Factors
 - Blocking of detoxification
 - Enhancement of activation

Critical Factors for SAR Consideration

- Mechanistic Factors
 - Electrophilic vs. receptor- mediated
 - Multistage process
- Physicochemical Factors
 - Molecular weight
 - Physical state
 - Solubility
 - Chemical reactivity

SAR Analysis

- Four modules
 - Organics
 - Metals
 - Polymers
 - Fibers
- Different rules and considerations programmed into each of the 4 modules

Running OncoLogic®: Organics Module

- Enter information on chemical identity
- Choose appropriate chemical class
- Select chemical class
 - 48 total
 - Description in Manual
 - Hit “F1” to view sample structures
- Enter chemical ID though name, CAS#, or drawing chemical structure
- Assessment based on known mechanisms of action for different types of functional groups and substitutions

Acylating Agents
Acyl and Benzoyl Halides
Acrylamides
Acrylates and Related Compounds
Aflatoxins and Microbial Toxins
Aldehydes
Aliphatic Azo and Azoxy Compounds
Alkanesulfonyl Esters
Alkenylbenzenes
Alkyl Sulfates and Alkyl Alkanesulfonates
Anhydride Compounds
Aromatic Amines
Arylazo Compounds
Aryldiazonium Salts
C-Nitroso Compounds and Oximes
Carbamates
Carbamyl Halides
Coumarins
Dicarbonyls
Direct-Acting Alkylating Agents
Direct-Acting Arylating Agents
Epoxides
Ethyleneimines
Furocoumarins
alpha-Haloalkylamines
alpha-/beta-Haloethers
Halogenated Aromatic Hydrocarbons
Halogenated Cycloalkanes and Cycloalkenes
Select the appropriate class.

Information Needed to Run the Metals Module

- Assessment based on:
 - Nature/form of the metal / metalloid
 - Organometal, metal powder
 - Type of chemical bonding (e.g., organic, ionic)
 - Dissociability / solubility
 - Valence / oxidation state
 - Crystalline or amorphous
 - Exposure scenario
 - Breakdown products (e.g., organic moieties)

Polymers Module

Information Needed to Evaluate Polymers

- Assessment Based on:
 - Percentage of polymer with molecular weight <500 and <1000
 - Percent of residual monomer
 - Identification of Reactive Functional Group(s)
 - Solubility
 - Special features
 - Polysulfation, "water-swellability"
 - Exposure route
 - Breakdown products (e.g., hydrolysis)

Fibers Module

Information Needed for Fibers

- Assessment Based on:
 - Physical dimensions
 - Diameter, length, aspect ratio
 - Physicochemical properties
 - High density charge, flexibility, durability, biodegradability, smooth and defect-free surface, longitudinal splitting potential
 - Presence of high MW polymer, low MW organic moiety, metals/metalloids
- Relevant manufacturing / processing / use information
 - Crystallization, thermal extrusion, naturally occurring, unknown method

OncoLogic® Justification Report

OncoLogic®(R) Justification Report

CODE NUMBER: Isodecyl Acrylate Example

SUBSTANCE ID: 1330-61-6

The final level of carcinogenicity concern for this acrylate when the anticipated route of exposure is inhalation or injection is MARGINAL.

JUSTIFICATION:

An acrylate is a potential alkylating agent which may bind, via Michael addition, to key macromolecules to initiate/exert carcinogenic action. The alkylating activity of acrylates can be substantially inhibited by substitution at the double bond, particularly by bulky or hydrophilic groups.....

OncoLogic® Interpreting Results

OncoLogic Concern	SF Concern	Definition	Proceed to Risk Screen?
Low	Low	Unlikely to be carcinogenic	No
Marginal	Further Research Needed	Likely to have equivocal carcinogenic activity	Additional information is needed
Low – Moderate	Moderate	Likely to be weakly carcinogenic	Yes
Moderate		Likely to be a moderately active carcinogen	Yes
Moderate – High	High	Highly likely to be a moderately active carcinogen	Yes
High		Highly likely to be a potent carcinogen	Yes