Performing a Cancer Hazard Assessment for Sustainable Futures

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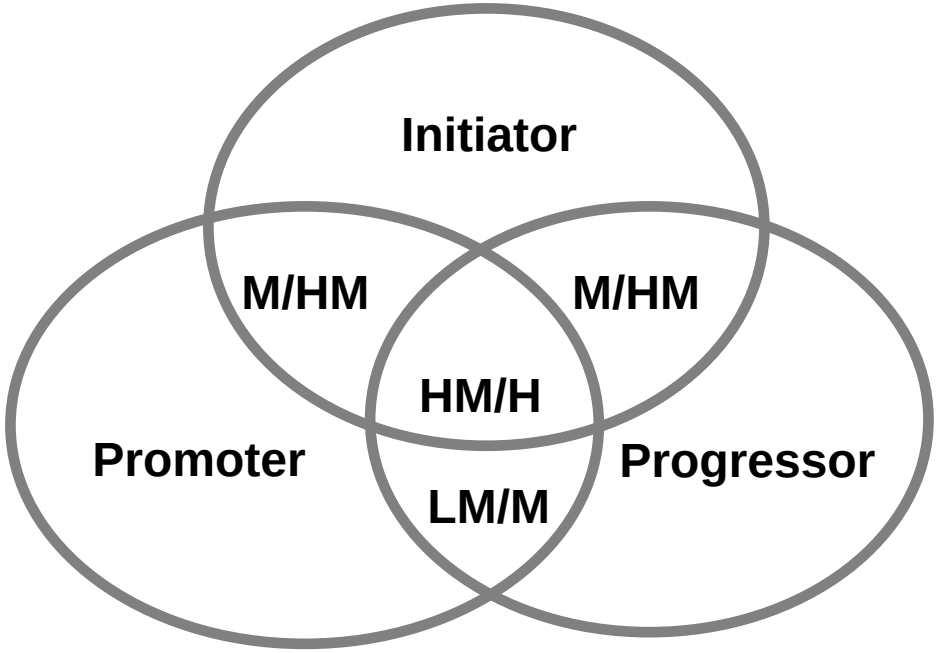
Introduction to the Cancer Endpoint

- Definitions
  - Uncontrolled dividing and growth of cells
  - Caused by mutations, ↑ cell proliferation, ↓ cell death, loss of homeostatic control, etc.
- Two general mechanisms by which a chemical can induce cancer
  - Genotoxic (default)
    - Interaction with DNA to cause mutation(s) in genes
  - Non-genotoxic
    - Variety of mechanisms



- Carcinongesis is a multistage/multistep process
  - **Initiation:** Mutation converts normal to preneoplastic cells
  - **Promotion:** Expansion of preneoplastic cells to benign tumors
  - **Progression:** Transformation of benign to invasive malignant tumors
- A potent carcinogen acts directly on all three stages
- A weak carcinogen acts directly on one stage and indirectly on other

	Initiation	Promotion	Progression
Main event(s)	Direct DNA binding Indirect DNA damage	Clonal expansion Cell proliferation Apoptosis Differentiation	Overcoming suppressions (e.g., *p53*, immune, angiogenesis)
Key mechanistic consideration	Electrophile, resonance stabilization, nature of DNA adduct	Receptor, cytotoxicity, gene expression	Free radical, receptor, gene suppression
		Signal transduction, homeostasis	
SAR/QSAR mechanistic descriptors	**Electrophilicity, HOMO/LUMO, delocalization energies, ……**	**2D, 3D, docking, biopersistence, methylation, ….**	**Reduction potential, 2D, 3D, ……**

Difficulties of (Q)SAR for carcinogenicity

- Complex, mechanism-dependent (Q)SAR
- Local vs. global models
- Data scarcity and variability
- Feedback and validation issues
- Need for integrative approach

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
  - Discussed in the Risk Assessment Presentation

Two Methods to Perform a Cancer Screen for Sustainable Futures

- Method 1. Use experimental data on the chemical or an analog
- Method 2. Use computer-based expert system (OncoLogic®) to predict carcinogenicity

Method 1.

Perform Cancer Screen Using Experimental Data on Chemical or Analog

Types of Experimental Data

- Laboratory studies
  - Study Design
  - Interpretation
- Epidemiology studies
  - Much more complex!

Laboratory Studies

- Studies conducted in controlled environment using laboratory animals
- Overview of study design
  - Animals dosed with test substance or with vehicle (e.g., water or corn oil) for majority of life
  - Tissues are examined for tumors at the end of the exposure period (or in animals that die prior to scheduled sacrifice)
  - Number of animals in treatment groups with tumors is compared with the number of animals in control group(s) with tumors in same tissue

Interpreting Experimental Data

- Indications of a positive study
  - Statistically significant increase in number of animals with cancer at one or more dose(s)
  - Statistically significant trend in number of animals with tumors
  - Presence of rare tumor(s)

Interpreting Experimental Data

- Are tumors in animals relevant to humans?
  - Default assumption is that tumors in animals are relevant to human health
  - Some exceptions exist
    - Best characterized example is that some kidney tumors in male rats are caused by protein that is not found in human kidneys at appreciable concentrations

Interpreting Experimental Data

- For a negative study, evaluate study adequacy using checklist below
  - Were sufficient number of animals dosed for a sufficient length of time?
  - Were animals given appropriate doses (ideal? MTD? Overly toxic?)?
  - Were enough tissues microscopically examined?
  - Is exposure route relevant (absorption)
  - Additional guidance for evaluating study adequacy can be found at http://www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/

Epidemiology Studies

- Conducted in human populations
  - Evaluate cancer incidences in human populations accidentally/inadvertently exposed to a substance compared with unexposed population
  - Often complicated to interpret results due to lifestyle and genetic differences

Sources of Experimental Data

- Some useful sources in finding cancer data
  - International Agency for Research on Cancer (IARC)
    - http://monographs.iarc.fr
  - National Toxicology Program (NTP)
    - http://ntp-server.niehs.nih.gov/
  - Environmental Health Criteria (EHC)
    - http://www.inchem.org/
  - TOXNET
    - http://toxnet.nlm.nih.gov/
  - Gold database
    - http://potency.berkeley.edu
  - Junghans et al. 2002. Cancer information resources: digital and online sources. Toxicology. 173(1-2): 13-34.

Method 2.

Use OncoLogic® to Predict Cancer Concern

OncoLogic: A mechanism-based expert system for predicting carcinogenic potential

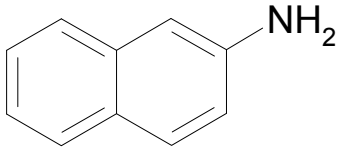
- Developed by domain experts in collaboration with expert system developer
- Knowledge from SAR on >10K chemicals
- Class-specific approach to optimize predictive capability
- Consider all relevant factors including biological input when possible
- Predictions with scientific rationale and semiquantitative ranking

Why is OncoLogic different than EpiSuite and ECOSAR?

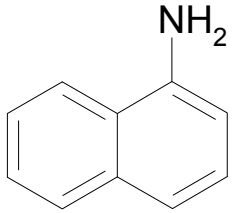
- Difficult to relate specific chemical/physical properties to carcinogenicity
  - Many properties have multiple possible effects on carcinogenicity
  - Multiple stages of carcinogenicity
  - Metabolism to carcinogenic intermediate
  - Isomers that have very similar properties may have dramatically different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

Challenges in Predicting Carcinogenicity (Cont.)

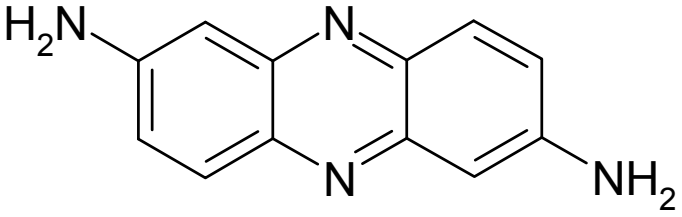
- Carcinogenicity of a chemical may be drastically different for chemicals with similar chemical/physical properties



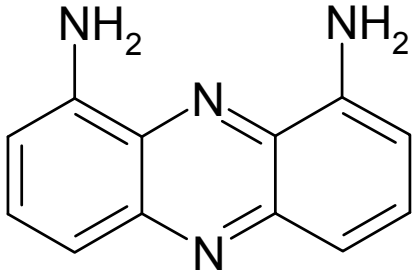
Potent human carcinogen



Marginal or inactive



Strong Mutagen



Approx. 10,000X less mutagenic

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

OncoLogic® - Benefits

- Allow non-experts to reach scientifically supportable conclusions
- Expedites the decision making process
- Allows sharing of knowledge
- Reduces/eliminates error and inconsistency
- Formalize knowledge rules for cancer hazard identification (SAT-style)

OncoLogic® - Concern Levels

OncoLogic Concern	Definition
Low	**Unlikely to be carcinogenic**
Marginal	**Likely to have equivocal carcinogenic activity**
Low – Moderate	**Likely to be weakly carcinogenic**
Moderate	**Likely to be a moderately active carcinogen**
Moderate – High	**Highly likely to be a moderately active carcinogen**
High	**Highly likely to be a potent carcinogen**

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

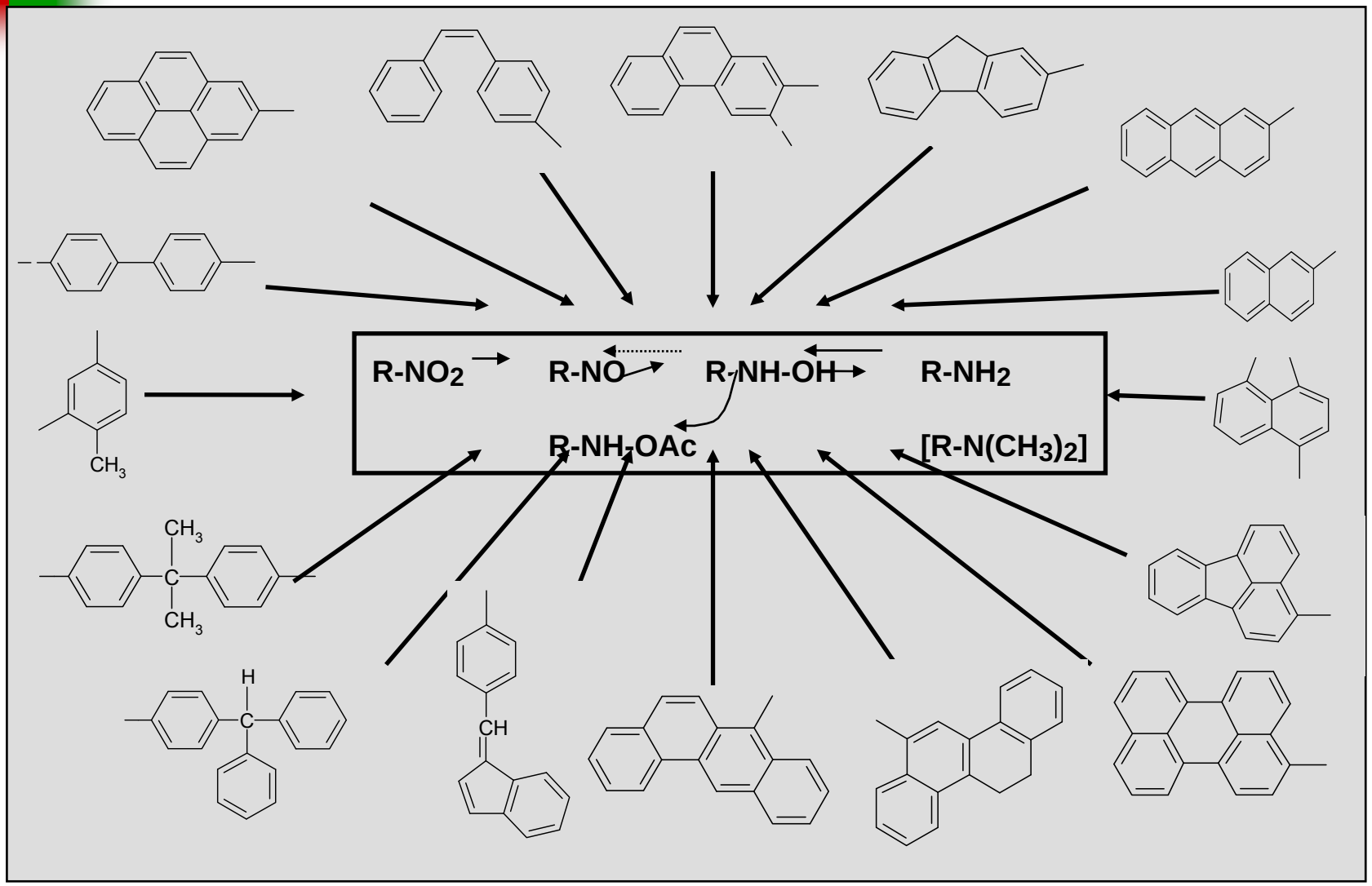
Critical Factors for SAR Consideration

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

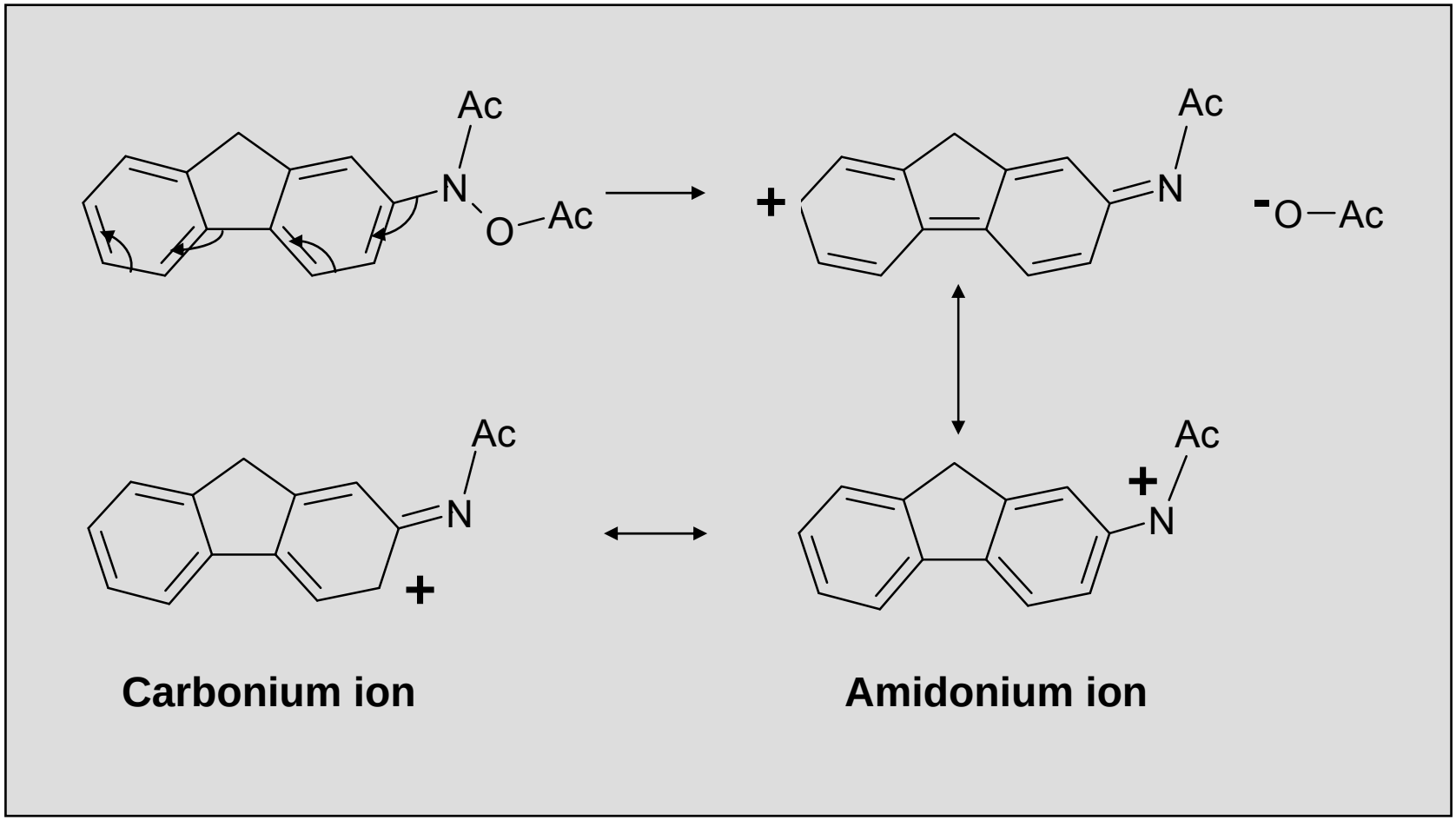
OncoLogic®
Factors Affecting Carcinogenicity of Aromatic Amines

- **Number of aromatic ring(s)**
- **Nature of aromatic ring(s) - homocyclic vs. heterocyclic - nature and position of heteroatoms**
- **Number and position of amino or amine-generating groups(s) - position of amino group relative to longest resonance pathway - type of substituents on amino group**
- **Nature, number, position of other ring substituent(s) - steric hindrance - hydrophilicity**
- **Molecular size, shape, planarity**

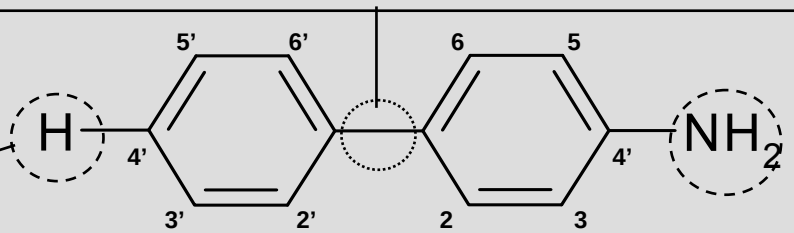
Some Hydrocarbon Moieties Present in Carcinogenic Aromatic Amines



Molecular Mechanism for Generation of Resonance-stabilized Reactive Intermediates from N-acyloxy Aromatic Amines



Synoptic Tabulation of Structural Requirements for Carcinogenic Activity of 4-Aminobiphenyl and Benzidine Derivatives



Very active if:
--F

Active if:
--NH_2
--NH•OC•CH_3
--NO_2

Weakly active if:
--C_6H_5

Inactive if:
--CH_3
--Cl
--Br

Active if:
--S--

Inactive if:
--NH--

For 4-aminobiphenyl:

Very active if:
3-methyl
3,2'-dimethyl
3,3'-dimethyl
3-fluoro
3'-fluoro

Active if:
3-chloro
3-methoxy
3,2,5'-trimethyl
3,2',4',6'-tetramethyl

Weakly active if:
3-hydroxy

Inactive if:
2-methyl
2'-methyl
2'-fluoro
3-amino

For benzidine:

Very active if:
2-methyl
3,3'-dihydroxy
3,3'-dichloro

Weakly active if:
3,3'-dimethyl
3,3'-dimethoxy

Inactive if:
2,2'-dimethyl
3,3'-bis-oxyacetic acid

Very active if:
--N(OC•CH3 / OH)

Active if:
--NH•OC•CH_3
--$N(CH_3)_2$
--NO_2
--OCH_3

Inactive if:
--F

R
|
--CH--
Transition to diphenylmethane and triphenyl-methane amines

--CH==CH--
Transition to amino-stilbenes

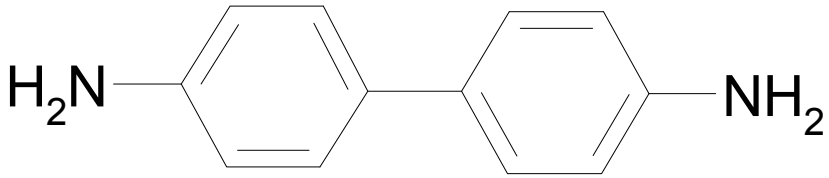
--N==N--
Transition to amino azo dyes

OncoLogic® Prediction vs. NTP Bioassays Aromatic Amines and Related Compounds

NTP #	Chemical	Bioassay Results			Oncologic® Evaluation
		Rat	Mouse	"Call"	
24	4,4'-Diamino-2,2'-stilbene disulfonic acid	N/N	N/N	--	L
42	p-Nitroaniline	NT	E/N	Eq	mar
26	p-Nitrobenzoic acid	N/S	N/N	+	mar
9	p-Nitrophenol	NT	N/N	--	LM
33	4-Hydroxyacetanilide	N/E	N/N	Eq	LM
32	2,4-Diaminophenol dihydrochloride	N/N	S/N	+	M
40	3,3'-Dimethylbenzidine	C/C	NT	+	HM
43	o-Nitroanisole	C/C	C/C	+	HM

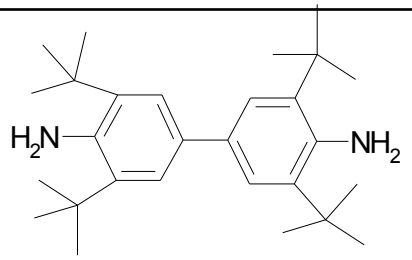
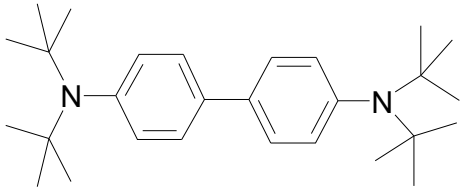
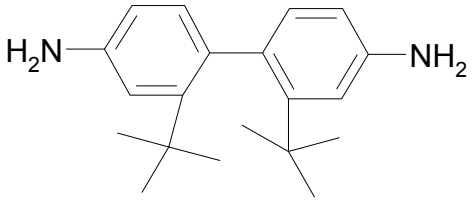
C = Clear evidence of carcinogenicity
S = Some evidence of carcinogenicity
N = No evidence of carcinogenicity
NT = Not tested
+ = At least one test = C or S
Eq = No C or S, and E must appear at least once
-- = No C, S, or E

Examples Knowledge Rules

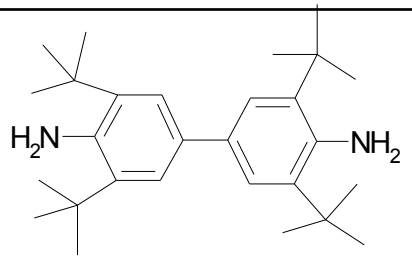
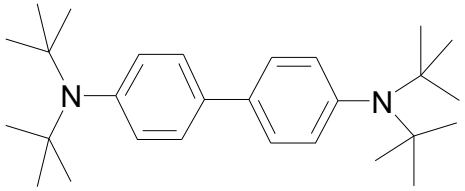
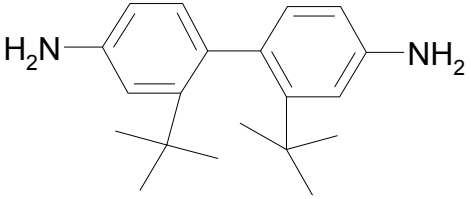


Benzidine
OncoLogic Cancer Concern = High

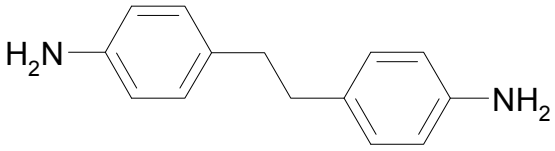
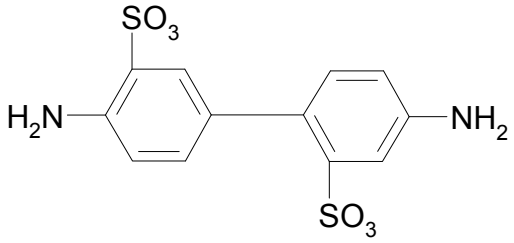
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential

Example	Action	Effect on Cancer Concern/Justification
	Introduce bulky substituent(s) ortho to amino / amine-generating group(s).	
	Introduce bulky N-substituent(s) to amino / amine-generating group(s).	
	Introduce bulky groups ortho to intercyclic linkages.	

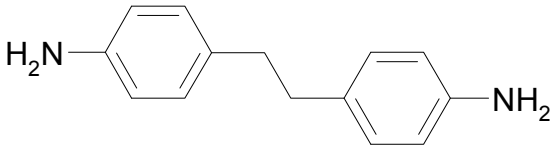
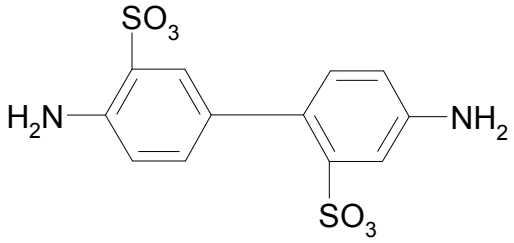
Molecular Design of Aromatic Amine Dyes with Lower Carcinogenic Potential

Example	Action	Effect on Cancer Concern/Justification
	Introduce bulky substituent(s) ortho to amino / amine-generating group(s).	Provide steric hindrance to inhibit bioactivation. Concern = Marginal
	Introduce bulky N-substituent(s) to amino / amine-generating group(s).	Make it a poor substrate for the bioactivation enzymes. Concern = Marginal
	Introduce bulky groups ortho to intercyclic linkages.	Distort the planarity of the molecule making 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
	Replace electron-conducting intercyclic linkages by electron-insulating intercyclic linkages.	
	Substitution with hydrophilic groups; 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
	Replace electron-conducting intercyclic linkages by electron-insulating intercyclic linkages.	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. Concern = Marginal
	Substitution of ring with hydrophilic and/or electron withdrawing groups	1. Render molecule more water-soluble thus reducing absorption and accelerating excretion. 2. Makes amines less nucleophilic Concern Level = Low

Conclusion from NTP Predictive Exercises

- Most of the best performers are predictive systems that incorporate human expert judgment and biological information
- OncoLogic was one of the best performers among more than 15 methods

External Validation by FDA Food Additives Section

	Sensitivity (# carcinogens identified / # tested)	Specificty (# noncarcinogens identified / # tested)
Bacterial rev. mutation	247/405 (61.0 %)	39/52 (75 %)
Mouse lymphoma	188/236 (79.7 %)	13/32 (41 %)
Chromosome aberration	195/298 (65.4 %)	20/44 (45 %)
Ashby-Tennant structural alert	415/569 (72.9 %)	46/81 (57 %)
Multi CASE ver. 3.1	445/530 (84.0 %)	46/62 (74 %)
OncoLogic ver. 4.1	297/325 (91.4 %)	16/29 (55 %)

SAR Analysis

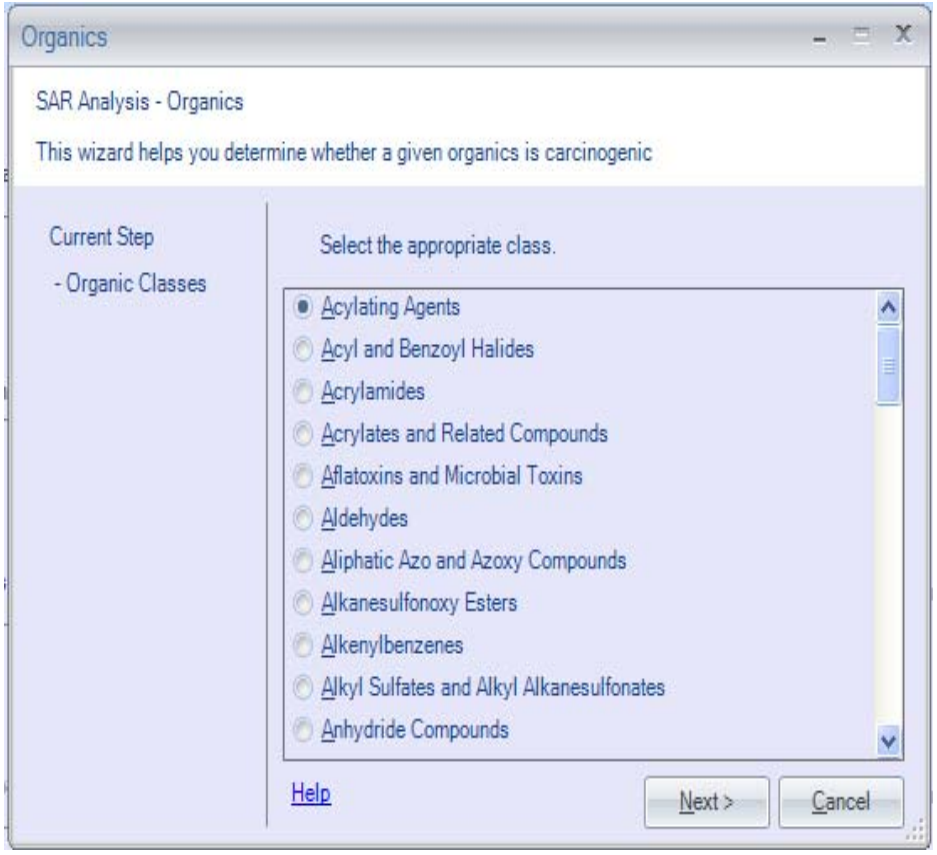
- Four modules
  - Organics
  - Metals
  - Polymers
  - Fibers
- Different method used to evaluate each type

Running OncoLogic® : Organics Module

- Organics
  - Enter information on chemical identity
  - Choose appropriate chemical class
  - Enter chemical name, CAS#, or chemical structure

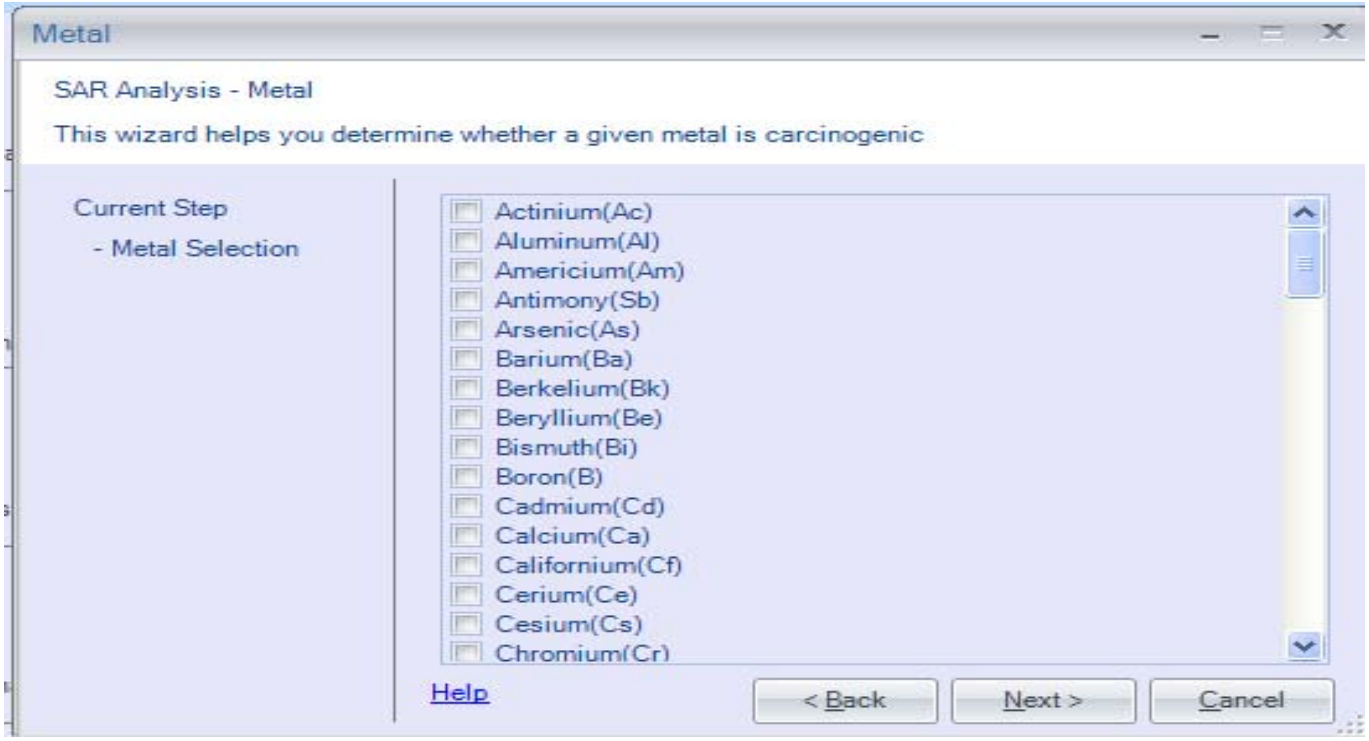
Running OncoLogic®: Organics Module

- Select chemical class
  - 48 total
  - Description in Manual
  - Select “Help” to view sample structures
- Absence of structure in OncoLogic provides suggestive, *but not definitive*, evidence of low cancer concern



Running OncoLogic®: Metals

- **Similar to running the organics module**
- **Pick the metal to be evaluated**
  - **OncoLogic® will then either ask a series of questions needed to evaluate the chemical or provide a database of related compounds**



Information Needed to Run the Metals Module

- 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)

Running OncoLogic®
Polymers

- Polymer must consist of covalently linked repeating units and have a number average molecular weight >1000
- OncoLogic® asks a series of questions designed to aid in evaluation of carcinogenicity of the polymer

Polymers Module:
Information Needed to Evaluate Polymers

- 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

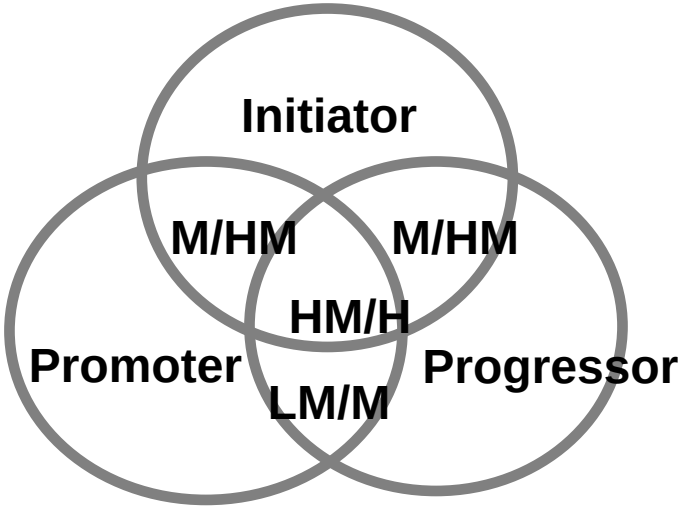
- Evaluations are based on physical dimensions and physicochemical properties
  - Physical dimensions
    - Diameter, length, aspect ratio
  - Physicochemical properties
    - High density charge, flexibility, durability, biodegradability, smooth and defect-free surface, longitudinal splitting potential
    - Presence of high MW polymer, low MW organic moiety, metals/metalloids

Fibers Module

- Relevant manufacturing / processing / use information
  - Crystallization, thermal extrusion, naturally occurring, unknown method

Use of Non-Cancer Data: Functional Arm Analysis

- Functional Arm predicts whether the chemical is likely to be a tumor initiator, promoter, and/or progressor
  - Possible relevance or contribution to the carcinogenesis process is indicated in the figure below



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

Major References on OncoLogic®

Woo, Y.-T., Lai, D.Y., Argus, M.F. and Arcos, J.C. Development of Structure Activity Relationship Rules for Predicting Carcinogenic Potential of Chemicals. Toxico. Lett. 79: 219-228, 1995.

Woo, Y.-T., Lai, D.Y., Argus, M.F. and Arcos, J.C. Carcinogenicity of Organophosphorous Pesticides/Compounds: An analysis of their Structure Activity Relationships. Environ. Carcino. & Ecotox. Revs. C14(1), 1-42, 1996.

Lai, D.Y., Woo, Y,-T., Argus, M.F. and Arcos, J.C.: Cancer Risk Reduction Through Mechanism-based Molecular Design of Chemicals. In:"Designing Safer Chemicals" (S. DeVito and R. Garrett, eds.), American Chemical Society Symposium series No. 640, American Chemical Society Washington, DC. Chapter 3, pp.62-73, 1996.

Major References - continued

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