Environmental Health Perspectives
Vol. 87, pp. 183–197, 1990

Mode of Action and the Assessment of Chemical Hazards in the Presence of Limited Data: Use of Structure-Activity Relationships (SAR) under TSCA, Section 5

by Charles M. Auer,* J. Vincent Nabholz,* and Karl P. Baetcke*

Section 5 of the Toxic Substances Control Act (TSCA) requires that manufacturers and importers of new chemicals must submit a Premanufacture Notification (PMN) to the U.S. Environmental Protection Agency 90 days before they intend to commence manufacture or import. Certain information such as chemical identity, uses, etc., must be included in the notification. The submission of test data on the new substance, however, is not required, although any available health and environmental information must be provided. Nonetheless, over half of all PMNs submitted to the agency do not contain any test data; because PMN chemicals are new, no test data is generally available in the scientific literature. Given this situation, EPA has had to develop techniques for hazard assessment that can be used in the presence of limited test data. EPA's approach has been termed "structure-activity relationships" (SAR) and involves three major components: the first is critical evaluation and interpretation of available toxicity data on the chemical; the second component involves evaluation of test data available on analogous substances and/or potential metabolites; and the third component involves the use of mathematical expressions for biological activity known as "quantitative structure-activity relationships" (QSARs). At present, the use of QSARs is limited to estimating physical chemical properties, environmental toxicity, and bioconcentration factors. An important overarching element in EPA's approach is the experience and judgment of scientific assessors in interpreting and integrating the available data and information. Examples are provided that illustrate EPA's approach to hazard assessment for PMN chemicals.

Background

A situation that commonly confronts the U.S. Environmental Protection Agency (EPA) involves evaluating the hazard potential of chemicals in the presence of little or no test data. This situation is encountered with many industrial chemicals as well as many of the substances identified in industrial effluents, hazardous waste sites, and in environmental monitoring surveys. A 1984 report by the National Research Council (*1*) explored the question of the availability of toxicity test data on industrial chemicals and concluded that such chemicals often have been subjected to a minimum of testing and, not infrequently, to no testing at all. Despite the limited available test data, there is a continuing demand on EPA to provide an evaluation of the potential hazards posed by these substances. EPA's approach to assessing chemical hazards in the presence of limited data will be discussed in this paper.

One of the areas that can serve to illustrate this discussion involves EPA's responsibility for the risk assessment of so-called new chemicals submitted to the agency under Section 5 of the Toxic Substances Control Act (TSCA) (*2*). New chemicals are those substances that do not appear on an inventory of existing TSCA chemicals; accordingly, notifications on these substances must be submitted for review by the EPA prior to commercial manufacture or importation. Pesticides, drugs, food additives, and certain other chemicals are controlled by other statutes and are not subject to TSCA. During the period from 1979 to 1988, over 12,000 Premanufacture Notifications, or PMNs, were submitted by the chemical industry to the agency for review (Fig. 1). Under the requirements of TSCA, Section 5, information such as chemical identity, uses, production volume, and exposure must be provided in the notification. The submission of test data, however, is not required,

*Health and Environmental Review Division (TS-796), Office of Toxic Substances, 401 M. St. SW, Washington, DC 20460-0001.

Address reprint requests to J. V. Nabholz, Health and Environmental Review Division (TS-796), Office of Toxic Substances, 401 M. St., SW, Washington, DC 20460-0001.

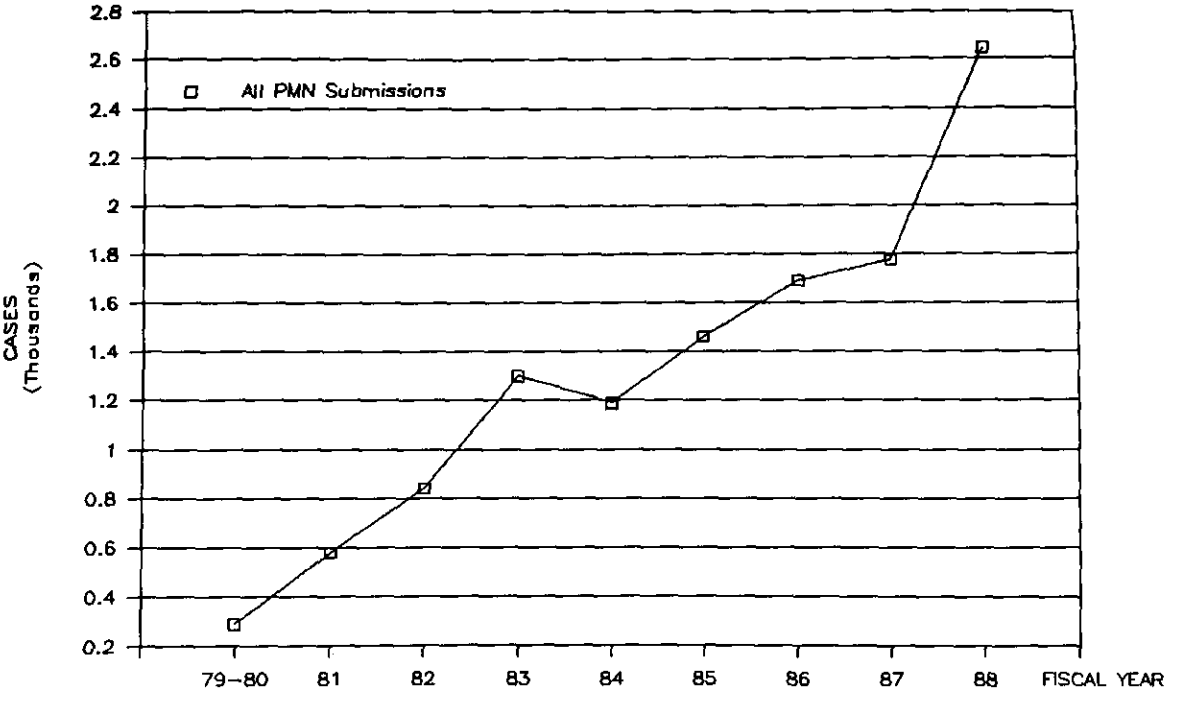

FIGURE 1. Annual receipt of PMNs (*51*). Fiscal year corresponds to the 1-year period starting October 1 and ending on September 30 of the following year.

although the submitter must provide any health or environmental effects information that is available at the time of submission. Since testing for health or environmental effects is not a requirement, slightly over half of all PMN chemicals submitted to the Agency do not include any test data; because these are new chemicals no data are generally available in the scientific literature. When test data are provided (Table 1), they most commonly consist of acute toxicity and skin and eye irritation studies in laboratory animals. Data generated from other types of testing such as mutagenicity, chronic animal toxicity, ecological toxicity, and environmental fate are seen in fewer than 15% of all PMN submissions (*3*).

The task before EPA under TSCA, Section 5 is to reach a judgment—despite the limited test data—as to whether or not the PMN chemical under its projected conditions of manufacturing, processing, use, and disposal may or will present an unreasonable risk of injury to human health or the environment. The term risk, as used in TSCA, is some function of hazard (i.e., toxicity) and exposure, and also includes consideration of economics. When EPA has concern for the potential risks of new chemicals, or in cases where the agency is uncertain of the potential toxicity but believes that production and exposure will be significant, it can take action to control potential risks pending the development of the test data needed to adequately assess the hazards of the chemical. EPA's regulatory actions under TSCA, Section 5 generally take the form of some control or limitation on the commercialization of the new chemical pending development of test data.

As was shown by NRC (*1*), the limited availability of test data on new chemicals is not unique; it is a characteristic shared by many of the chemicals for which EPA has regulatory responsibilities. For example, only limited data are available on many of the over 700 chemicals found on the Superfund list of hazardous sub-

Table 1. Test data submitted with premanufacture notices.

	Percent of PMN[a]		
Type of test data	All	Nonpolymer	Polymer
No test data of any type	51	38	68
Health data			
Acute toxicity			
Oral	38	50	22
Dermal	23	29	14
Inhalation	11	14	7
Local toxicity			
Eye irritation	36	47	21
Dermal irritation	38	50	22
Sensitization	11	17	5
Mutagenicity	15	23	6
Other[b]	11	16	4
Ecotoxicological data			
Acute lethal vertebrate	6	9	3
Acute lethal invertebrate	3	3	2
Environmental fate data			
Biodegradation	6	8	2
Log P	4	5	1

[a] Based on the PMNs received through September 1985 from data printouts retrieved from PENTA, an EPA in-house PMN information system.

[b] The other health data category includes acute toxicity studies by other routes (IP, IV, etc.), repeated-dose toxicity studies (generally 28 days or less in duration), developmental toxicity, phototoxicity, neurotoxicity, and a variety of other toxicity studies.

stances (4); despite this limitation EPA is called on to evaluate the hazards and risks of these chemicals at Superfund sites.

General Discussion of Approach

The approach that EPA's Office of Toxic Substances (EPA/OTS) has developed for assessing the hazards of chemicals in the presence of limited test data has been termed structure-activity relationships (SAR) and involves the following three major components: *a*) critical evaluation of available data, if any, on the chemical; *b*) identification and selection of potential analogues and/or chemicals predicted to be key metabolites, followed by an evaluation of the test data available on these compounds; and c) use of quantitative structure-activity relationships (QSARs).

As previously discussed, few data are generally available for most PMN chemicals, thus the incorporation of inputs and understanding from the latter two components is critical in assessing the hazards of new chemicals. These components are detailed later. Implementation of analogue analysis and QSAR techniques in assessing the hazards of PMN chemicals requires the experience and judgment of scientific assessors in interpreting and integrating the data and information from these components. This is critical in assessing the relevance and use of analogues as indicators of the potential hazards of PMN chemicals and in selecting the proper QSARs and evaluating the associated predictions. The importance of expert judgment in implementing this approach cannot be minimized, and its role is clearly defined in the following discussions.

Identification and Evaluation of Analogues and Potential Metabolites

This component represents one of the unique aspects of EPA's approach. [A more detailed discussion of this component can be found in Auer and Gould (*3*).] In order for an analogue to be useful to EPA, it must resemble the chemical of interest in one or more critical aspects (i.e., structurally, substructurally, physicochemically, etc.) and have pertinent toxicologic or other data available in the literature. A major constraint on the usefulness of any potential analogues, however, is the generally limited availability of test data on TSCA existing chemicals (*1*).

Analogues can be suggested by EPA staff or be retrieved from several publicly available automated chemical substructure and nomenclature search systems [e.g., Structure and Nomenclature Search System (CIS, Inc., Baltimore, MD) or CAS-Online (Chemical Abstract Service, Columbus, OH)]. Analogues recommended by technical staff also often provide very useful inputs to the assessment effort. These individuals also provide expert guidance in constructing the strategy for the automated analogue searches. The guidance consists of the identification, based on consideration of chemistry, metabolism, possible mechanisms of toxicity, etc., of key structural features in the chemical that may be associated with toxic action or be subject to activation or deactivation. Potential analogues resembling the chemical in the structure or function of these key features are then identified using automated substructure searching techniques. [A functionally similar chemical is one which, despite substructural differences, can be considered a functional equivalent of the chemical of interest. Examples of functional equivalents could include an aromatic amine substituent in lieu of an aromatic nitro or *C*-nitroso group, based on the expectation of biotransformation (*5*); or an α, β-unsaturated ketone in lieu of an α, β-unsaturated ester, based on similar mechanistic possibilities (*6*).] Once a set of potential analogues has been identified, a variety of factors (for example, physical chemical properties, possible steric effects, molecular topology, etc.) become important considerations in selecting the analogues that will be subjected to a literature search for toxicological data. Only analogues that yield relevant information are used in the assessment process.

The second major aspect of this component to identify related chemicals that may be useful in the assessment involves identifying possible biotransformation products of the target chemical. Metabolism studies are rarely available on industrial chemicals and, thus, the effort focuses on assessing the metabolic potential of the chemical with the objective of identifying likely activation and deactivation pathways. In assessing metabolic potential, EPA considers established principles of xenobiotic metabolism as well as any available metabolism studies on chemical analogues (*3*,*7*,*8*). Potential metabolites are then subjected to analogue search and literature search as described above.

The successful application of this component requires the experience and judgment of scientific assessors in interpreting and integrating the available information. In evaluating the use of different analogues, the assessor considers the similarity of the analogue to the PMN chemical. This assessment is performed using a variety of factors that include comparisons of physical chemical properties, absorption potential, metabolic potential, the presence and positioning of reactive functional groups, and possible mechanisms of toxicity. Metabolites are evaluated in terms of the likelihood and significance of their formation. For example, if a postulated metabolite is projected to require a series of biotransformation steps for its formation or can be formed only via an unusual or theoretical pathway, these points will be considered in reaching assessment conclusions.

Use of QSARs

The third component entails the use of QSARs, which are used extensively in assessing the hazards of chemicals lacking adequate data. At this time, however, the use of QSARs is limited to estimating certain physicochemical properties (*9*), ecological toxicology, and fish bioconcentration factors (*10*). EPA/OTS approaches in estimating the latter two items are discussed below.

A QSAR is a quantitative relationship between chemical structure and a specific biological effect and is derived using information on a series of related chemicals (*11*). The chemical structure component can include one or more structural or physicochemical attributes, while the activity component can include any measurable effect or biological fate attribute observed following acute or chronic exposure to a chemical. For example, a common effect is acute toxicity to fish, often expressed as the 96-hr LC_{50} (the aqueous concentration needed to kill 50% of a population within 96 hr). A common biological fate attribute is the bioconcentration factor (BCF), which is the equilibrium concentration of a chemical in an organism following aqueous and/or oral exposure. Statistical methods are used to determine the relationship between structural attribute(s) and effect. Regression analysis is one of the more frequently used methods and has been relied on extensively by the EPA/OTS in developing QSARs. The predictive power of a QSAR varies with the amount of data, the mode(s) of toxic action, and any interactions between the modes of toxic action and bioavailability. Driven by assessment and regulatory needs under the various sections of TSCA, EPA/OTS has developed QSARs based on as little as one datum and assumptions about the nature of the relationship between a chemical class and its toxicity. However, EPA/OTS prefers to use QSARs that are based on as much data as possible and, accordingly, new data are added whenever they become available.

In theory, a variety of physicochemical properties have been used to develop QSARs including octanol/water partition coefficient (K_{ow}); hydrophobicity; hydrophilicity; degree of dissociation of an ionizable functional group (pK_a); chemical reactivity; steric attributes such as molecular size, molecular volume, and molecular shape; hydrophile-hydrophobe balance; and surface tension (*9,12–15*). In practice, however, given the unavailability of environmental testing results on most industrial chemicals, the EPA/OTS has relied on those structural and physicochemical properties that can be easily determined from chemical structure, i.e., K_{ow}; molecular weight (MW); number of carbons in the hydrophobic alkyl chain of a surfactant and/or number of ethoxylate units in a surfactant; and charge density of polycationic polymers (e.g., percent of amine-nitrogen, or more generally, the number of cationic charges per unit MW).

EPS/OTS uses QSARs extensively in the environmental hazard assessment of new chemicals. Clements et al. (*10*) presented 49 QSARs currently used by EPA/OTS to predict the toxicity of industrial organic chemicals to aquatic organisms. These QSARs apply to about 40 classes and subclasses of organic chemicals and are grouped into three broad types based on the mode of toxic action: neutral organic chemicals that are assumed to act with a narcotic mechanism; organic chemicals that are assumed to have a more specific mode of toxic action and show excess toxicity in addition to narcosis; and organics that are surface active agents. In addition, there are a number of chemical classes where EPA/OTS has tried but not succeeded in attempts to develop environmental toxicity QSARs.

Neutral Organic Chemicals

Neutral organic chemicals are nonelectrolyte and nonreactive compounds (e.g., solvents) that act like anesthetics or narcotics. Descriptions of narcotic toxicity can be found in Lipnick (*16*), Franks and Lieb (*17*), and Veith and Broderius (*18*) in these proceedings. Classes of organic chemicals having neutral organic QSARs according to Clements et al. (*10*) include: alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, sulfides, and disulfides. There are nine QSARs for the neutral organic chemical class and most of these QSARs are effect specific (e.g., 96-hr LC_{50}, 16-day EC_{50}, and fish BCF). In general, QSARs are specific with respect to the effect modeled, but may or may not be specific with respect to species (e.g., the QSAR for fish BCF includes data from 15 species). Excellent predictive power can be obtained using K_{ow} alone.

Chemicals Having Excess Toxicity

Organic chemicals with a more specific mode(s) of toxic action may contain reactive functional groups (e.g., groups known to be electrophilic or nucleophilic that act directly or following metabolic activation (*16*); ionizable functional groups (e.g., phenols and anilines); specific structural attributes having specific toxic mechanisms (e.g., acetylcholinesterase inhibition by alkyl phosphate esters); and/or any number of specific structural and mechanistic attributes. What distinguishes these classes of chemicals is that when toxicity test results are compared with toxicity values predicted by neutral organic (narcotic) QSARs, each of these classes of chemicals exhibit toxicity greater than that predicted by the narcotic QSARs (the EPA/OTS currently considers the latter to represent baseline toxicity for aquatic organisms). Chemical classes having excess toxicity include: acrylates, methacrylates, aldehydes, anilines, benzotriazoles, esters, phenols, and epoxides. Each chemical class in this group has its own QSAR (*10*) and, it is assumed, its own specific mode(s) of toxic action in addition to narcosis. However, just what these specific toxic mechanisms may be is presently poorly understood. For example, acrylates are known to be more toxic to fish than predicted by the neutral organic QSARs, but the cause of this excess toxicity is not known exactly. Russom et al. (*19*) and Reinert (*20*) discussed six possible toxic mechanisms for acrylates. Furthermore, Veith et al. (*21*) did not discuss a specific mechanism for the excess fish toxicity observed for simple esters, although a form of narcosis may be associated with monoesters as suggested by Veith and Broderius (*18*) in these proceedings. This suggests that knowing the mode of toxic action for a chemical class is not necessary for QSAR analysis. Selecting the proper QSAR for a given chemical is, however, a crucial element in

SAR analysis; the factors considered in making such judgments are discussed at a later point in this paper and by Clements et al. (*10*). Currently, only K_{ow} is used to predict aquatic toxicity for this group of chemicals, even though it is known that the predictive power of these QSARs could be improved by adding a second physicochemical attribute, e.g., reactivity, ionization constant (pK_a), or steric descriptor. However, EPA/OTS in its evaluation of new chemical is limited to those physicochemical attributes that can be easily predicted from chemical structure alone and, outside of laboratory experimentation, there are currently no easily utilized methods for predicting these descriptors directly from chemical structure.

Surface-Active Agents

The last broad group of QSARs (*10*) are those for surface active agents (i.e., chemicals that damage the respiratory membranes of aquatic organisms), and this group is composed of two subgroups: surfactants that can be absorbed through respiratory membranes and charged polymers that cannot be absorbed. While surfactants are thought to act primarily as surface-active agents during acute exposures by destroying respiratory (i.e., gill) membranes, it is assumed that they act primarily as systemic toxicants during chronic exposures because they can be absorbed through respiratory membranes. Charged polymers are thought to only act through a surface active toxic mechanism since their MWs (generally greater than 1000 amu) prevent them from being absorbed to a significant degree (*22*). Surfactant QSARs in Clements et al. (*10*) include anionic surfactants, specifically, linear alkyl benzene sulfonates (LAS); nonionic surfactants, specifically, alcohol ethoxylates and ethoxylated β-amine surfactants (ETHOMEEN); and cationic surfactants, specifically, linear *N*-alkyl quaternary ammonium compounds. The QSARs for all of these surfactants are parabolic, i.e., toxicity is related to the size of the hydrophobic component in a parabolic manner when the size of the hydrophilic component remains constant. The size of the hydrophobic component, usually a linear alkyl carbon chain, is estimated by simply counting the number of carbons in the hydrophobic alkyl chain. In all the surfactant QSARs used by EPA/OTS, maximum toxicity (or lowest effective concentration or EC_{50} value) occurs when there are about 16 to 17 carbons in the hydrophobic alkyl chain. Toxicity for the nonionic surfactants is also affected by the number of ethoxylate units, and this must be known before accurate predictions can be made.

The only QSAR for charged polymers is for polycationic polymers (polycationic polymers include polymers with primary amines, secondary amines, tertiary amines and/or quaternary ammoniums, polyphosphoniums and polysulfoniums). The mode of toxic action for these polymers is specifically surface activity, in that they are known to destroy the respiratory membranes of aquatic organisms (unpublished observations from EPA/OTS New Chemical Review Program, Washington, DC). The molecular descriptor used to predict toxicity for these polymers is equivalent charge density as determined from chemical structure, i.e., percent amine-nitrogen or number of cationic charges per 1000 MW. If the polymer is based on nitrogen moieties that can be protonated and/or are quaternarized, then percent amine-*N* or number of cationic charges per 1000 MW can be used as a molecular descriptor. In a similar fashion, if the cations are based on polyphosphoniums or polysulfoniums, the number of cationic charges can also be used. These QSARs are appropriate for use when the number average MWs are over 1000 amu (i.e., the mode of the distribution of MWs is greater than 1000); in addition, polymers must be water soluble and/or dispersible. These QSARs are biphasic with toxicity increasing exponentially with charge density from 0.1 to 2.4 cations/1000 MW, thereafter becoming asymptotic.

Some Chemical Classes Lacking QSARs

EPA/OTS has no QSARs for polyanionic polymers, cationic dyes, acid dyes, inorganic chemicals, and for most classes of pesticides. Two classes of polyanionic polymers are known to be toxic to aquatic organisms: poly(aromatic sulfonic acids) that are most toxic to fish and polycarboxylic acids that are toxic only to green algae. These polymers are not expected to be absorbed due to their high MWs and are assumed to be surface active in their toxic mechanism, but anionic charge density is not correlated to toxicity (unpublished observation of EPA/OTS New Chemical Review Program) and no other meaningful molecular descriptors are known.

Cationic dyes are toxic to aquatic organisms and, like surfactants, are probably primarily surface active agents during acute exposures but primarily systemic toxicants during chronic exposures since they can be absorbed. Attempts have been made to relate effective concentrations for aquatic organisms within subclasses of cationic dyes (R. D. Platz, E. W. Odenkirchen, and J. V. Nabholz, unpublished observations). It has been hypothesized that dyes with delocalized cationic charges are more toxic, followed by dyes with four localized charges, then three localized charges, etc. However, correlations were weak and as more dyes were included in the analysis the relationship became progressively worse. The most probable explanation is the intrinsic impurity of commercial dyes. All of the dyes analyzed were commercial dyes with varying amounts of impurities, which is typical of dyes submitted under TSCA, Section 5. While a strong correlation might be observed if pure dyes were tested, too much inherent variability exists in commercial dyes for QSAR analysis. As a result, each such commercial cationic dye has to be tested to determine its aquatic toxicity.

Acid dyes are toxic only to green algae (J. V. Nabholz, unpublished observations). Analysis of available data suggested that effects to algae were not direct effects of the acid dye but represented indirect effects from shading. Perez et al. (*23*) partially confirmed this con-

clusion while studying the effects of the blue acid dye, Direct Blue 15, in a site-specific marine microcosm. It appeared that the potency of the indirect effect was related to the color of the dye and the intensity of light shading. Therefore, blue dyes should have a stronger effect than orange or yellow dyes, and deep blue dyes should have a stronger effect than light blue dyes. EPA/OTS has not been able to test this hypothesis because absorption maxima are generally not included in PMN submissions and because of the difficulty of predicting light absorption from chemical structure. In practice, light absorbance attributed to dye color and intensity for a series of acid dyes will have to be measured concurrently with toxicity studies if any QSAR is to be developed.

Factors To Be Considered in Selecting and Evaluating a QSAR

Prior to using a QSAR, specific information must be gathered about a chemical so that the proper model can be identified and selected. The major factors considered by EPA/OTS (*10*) in conducting a QSAR-based analysis of a poorly studied chemical include chemical class, K_{ow}, MW, physical state and water solubility, number of carbons and/or ethoxylates, and percent amine-nitrogen and/or number of cationic charges per 1000 MW. These factors are discussed in detail by Clements et al. (*10*); they are set up as a user-directed guide to the proper selection and use of the available QSARs.

The initial determination of chemical class is probably the most important, because QSARs currently are chemical class specific. Clements et al. (*10*) have an alphabetical listing of chemical classes for which there are QSARs. Users simply find the chemical class to which the compound in question belongs and identify the proper QSAR.

QSARs for neutral organic chemicals, which act as narcotics, and chemicals with excess toxicity are based on Kow. QSARs predicting acute toxicity to aquatic organisms (96-hr LC_{50} values) are valid when log K_{ow} values are less than five. When organic chemicals have log K_{ow} values of greater than five, then longer exposures are necessary to attain 50% lethality. If log K_{ow} exceeds eight for a chemical, no adverse effects are expected at saturation even under long-term exposures. QSARs for chronic (long-term) exposure (greater than 20 days for daphnids and the early life stages of fish, and 4 days for green algae) are valid for log K_{ow} values of eight or less (*10*).

QSARs for organic chemicals that show excess toxicity are generally valid for log K_{ow} values of about five or less. Excess toxicity decreases with increasing log K_{ow} until it becomes indistinguishable from baseline (narcotic) toxicity at about a log K_{ow} of five. This pattern has been demonstrated for esters by Veith et al. (*21*); epoxides by Deneer et al. (*24*); acrylates and methacrylates by J. V. Nabholz and R. D. Platz (unpublished observations) and Russom et al. (*19*); aldehydes by Deneer et al. (*25*) and R. G. Clements and J. V. Nabholz (unpublished observations); and benzotriazoles by J. V. Nabholz (unpublished observations).

MW is needed to convert moles/L to mg/L since most QSARs are generally determined based on a moles/L basis. In addition, MW is used to set absorption cutoffs for aquatic organisms. Absorption through biological membranes is considered possible for molecules with MWs of less than 600 (*22*); absorption decreases significantly with MWs greater than 600. Chemicals with MWs of greater than 1000 are assumed to have negligible (i.e., biologically insignificant) absorption. For surface active compounds, there is no MW limit since toxicity from surface active compounds does not require absorption.

A determination of physical state (liquid, solid, or gas) for a compound is important for a proper QSAR application. QSARs used by EPA/OTS were developed almost entirely on organic chemicals that are liquids at room temperature. If an organic chemical is a solid at room temperature, then the melting point needs to be known or estimated because of the effect it has on water solubility, i.e., assuming K_{ow} is constant, the higher the melting point of a neutral organic chemical, the lower its water solubility (*26*). Whenever EPA/OTS predicts the aquatic toxicity for an organic chemical using QSARs, regardless of its physical state, the predicted effective concentration is compared with the water solubility. When the effective concentration is significantly higher than the measured or predicted maximum water solubility, then the effect is not expected to occur. In practice, prediction of water solubility and/or dispersibility in water is often difficult to obtain from chemical structure. In many cases, water solubility and dispersibility are considered essentially synonymous.

The number of carbons and/or ethoxylate units in an organic compound is needed only when the compound is expected to act like a surfactant, and a surfactant QSAR is to be used to predict aquatic toxicity. All of the surfactant QSARs used by EPA/OTS are based on surfactants where the hydrophobic component is composed of a single aliphatic carbon chain and/or linear chains of ethoxylate units. Surfactants that have complex hydrophobic components are assessed by calculating the K_{ow} of the complex hydrophobic component alone and determining which aliphatic alkyl (carbon) chain has an equivalent K_{ow}. Toxicity predictions are based on this equivalent chemical structure. Finally, the need to calculate the percent amine-nitrogen and/or number of cationic charges per 1000 MW is necessary when the toxicity of a polycationic polymer (*10*) is to be predicted.

Illustrative PMN Hazard Assessments

As discussed above, the first step in EPA/OTS analysis of the potential hazards of new chemicals involves critical analysis of any submitted test data. Given the prevailing circumstances of limited test data, however,

EPA/OTS has come to rely on an SAR approach, as described above, to supplement available data and to support the development of scientific evaluations of the potential hazards posed by data-poor chemicals. The goal of EPA/OTS PMN hazard analysis is to identify and assess the potential hazards and then to determine the further testing that is needed to permit an adequate assessment of the chemical. It is important to recognize that while SAR is being used by the Agency as an assessment tool, SAR approaches are not viewed as a replacement for test data. When one is dealing with data-poor chemicals, however, EPA believes that SAR can be a useful tool in evaluating the potential hazards of such chemicals and for identifying assessment or testing needs and priorities. Examples of several PMN hazard assessments follow and can serve to illustrate EPA/OTS application of the approaches described above. The first two examples focus on assessment of human health hazards, while the balance of the examples are focused on assessment of environmental effects. Note that although the examples are limited to discussion of either health or environmental effects, in practice under the new chemicals program EPA/OTS assesses both health and environmental effects for all PMN chemicals submitted to the agency. Statements not supported by references represent the assessment of EPA/OTS scientists.

Health Hazard Assessments

Example 1: An Azo Dye. This PMN chemical (I; Fig. 2) is intended for use as a dye for fabric. Submitted toxicological data were as follows: rat oral $LD_{50} > 5$ g/kg; rabbit dermal $LD_{50} > 2$ g/kg; mild eye irritant in the rabbit; Salmonella/microsome assay (Ames Test), positive with or without activation; mouse lymphoma L5178Y assay, positive with or without activation.

HAZARD ASSESSMENT. PMN chemical I, a lipophilic azo dye, was expected to be absorbed via the gastrointestinal tract and lungs and to a lesser extent via the skin. If absorbed intact, chemical I may be subject to reductive cleavage in the liver which would release 5-nitro-2-aminothiophene (II; Fig. 2) and a substituted para-phenylenediamine (III; Fig. 2). In addition, the dye was subject to azo cleavage in the gut by intestinal flora (*27*). If azoreduction occurred in the gut, metabolites II and III would be more readily absorbed systemically than the parent compound. Wang et al. (*28*) pointed out the great similarity in structure and chemical properties between 5-nitrofurans and 5-nitrothiophenes and discussed the metabolism of these compounds. Both 5-nitrofurans and 5-nitrothiophenes appeared to undergo nitroreduction and binding to macromolecules; activation and binding was proposed to involve formation of a free radical or nitroso derivative.

Submitted genotoxicity data indicated that the chemical was mutagenic *in vitro* (the Ames Test response was 20 × background with activation). Numerous 5-nitrofurans and 5-nitrothiophenes have been demonstrated to be mutagenic in the Ames Test (*29,30*), and many of these compounds are also known to be carcinogenic (*29,31*). Arcos and Argus (*32*) discussed the importance of the nitro group and the influence of aryl substitution on the carcinogenic activity of 5-nitrofurans. The authors noted the marked aromatic behavior of furan and that the aryl group at the 2-position is conjugated with the nitro in position 5 on the ring. Removal of the nitro group abolished activity.

Aryl conjugated 5-nitrothiophenes possess carcinogenic potential similar to that of the 5-nitrofurans; this was confirmed in studies performed on a series of 5-nitrothiophenes (including IV and V depicted in Fig. 2) by Cohen et al. (*29*). No statistically significant increase in tumor incidence was reported for the thiophene compound (VI; Fig. 2) lacking the nitro group. Finally, results for compound VII (Fig. 2), which contained a conjugated intercyclic vinyl moiety, indicated that the nitrofuran ring and the second aromatic ring need not be directly connected for the compound to be carcinogenic (*29*).

Based on the submitted evidence of mutagenic activity plus the discussion developed above regarding the carcinogenic potential of 5-nitrothiophenes and the analogous 5-nitrofurans, the PMN chemical was concluded to be mutagenic and was judged likely to be carcinogenic. Accordingly, it was recommended that, if exposures were significant, the PMN chemical should be tested in a lifetime animal bioassay to determine its carcinogenic activity.

ANALOGUE SEARCH. The Structure and Nomenclature Search System in the Chemical Information System (SANSS/CIS; CIS, Inc., Baltimore, MD) was used in the analogue search described for this case. Three query structures (VIII, IX, X), were developed and used in the SANSS/CIS substructure search and are shown in Figure 3. Query structure VIII simulated chemicals analogous to the intact PMN chemical and allows the identification of both thiophene and furan analogues (i.e., 5-nitro(thiophene/furan)-2-yl azo compounds). Query structure IX identified thiophene/furan analogues of I that have an aromatic substituent at position 2, while query structure X identified analogues having an intercyclic vinyl moiety at position 2. After the search with the query structures was completed, the search results were intersected with a selected subset of the pointers in SANSS/CIS to assist and speed the analogue identification process. [Pointers in SANSS/CIS are used to identify or point to specific data files or information sources (e.g., computerized databases, secondary reference works, etc.), which are indexed by chemical in the information system.] In this example, a carcinogenicity/genotoxicity pointer file was created in SANSS/CIS by merging the chemicals included in the following pointer files: International Agency for Research on Cancer, Monographs on the evaluation of the carcinogenic risk of chemicals to humans (*33*); U.S. Public Health Service, survey of compounds that have been tested for carcinogenic activity (*34*); and GENETOX, a genetic toxicity data base developed by EPA (*35,36*). In a similar fashion, pointer

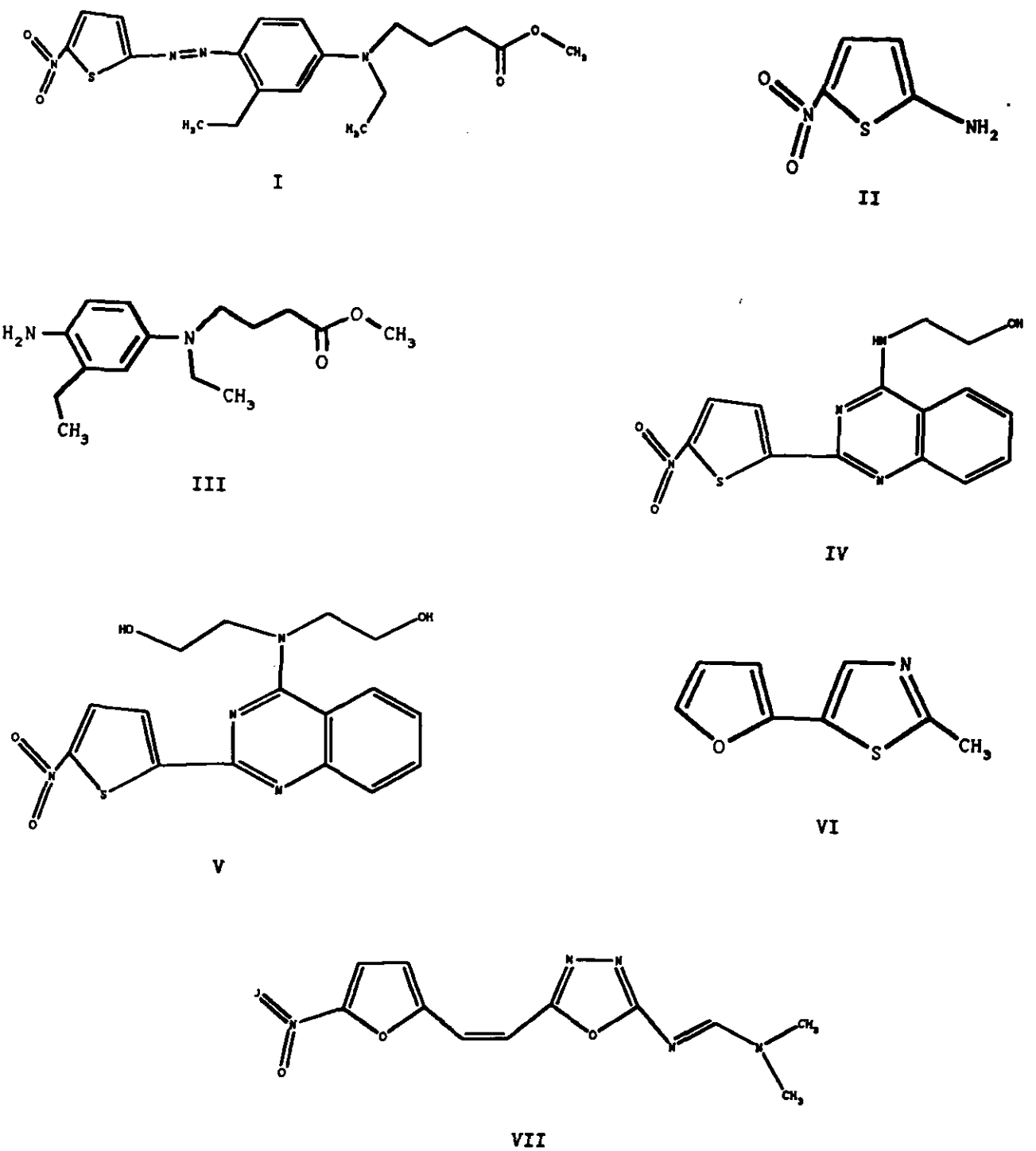

FIGURE 2. Chemical structures cited in Example 1.

files for other end points (general toxicity, aquatic toxicity, etc.) can also be set up and be used in SANSS/CIS. The results of the intersection of the substructure search files for the three query structures with the entire SANSS/CIS data base and the carcinogenicity/genotoxicity pointer files are shown in Table 2 (note that double counting has not been excluded).

The output from the query structure searches was examined to identify chemicals that had the greatest similarity to the PMN chemical (considering the factors described earlier) and that appeared to have carcinogenicity/genotoxicity test data available (as shown by SANSS/CIS pointers). The potential analogues that best met the needs of the review were selected and then

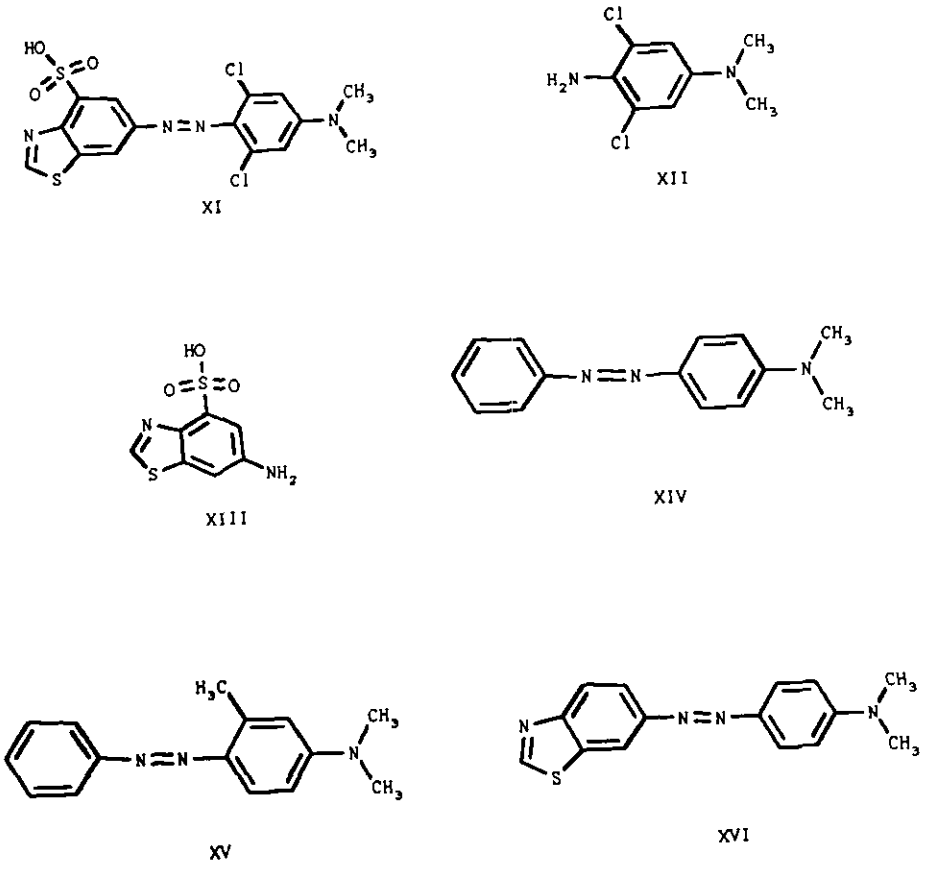

FIGURE 3. Query structures cited in Example 1. R = S or O. X = Any aromatic moiety.

Table 2. Results of intersecting the substructure search files with the SANSS/CIS data base and the carcinogenicity/genotoxicity pointer files.

Pointer files	Number of chemicals		
Query structure	VIII	IX	X
Total SANSS/CIS hits	3	10	19
Carcinogenicity/genotoxicity file hits	0	6	7

subjected to a literature search to identify possibly relevant articles. Note that query structure VIII did not yield any data-containing analogues.

Example 2. An Azoheterozole. The PMN chemical (XI; Fig. 4) was a monoazo compound. No toxicological data were submitted by the manufacturer and the intended use of the chemical was claimed confidential business information by the submitter.

HAZARD ASSESSMENT. Little or no absorption of the PMN chemical was expected through the skin, while some absorption of the chemical was expected in the lungs and gastrointestinal tract. Azoreduction was expected to occur in the gastrointestinal tract (*27*), releasing the individual monocyclic (XII; Fig. 4) and heterocyclic (XIII; Fig. 4) moieties of the chemical. The azoreduction products of the PMN chemical were expected to be more readily absorbed (especially metabolite XII) than the intact chemical. Sulfonation of the PMN chemical increased its water solubility, which was expected to increase the extent of azoreduction in the gastrointestinal tract and to reduce absorption of the intact molecule and metabolite XIII.

The PMN chemical was identified as a prime ring analogue of *N*,*N*-dimethylaminoazobenzene (DAB; XIV; Fig. 4), a potent hepatocarcinogen (*37*), and derivatives thereof. One derivative of DAB, 2-methyl-DAB (XV; Fig. 4), has been shown to be carcinogenic only following partial hepatectomy and dietary administration of the chemical (*38*). The chemical 6-*p*-dimethylaminophenylazobenzothiazole (6-BT; XVI; Fig. 4), a close structural analogue of the intact PMN chemical, has also been shown to be a potent rodent hepatocarcinogen (*39,40*).

DAB and 6-BT both produced positive responses in mutagenicity assays with Salmonella (*41*). In addition, 6-BT produced positive results in an *in vivo* rat liver unscheduled DNA synthesis (UDS) bioassay (*42*).

It has been demonstrated that MAB (the monomethyl analogue of DAB) binds to DNA as an intact moiety (*43*). Bioactivation of DAB has been suggested to involve *N*-demethylation followed by *N*-oxidation and conjugation of the derived hydroxylamine to form a reactive species capable of binding to DNA (*44*). A similar activation mechanism has been proposed for 6-BT (*45*).

The carcinogenicity of DAB is known to be reduced

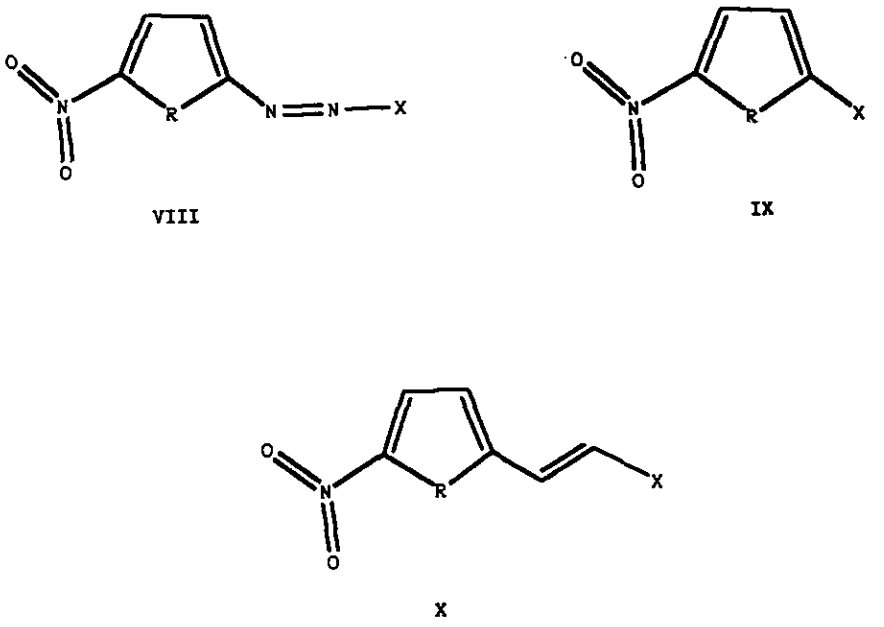

FIGURE 4. Chemical structures cited in Example 2.

by diets high in riboflavin content (Vitamin B2), and the reduction in carcinogenic activity has been attributed to an increase in azoreduction activity (*46*). Azoreduction of 6-BT was reduced by 90% in rat liver tissue when the rats were placed on a low riboflavin diet (*47*). It has also been shown that the azoreduction products of 6-BT were nonmutagenic in Salmonella (*48*). Although the phenylene diamine portion of the PMN chemical has substituents not found in DAB or 6-BT, no data were available to suggest that metabolite XII may be potentially carcinogenic; on the contrary, 2,6-substitution on DAB may be inferred to eliminate or minimize carcinogenic potential (*37*).

Miller et al. (*37*) proposed that for DAB and analogues thereof to be carcinogenic, the 2-position must be unsubstituted. These authors demonstrated that when the 2-position and the equivalent 6-position of DAB were substituted with fluorines, carcinogenic potential was lost, based on results observed with 1-year studies on 3 fluorinated derivatives of DAB. 2-Methyl-*N*-methyl aminoazobenzene was found to be, at best, weakly carcinogenic (1/16 animals had a liver tumor at 11 months). Arcos and Argus (*38*) proposed that all carcinogenic azo compounds were probably *trans* and coplanar, and that bulky ring substituents *ortho* to the azo linkage disrupted this configuration. Since coplanarity and conjugation across the molecule were believed to be important factors in the carcinogenicity of DAB-type compounds and substitution at the 2-position disrupted the configuration, it was possible that the PMN chemical, which was halogenated at the 2,6-position, would exhibit minimal activity, relative to DAB.

Overall, there was a concern for the carcinogenic potential of the PMN chemical based on structural analogies to DAB and 6-BT. The concern for carcinogenic potential was mitigated by evidence that 2,6-substitution was likely to reduce or eliminate carcinogenic activity; a lack of evidence suggesting the azoreduction products of XI were likely to be carcinogenic; and estimates that only limited absorption of the intact PMN chemical would occur. However, since evidence for the low carcinogenic potential of 2,6-disubstituted analogues of the PMN chemical was based on less-than-lifetime studies, it was recommended that if exposure potential was sufficient, genotoxicity tests (Ames test and an *in vivo* mouse micronucleus assay) should be performed on the PMN chemical. 6-BT should be used as a positive control in both assays. The results obtained with the PMN chemical would be evaluated independently and compared with those obtained with 6-BT to test the analogy between the two substances. If results of these assays were positive, and especially if the results were qualitatively consistent with those for 6-BT, an oncogenicity bioassay should be performed.

Environmental Effects Assessments

Example 3. An Aliphatic Ketone. The PMN chemical (XVII; Fig. 5) is intended for use as a solvent for paints. No environmental toxicological data were submitted by the manufacturer, however, the following physical/chemical properties were listed: log K_{ow} = 2.9 at 20°C; molecular weight, 142; water solubility, 714 mg/L at 20°C; and liquid at room temperature.

HAZARD ASSESSMENT. The PMN chemical is nonreactive and is a nonelectrolyte. It is expected to exhibit only narcosis (and thus baseline) toxicity to aquatic organisms and is expected to be easily absorbed from water. Toxicity predictions based on the neutral organic QSARs (in mg/L) are as follows:

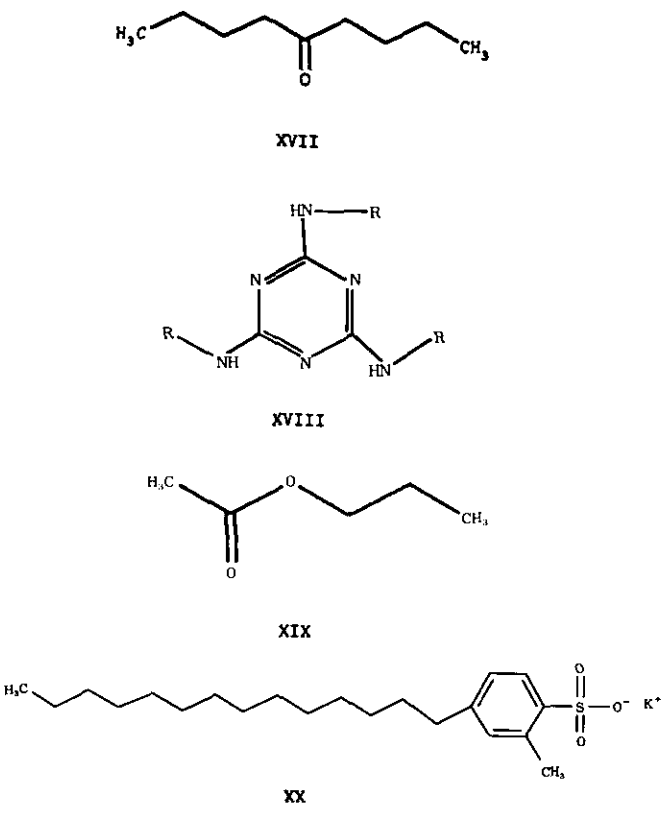

FIGURE 5. Chemical structures cited in Examples 3–6. For structure XVIII, R = linear or branched aliphatic ethers and alcohols.

Freshwater green algae 3-hr EC_{50} for inhibition of photosynthesis	> 10,000.0
Freshwater fish 14-day LC_{50}	31.0
Daphnid 48-hr LC_{50}	17.0
Freshwater fish 96-hr LC_{50}	16.0
Freshwater green algae 96-hr LC_{50} for growth inhibition	7.7
Marine fish 96-hr LC_{50}	5.0
Daphnid 16-day LC_{50}	3.6
Mysid shrimp (marine) 96-hr LC_{50}	2.2
Daphnid 16-day LC_{50} for reproductive inhibition	1.3
Freshwater green algae 96-hr no effect concentration (NEC)	1.9

[NEC is equivalent to the chronic value (ChV) or the geometric mean of the maximum allowable toxicant concentration (GMATC); the MATC is the range of concentrations between the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC).] The 28-day BCF for fish is predicted to be 79.

All of the QSARs, except for the 96-hr EC_{50} and NEC for freshwater green algae, used in the analysis came from Clements et al. (*10*); the algal 96-hr ECs came from a QSAR developed from Calamari et al.(*49*), and it is a future addition to Clements et al. (*10*) when it is revised.

The QSAR-based toxicity profile for aquatic organisms indicates that toxicity is not limited by water solubility; marine fish and invertebrates (i.e., mysid shrimp) are slightly more sensitive than freshwater fish and invertebrates (i.e., daphnids); and freshwater green algae appear to be more sensitive than freshwater fish and invertebrates. Note, however, that the 96-hr bioassay for green algae is actually a chronic toxicity test relative to algae because this test is multi-generational (i.e., 96 hr allows for the attainment of exponential growth and the production of about eight generations of algae) and the effect is a sublethal effect, i.e., inhibition of population growth. Also, the algal 96-hr EC_{50} and NEC values compare closely with the daphnid 16-day LC_{50} and EC_{50} values that are estimates of chronic toxicity for daphnids. Overall, this solvent is considered only moderately toxic to aquatic organisms because the base set of toxicity values (i.e., fish 96-hr LC_{50} values, aquatic invertebrate acute LC_{50} values and the algal 96-hr EC_{50} values) are between 1 and 100 mg/L. Finally, concerns for the bioconcentration potential of this solvent are low because the fish BCF is less than 250, and the BCF values for aquatic invertebrates and algae are expected to be equal to or less than the BCF for fish.

Example 4. A Substituted Melamine. This PMN chemical is a substituted melamine (XVIII; Fig. 5) containing various branched and linear aliphatic ethers and alcohols. Its use was claimed confidential by the manufacturer. The following data were submitted with the PMN:

Average number MW = 1300
Percent < 1000 MW = 20%
Percent < 500 MW = 10%
Water solubility = 0.300 mg/L (measured)
Freshwater fish 24-hr LC_{50} = 50 mg/L
Freshwater fish 96-hr LC_{50} = 20 mg/L
Freshwater fish 96-hr LOEC = 10 mg/L
Daphnid 24-hr LC_{50} > 100 mg/L
Daphnid 48-hr LC_{50} = 90 mg/L
Daphnid 48-hr NEC = 42 mg/L
Freshwater green algal 96-hr NOEC = 0.300 mg/L
Liquid at room temperature

Toxicity test data were based on 100% active ingredient, static aqueous exposures where a carrier solvent and dispersant were used for dissolution, and nominal (i.e., unmeasured) toxicant concentrations.

HAZARD ASSESSMENT. The PMN chemical is nonreactive and is a nonelectrolyte. It is expected to exhibit only narcosis and baseline toxicity to aquatic organisms and is not expected to be easily absorbed from water because the MW is greater than 1000. QSAR analysis (*10*) indicated that this chemical should not exhibit any adverse effects at saturation; however, lethality to fish and aquatic invertebrates was observed during testing. Acute effective concentrations were higher than measured water solubility by at least 30 times. The algal toxicity test results were consistent with QSAR analysis. In this test, the maximum treatment concentration was at the limit of water solubility and no adverse effects were observed. It was concluded that the toxicity seen in the acute fish and aquatic invertebrate tests

could not be attributed to the carrier solvent and dispersant because these carriers were also present in the algal bioassay, and algae are generally more sensitive to dispersants than fish and daphnids. If no toxicity data had been submitted with the PMN, EPA would not have identified concerns for potential hazard to the aquatic environment. However, since effects were observed during the acute toxicity tests with fish and aquatic invertebrates and it did not appear to be due to the carriers, it was recommended that chronic testing with daphnids and fish be done with the highest treatment concentration set at 300 μ/L or the maximum water solubility, if higher.

After consultation with EPA/OTS, the submitter conducted a fish 14-day toxicity test at 1 mg/L (the apparent maximum water solubility under testing conditions) without any carriers. Test conditions included flow-through conditions with measured concentrations and 100% active ingredient. In addition, the submitter also tested the carriers in fish acute toxicity tests. The results of these tests were no observed effects at saturation during the fish 14-day toxicity test and very low toxicity (i.e., acute values greater than 1000 mg/L) for the carriers. Since these test results were consistent with the EPA/OTS hazard assessment, no additional steps were taken with the PMN chemical.

This example illustrates how QSAR analysis can assist in interpreting and validating environmental toxicological testing results and also demonstrates EPA/OTS belief that SAR analysis, while of great value, is not to be relied upon as a substitute for test data. Another important point to recognize is that testing such as that conducted under EPA/OTS New Chemical Review Program can subsequently be used to improve EPA/OTS SAR predictions.

Example 5. A Simple Ester. This PMN chemical is propyl acetate (XIX; Fig. 5). Its use has been declared confidential business information by the manufacturer. No environmental toxicological data were submitted by the manufacturer, however, the following physical/chemical properties were listed: purity, 99%; MW, 102; melting point, −59°C; density, 0.888 mg/mL at 20°C.

HAZARD ASSESSMENT. The PMN chemical is a nonelectrolyte but is expected to be more toxic than what would be predicted from the neutral organic (narcotic) QSARs (at least in some species) as has been shown with other simple esters (*21*). The log K_{ow} was calculated to be 1.2 using the computer program, CLOGP, Version 3.3 (*50*). Toxicity (in mg/L) and BCF predictions based on QSAR analysis are as follows:

Daphnid 48-hr LC_{50}	425.0
Green algal 96-hr EC_{50} for inhibition of growth	200.0
Green algal 96-hr NEC for growth inhibition	50.0
Fish 96-hr LC_{50}	41.0
Daphnid 16-day LC_{50}	32.0
Daphnid 16-day LC_{50} for reproductive inhibition	15.0
Fish BCF	3.0

All of the QSARs, except for the toxicity values for green algae came from Clements et al. (*10*); the algal predictions came from QSAR developed from Calamari et al. (*49*). The fish acute value, which was derived from the QSAR for simple esters (*21*) and cited in Clements et al. (*10*), exhibit the expected excess toxicity. Comparison to the neutral organic QSAR for acute toxicity for fish indicated that this ester is expected to be about 10 times more toxic than an equivalent neutral organic chemical with the same K_{ow} and MW (i.e., the fish 96-hr LC_{50} = 419 mg/L based on the neutral organic QSAR).

The QSAR predictions for daphnids were based on the neutral organic QSARs. This ester was not expected to show excess toxicity to daphnids. The support for this conclusion lies in analysis of the phthalate ester QSARs (*10*) for fish and daphnids. Phthalate esters are simple esters and were included in the simple ester QSAR for fish acute toxicity by Veith et al. (*19*). Therefore, when a fish toxicity prediction for a phthalate ester is needed, Clements et al. (*10*) direct the user to the ester QSAR. However, when the available acute and chronic toxicity data for phthalate esters for daphnids were compared to the acute and chronic toxicity predictions for daphnids using the neutral organic QSARs (J. V. Nabholz, unpublished observations), there was no evidence of excess toxicity. Accordingly, Clements et al. (*10*) direct the user to use the neutral organic QSARs for daphnid acute and chronic toxicity when toxicity predictions are needed for phthalate esters and other simple esters. Finally, no excess toxicity from simple esters and phthalate esters is expected for freshwater green algae as well, since the available empirical data for simple phthalate esters do not indicate excess toxicity for green algae when compared to neutral organic QSAR predictions (J. V. Nabholz, unpublished observations).

This toxicity profile for aquatic organisms indicates that: *a*) fish are expected to be the most sensitive species; *b*) the excess toxicity predicted for fish is because of some specific mode of toxic action unique to fish, e.g., fish may have some metabolic pathway that is especially sensitive to interference by esters and a pathway that aquatic invertebrates may not have; *c*) there is a high probability that this ester could be a cumulative toxicant in fish, i.e., the ratio of the 24-hr LC_{50} value divided by the 96-hr LC_{50} value is expected to be greater than two; *d*) this cumulative toxicity is expected to be because of an accumulation of damage or injury and not to bioconcentration (increasing residues in fish) of the chemical because the bioconcentration potential of this ester is very low (thus there is also a low concern for bioconcentration in fish); and *e*) the fish chronic value (i.e., the NEC or GMATC) is expected to be more than 10 times lower (i.e., more toxic) than the fish acute (96-hr) toxicity value because the cumulative toxicity ratio is expected to be greater than two. Overall, the concern for hazard to the aquatic environment is moderate because of the excess acute toxicity and the expected high degree of chronic toxicity in fish.

Example 6. A Linear Alkyl Toluene Sulfonate (LTS). This LTS (XX; Fig. 5) is an anionic surfactant with 14 carbons in the alkyl chain (or the hydrophobic component). It is intended for use in laundry detergents and hard-surface cleaners. The following information was submitted with the PMN: formulated as potassium salt; miscible (or dispersible) in water; PMN to be sold as 50% dispersion in water; pH of product = 7.6; fish 56-day BCF = 14.

HAZARD ASSESSMENT. The PMN is an anionic surfactant and is analogous to linear alkyl benzene sulfonates (LAS). Toxicity predictions (in mg/L) were based on the LAS QSARs from Clements et al. (*10*) and are as follows:

Fish 96-hr LC_{50}	0.31
Daphnid 48-hr LC_{50}	0.31
Green algal 96-hr LC_{50} for inhibition of growth	0.16
Green algal 96-hr NEC for growth inhibition	0.11
Fish 28-day NEC for early life stages	0.05
Daphnid 21-day NEC for reproductive inhibition	0.05

The predicted toxicity values for the PMN surfactant are expected to be very close to the actual test data if the chemical were to be tested because the LAS QSAR is based on a homologous series of LAS surfactants and has high predictive power. Any differences in toxicity between a linear alkyl benzene sulfonate and a linear alkyl toluene sulfonate (i.e., differences due to the presence of the methyl group on the LTS benzene ring) are expected to be insignificant. This QSAR-based toxicity profile for aquatic organisms indicates that *a*) toxicity is not expected to be limited by water solubility or dispersibility; *b*) while freshwater green algae are predicted to be slightly more sensitive during exposures of 96-hr or less, daphnids are predicted to be more sensitive during longer exposures; *c*) damage to algal cell membranes and gill membranes in fish and daphnids is expected during the 48 to 96-hr exposures, but not during the longer term exposure (21 to 28 days) tests; and *d*) toxicity during chronic exposures to daphnids and fish is expected to be attributed to an accumulation of systemic damage or injury rather than accumulation of the PMN chemical in the body (i.e., body residues), because the bioconcentration potential has been shown to be low. Overall, the hazard concern for the aquatic environment is high because the base set of aquatic toxicity tests are predicted to have effective concentrations (EC_{50} values) of less than one. As a result, acute and chronic toxicity testing was recommended for this PMN. The testing recommendations consisted of the fish acute toxicity test, the daphnid acute toxicity test, and the green algal toxicity test; and the fish early life-stage toxicity test and the daphnid chronic toxicity test, if triggered by the base set toxicity tests. Chronic toxicity testing would be required if the fish and/or daphnid acute toxicity values are less than 1 mg/L or if the fish and/or daphnid acute toxicity values are between 1 mg/L and 100 mg/L and there are indications of cumulative toxicity (i.e., the ratio of the fish 24-hr LC_{50} value to the 96-hr LC_{50} value or the ratio of the daphnid 24-hr LC_{50} value to the 48-hr LC_{50} value is greater than two).

If test data are eventually obtained for this LTS, these data will be compared to the predictions obtained from the LAS QSAR and incorporated into the LAS QSAR in order to improve predictions for these anionic surfactants in the future.

Summary

The limited availability of test data on the majority of industrial chemicals and environmental contaminants confronting the U.S. EPA has resulted in the development by the Agency of SAR-based approaches for hazard analysis. The major components of the analysis (which were discussed and illustrated in detail in this paper), involved review of available test data; application of test data available on analogous substances or potential metabolites of the target chemical; and the use of QSARs to predict physical-chemical properties, environmental effects, and bioconcentration factors. A key aspect in the application of the latter two components is the experience and scientific judgment of assessors in interpreting and integrating the information; these are critical in identifying structural or functional analogues or in selecting appropriate QSARs. The identification of possible modes of toxicity is an integral part of the overall approach. In many cases, the available information does not extend beyond chemical structure and basic physical-chemical properties that can be calculated from structure. Thus, the importance is evident of the EPA/OTS of this workshop, with its emphasis on predicting modes of toxicity from chemical structural information.

The authors gratefully acknowledge the support of A. Christine Watson and Bobbe Ward in the careful typing of the manuscript and of Vivian L. Hart in the preparation of the figures.

The article has been reviewed by the Office of Toxic Substances, U.S. EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

1. National Research Council (NRC). Toxicity Testing: Strategies to Determine Needs and Priorities. National Academy Press, Washington, DC, 1984.
2. "Toxic Substances Control Act" (TSCA). Public Law 94-469, 90 Stat. 2003, October 11, 1976.
3. Auer, C. M., and Gould, D. H. Carcinogenicity assessment and the role of structure-activity relationships (SAR) analysis under TSCA Section 5. Environ. Carcinog. Rev. C5(1): 29-71 (1987).
4. "Comprehensive Environmental Response, Compensation, and Liability Act" (CERCLA). Public Law 96-510, 42 Stat. 9601 (section 101), December 11, 1980.
5. Williams, D. A. Drug metabolism. Principles of Medicinal Chemistry (W. O. Foye, Ed.), Lea and Febiger, Philadelphia, 1976, p. 111.
6. Eder, E., Henschler, D., and Neudecker, T. Mutagenic properties of allylic and alpha, beta-unsaturated compounds: consideration of alkylating mechanisms. Xenobiotica 12(12): 831-848 (1982).
7. DiCarlo, F. J., Bickart, P., and Auer, C. M. Structure-metabo-

lism relationships (SMR) for the prediction of health hazards by the Environmental Protection Agency. I. Background for the practice of predictive toxicology. Drug Metab. Rev. 17: 171–186 (1986).
8. DiCarlo, F. J., Bickart, P., and Auer, C. M. Structure-metabolism relationships (SMR) for the prediction of health hazards by the Environmental Protection Agency. II. Application to teratogenicity and other toxic effects caused by aliphatic acids. Drug Metab. Rev. 17: 187–220 (1986).
9. Lyman, W. J., Reehle, W. F., and Rosenblatt, D. H. Handbook of Chemical Property Estimation Methods. McGraw Hill, New York, 1982.
10. Clements, R. G., Ed., Johnson, D. W., Lipnick, R. L., Nabholz, J. V., and Newsome, L. D. Estimating Toxicity of Industrial Chemicals to Aquatic Organisms Using Structure Activity Relationships. EPA-560-6-88-001. U.S. Environmental Protection Agency, Washington, DC, 1988. (Available from NTIS, Springfield, VA, PB89-117592.)
11. Franke, R. Theoretical Drug Design Methods. Elsevier, New York, 1984.
12. Golberg, L., Ed. Structure Activity Correlation as a Predictive Tool in Toxicology: Fundamentals, Methods, and Applications. Hemisphere Publishing Corporation, Washington, DC, 1983.
13. Hansch, C., and Leo, A. Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, New York, 1979.
14. Sexton, W. A. Chemical Constitution and Biological Activity, Third ed. Van Nostrand, New York, 1963.
15. Albert, A. Selective Toxicity. The Physico-Chemical Basis of Therapy, Sixth ed. Chapman and Hall, London, 1979.
16. Lipnick, R. L. Validation and extension of fish toxicity QSARs and interspecies comparisons for certain classes of organic chemicals. QSAR in Toxicology and Xenobiochemistry, Vol. 8 (M. Tichy, Ed.), Elsevier, New York, 1985, pp. 39–52.
17. Franks, N. P., and Lieb, W. R. Mechanisms of general anesthesia. Environ. Health Perspect. 87: 199–205 (1990).
18. Veith, G. D., and Broderius, S. J. Rules for distinguishing toxicants that cause type I and type II narcosis syndromes. Environ. Health Perspect. 87: 207–211 (1990).
19. Russom, C. L., Drummond, R. A., and Hoffman, A. D. Acute toxicity and behavioral effects of acrylates and methacrylates to juvenile fathead minnows. Bull. Environ. Contam. Toxicol. 41: 589–596 (1988).
20. Reinert, K. H. Aquatic toxicity of acrylates and methacrylates: quantitative structure-activity relationships based on Kow and LC_{50}. Regulatory Toxicol. Pharmacol. 7: 384–389 (1987).
21. Veith, G. D., DeFoe, D., and Knuth, M. Structure-activity relationships for screening organic chemicals for potential ecotoxicity effects. Drug Metab. Rev. 15: 1295–1303 (1984).
22. Zitko, V. Uptake and excretion of chemicals by aquatic fauna. Ecotoxicology and the Aquatic Environment (P. M. Stokes, Ed.), Pergamon Press, New York, 1981, pp. 67–78.
23. Perez, K. T., Davey, E. W., Morrison, G. E., Soper, A. W., Lackie, N. F., Winslow, D. W., Blasco, R., Johnson, R., and Marino, S. Environmental assessment of a substituted benzidine based azo dye, Direct Blue-15, in experimental marine microcosms. ERL-N contribution No. 882. U.S. Environmental Protection Agency, Narragansett, RI.
24. Deneer, J. W., Sinnige, T. L., Seinen, W., and Hermens, J. L. M. A quantitative structure-activity relationship for the acute toxicity of some epoxy compounds to the guppy. Aquat. Toxicol. 13: 195–204 (1988).
25. Deneer, J. W., Seinen, W., and Hermens, J. L. M. The acute toxicity of aldehydes to the guppy. Aquat. Toxicol. 12: 185–192 (1988).
26. Banerjee, S., Yalkowsky, S. H., and Valvani, S. C. Water solubility and octanol/water partition coefficients. Limitations of the solubility-partition coefficient correlation. Env. Sci. Tech. 14: 1227–1229 (1980).
27. Walker, R. The metabolism of azo compounds: a review of the literature. Food Cosmet. Toxicol. 8: 659–676 (1970).
28. Wang, C. Y., Chiu, C. W., and Bryan, G. T. Nitroreduction of carcinogenic 5-nitrothiophenes by rat tissues. Biochem. Pharm. 24: 1563–1568 (1975).
29. Cohen, S. M., Enturk, E., and Bryan, G. T. Comparative carcinogenicity of 5-nitrothiophenes and 5-nitrofurans in rats. J. Natl. Cancer Inst. 57: 277–282 (1976).
30. Wang, C. Y., Muraoka, K., and Bryan, G. T. Mutagenicity of nitrofurans, nitrothiophenes, nitropyrroles, nitroimidazoles, aminothiophenes, and aminothiazoles in *Salmonella typhimurium*. Cancer Res. 35: 3611–3617 (1975).
31. Bryan, G. T., Ed. Carcinogenesis—A Comprehensive Survey. Vol. 4, Nitrofurans. Raven Press, New York, 1987.
32. Arcos, J., and Argus, M. Structure-activity relationships of chemical carcinogens. Effect of chemical reactivity, molecular geometry, and metabolism on carcinogenic activity. In: Chemical Induction of Cancer, Vol. II B. Academic Press, New York, 1974, p. 37.
33. International Agency for Research on Cancer (IARC). Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon, France, Vol. I (1972) through Vol. 41 (1986).
34. U.S. Public Health Service. Survey of compounds which have been tested for carcinogenic activity. National Cancer Institute, Bethesda, MD. Publication No. 149, beginning with Vol. I in 1951.
35. Green, S., and Auletta, A. Editorial introduction to the reports of the Gene-Tox Program. An evaluation of bioassays in genetic toxicology. Mutat. Res. 76: 165–168 (1980).
36. Waters, M. D., and Auletta, A. The Gene-Tox Program: genetic activity evaluation. J. Chem. Info. Computer Sci. 21: 35–38 (1981).
37. Miller, J. A., Miler E. C., and Finger, G. C. Further studies on the carcinogenicity of dyes related to 4-dimethylamino-azobenzene; the requirement for an unsubstituted 2-position. Cancer Res. 17: 387–398 (1957).
38. Arcos, J., and Argus, M. Structure-activity relationships of chemical carcinogens. Effect of chemical reactivity, molecular geometry, and metabolism on carcinogenic activity. In: Chemical Induction of Cancer, Vol. II B. Academic Press, New York, 1974, pp. 149–154.
39. Brown, E., and Sanchorwala, C. J. Carcinogenicity activity of analogs of p-dimethylaminoazobenzene. VI. Activity of the benzimidazole and benzthiazole analogs. J. Med. Chem. 11: 1074–1075 (1968).
40. Elliot, B. M., Robinson, M., and Ashby, J. 6-*p*-Dimethylaminophenylazobenzothiazole: a potent hepatocarcinogen in the rat. Cancer Lett. 21: 69–76 (1983).
41. Ashby, J., Lefevre, P. A., Styles, J. A., Charlesworth, J., and Paton, D. Comparisons between carcinogenic potency and mutagenic potency to Salmonella in a series of derivatives of 4-dimethylaminoazobenzene (DAB). Mutat. Res. 93: 67–81 (1982).
42. Ashby, J., and Bekje, B. Use of an *in vivo/in vitro* rat liver DNA repair assay to predict the relative rodent hepatocarcinogenic potency of 3 new azo mutagens. Carcinogenesis 6: 611–615 (1985).
43. Tullis, D. L., Dooley, K. L., Miller, D. W., Baetcke, K. P., and Kadlubar, F. F. Characterization and properties of the DNA adducts formed from *N*-methyl-4-aminoazobenzene in rats during a carcinogenic treatment regimen. Carcinogenesis 8: 577–583 (1987).
44. Lin, J. K., and Wu, J. R. Synthesis, toxicities and carcinogenicities of carcinogenic bifunctional aminoazodyes. Cancer Res. 34: 2274–2282 (1974).
45. Ashby, J., Callander, R. D., Lefevre, P. A., Paton, D., and Fishwick, B. Mutagenicity to Salmonella of the monomethylamino and *N*-cyanoethyl analogues of 4-dimethylaminoazo-benzene (DAB) and 6-dimethylaminophenylazobenzothiazole (6BT). Mutat. Res. 128: 11–16 (1984).
46. Miller, J. A., and Miller E. C. The carcinogenic aminoazo dyes. Adv. Cancer Res. 1: 339–396 (1953).
47. Elliot, B. M. Azoreductase activity of Sprague-Dawley and Wistar-derived rats towards both carcinogenic and non-carcinogenic analogues of 4-dimethylaminoazobenzene (DAB). Carcinogenesis 5: 1051–1055 (1984).
48. Ashby, J., Lefevre, P. A., and Callander, R. D. The possible role of azoreduction in the bacterial mutagenicity of 4-dimethylami-

noazobenzene (DAB) and two of its analogues (6BT and 5I). Mutat. Res. 116: 271–279 (1983).
49. Calamari, D., Galassi, S., Setti, F., and Vighi, M. Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12: 253–262 (1983).
50. Leo, A., and Weininger, D. CLOGP Version 3.3: Estimation of the *n*-Octanol/Water Partition Coefficient for Organics in the TSCA Industrial Inventory. Pomona College, Claremont, CA, 1985.
51. PENTA (an IPA in-house PMN information system). Office of Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC, 1985.