13 Structure–Activity Relationships: Computerized Systems

Michael D. Waters, Ann M. Richard, James R. Rabinowitz*
U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

H. Frank Stack and Neil E. Garrett
Environmental Health Research and Testing, Inc., Research Triangle Park, North Carolina, USA

Paul H. M. Lohman
University of Leiden, The Netherlands

Herbert S. Rosenkranz
Case Western Reserve University, Cleveland, Ohio, USA

13.1 INTRODUCTION

Applications of computational methods to study relationships between chemical structure and biological activity have contributed substantially to our current understanding of the interactions of chemicals with biological systems. The number and sophistication of such applications have increased, and will continue to increase, with advances in computer hardware and software, with the availability of larger biological databases, and with increased understanding of molecular-level interactions in biological systems. Such advances have enhanced both the scope and detail of the biological problems that can be successfully treated by computational structure–activity relationships (SAR) techniques.

This paper presents important issues relative to the application of computational

*Although the research described in this paper has been reviewed and approved for publication by the Health Effects Research Laboratory, US Environmental Protection Agency, such approval does not signify that the contents necessarily reflect the views and policies of the Agency; and the mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Methods to Assess DNA Damage and Repair: Interspecies Comparisons
Edited by R. G. Tardiff, P. H. M. Lohman and G. N. Wogan

SAR techniques to problems in environmental chemical hazard evaluation. Such problems are most often characterized by not only a lack of biological activity data for the chemicals of concern but also a lack of understanding concerning molecular-level mechanisms of activity. The goal is to develop SAR models that can rely on existing biological activity data on chemical analogs to estimate the toxicity of the chemicals of interest. SAR models such as these may play important roles in preliminary assessments, and may help direct future experimental and theoretical investigations.

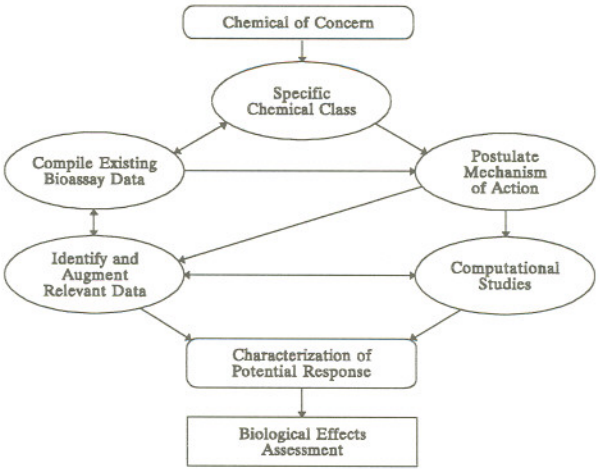

Figure 13.1 The process of developing information for the structure–activity assessment of the potential biological effects of a chemical or chemical class

This paper presents important generalizations relative to the application of computational SAR techniques, problems particular to the SAR modelling of genotoxicity and cancer endpoints, and an illustrative analysis of a chemical class—the organic halides. Additional issues relating to database development, biologically based comparative assessment approaches, and a weight-of-evidence evaluation scheme are also discussed.

13.2 SAR AS A PROCESS

The relationship among various components of an SAR study is complex, as illustrated in Figure 13.1. The initial stages involve specification of the chemical class and compilation of bioassay data for the biological activity endpoint of interest. Specification of these components limits and defines the scope of the SAR modelling study. This information together with knowledge of chemical properties

and potential interactions with biomolecular targets contributes to the derivation of possible mechanisms of toxicity. Mechanistic hypotheses can suggest the type of computational study that would be most useful, and the results of such a study can provide a quantitative basis for the organization and differentiation of chemicals within a class. Continuing feedback may occur between computational studies, knowledge concerning the mechanisms of action, and the assembly of a relevant bioassay database for the chemical class. Postulating mechanisms of action can be the basis of experimental bioassay strategies, and may suggest a means to extrapolate the results of one type of bioassay to another in order to estimate toxic endpoints of interest. Ultimately, SAR modelling of potential biological responses based on the current state of understanding of molecular-level mechanisms should provide a rational basis to extrapolate existing information to untested chemicals.

The feedback loops indicated in Figure 13.1 can greatly enhance the relative contributions of each component in the SAR study. This situation, in turn, can lead to a improved understanding of the fundamental processes involved in a biological process, and can improve the ability to characterize potential responses of untested members of the chemical class. Generally, such feedback requires closer interactions between theoretical chemists, synthetic organic chemists, and experimental biologists. For example, an underutilized yet potentially very powerful approach involves the identification of one or more particular compounds that may have no environmental significance, but which, if tested, could be extremely informative in refining mechanistic hypotheses.

The SAR model derived from Figure 13.1 pertains to the underlying processes specific to the particular bioassay and chemical class chosen. Frequently, however, a chemical class is specified based on little or no knowledge concerning the mechanisms of toxic action of its members, but rather by consideration of common chemical structural and reactivity characteristics. Incomplete class specification or the inclusion of chemicals that act through alternate mechanisms can confuse and limit the utility of the resulting SAR model.

As studies progress and additional information becomes available, efforts should continue towards revising and updating chemical class specifications to incorporate expanding knowledge. In addition, alternative means to specify chemical classes from a biological perspective should be investigated. Furthermore, an SAR study requires a consensus concerning the activity assignment of a specific chemical in a given bioassay. Response criteria required to distinguish a biologically active molecule from an inactive one may be subject to controversy. This problem is particularly acute for complex endpoints such as carcinogenicity. Hence, weight-of-evidence schemes, that attempt to formulate a consensus outcome from such data, can provide a useful starting point for an SAR study. Other types of SAR approaches could seek to incorporate an array of bioassay data into the modelling of a complex biological endpoint. For example, a select group of genetic toxicity bioassays might provide a useful basis for SAR modelling of carcinogenicity. These characteristics are examples of only a few of the preliminary considerations that impact on the selection of any SAR modelling method and its determination

of predictive value.

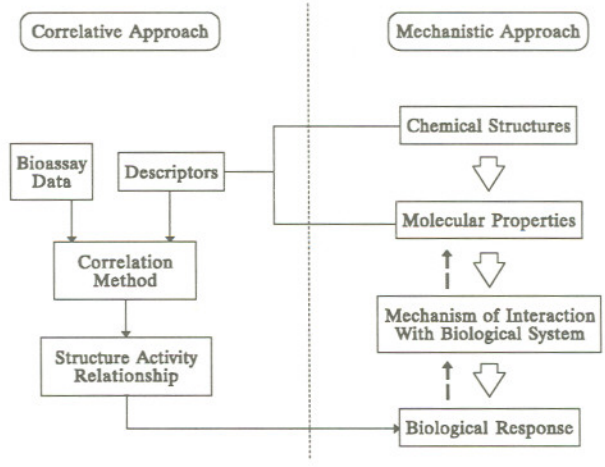

Figure 13.2 Generic components of a structure-activity study

13.3 CORRELATIVE AND MECHANISTIC APPROACHES

Two extreme but complementary SAR approaches exist which attempt to model the relationship between chemical structure and biological response: one from a correlative and the other from a causal perspective (Figure 13.2). In each case, the chemical structure is responsible for a molecule's physical properties (such as the molecular electrostatic potential, electrophilicity or nucleophilicity, solubility, and partition coefficient). These properties determine the biological response through specific biomolecular interactions. In the causal approach, understanding the mechanism of action and the underlying molecular-level interactions that determine activity is the key step to estimating possible biological responses of untested class members. The tools of computational chemistry, such as quantum mechanical and semi-empirical programs to compute minimum energy structural configurations, atomic charges, electronic properties, and relative molecule energies, are used to probe and model the molecular-level basis for biological activity in these approaches. Since studies used to validate the mechanisms of action are critical and may require additional investigation of related chemicals, interactions among experimentalists and theoreticians are extremely valuable in this approach (Figure 13.1).

The left half of Figure 13.2 represents the strictly correlative approach, in which little or no knowledge concerning the causal basis for activity is presumed. A large number of descriptors based on chemical structure and molecular properties is computed for each member of a class of chemicals. This descriptor set is then

merged with biological activity information, and a statistical or pattern recognition technique is used to identify relevant descriptors that are significantly correlated with a particular biological response. These descriptors, and in some cases the quantitative weights associated with their contributions, are the basis of the SAR model for the prediction of biological response.

In practice, these two approaches (Figure 13.2) are rarely separate, and SAR model development ideally should incorporate elements of each. Mechanistic considerations can and should influence the types of molecular descriptors that are considered in a correlative SAR study. Likewise, the results of a purely correlative study have limited utility if considered independent from mechanistic understanding. By considering the descriptors that account for activity correlations and developing insight concerning their relationship to possible mechanisms of activity, the validity of the SAR model and confidence in its predictions are enhanced. Computational SAR models incorporating elements of both the causal and correlative approaches likely will play an increasingly important role in identifying the features of chemical structure conditionally required for the biological activity or inactivity within a series of compounds.

13.4 CORRELATIVE SAR TECHNIQUES

The allure of the correlative SAR approach is that little prior knowledge of mechanisms is required. Several such approaches have been applied to problems in genotoxicity and carcinogenicity, that are most often characterized by large amounts of data but very little molecular-level understanding of mechanisms of activity. Since the application of such approaches is likely to increase in conjunction with extensive data collection efforts currently under way, a sampling of available approaches is considered and contrasted. General reviews on correlative methods are contained in Jurs *et al.* (1985), McKinney (1985), Enslein *et al.* (1983, 1987), Golberg (1983), and Frierson *et al.* (1986). The reader is referred to the literature for a more detailed discussion of each method and its specific applications: ADAPT (Jurs *et al.*, 1983; Stouch and Jurs, 1985), SIMCA (Wold and Sjostrom, 1977; Dunn and Wold, 1980, 1981), CASE (Klopman, 1985; Klopman *et al.*, 1985a, 1987a), and TOPKAT (Enslein and Craig, 1982; Enslein *et al.*, 1983, 1987). For illustrative purposes, one technique, the CASE program, will be considered in more detail, and applied to a sample analysis of the organic halides as a class.

Input to a correlative SAR program usually consists of graphical or coded molecular structures of the chemical of interest, and qualitative or quantitative measures of biological activity corresponding to a common biological endpoint. This information constitutes the database for study. Molecular descriptors are then either input from external sources or calculated internally. The term "molecular descriptor" refers to any physico-chemical or substructural parameter, either experimentally measured or calculated, that attempts to encode an aspect of molecular structure. Descriptors fall into four major categories—topological,

geometrical, physicochemical, and electronic—and pertain either to the entire molecule or a local region of a molecule. Specific examples include: connectivity indices (Randic, 1975); fragment substructures; moments of inertia; molecular volume; partition coefficients; and HOMO or LUMO (highest occupied or lowest unoccupied molecular orbital) energies. Descriptors are discarded that have either linear dependencies on other descriptors already included in the analysis, or that account for little activity variation within the database. A correlation step employs a statistical or pattern recognition method to determine associations between remaining descriptors and activity variation within the database. The final set of molecular descriptors, and in some cases the statistical weights associated with these descriptors are the basis of the SAR model used for subsequent activity predictions.

Correlative SAR programs vary with regard to the number and type of molecular descriptors considered, the degree of user input in the selection of descriptors, the analysis used to determine which descriptors are relevant to activity, and the final form of the SAR prediction model. In addition, many programs operate in more than a single mode (SAR or QSAR) and with more than one type of correlation or pattern recognition method. A wide variety of molecular descriptors can be supplied for a TOPKAT, SIMCA, or ADAPT analysis—some are calculated internally and others must be supplied from external sources. A CASE analysis, by contrast, is based on a single type of descriptor—molecular skeletal fragments. Two problems are associated with the use of a large number of descriptors in correlative SAR approaches. When any type of regression statistics is used, strict limits exist on the number of initial descriptors that can be considered in relation to the number of molecules in the database to avoid chance correlations and meaningless results (Topliss and Edwards, 1979). Second, while numerous diverse descriptors relate to molecular structure in a general sense, their relation to each other or to specific physico-chemical processes is often unclear. Hence, to assess their causal significance, which ultimately limits the utility of the model, may be difficult (Frierson *et al.*, 1986; Rosenkranz *et al.*, 1990). Hence, SAR models that attempt to restrict the range of possible descriptors based on general mechanistic relevance have some advantages over unrestricted methods. The descriptor generation and selection processes in TOPKAT are semi-automated, and involves calculation and consideration of several descriptors. An ADAPT or SIMCA analysis, by contrast, requires greater user expertise and user involvement in generating and selecting descriptors. The CASE descriptor selection process is fully automated, and involves an exhaustive calculation of all possible descriptors. However, in this method, the descriptors are all the same type. Although the number or size of fragments needed to model a relevant molecular parameter could be exceedingly large, an advantage of the CASE approach is that molecular fragments may provide more insight into a possible mechanistic interpretation than a combination of dissimilar descriptors.

The specific correlative SAR methods also differ with regard to the type of correlation processes used to evaluate and select relevant descriptors. TOPKAT

uses a multivariate regression method to determine the smallest set of descriptors that best reproduces the activity variation within the database. The resulting quantitative SAR (QSAR) equation is based on the premise that a discrete increment of activity can be associated with any type of molecular descriptor. The most common application of CASE makes qualitative activity predictions based on the presence or absence of one or more significant fragments. Significance is determined by deviation of the fragment incidence distribution within the database from a random binomial distribution among activity classes. SIMCA and ADAPT are classified as pattern recognition approaches, and use linear discriminate or principle components clustering analysis methods to derive an SAR model. Molecules represented by a string of descriptor values are positioned in a multi-dimensional space, where each descriptor is represented by a separate axis in this space. Molecules that have similar descriptor values (presumably chemically and biologically similar) tend to cluster in the same regions of this descriptor space. If the position of a molecule within this space meaningfully correlates with activity, then a qualitative activity prediction is based on the position of a new compound within the delineated active or inactive regions of this space. The linear discriminant analysis used most commonly in ADAPT attempts to determine the descriptor space that gives the best planar separation of the entire data set into an inactive and active molecular region. The principle components cluster approach used by SIMCA searches instead for significant clustering of similar molecules associated with either activity or inactivity. In this approach, one or more significant clustering patterns can be associated with each activity category, where the category can include different measures of activity (such as active versus inactive) or different mechanisms of activity (Dunn and Wold, 1981).

13.4.1 THE CASE SYSTEM

Since the CASE method will be applied to a sample analysis for illustrative purposes, it is considered in greater detail. The CASE methodology has been described on several occasions (Klopman 1984; Klopman and Macina, 1987; Klopman *et al.*, 1985b, 1987a; Rosenkranz and Klopman, 1989). CASE is fully automated and selects its own molecular fragment descriptors from a user selected "training" set composed of active and inactive molecules and their associated activities. The descriptors are easily recognizable single, continuous structural fragments, of length two to ten non-hydrogen atom units embedded in the complete molecule. The descriptors are classified as either activating (biophore) or inactivating (biophobe) fragments. The ability of CASE to select biophores readily recognized as being part of a molecule is a major advantage of the method. Chemical "intuition" traditionally has been used to associate the presence or absence of particular functional groups with biological activity. Indeed, this approach was taken recently by Ashby and Tennant (1988) and Ashby *et al.* (1989) in identifying "structural alerts" for carcinogenesis. Enzymes and other biological

receptors recognize moieties much larger than the groups usually considered. While CASE is limited in its ability to predict important steric or 3-dimensional conformational requirements for activity, it can generate substructures of intermediate size that may be associated with biological activity. Since a major aim is to elucidate the basis of the action of toxicologically active molecules, the identification by CASE of structural components embedded in the molecule offers a tool that may be exploited to investigate structural sites of metabolism or receptor binding or to test other hypotheses.

Once a "training set" of chemicals has been assimilated, CASE can be queried regarding the expected activity of molecules of unknown activity. On the basis of the presence or absence of the previously identified biophores and bioprobes, CASE estimates activity or lack thereof. In addition, CASE can use the descriptors to perform an *ad hoc* multivariate regression analysis (QSAR) that results in a projected potency (Frierson *et al.*, 1986; Klopman *et al.*, 1987a, b; Rosenkranz and Klopman, 1989). Experience with CASE indicates that databases consisting of at least 30 to 50 congeneric (structurally similar) chemicals distributed among inactive, marginally active, and active chemicals are required for a correlative analysis.

13.4.2 CHEMICAL CLASSIFICATION AND REPRESENTATION

An important aspect of SAR modelling that has particular relevance to a correlative SAR study is the initial database compilation. The statistical nature of such methods imposes numerical and representation requirements on the database under consideration. The composition of the database is generally restricted to a chemical class of structurally, and presumably mechanistically related compounds. This chemical classification usually imposes limits on the structural diversity of the compounds under consideration, with structural features common to all members of a chemical class considered necessary but not sufficient requirements for activity. Hence, even in a strictly correlative study, some structure-mediated aspects of an assumed common mechanism of action are usually implicitly incorporated before analysis.

An alternative means to assess chemical class membership from a strictly biological perspective has been suggested by Garrett *et al.* (1984, 1986) and Waters *et al.* (1988c), using pattern recognition techniques. This approach classifies chemicals according to a similar overall pattern of genetic or other biological activity based upon biologically effective dose. Thus, groups of chemicals may be identified that display qualitatively and quantitatively similar biological responses across species without knowledge of their chemical structures. Such classifications may result in novel or unusual groupings of chemicals that appear to be structurally unrelated, but that may exert their biological activity through similar mechanisms. Using computerized profile matching techniques, rapid identification of subsets of chemicals is possible from large databases with similar profiles for such

classification purposes. Correlative SAR techniques, such as CASE, may then be used to ascertain those structural components that may be responsible for biological activity or inactivity.

Some correlative SAR approaches have a limited capacity to evaluate structurally diverse databases. This capability strongly depends, however, on whether each mechanistically distinct chemical class is adequately represented within the database. Careful analysis of such databases could facilitate the identification of mechanistically distinct chemical classes that cross traditional class boundaries. However, any further analysis of such data should proceed on the smaller circumscribed data classes. A danger is that large, diverse databases are being analyzed indiscriminately, and used to satisfy numerical representation requirements for SAR without being examined subsequently to see if they have satisfied important class representation requirements. A CASE or TOPKAT analysis, in principle, is capable of yielding independent sets of descriptors when analyzing a combined database of mechanistically distinct classes subject, however, to the above precautions. Also, a principle-components-clustering approach, such as used in SIMCA, is particularly well suited to distinguishing separate patterns of activity within a database.

In addition to limits imposed on the nature of chemicals that can be included in a particular database, data representation requirements dictate the type and number of compounds that should be included for a correlative SAR study. Confident estimates can be made only for chemicals whose descriptor values lie within the bounds of the model. In addition, sufficient numbers of compounds in each activity category and descriptor class should be included to provide adequate statistics and confidence levels for reliable estimations. In general, the more diverse the database, the more descriptors are needed to span the variation within the database. In practice, few of these conditions are adequately met for most classes of compounds of interest. Data gaps and deficiencies are a common and sometimes serious problem associated with correlative SAR studies. Such studies are dependent ultimately on the availability of high quality biological databases, and, as a result, have been limited to a few particular bioassays and endpoints.

13.4.3 SAR MODELLING OF GENOTOXICITY AND CARCINOGENESIS

Several features and important unresolved issues pertaining to the modelling of genotoxicity and carcinogenicity endpoints that impact on SAR studies remain. On first inspection, an abundance of data available for use in SAR modelling studies appears to exist. On closer examination, however, several serious deficiencies and problems become apparent. The range of bioassays that have been used to evaluate thousands of chemicals for genotoxicity is wide. However, the diversity of chemical structures represented is tremendous, and very few chemicals have been tested in the full range of bioassays. Only a few select bioassays, such as the *Salmonella* test, exist for which sufficient data representation requirements are met within a wide variety of chemical classes. Such bioassays were designed as

experimental surrogates of specific processes thought to be important components in mechanisms of carcinogenicity. However, the structural determinates for activity within a particular bioassay, the relationships among bioassays, and the relationships among bioassays and the processes being modelled are often unclear and subject to controversy.

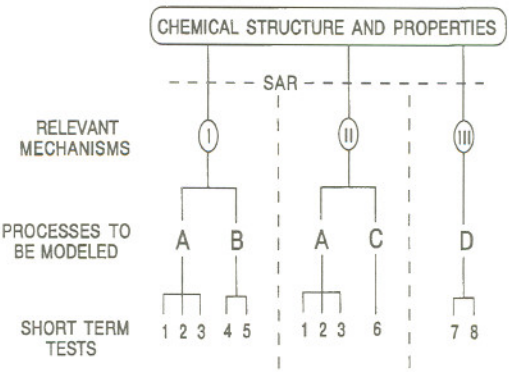

Figure 13.3 A paradigm using the relationships between chemical structure and carcinogenesis to select a short-term test battery for a specific class of chemicals

The complex nature of the carcinogenicity endpoint in relation to the underlying multi-step processes and surrogate short-term tests is illustrated schematically in Figure 13.3. Ideally, a battery of short-term tests (1 to 8) for the evaluation of a given chemical should address the relevant processes (A, B, C, and D) within the array of possible mechanisms (I, II, and III). Several postulated mechanisms for carcinogenesis have been reported (Huberman and Barr, 1985), and more than one may apply depending on the chemical class under consideration. These mechanisms often involve multi-step processes, with DNA damage and repair being important components. Short-term tests attempt directly or indirectly to model the processes of interest. In practice, however, available short-term tests are imperfect predictors of the processes being modelled, and the processes themselves are often neither known nor clearly understood. Hence, major uncertainties are present at every level in Figure 13.3. SAR modelling studies can also be involved at every level (Figure 13.3) in trying to reduce these uncertainties and to establish meaningful correlations and mechanistic hypotheses. Correlative SAR modelling studies that attempt to identify relevant structural features can be performed at the first level using carcinogenicity as the endpoint, and at the last level using the particular bioassay data as the endpoint. At the second level, specific processes thought to be relevant to carcinogenicity, such as DNA-adduct formation, can be modelled at the molecular level for specific chemicals and chemical classes using the tools of theoretical chemistry. At the last level, SAR modelling can also help

elucidate the underlying basis for activity in a particular short-term bioassay. Also at this level, profitable interactions between theoreticians and experimentalists to generate new data for mechanistic hypothesis refinement are feasible. This knowledge, in turn, can be used to define the combination of short-term tests likely to estimate most accurately the chemical processes of concern.

In the absence of information concerning the optimal selection of short-term tests to model the carcinogenicity endpoint, the current strategy must incorporate redundant assays for processes that are imperfectly modelled and independent tests to adequately model each unique process. Judgment concerning which tests are independent or redundant can be made only after a careful evaluation of all data. Finally, the selection of tests should depend on a proven capability of each test to model structural analogues within a chemical class of interest.

The remainder of this monograph will consider in greater detail the composition of available databases, methods to organize and represent such data in terms of profiles, and the sample evaluation of the class of organic halides.

13.5 GENETIC TOXICOLOGY DATABASES: THE GENE-TOX AND NTP DATABASES

Currently, more than 180 test systems or bioassays exist to evaluate agents that induce genetic or related effects, and these test systems may be conveniently organized into a series of phylogenetic endpoint categories (IARC, 1987a; Waters *et al.* 1988a). The number of chemicals for which data exist is greater than 18200, with more than 60000 references in the database at the Environmental Mutagen Information Centre (EMIC) at Oak Ridge National Laboratory (Wassom, 1980).

Three major peer-reviewed genetic toxicology databases are currently under development in the US One is the database on short-term *in vitro* and *in vivo* genetic tests being developed by the National Toxicology Program (NTP). The other two genetic toxicology databases, being assembled by the US Environmental Protection Agency (EPA), are the Gene-Tox database and the Genetic Activity Profile (GAP) described under "Genetic Activity Profiles."

The NTP database uses standardized laboratory protocols and blind testing procedures in selected laboratories. While relatively few short-term tests have been employed by the NTP, the number of chemicals evaluated to date, particularly in the Ames test, is substantial. The NTP database offers advantages of internal consistency and comparability; however, the representation of chemical analogs in the NTP database at its current size is sparse, except for Ames test data. Although the requirements of SAR analysis are not considered by the NTP in chemical selection at the present time, the NTP's databases for Salmonella mutagenicity and the rodent carcinogenicity have been utilized extensively for SAR studies. An advantage of the NTP databases is that each chemical is jointly tested for carcinogenicity and mutagenicity in *Salmonella* and for the presence of "structural alerts" for genotoxicity (Ashby and Tennant, 1988; Ashby *et al.*, 1989). Thus, the

determination of whether the chemicals associated with specific CASE biophores are also mutagens is relatively straightforward.

The largest peer-reviewed and publicly available database has been developed through the EPA Gene-Tox Program (Waters and Auletta, 1981). Gene-Tox data are available for more than 4000 chemicals evaluated in 73 different short-term bioassays. About 330 chemicals have been tested in five or more different kinds of bioassays. The Gene-Tox Program was designed to assess the current status of genetic bioassays rather than to evaluate individual chemicals. However, all chemicals tested in the bioassays under evaluation are represented by overall positive or negative classification for the given chemical bioassay. These classifications are rendered by peer review committees in the process of periodically considering the accumulated data for an individual test system. The qualitative Gene-Tox data have recently been combined with the mutagenicity database of the Registry of Toxic Effects of Chemical Substances (RTECS) that includes information on dose. RTECS is searchable on-line through the US National Library of Medicine's Toxicology Data Network (TOXNET). The mutagenicity data in RTECS have not been peer reviewed, and do not correspond to those data reviewed by Gene-Tox committees.

The Gene-Tox Program also has reviewed the animal cancer bioassays for 506 compounds, and has classified the results qualitatively as follows: 252 as Sufficient Positive; 99 as Limited Positive; 40 as Sufficient Negative; 21 as Limited Negative; one as Equivocal; and 93 as Inadequate (Nesnow *et al.*, 1986). The preponderance of positive data in Gene-Tox for both mutagenicity and carcinogenicity has restricted assessments of test and battery performance largely to issues of sensitivity and positive estimation (Nesnow and Bergman, 1988). Despite these limitations, data from the Gene-Tox Program have been used in conjunction with ADAPT, CASE, and TOPKAT in the SAR assessment of genetic toxicity and potential carcinogenicity (Klopman *et al.*, 1985a; Enslein *et al.*, 1987). These correlative SAR programs have relied heavily on the results of the Ames test that represents by far the largest number of chemicals in the Gene-Tox database.

From the point of view of test battery selection, analyses of the Gene-Tox database have been performed by Rosenkranz *et al.* (1984) and Ray *et al.* (1987). The Gene-Tox report of Ray *et al.* (1987) deals specifically with the identification of specialized batteries of bioassays applicable to specific chemical classes. The report concludes that the small number of chemicals within any chemical class for which more than two or three assays have been performed severely limits definitive conclusions regarding chemical class-specific test batteries. Ideally, if data from short-term test batteries were available for a large number of compounds, the data sets could be used to investigate common or different mechanisms of chemical action among various tests in a battery, and would be more useful for SAR investigations.

13.5.1 GENETIC ACTIVITY PROFILES

To enhance the utility of genetic bioassay data in the evaluation of individual chemicals, a quantitative presentation format has been developed which incorporates Gene-Tox and NTP as well as other published data (Garrett *et al.*, 1984; IARC, 1987a; Waters *et al.*, 1988b). The GAP is a bar graph (Figure 13.4; symbols used: (-) = no exogenous activation; (^) with exogenous activation; majority response (+/-) = a solid vertical line to mean LDU value; conflicting data = dashed vertical line from the origin through all conflicting data points) that displays information on the various genetic and related tests that have been performed using a given chemical, including: (a) the phylogenetic levels of the test systems; (b) the genetic endpoints that the tests represent; and (c) the dose of the chemical that has yielded a positive or negative response in each test. Thus, a profile permits direct visual assessment of the responses of an array of short-term tests applied to a chemical and facilitates a computer-based comparison of genetic activity for chemical selection and SAR model development (Waters *et al.*, 1988c). GAP matching techniques can indicate biological similarities across chemical structural classes or differences within classes which could have mechanistic significance (Garrett *et al.*, 1984, 1986; Waters *et al.*, 1988c). They can also assist in pinpointing differences in genotoxic responses among species.

The current GAP database includes profiles and data listings for approximately 350 agents tested in nearly 200 different genetic bioassays. Many agents represented in the GAP database (IARC, 1987a) have been evaluated in animal cancer tests and in epidemiological studies (ATSDR, 1986; IARC, 1987b). Because of its quantitative component, the GAP database should be useful in quantitative SAR studies. Limitations of the GAP database include size, chemical class representation, and a preponderance of positive data. Fortunately, for purposes of the sample SAR investigation, both the GAP and Gene-Tox databases contain a large proportion of negative results for the organic halides.

13.5.2 PROBLEMS WITH SOME EXISTING DATABASES

Two significant shortcomings of available databases are the lack of both standardization of test protocols and consensus interpretation of the data. Thus, several analyses have been performed comparing the Gene-Tox and US National Toxicology Program data compilations (Klopman *et al.*, 1990; Rosenkranz and Klopman, 1990a; Rosenkranz *et al.*, 1990). In a detailed analysis of the *Salmonella* mutagenicity results, a significant overlap was found between the structural determinants identified in each data set, even though the specific chemicals in these databases did not overlap greatly (Klopman *et al.*, 1990; Rosenkranz and Klopman, 1990a). This finding implies a common mechanistic basis for the activity of the two sets of compounds. However, a comparison of the CASE results derived from the NTP and Gene-Tox databases for sister chromatid exchange as well as chromosomal aberrations revealed very little commonality between the CASE substructures identified in the NTP versus the Gene-Tox database for each assay

(Rosenkranz *et al.*, 1990). Since the chemical class representation within the two databases differs, this result could be a reflection of class-specific mechanisms of activity for these assays. The two databases were developed differently: The NTP data were generated using coded chemicals of known purity, following a rigid experimental protocol and statistical criteria for the consistent interpretation of the results, whereas the Gene-Tox results were abstracted from the published literature and submitted to individual peer panels for their expert judgment and overall assessment.

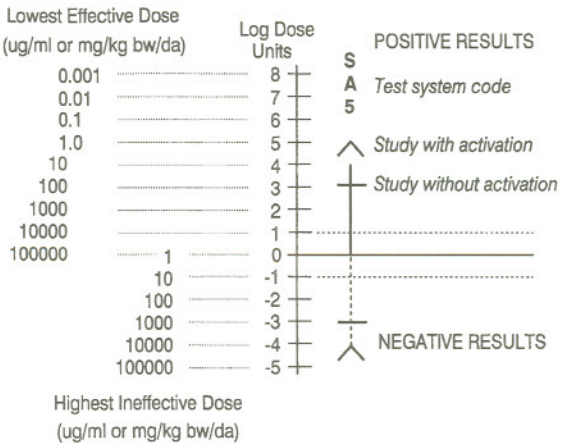

Figure 13.4 Genetic activity profile: LED and HID converted to logarithmic dose units (LDU).

Another problem with these databases relates to the standardization of carcinogenicity results. Thus, the compilation of Gold *et al.* (1984, 1986, 1987) theoretically is well suited for the study of the structural basis of carcinogenicity, since it also provides a measure of carcinogenic potency in the most sensitive species. Many chemicals studied under the aegis of the NTP were included in the compilation of Gold *et al.* (1984, 1986, 1987, 1989). On several occasions, a chemical listed as non-carcinogenic by the NTP was given a potency (TD_{50}) value by Gold *et al.*, and hence was interpreted by CASE as being carcinogenic in the analysis of the latter data. Thus, when the two databases were used independently as training sets for CASE, different predictions resulted. When, however, the Gold *et al.* TD_{50} values were used for the chemicals designated as positive carcinogens by NTP, excellent quantitative correlations with carcinogenic potency were obtained (Nesnow, 1990).

The NTP database is used for most of the present CASE analyses, because both carcinogenicity and short-term test data are available for the class of organic halides under consideration. Generally, however, given the paucity of data available,

agreement on the interpretation of multiple species/sex carcinogenicity data are needed to facilitate not only SAR studies but also the validation of short-term test data performance.

13.6 INTEGRATION OF STRUCTURAL CONCEPTS WITH DATABASE EVALUATION

A plethora of information exists about the genotoxic properties of molecules and, to some extent, their carcinogenicity. Furthermore, some SAR methodologies have limited ability to investigate non-carcinogenicity data sets with respect to the structural basis of mutagenicity, genotoxicity, and carcinogenicity subject to data representation requirements (Klopman *et al.*, 1990; Rosenkranz and Klopman, 1990a, b). The feasibility of integrating some of these techniques is discussed below. To accomplish this, the CASE methodology is used, because it has proven especially effective for comparing the structural basis of various biological endpoints (Klopman *et al.*, 1990; Rosenkranz and Klopman, 1990b). The advantages of the CASE program are augmented by the availability of GAPs from the GAP database and the use of profile-matching techniques.

The first step in the process of integrating these methodologies is database compilation. A qualitative genetic toxicology database on the organic halides, derived from the larger Gene-Tox and GAP databases is used as an illustration. Each organic halide has been evaluated in 10 or more short-term tests; the class has been divided into aryl halides, saturated alkyl halides, and unsaturated alkyl halides. Some compounds span more than one chemical class. Organic halides, that represent a general class of chemicals for which the evaluation of the potential carcinogenicity in terms of short-term test data is particularly difficult, provide a major challenge for the application of various computerized SAR and comparative biological assessment tools. Approximately one-third of these organic halides produce primarily positive responses in short-term tests; about one-half are essentially negative in the Ames *Salmonella* tester strains; and about one-third are negative in most of the other short-term tests. Approximately one-half of these halides are positive in one or more rodent cancer bioassays. Of the remaining one-half, a majority are equivocal for carcinogenicity or have been inadequately evaluated for carcinogenicity. Only a few of these chemicals are considered to be negative for carcinogenicity in animal studies.

13.7 SAR ESTIMATIONS

Using a training set consisting of 254 diverse chemicals derived from the NTP carcinogenicity test results, CASE identified major biophores found in organic halides that are associated significantly with carcinogenicity in rodents (Rosenkranz and Klopman, 1990c). These biophores were used by CASE to estimate the

carcinogenicity of a group of halogenated chemicals that were not included in the original training set (Table 13.1). When the Gold *et al.* (1984, 1986, 1987, 1989) database was used to derive the CASE fragments, the resulting predictions were reasonably different, i.e., the CASE predictions were in agreement for 75% of the chemicals (Table 13.1). Based upon the results of the *Salmonella* mutagenicity assays for the same chemicals, the mutagenicity of these chemicals was also estimated (Table 13.2).

Table 13.1 Some significant biophores associated with the probability of carcinogenicity of halogenated chemicals in rodents

Fragment size = *** 1 2 3 4 5 6 7 8 9 10 SUB	Number	Inactives	Marginals	Actives	Probability
Cl -CH =	4	0	0	4	0.063
Cl -CH_2-	19	5	2	12	0.058
Br -CH-	3	0	0	3	0.125
Br -CH_2-	3	0	0	3	0.125
O -CH_2-CH-	6	0	1	5	0.031
Cl -C^n-Cl	5	1	0	4	0.109
Cl -CH -Cl	3	0	0	3	0.125
Cl -C -C <2-Cl>	9	2	1	6	0.090
C =CH -C =C -	49	11	6	32	0.001
CH =C -C =CH - 54	14	7	33		0.005
CH =CH -C.=C.- 3	0	0	3		0.125
O -PO -O -CH_3 <3-O>	4	0	1	3	0.125
CH =CH -C =C - <3-O>	7	0	0	7	0.008
Cl -C =C -C =CH - <4-Cl>	4	0	0	4	0.063

C. = a carbon atom shared by two rings.
<2-Cl> = a chlorine atom at position 2 from the left.

During these analyses, chemicals were identified (Table 13.3) that are estimated to be carcinogenic in only one species but not in the other. This conclusion follows from the CASE identification of species-specific biophores. Furthermore, for halothane, a chemical may possess a biophore for one species yet may have a biophore for the other.

The accuracy of SAR estimates is very much a function of the quality and composition of the databases employed. Of the 60 organic halides evaluated in one

or more rodent carcinogenicity bioassays, 54 are reported in the NTP, IARC, and Gene-Tox databases, and a "consensus judgment" has been assigned. The 54 organic halides for which carcinogenicity data exist have been listed in order from negative to positive according to the consensus judgment. Although this group is incomplete with respect to the carcinogenicity of the 60 halides, substantial agreement exists for data generated by the NTP and the evaluations of IARC and Gene-Tox. Where adequate positive data exist (i.e., NTP, IARC, or Gene-Tox display classifications of LP, CE, S, or SP consensus judgment), the CASE program accurately estimates the carcinogenicity of organic halides. For those halides where inadequate or limited carcinogenicity data exist and for halides negative for carcinogenicity, CASE predictions are much worse. In fact, when the NTP carcinogenicity data are used to train CASE, several possible carcinogens not adequately represented in the training set (i.e., those labelled LP, CE, S, or SP consensus judgment) are not predicted by CASE. These agents include acridine mustard, benzyl chloride, vinylidene chloride, and pentachlorophenol. The correlation of carcinogenicity based on the consensus judgment with *Salmonella* mutagenicity is very poor for the halides studied, with only 51% concordance.

13.7.1 PROFILE MATCHING

To determine patterns or similarities in genetic activity profiles for a group of chemicals, the data for each possible pair of chemicals was examined by computer over the entire series of tests to find common test results (Figure13.5; j_a (abbreviated a) and j_b (abbreviated b); bioassays 2 and 4 are common to both chemicals; however, results are positive in test 4 for chemical b and negative for chemical a). The comparative evaluation of chemicals used the binomial distribution to determine the probability (p) that concordant results would occur by chance among the common tests in two different profiles. The p value was the primary function for evaluating the significance of a qualitative match between a pair of chemicals. The agreement in the relative magnitudes of common profile lines was determined by calculating the dose-related function (DRF). A DRF was computed for common concordant test results only, and is termed the DRF_c. The p and the DRF_c value may be used as screening tools to obtain matched profiles at preselected levels of significance and quantitative agreement.

Using a p value cutoff of $p \leq 0.05$, two highly statistically significant matches were obtained for the aryl halides. The matches were between the structural analogue pairs 2,4-D and 2,4,5-T and between p,p′-DDD and p,p′-DDE. For the saturated alkyl halides, four matches of the previously stated quality were found. Matches were found for carbon tetrachloride with halothane and with 1,1,2,2-tetrachloroethane, and ethylene dibromide was matched with dibromochloropropane and with epichlorohydrin.

Table 13.2 Estimation of carcinogenicity in rodents and comparison of estimations for two *Salmonella* mutagenicity databases for halogenated compounds

CAS no.	Chemical	C/R	Mutagenicity Overall	Mutagenicity %
00071-55-6	1,1,1-Trichloroethane	+	+	67.0
00079-34-5	1,1,2,2-Tetrachloroethane	+	-	20.0
00096-12-8	1,2-Dibromo-3-chloropropane	+	+	82.9
00106-93-4	1,2-Dibromoethane	+	+	80.0
00107-06-2	1,2-Dichloroethane	+	+	66.0
00078-87-5	1,2-Dichloropropaned	+	+	74.0
01746-01-6	2,3,7,8-TCDD	+	-	7.0
00093-76-5	2,4,5-Trichlorophenoxyacetic	+	-	7.0
00088-06-2	2,4,6-Trichlorophenol	+	-	38.0
00094-75-7	2,4-Dichlorophenoxyacetic acid	+	+	38.0
00151-67-7	2-Bromo-2-chloro-1,1,1-trifluor\	m	+	63.0
00101-14-4	4,4′-Methylenebis(2-chloroben\	+	-	86.7
00051-21-8	5-Fluorouracil	+	-	0
16238-56-5	7-Bromomethyl-12-methylBA	+	+	63.0
24961-39-5	7-BromomethylBA	+	+	63.0
00309-00-2	Aldrin	+	-	7.0
01912-24-9	Atrazine	+	-	0
00100-44-7	Benzyl chloride	-	+	63.0
00314-40-9	Bromacil	+	+	67.0
00075-25-2	Bromoform	+	+	63.0
00133-06-2	Captan	+	m	57.0
00056-23-5	Carbon tetrachloride	+	-	0
00056-75-7	Chloramphenicol	m	m	55.6
00108-90-7	Chlorobenzene	-	-	25.0
00075-45-6	Chlorodifluoromethane	-	-	0
00067-66-4	Chloroform	+	-	36.0
00439-14-5	Diazepam	-	-	36.2
00072-55-9	Dichlorodiphenyldichloroethylene	+	-	38.0
00075-09-2	Dichloromethane	+	+	66.0
00062-73-7	Dichlorvos	+	m	57.0

GAPs showed that the compounds named above are negative in the Ames test, but positive in *Saccharomyces cerevisiae* for gene mutation, mitotic recombination and gene conversion. No highly significant matches were obtained for the unsaturated alkyl halides. However, at lower levels of significance ($p \leq 0.05$), some structurally related compounds (e.g., aldrin, dieldrin, heptachlor) displayed matches. If the stringency of matching criteria are relaxed ($p \leq 0.05$) and the focus is placed on matches based on positive test results, matches from some of the saturated alkyl halides become apparent. According to this criterion, ethylene dibromide matches with dibromochloropropane, epichlorohydrin, benzyl chloride, tris (2,3-dibromo-

Table 13.2 (Continued)

CAS No.	Chemical	C/R	Mutagenicity Overall	Mutagenicity Per Cent
00060-57-1	Dieldrin	m	-	7.0
00072-20-8	Endrin	m	-	7.0
00106-98-8	Epichlorohydrin	+	+	84.7
00074-96-4	Ethyl bromide	+	+	80.0
00133-07-3	Folpet	m	m	57.0
00076-44-8	Heptachlor	+	-	7.0
00146-59-8	ICR 170	-	m	43.5
17070-44-9	ICR 191	-	+	63.6
00054-42-2	Iododeoxyuridine	m	-	0
00058-89-9	Lindane	-	-	0
00094-74-6	MCPA	+	-	38.0
00072-43-5	Methoxychlor	+	-	0
00074-83-9	Methyl bromide	NT	NT	0
00150-66-6	Monuron	+	-	33.3
00303-47-9	Ochratoxin A	+	-	0
00072-55-9	p,p′-DDE	+	-	38.0
00082-68-8	Pentachloronitrobenzene	-	-	13.3
00087-86-5	Pentachlorophenol	-	-	7.0
67774-32-1	Polybrominated biphenyl	+	-	0
00709-98-8	Propanil	+	-	10.1
00072-54-8	Tetrachlorodiphenylethane	+	-	25.6
00127-18-4	Tetrachloroethylene	+	-	30.0
00052-68-6	Trichlorfon	+	-	0
00079-01-6	Trichloroethylene	+	-	0
01582-09-8	Trifuralin	+	+	70.0
00126-72-7	Tris(2,3-dibromopropyl)phosph\	+	+	74.4
00075-01-4	Vinyl chloride	+	-	0
00075-35-4	Vinylidene chloride	-	-	0

C/R = carcinogenicity in rodents

propyl)-phosphate, and dichloromethane. These pair-wise matches can be displayed graphically. Clearly the more structurally complex compounds such as tris(2,3-dibromopropyl)phosphate, that have greater structural diversity than most of the saturated alkyl halides identified as matches, will likely belong in more than one chemical class. The results of profile matching are consistent with the presence in each of these compounds of the CASE *Salmonella* biophore [(*X*)-CH_2-] where *X* = Cl, Br, or I. Where marginal matches (0.05) were included, all compounds with the (*X*)-CH2- biophore were identified. Structural alerts [(*X*)-CH_2- and/or C(*X*)$_4$ where *X* = H, F, Cl, Br, or I in any combination] have also been reported by Ashby *et al.* (1989). A computerized SAR method, a biological comparative assessment approach, and expert opinion lead to similar conclusions in this example.

Table 13.3 Identification of chemicals estimated to be carcinogenic in one species but not in another

CAS no.	Chemical	Carcinogenicity Rat	Mouse
00079-34-5	1,1,2,2-Tetrachloroethane	-	m
00078-87-5	1,2-Dichloropropane	-	+
00151-67-7	2-Bromo-2-chloro-1,1,1-trifluoroethane	-	+
00309-00-2	Aldrin	-	m
00075-25-2	Bromoform	-	+
00133-06-2	Captan	-	m
00056-23-5	Carbon tetrachloride	-	m
00067-66-4	Chloroform	-	+
00072-55-9	Dichlorodiphenyldichloroethylene	-	+
00133-07-3	Folpet	-	m
00076-44-8	Heptachlor	-	m
00094-74-6	MCPA	-	+
00072-55-9	p,p′-DDE	-	+
00127-18-4	Tetrachloroethylene	-	+
00079-01-6	Trichloroethylene	-	+
01582-09-8	Trifuralin	-	m
00075-01-4	Vinyl chloride	-	+
00101-14-4	4,4′-Methylenebis(2-chlorobenzene)	+	-
00051-21-8	5-Fluorouracil	+	-
00106-89-8	Epichlorohydrin	+	-
00150-66-6	Monuron	+	-
00709-98-8	Propanil	+	-

- = negative; + = positive; m = marginal

13.8 WEIGHT-OF-EVIDENCE SCORING SYSTEM

Committee 1 of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) has been involved for several years in the development of a computer-based methodology to assess the evidence from short-term genetic tests that a chemical is a mutagen (Lohman *et al.*, 1990). The evaluative approach selected by ICPEMC Committee 1 is based on a "weighted test" scoring system. Input data for this methodology have been obtained from the GAP database described above. Data are first combined into a test score. The test score is the product of several factors representing dose, the sign of the test result (+ or -), and the use of metabolic activation. This process is repeated for each type of test included in the battery, and the results are combined and weighted over a sequence of data reduction steps. The results from each test (and replicate) are used in generating the overall assessment of the chemical being evaluated. The analysis is continued through the hierarchy of information, agglomerating data from individual tests into results for classes representing similar kinds of tests into

overall *in vitro* or *in vivo* values and then combining these into a final score for the agent.

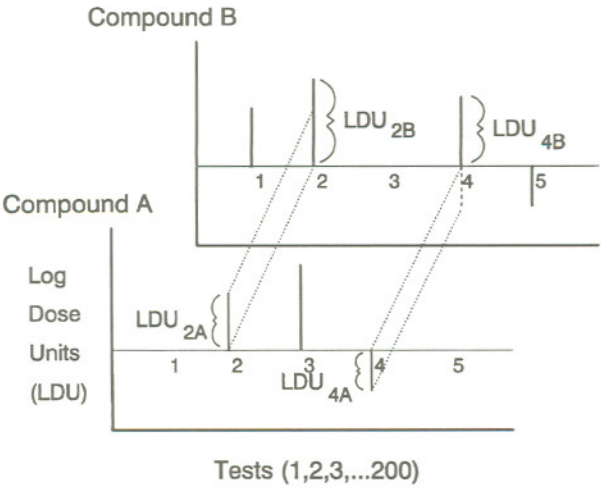

Figure 13.5 Genetic activity spectra for five bioassays of two chemicals

Table 13.4 illustrates the final score from the Committee 1 evaluation compared to the present "consensus judgment" for carcinogenicity. The three compounds found negative in carcinogenicity testing (pentachloronitrobenzene, halothane, and methoxychlor) are at the negative end of the ICPEMC ranking. The ranking clearly differentiates chemicals that display quantitative differences in genotoxicity. The last eight compounds (on the positive end) of the ICPEMC weight-of-evidence ranking (Table 13.4) are detected, and grouped by profile matching techniques. Five compounds (1,2-dichloroethane, dibromo-chloropropane, ethylene dibromide, tris(2,3-dibromopropyl)PO_4, and epichlorohydrin) are considered to have sufficient evidence of carcinogenicity. Also a number of the sufficient positive carcinogens are not detected in genotoxicity assays, and, therefore, display an overall negative score.

ICPEMC Committee 1 is investigating a consensus view with carcinogenicity as the endpoint of choice (Nesnow, 1990). For practical use, the test weights may be subject to adjustment following internal and/or external standardization. For such a standardization, data on long-term carcinogenicity studies in rodents, epidemiological data, or SAR may be used.

In addition to its utility in weight-of-evidence evaluations, the ICPEMC Committee 1 scoring method is intended to serve as a mechanism to evaluate test system consensus for diverse kinds of tests. As illustrated by Figure 13.6, the combined agent score for 34 halogenated organic compounds may be plotted versus the score for the test class. Figure 13.6 shows results for sister-chromatid

exchange, chromosomal aberrations, and cell transformation in mammalian cells *in vitro*. These results indicate that test class scores for sister-chromatid exchange and chromosomal aberrations are most consistent with consensus. Although cell transformation assays are inconsistent with consensus, these assays display a positive class score for so-called non-genotoxic carcinogens such as chloroform and carbon tetrachloride. The *in vivo* test classes that are most consistent with consensus are (a) heritable damage in insects; and (b) chromosomal aberration in mammals (data not shown). With respect to carcinogenicity, *in vivo* mammalian test classes (especially micronuclei and dominant lethal) have yielded negative scores for limited and sufficient carcinogens more frequently than did *in vitro* test classes (data not shown).

An expert system such as CASE can be based as easily on weight-of-evidence or consensus evaluations as any other biological endpoint. In fact, in the present instance such consensus evaluation was found to improve the CASE prediction accuracy. As mentioned earlier, while CASE was only partially successful in performing the QSAR portion of the program on the TD_{50}-based carcinogenicity database assembled by Gold *et al.* (1984, 1986, 1987, 1989), it was very successful in doing so when the TD_{50} values were modified by a weight-of-evidence carcinogen ranking scheme (Nesnow, 1990). Furthermore, CASE was also readily able to learn the human intelligence-based rules for identifying "structural alerts" of genotoxicity. Thus, the databases compiled using human intelligence/intuition weight-of-evidence appeared to provide a valuable resource and adjunct to purely computer-driven algorithms.

13.9 SUMMARY AND DISCUSSION

This chapter has discussed several general issues relating to the application of computational SAR methods, contrasted a few particular correlative SAR methods, considered general problems relating to indirect or direct SAR modelling of the carcinogenicity endpoint, and discussed database considerations. The role of SAR and biologically based techniques in chemical classification and test selection, and a weight-of-evidence scoring scheme have also been discussed.

A database on 60 organic halides, a general class considered difficult to test and evaluate for carcinogenicity, served as a vehicle for discussion of specific methods. Organic halides have frequently proven to be carcinogenic in animal studies. However, no existing mechanistic model adequately accounts for this activity, and several compounds are considered to be non-genotoxic carcinogens.

The so-called "genotoxic" and "non-genotoxic" carcinogens appear to fall into two groups: "genotoxic" carcinogens generally cause cancers in multiple species, in both sexes and at multiple sites, while "non-genotoxic" carcinogens appear to be greatly restricted in their specificity (Ashby and Tennant, 1988; Gold *et al.*, 1989). Additionally, "genotoxic" carcinogens generally display an enhanced carcinogenic potency (Rosenkranz and Ennever, 1990) and a decreased lipophilicity. The vast

majority of recognized human carcinogens are genotoxicants (Ennever *et al.*, 1987; Shelby, 1988; Bartsch and Malaveille, 1989), while presumed human non-carcinogens appear to be non-genotoxic (Ennever *et al.*, 1987).

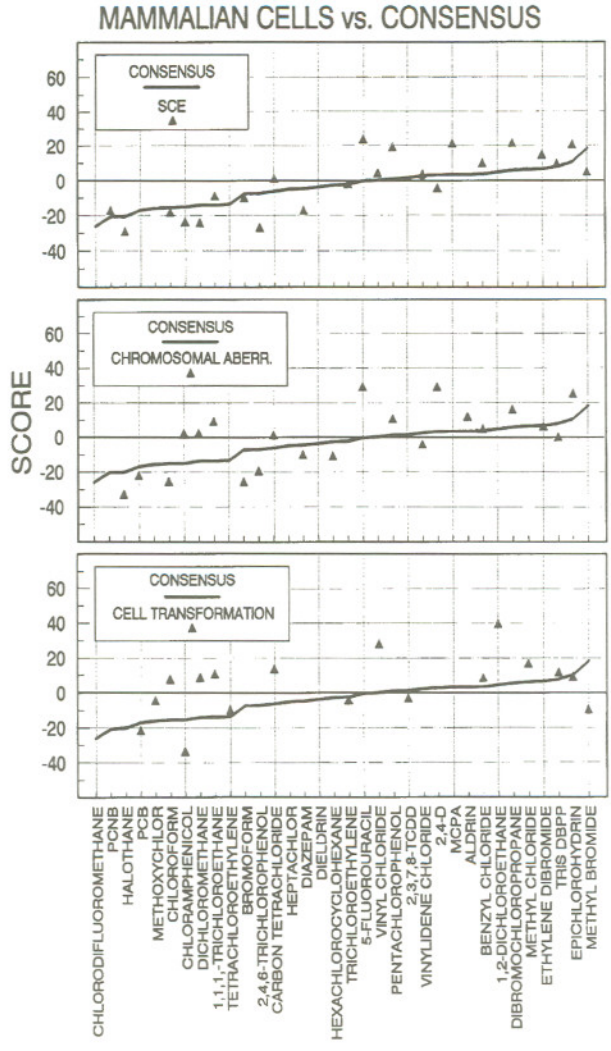

Figure 13.6 Scores for primary DNA damage and overall consensus judgment using ICPEMC Committee 1 approach

The genotoxicity of many of the halogenated organic compounds considered is undetected in the Ames test. Furthermore, poor correspondence exists between the

results of the Ames test when compared with the "consensus judgment" for carcinogenicity. The CASE programme was able to estimate accurately the carcinogenicity of many organic halides. Where only inadequate or limited data were available and for halides the data were negative for carcinogenicity, CASE estimates were less accurate. Even when NTP carcinogenicity data were used to train CASE, several agents labelled by consensus judgment as "limited positive," "clear evidence," "sufficient positive" were not estimated by CASE to be carcinogenic. Perhaps part of the explanation for the dichotomy in the predicted versus the actual carcinogenicity response lies in the dose necessary to cause a response in a short-term test versus that required to produce cancer in a rodent: The dose required for carcinogenicity is frequently 100 times that necessary to produce a response in a short-term test. Data presented in the GAP format for ethylene dibromide (Figure 13.7) illustrate that even among short-term tests, the doses needed to produce various responses range over several orders of magnitude. Thus, the mere presence of a chemical fragment or substituent may be insufficient to model chemical reactivity in a particular reaction mechanism that is important for carcinogenicity. Additional investigation is needed to determine the quantitative relationships between chemical structure, reactivity, and carcinogenicity for this chemical class.

The ICPEMC Committee 1 weight-of-evidence scheme may be useful to assess quantitative genotoxicity data and their relationship to carcinogenicity. In the enclosed illustration, the scheme was successful at separating many of the positive genotoxic carcinogens from known putative non-carcinogens. The scheme could not, however, deal effectively with the non-genotoxic carcinogens. Evidence presented elsewhere (McCoy *et al.*, 1990; Rosenkranz and Klopman, 1990b) suggests that CASE may be able to recognize structural fragments associated with some non-genotoxic carcinogens.

Prospectively, a useful approach may involve the application of biologically based comparative techniques (profile matching) and weight-of-evidence schemes for the subsetting of chemicals within a large quantitative genetic toxicology database. In this way, correlative SAR methods may be more effectively applied to identify the substructural elements responsible for particular biological responses and to suggest biologically plausible mechanisms of action. Clearly, additional investigation along the lines discussed in this chapter is necessary to further the utility of the various computerized techniques that may be applicable in the study of SAR across species.

Table 13.4 Carcinogenicity of 34 halides ranked using the ICPEMC Committee 1 approach

Compound	Carcinogenicity evaluation	ICPEMC score
Chlorodifluoromethane	L	-26.05
Pentachloronitrobenzene	LN	-20.45
Halothane	LN	-20.21
Polychlorinated biphenyls	S	-16.88
Methoxychlor	SN	-15.71
Chloroform	S	-15.28
Chloramphenicol	L	-15.11
Dichloromethane	S	-13.85
1,1,1-Trichloroethane	I	-13.81
Tetrachloroethylene	S	-13.48
Bromoform	CE	-7.29
2,4,6-Trichlorophenol	S	-7.22
Carbon tetrachloride	S	-6.18
Heptachlor	LP	-5.06
Diazepam	I	-4.68
Dieldrin	L	-3.67
Hexachlorocyclohexane	LP	-2.70
Trichloroethylene	LP	-2.34
5-Fluorouracil	I	-0.26
Vinyl chloride	S	0.20
Pentachlorophenol	CE	1.09
2,3,7,8-TCDD	S	1.38
Vinylidene chloride	LP	2.59
2,4-D	L	3.14
MCPA	L	3.29
Aldrin	LP	3.34
Benzyl chloride	LP	3.58
1,2-Dichloroethane	S	4.58
Dibromochloropropane	S	5.71
Methyl chloride	I	6.37
Ethylene dibromide	S	6.60
Tris(2,3-dibromopropyl)PO_4	S	7.77
Epichlorophydrin	S	10.60
Methyl bromide	L	18.33

CE = clear evidence of carcinogenicity; I = inadequate evidence of carcinogenicity; L = limited evidence of carcinogenicity; LN = limited negative;

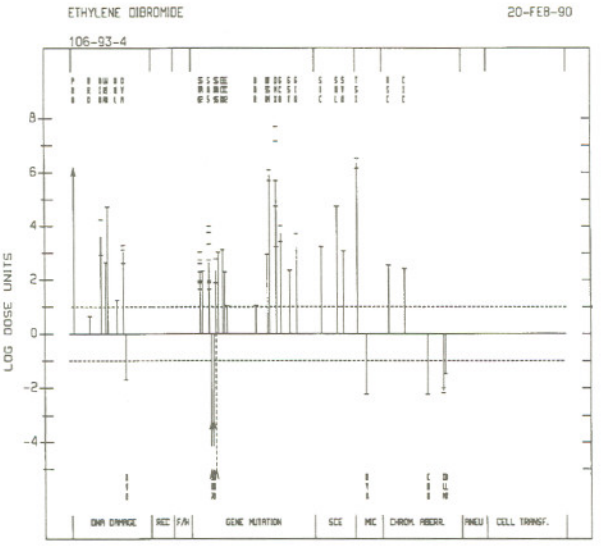

Figure 13.7 GAP of ethylene dibromide

13.10 REFERENCES

Ashby, J. and Tennant, R.W. (1988) Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutat. Res.* **204**, 17–115.

Ashby, J., Tennant, R.W., Zeiger, E., and Stasiewicz, S. (1989) Classification according to chemical structure, mutagenicity to *Salmonella* and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the US National Toxicology Program. *Mutat. Res.* **223**, 73–103.

ATSDR (1988) Toxicological Profile Series, Agency for Toxic Substances and Disease Registry, US Public Health Service, Atlanta, Ga.

Bartsch, H. and Malaveille, C. (1989) Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series. *Cell Biol. Toxicol.* **5**, 115–127.

Dunn III, W.J. and Wold, S. (1980) Relationships between chemical structure and biological activity modeled by SIMCA pattern recognition. *Bioorganic Chem.* **9**, 505–523.

Dunn III, W.J. and Wold, S. (1981) The carcinogenicity of *N*-nitroso compounds: A SIMCA pattern recognition study. *Bioorganic Chem.* **10**, 29–45.

Ennever, F.K., Noonan, T.J., and Rosenkranz, H.S. (1987) The predictivity of animal bioassays and short-term genotoxicity tests for carcinogenicity and non-carcinogenicity to humans. *Mutagenesis* **2**, 73–78.

Enslein, K. and Craig, P.N. (1982) Carcinogenesis: A predictive structure–activity model. *J. Toxicol. Environ. Health* **10**, 521–530.

Enslein, K., Lander, T.R., Tomb, M.E., and Landis, W.G. (1983) Mutagenicity (Ames): A

structure–activity model. *Teratog. Carcinog. Mutagen.* **3**, 503–514.

Enslein, K., Borgstedt, H.H., Tomb, M.E., Blake, B.W., and Hart, J.B. (1987) A structure–activity prediction model of carcinogenicity based on NCI/NTP assays and food additives. *Toxicol. Industr. Health* **3**, 267–287.

Frierson, M.R., Klopman, G., and Rosenkranz, H.S. (1986) Structure–activity relationships (SARs) among mutagens and carcinogens: A review. *Environ. Mutagen.* **8**, 283–327.

Garrett, N.E., Stack, H.F., Gross, M.R., and Waters, M.D. (1984) An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. *Mutat. Res.* **134**, 89–111.

Garrett, N.E., Stack, H.F., and Waters, M.D. (1986) Evaluation of the genetic activity profiles of 65 pesticides. *Mutat. Res.* **168**, 301–325.

Golberg, L. (Ed) (1983) *Structure–Activity Correlation as a Predictive Tool in Toxicology: Fundamentals, Methods, and Applications*. Hemisphere Publ., Washington, DC.

Gold, L.S., Sawyer, C.B., Magaw, R., Backman, G.M., de Veciana, M., Levinson, R., Hooper, N.K., Havender, W.R., Bernstein, L., Peto, R., Pike, M.C., and Ames, B.N. (1984) A carcinogenic potency database of the standardized results of animal bioassays. *Environ. Health Perspect.* **58**, 9–319.

Gold, L.S., de Veciana, M., Backman, G.M., Magaw, R., Lopipero, P., Smith, M., Blumenthal, M., Levinson, R., Bernstein, L., and Ames, B.N. (1986) Chronological supplement to the Carcinogenic Potency Database: Standardized results of animal bioassays published through December 1982. *Environ. Health Perspect.* **67**, 161–200.

Gold, L.S., Slone, T.H., Backman, G.M., Magaw, R., Da Costa, M., Lopipero, P., Blumenthal, M., and Ames, B.N. (1987) Second chronological supplement to the carcinogenic potency database: Standardized results of animal bioassays published through December 1984 and by the National Toxicology Program through May 1986. *Environ. Health Perspect.* **74**, 237–329.

Gold, L.S., Bernstein, L., Magaw, R., and Slone, T.H. (1989) Interspecies extrapolation in carcinogenesis: Prediction between rats and mice. *Environ. Health Perspect.* **81**, 211–219.

Huberman, E. and Barr, S.H. (1985) *Carcinogenesis—A Comprehensive Survey: The Role of Chemicals and Radiation in the Etiology of Cancer*. Raven Press, New York, 538 pp.

IARC (1987a) IARC Monographs on the evaluation of carcinogenic risks to humans: *Genetic and Related Effects*: An update of selected IARC Monographs from Volumes 1 to 42, Supplement 6, International Agency for Research on Cancer, Lyon, France.

IARC (1987b) IARC Monographs on the evaluation of carcinogenic risks to humans: *Carcinogenicity*: An update of selected IARC Monographs from Volumes 1 to 42, Supplement 7, International Agency on Cancer, Lyon, France.

Jurs, P.C., Hasan, M.N., Henry, D.R., Stouch, T.R., and Whalen-Pedersen, E.K. (1983) Computer-assisted studies of molecular structure and carcinogenic activity. *Fund. Appl. Toxicol.* **3**, 343–349.

Jurs, P.C., Stouch, T.R., Czerwinski, M., and Narvaez, J.N. (1985) Computer-assisted studies of molecular structure–biological activity relationships. *J. Chem. Informat. Comput. Sci.* **25**, 296–308

Klopman, G. (1984) Artificial intelligence approach to structure–activity studies. Computer automated structure evaluation of biological activity of organic molecules. *J. Am. Chem. Soc.* **106**, 7315–7321.

Klopman, G. (1985) Predicting toxicity through a computer automated structure evaluation program. *Environ. Health Perspect.* **61**, 269–274.

Klopman, G., and Macina, O.T. (1987) Computer automated structure evaluation of antileukemic 9-anilinoacridines. *Mol. Pharmacol.* **31**, 457–476.

Klopman, G., Contreras, R., Rosenkranz, H.R., and Waters, M.D. (1985a) Structure–genotoxic activity relationships of pesticides: Comparison of the results from several short-term assays. *Mutat. Res.* **147**, 343–356.

Klopman, G., Frierson, M.R., and Rosenkranz, H.S. (1985b) Computer analysis of toxicological databases: Mutagenicity of aromatic amines in *Salmonella* tester strains. *Environ. Mutagen.* **7**, 625–644.

Klopman, G., Kalos, A.N., and Rosenkranz, H.S. (1987a) An artifical intelligence study of the structure–mutagenicity relationships of non-fused-ring nitroarenes and related compounds. *Mol. Toxicol.* **1**, 61–81.

Klopman, G., Macina, O.T., Levinson, M.E., and Rosenkranz, H.S. (1987b) Computer automated structure evaluation of quinolone antibacterials. *Antimicrob. Agents Chemotherapy* **31**, 1831–1840.

Klopman, G., Frierson, M.R., and Rosenkranz, H.S. (1990) The structural basis of the mutagenicity of chemicals in *Salmonella typhimurium*: The Gene-Tox database. *Mutat. Res.* **228**, 1–50.

Lohman, P.H.M., Mendelsohn, M.L., Moore II, D.H., Waters, M.D., and Brusick, D.J. (1990) The assembly and analysis of short-term genotoxicity test data—An ICPEMC Committee 1 working paper. In: Mendelsohn, M.L. and Albertini, R.J. (Eds) *Mutation and the Environment*, pp. 283-294. Wiley-Liss, New York.

McCoy, G.D., Rosenkrantz, H.S., and Klopman, G. (1990) Non-mutagenic carcinogens are primarily hydrophobic. *Carcinogenesis* **11**, 1111–1117.

McKinney, J.D. (Ed) (1985) Monograph on structure–activity correlation in mechanism studies and predictive toxicology. *Environ. Health Perspect.* **61**, 1–349.

Nesnow, S. (1990) International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper 1/2. A multi-factor ranking scheme for comparing the carcinogenic activity of chemicals. *Mutat. Res.* **239**, 83–115.

Nesnow, S. and Bergman, H. (1988) An analysis of the Gene-Tox carcinogen database. *Mutat. Res.* **205**, 237–253.

Nesnow, S., Argus, M., Bergman, H., Chu, K., Frith, C., Helmes, T., McGaughy, R., Ray, V., Slaga, A.T.J., Tennant, R., and Weisburger, E. (1986) Chemical carcinogens. A review and analysis of the literature of selected chemicals and the establishment of the Gene-Tox carcinogen database. *Mutat. Res.* **185**, 1–195.

Randic, M. (1975) On characterization of molecular branching. *J. Am. Chem. Soc.* **97**, 6609–6615.

Ray, V.A., Kier, L.D., Kannan, K.L., Haas, R.T., Auletta, A.E., Wassom, J.S., Nesnow, S., and Waters, M.D. (1987) An approach to identifying specialized batteries of bioassays for specific classes of chemicals: Class analysis using mutagenicity and carcinogenicity relationships and phylogenetic concordance and discordance patterns. 1. Composition and analysis of the overall database. *Mutat. Res.* **185**, 197–241.

Rosenkranz, H.S. and Ennever, F.K. (1990) An association between mutagenicity and carcinogenic potency. *Mutat. Res.* **244**, 61–65.

Rosenkranz, H.S. and Klopman, G. (1989) Structural basis of the mutagenicity of phenylazoaniline dyes. *Mutat. Res.* **221**, 217–239.

Rosenkranz, H.S. and Klopman, G. (1990a) The structural basis of the mutagenicity of chemicals in *Salmonella typhimurium*: The National Toxicology Program database. *Mutat. Res.* **228**, 51–80.

Rosenkranz, H.S. and Klopman, G. (1990b) Structural basis of carcinogenicity in rodents of genotoxicants and non-genotoxicants. *Mutat. Res.* **228**, 105–124.

Rosenkranz, H.S. and Klopman, G. (1990c) Natural pesticides present in edible plants are predicted to be carcinogenic. *Carcinogenesis* **11**, 349–353.

Rosenkranz, H.S., Klopman, G., Chankong, V., Pet-Edwards, J., and Haimes, Y.Y. (1984) Prediction of environmental carcinogens: A strategy for the mid-1980s. *Environ. Mutagen.* **6**, 231–258.

Rosenkranz, H.S., Ennever, F.K., Dimayuga, M., and Klopman, G. (1990) Significant differences in the structural basis of the induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. *Environ. Mol. Mutagen.* **16**, 149–177.

Shelby, M.D. (1988) The genetic toxicity of human carcinogens and its implications. *Mutat. Res.* **204**, 3–15.

Stouch, T.R. and Jurs, P.C. (1985) Computer-assisted studies of molecular structure and genotoxic activity by pattern recognition techniques. *Environ. Health Perspect.* **61**, 329–343.

Topliss, J.G. and Edwards, R.P. (1979) Chance factors in studies of quantitative structure–activity relationships. *J. Med. Chem.* **22**, 1238–1244.

Wassom, J.S. (1980) The storage and retrieval of chemical mutagenesis information. In: Marija Alacevic (Ed), *Progress in Environmental Mutagenesis*, pp. 313-330. Elsevier, Amsterdam.

Waters, M.D. and Auletta, A. (1981) The GENE-TOX program: Genetic activity evaluation. *J. Chem. Informat. Comput. Sci.* **21**, 35–38.

Waters, M.D., Bergman, H.B., and Nesnow, S. (1988a) The genetic toxicology of Gene-Tox non-carcinogens. *Mutat. Res.* **205**, 139–182.

Waters, M.D., Stack, H.F., Brady, A.L., Lohman, P.H.M., Haroun, L., and Vainio, H. (1988b) Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. *Mutat. Res.* **205**, 295–312.

Waters, M.D., Stack, H.F., Rabinowitz, J.R., and Garrett, N.E. (1988c) Genetic activity profiles and pattern recognition in test battery selection. *Mutat. Res.* **205**, 119–138.

Wold, S. and Sjostrom, M. (1977) SIMCA: A method for analyzing chemical data in terms of similarity and analogy, chemometrics: Theory and application. *ACS Symposium Series* **52**, 243–282.