Structure−Activity Relationship Studies of Chemical Mutagens and Carcinogens: Mechanistic Investigations and Prediction Approaches

Romualdo Benigni*

Istituto Superiore di Sanita', Experimental and Computational Carcinogenesis, Department of Environment and Primary Prevention, Viale Regina Elena 299-00161 Rome, Italy

Received June 9, 2004

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* To whom correspondence should be addressed. E-mail: rbenigni@iss.it.

1. Introduction

Carcinogenicity and mutagenicity are among the toxicological endpoints that pose the highest concern. The environment—in its broadest sense, as opposed to genetics—is the major determinant of cancer, and it is responsible for up to 80% of tumor cases.[1,2] Environment includes lifestyle, diet, smoking, pollution, workplace exposures, etc.; an essential component of all this is the chemicals. Society in general, and the discipline of toxicology in particular, face the parallel tasks of performing safety evaluations for the uses of new chemicals before human exposure is permitted and assessing the potential hazards posed by exposure to chemicals that lack safety evaluations. In addition, the accelerated pace of chemical discovery and synthesis has heightened the need for efficient prioritization and toxicity screening methods. Whereas the mutagenic potential of chemicals can be assessed with relatively simple test methods, the standard bioassay in rodents used to assess the carcinogenic potential of chemicals is extremely long and costly and requires the sacrifice of large numbers of animals. For these reasons, chemical carcinogenicity has been the target of numerous attempts to create alternative predictive models, ranging from short-term biological assays (e.g., the mutagenicity tests themselves) to theoretical models. Among the theoretical models, the application of the science of structure−activity relationships (SARs) has earned special prominence since it permits the exploitation of the huge wealth of existing chemical knowledge in understanding the interactions between chemicals and living organisms. The use of SAR concepts obviously applies also to the rationalization and prediction of the mutagenic properties of chemicals.

Principles and concepts of chemical induction of mutations and cancer are covered by a large body of literature; a brilliant example is represented by the series on *Chemical Induction of Cancer* (see, for example, ref 3). A short and clear introduction to the subject is discussed earlier in ref 4.

From the point of view of the mechanism of action, the carcinogens can be classified into (a) genotoxic carcinogens, which cause damage directly to DNA.

10.1021/cr030049y CCC: $53.50
Published on Web 03/15/2005

Romualdo Benigni received his education in chemistry at the University of Rome "La Sapienza". He then joined the Istituto Superiore di Sanita' (Italian National Institute of Health) where he got a permanent position in 1977 and where he remained except for two sabbaticals, at the New York University in 1988 and at the Jawaharlal Nehru University in New Delhi in 2000. He worked experimentally in the field of molecular biology and environmental chemical mutagenesis. In the 1980s, he turned his attention to the statistical analysis and modeling of toxicological data and to the study of the relationships between the structure of organic compounds and their toxicological properties (mainly mutagenesis and carcinogenesis). Dr. Benigni has published over 100 journal articles and book chapters, applying a wide variety of quantitative analysis techniques, including QSAR, to the examination of chemical toxicity information.

Many known mutagens are in this category, and often mutation is one of the first steps in the development of cancer;[5] and (b) epigenetic carcinogens, which do not bind covalently to DNA, do not directly cause DNA damage, and are usually negative in the standard mutagenicity assays.[6] Whereas the epigenetic carcinogens act through a large variety of different and specific mechanisms, the genotoxic carcinogens have the unifying feature that they are either electrophiles per se or can be activated to electrophilic reactive intermediates. The activation can be chemically, photochemically, or metabolically mediated.[7] This idea, as well as the observation of many different cases, has led to the identification of several chemical functional groups and substructures (structural alerts, SAs) for genotoxic carcinogens. Well-known SAs are the carbonium ions (alkyl-, aryl-, benzylic-), nitrenium ions, epoxides and oxonium ions, aldehydes, polarized double bonds (α,β-unsaturated carbonyls or carboxylates), peroxides, free radicals, and acylating intermediates.[8,9] On the contrary, the recognition of SAs for the nongenotoxic carcinogens is far behind, basically because no unifying theory provides scientific support.

Together with the recognition of the SAs, much evidence has pointed to a number of factors that are critical for the structure−activity analysis of chemical carcinogens.[4] Among the physicochemical factors, there are (1) molecular weight (MW), chemicals with very high MW and size have little chance of being absorbed in significant amounts; (2) physical state, which influences the capability of the compounds to reach critical targets; (3) solubility: in general highly hydrophilic compounds are poorly absorbed and, if absorbed, are readily excreted; (4) chemical reactivity: compounds that are "too reactive" may not be carcinogenic because they hydrolyze or polymerize spontaneously or react with noncritical cellular constituents before they can reach critical targets in cells. Another critical factor is the geometry of the chemical compounds: many potent carcinogens and mutagens [e.g., polycyclic aromatic hydrocarbons (PAHs), aflatoxin B1, etc.] are planar molecules, with an electrophilic functional group and favorable size, so that they can intercalate properly into DNA. One other critical factor is metabolism. Metabolism can both activate and detoxify chemical carcinogens: substitutions at critical molecular regions can influence one metabolic pathway at the expense of others. Thus, knowledge of the metabolic pathway of a chemical can substantially enhance the accuracy of structure−activity analyses.

The recognition of SAs and the critical structural factors has been a very important scientific advancement since it has contributed to the design of safer chemicals[10] and to the assessment of the toxic potential of chemicals devoid of appropriate toxicological data.[4,9]

Obviously, the final toxicological effect of a certain SA in a molecule depends heavily on the general structure/properties of the molecule, both in terms of potency and of yes/no activity. The recognition of SAs has a strong statistical value as an indicator of the propensity for a given chemical to be a mutagen and/or a carcinogen; however, a more efficient assessment of the toxicological properties of the chemicals requires the more powerful tools provided by the quantitative structure−activity relationships (QSARs) methods. Thus, a very specialized section of the structure−activity analyses on chemical mutagens and carcinogens has been developed through the use of concepts from the QSAR science and technology.

QSAR is a widely developed branch of chemical research. Its foundation came about 40 years ago when Hansch found the way to bring together two areas of science that had seemed far apart for many years: physical organic chemistry and the study of chemical ↔ life interaction. A cornerstone of physical organic chemistry is the Hammett equation:

$$\log k = \rho\sigma + \text{constant}$$

The Hammett equation models the reaction mechanisms of organic chemicals: k is a rate or equilibrium constant; ρ is a measure of the sensitivity of the reaction to substituents changes; σ is a parameter characteristic of each chemical. The Hammett equation was subsequently extended by Taft who also considered steric factors. Next, Hansch showed that this type of model can also be used for chemicobiological reactions by introducing a hydrophobic parameter:

$$\log k = f(\text{electronic, steric, hydrophobic})$$

This approach was developed from, and meant to be applied to, sets of congeneric chemicals, i.e., chemicals structurally similar, and acting through the same mechanism of action (better, the same rate-limiting step): for optimal applications, QSAR analysis requires a very clear definition of the applicability domain of the model (i.e., the type of chemicals to which it applies). Finally, the model is derived from

the statistical analysis of a (training) set of chemicals; thus its character is fundamentally empirical. This model worked for an enormous number of biological problems, and its success is demonstrated clearly by its widespread diffusion. In the years after the 1960s, the need to solve new problems, together with the contributions of many other investigators, generated thousands of variations of the Hansch approach, as well as approaches that are formally completely new. However, the QSAR science still maintains a fundamental unity, founded on the systematic use of mathematical models and on the multivariate point of view. At present, the QSAR science is one of the basic tools of modern drug and pesticide design and has an increasing role in environmental sciences.[11−15]

The strength of QSAR applications to toxicology derives from the fact that it contributes to the identification of the molecular determinants of the biological action of the chemicals and provides mathematical models to predict the activity of chemicals not tested experimentally, provided that information on similar chemicals, acting with the same mechanisms, is available.

This review paper focuses first on QSAR applications; however, because of the contribution that more qualitative approaches have given, especially to the field of risk assessment, this area of research also will be considered. Thus, the next section (Section 2) presents the rigorous applications of QSAR concepts to individual chemical series of mutagens and carcinogens. All these are characterized by the genotoxic mechanism of action. Section 3 reports on the less populated area of nongenotoxic carcinogens. For these chemicals, the QSAR applications are quite scarce. Section 4 focuses on a wide series of applications of a very different nature. Some derive from QSAR itself, other are very qualitative in nature; their practical implementations are extremely different as well. What they have in common is that they are all aimed at dealing with large samples of chemicals with different structures and action mechanisms (noncongeneric chemicals). These are the methods most commonly used for risk assessment.

2. QSARs for Congeneric Series of Chemical Mutagens and Carcinogens

The QSAR approach, mainly in its original Hansch formulation, has been applied to numerous congeneric classes of mutagens and carcinogens. A number of reviews have already appeared on this subject.[11,16−20]

One factor that has sometimes discouraged investigators from applying QSAR analysis to chemical carcinogenesis has been the idea that this biological process is too complicated and involves too many steps to be successfully modeled. The complexity of the situation has been thoroughly discussed in the case of skin carcinogenicity induced by PAHs.[21] In the first step, the carcinogen must move through the skin (either through or around the cells) and into the cell where it is to be activated by the P-450 metabolic enzymes. This step is likely to be highly dependent on hydrophobicity, with little dependence on electronic or steric properties. In the following step, binding to P-450 is again dependent on hydrophobic, and possibly steric factors. The next steps, involving oxidation of the P-450-bound carcinogen, are expected to be sensitive to the carcinogen's electronic and steric factors. After activation, the product must move within the cell, or into a nearby cell, to the DNA: this step will depend on hydrophobic and steric factors since the electronic characteristics of the diol epoxide will determine the lifetime of this intermediate and its tendency to undergo deactivating side reactions before reaching the DNA. In the generation of reaction products with DNA, all three physicochemical factors are likely to be important. Of course, reaction of the carcinogen with DNA is far from being the last step in the carcinogenesis problem; there is the potential for the organism to repair the DNA damage to be taken into account. Moreover, the "promotion" by other chemicals (in which pre-neoplastic cells are expressed into individual tumor cells) and "progression" (involving progress to malignancy by hystopathologic criteria) have to be considered.

Thus, many steps are likely to occur, with different weights for the hydrophobic, electronic, and steric factors. However, as it will be clear from the following sections, a large number of successful QSAR analyses of chemical mutagens and carcinogens have been generated during the past 40 years of QSAR research. For a final QSAR of some value to emerge, one must assume that only one step is rate limiting for all the congeners under consideration, or that the different weights of the physicochemical factors are linearly combined. It must be assumed also that all congeners are acting via the same mechanism. At present, this is largely unknowable and is usually dealt with by discarding very poorly fit congeners.[21] Fortunately, it appears that the above assumptions are often realistic and that QSAR analysis is able to model the rate limiting step(s) of mutations and cancer induced by various chemical classes, thus providing models that are both mechanistically valid and useful for making predictions.

The following sections will summarize QSAR analyses on individual classes of chemical mutagens and carcinogens. A crucial issue that affects the quality of the results is the quality of the experimental data. This also includes the nature of the biological endpoint measured. For example, the number of existing mutagenicity tests is quite wide, but only a restricted number of them have been recognized as being validated enough. Moreover, the measures obtained from the mutagenicity tests can be both quantitative (potency of the mutagenic chemicals) and qualitative (mutagenic versus nonmutagenic chemicals). Even more complicated is the situation with carcinogenicity, in which different endpoints can be measured: overall yes/no response, response of the individual animal experimental groups, carcinogenic potency (e.g., TD_{50}), profile of tumors induced, etc. For a discussion of this point, see ref 22. A related issue is that of the availability of data from public sources (including existing websites) and how this influences the practical feasibility of QSAR modeling. This is discussed in detail in refs 23 and 24.

2.1. Aromatic Amines

Aromatic amines represent one of the most important classes of industrial and environmental chemicals. They have a wide variety of uses in many industries, i.e., the manufacture of polymers, rubber, agricultural chemicals, dyes and pigments, pharmaceuticals, and photographic chemicals. Many aromatic amines have been reported to be powerful carcinogens and mutagens and/or hemotoxicants. Exposure to aromatic amines occurs in different industrial and agricultural activities as well as in tobacco smoking. Moreover, several types of aromatic amines are generated during cooking.[25−29]

The aromatic amines have to be metabolized to reactive electrophiles to exert their carcinogenic potential. For aromatic amines and amides, this typically involves an initial N-oxidation to *N*-hydroxy arylamines and *N*-hydroxyarylamides, which in rat liver is mediated primarily by cytochrome P-450 isozyme *c* (BNF-B) and *d* (ISF-G).[30,31] Moreover, hydroxylamino, nitro, and nitroso groups are able to generate amine groups (due to metabolic interconversion).

The initial activation of nitroaromatic hydrocarbons is likewise through the formation of an *N*-hydroxyarylamine, a reduction catalyzed by both microsomal and cytosolic enzymes.[25,31] Microsomal nitroreduction too appears to depend on cytochrome P-450 complex, in particular rat liver isozymes *c*, *d*, *b* (PB-B) and *e* (PB-D). Cytosolic nitroreductase activity is associated with a number of enzymes, including DT-diaphorase, xanthine oxidase, aldehyde oxidase, and alcohol dehydrogenase.[31] In addition to the reactions of nitrogen oxidation and reduction (main activation pathways), certain aromatic amines and nitroaromatic hydrocarbons are converted into electrophilic derivatives through ring-oxidation pathways. *N*-Hydroxyarylamines, iminoquinones, and epoxide derivatives are directly electrophilic metabolites, while *N*-hydroxy arylamides require esterification before becoming capable of reacting with DNA.[32]

2.1.1. QSARs for the Mutagenic Activity of the Aromatic Amines

Because of their environmental and industrial importance, the aromatic amines are the single chemical class most studied for their ability to induce mutations and cancer. For example, in a database of experimental carcinogenicity results collected in our laboratory, about one-fourth (200 out of 800) of the chemicals were aromatic amines. Because of the shortcomings of the rodent carcinogenicity bioassay (long times, high price, sacrifice of large numbers of animals), the number of studies in short-term mutagenicity assay, notably, the *Salmonella typhimurium* (Ames test) bacterial assay, is far higher than that in the rodent carcinogenicity bioassay. This assay is also used as prescreen for rodent carcinogenicity.[33−35]

The large database of mutagenicity results for the aromatic amines has been studied with QSAR approaches by several authors. Major reviews have appeared on this topic.[36−38]

Trieff et al.[39] studied the *S. typhimurium* mutagenicity of 19 aromatic amines tested in the strains TA98 (frame shift mutations) and TA100 (base pair mutations), with the addition of S9 metabolizing fraction from Aroclor 1254-induced rat liver. The QSAR models for the two strains were

$$\log \text{BR-TA98} = -1.639(\pm 0.399) + 0.816(\pm 0.127)\log P - 0.752(\pm 0.174)I_1 + 0.377(\pm 0.174)I_2 \quad (1)$$
$$s = 0.78,\ n = 19,\ r^2 = 0.78$$

$$\log \text{BR-TA100} = -1.559(\pm 0.282) + 0.784(\pm 0.090)\log P - 0.735(\pm 0.123)I_1 + 0.496(\pm 0.123)I_2 \quad (2)$$
$$s = 0.80,\ n = 19,\ r^2 = 0.88$$

The bacterial mutagenic potency was defined as BR = 1 + NR/nmol, where NR is the net revertant number. The revertants are the cells that underwent mutation. The indicator variable I_1 was 1.0 if the amine or acetamido group was proximal (adjacent) to the juncture (i.e., the carbon atom connecting the substituted ring with the rest of the molecule). I_2 related to whether the amine group was free ($I_2 = 1$) or acetylated ($I_2 = 0$). Equations 1 and 2 are quite similar and show that mutagenicity increased with lipophilicity. On the other hand, mutagenicity was reduced when the amine or acetamido group was ortho to the juncture because of steric hindrance in its biotransformation. Mutagenic potency was also decreased by the acetylation of the amino group, probably because, according to the authors, the acetyl group should be first split off prior to oxidation of the amine group. However, this interpretation may be controversial since, at least in mammalian systems, N-acetylation, followed by N-hydroxylation, N,O-transacetylation, and departure of acetoxy group, is an established metabolic activation pathway.

Ford and Griffin[40] related the mutagenicity of a variety of heteroaromatic amines present in cooked foods with the stabilities of the corresponding nitrenium ions. The stability of the nitrenium ions was measured by the calculated energy ($\Delta\Delta H$) of the process

$$\mathrm{ArNH_2} + \mathrm{PhN^+H} \rightarrow \mathrm{ArN^+H} + \mathrm{PhNH_2}$$

$\Delta\Delta H$ was calculated using the semiempirical AM1 molecular orbital procedure. The mutagenic potencies (m) in three *S. typhimurium* strains (TA98, TA100, and TA1538) correlated with the $\Delta\Delta H$ values:

$$\log(\text{m})\ \text{TA98} = -0.181(\pm 0.043)\Delta\Delta H + 0.227(\pm 0.2792) \quad (3)$$
$$s = 0.966,\ r^2 = 0.593,\ n = 14$$

$$\log(\text{m})\ \text{TA100} = -0.147(\pm 0.024)\Delta\Delta H - 0.1619(\pm 0.450) \quad (4)$$
$$s = 0.540,\ r^2 = 0.770,\ n = 13$$

$$\log(\text{m})\ \text{TA1538} = -0.2417(\pm 0.0353)\Delta\Delta H - 0.801(\pm 0.765) \quad (5)$$
$$s = 0.245,\ r^2 = 0.922,\ n = 6$$

The above equations show that in the three Salmonella strains, the mutagenic potency was highly correlated with the stability of the hypothesized intermediate metabolite nitrenium ion.

Ford and Herman[41] studied the relative energetics ($\Delta\Delta H$) of arylamine N-hydroxylation and N−O heterolysis (ArNH2 → ArNHOH → $ArNH^+$) for condensed systems of two, three, and four rings using the semiempirical AM1 molecular orbital theory. Limited correlations between the energetics of nitrenium ion formation and experimental TA98 and TA100 mutagenicities were found.

Debnath et al.[42] studied a large database of chemicals with different basic structures (e.g., aniline, biphenyl, anthracene, pyrene, quinoline, carbazole, etc..) tested in *S. typhimurium* TA98 and TA100 strains, with S9 metabolic activation. The mutagenic potency is expressed as log(revertants/nmol). The AM1 molecular orbital energies are given in eV. The mutagenic potency in TA98 + S9 was modeled by

$$\log \text{TA98} = 1.08(\pm 0.26)\log P + 1.28(\pm 0.64)\text{HOMO} - 0.73(\pm 0.41)\text{LUMO} + 1.46(\pm 0.56)I_L + 7.20(\pm 5.4) \quad (6)$$
$$n = 88,\ r = 0.898,\ s = 0.860$$

where HOMO is the energy of the highest occupied molecular orbital, LUMO is the energy of the lowest unoccupied molecular orbital, I_L is an indicator variable that assumes the value of 1 for compounds with three or more fused rings. The electronic terms HOMO and LUMO, though statistically significant, accounted for only 4% of variance, whereas log P alone accounted for almost 50%. The most hydrophilic amines ($n = 11$) could not be treated by eq 6 and were modeled by a separate equation containing only log P (with negative sign), thus suggesting that these amines may act by a different mechanism.

The mutagenic potency in the *S. typhimurium* strain TA100 + S9 was modeled by

$$\log \text{TA100} = 0.92(\pm 0.23)\log P + 1.17(\pm 0.83)\text{HOMO} - 1.18(\pm 0.44)\text{LUMO} + 7.35(\pm 6.9) \quad (7)$$
$$n = 67,\ r = 0.877,\ s = 0.708$$

Also in this case, a different equation (with negative log P) was necessary for the most hydrophilic amines ($n = 6$). Overall, the principal factor affecting the relative mutagenicity of the aminoarenes was their hydrophobicity. Mutagenicity increased with increasing HOMO values: this positive correlation seems reasonable since compounds with higher HOMO values are easier to oxidize and should be readily bioactivated. For the negative correlation with LUMO, on the other hand, no simple explanation could be offered by the authors. A remarkable difference between the models for the two *S. typhimurium* strains was that the TA100 QSAR lacked the I_L term present in the TA98 model. It was hypothesized that larger amines are more capable of inducing frame shift mutations (TA98 is specific for frame shift mutations, whereas TA100 is specific for base pair substitution mutations) and that this effect is not accounted for by the increase of log P at increasing size of the molecules.[42]

The above QSARs are quite good in modeling mutagenic potency, whereas they are less satisfactory when one wants to predict the activity of the nonmutagenic amines. Using eqs 6 and 7, several inactive compounds are incorrectly predicted to be highly mutagenic.[42] Benigni et al.[43] studied the discrimination between mutagenic and nonmutagenic amines for the same set of compounds considered by Debnath et al.[42] Lipophilicity alone had no discriminating power in TA98 and TA100, which is at odds with the major role played in the modulation of potency within the group of active compounds. Discriminant functions separating mutagenic from nonmutagenic amines were based mainly on electronic and steric hindrance factors. The overall reclassification rate was around 70% accurate. In a following paper, Benigni et al.[44] split the amines into structural subclasses and then applied discriminant analysis separately to each subclass. The single ring amines were best separated by electronic factors (first HOMO and second LUMO, in decreasing order of importance) (correct reclassification rate around 70%). This result confirmed the central role of metabolic transformation in the mutagenic activity of these chemicals. The diphenyl methanes were modeled by the contribution to molar refractivity (MR) of the substituents in the ortho position to the functional group, thus indicating the negative effect of steric hindrance on the accessibility of the metabolizing system (correct reclassification rate: 87% for TA98; 93−100% for TA100). Steric factors, as measured by a similarity index, were also a key factor in the discrimination of biphenyls. The fused-rings amines were all mutagenic, so no discriminant model was necessary.

A mechanistically based QSAR experiment was performed by Glende et al.[45] They synthesized a number of alkyl-substituted (ortho to the amino function) derivatives of 2-aminonaphthalene, 2-aminofluorene, and 4-aminobiphenyl not included in the Debnath et al.[42] compilation. The measured mutagenic activity was compared with that predicted through the QSARs (eqs 6 and 7) for TA98 and TA100. The mutagenicity of the ethyl-substituted compounds was decently predicted, whereas with growing steric demand of the alkyl groups (*n*-butyl, isopropyl), the predicted and experimental values differed considerably. The bulky alkyl substituents decreased the mutagenicity of the arylamines. The authors argued that this was due to the steric hindrance of the metabolic oxidation of the amino group by the enzymes. This effect was not modeled by eqs 6 and 7 since the databases used to build the equations did not include a representative sample of the ortho-substituted compounds.

Klopman et al.[46] analyzed a set of approximately 100 aromatic amines using the computer automated structure evaluation (CASE) software. The CASE

methodology is a software that selects its descriptors (molecular substructures) automatically from a learning set of molecules. It identifies single, continuous structural fragments that are embedded in the complete molecule and selects those that are statistically associated with activity or nonactivity or with increasing potency. Normally, the program screens the molecules for all the possible fragments ranging from 2 to 10 heavy (nonhydrogen) atoms. The program was used to examine mutagenicity in *S. typhimurium* strains TA98 and TA100 (with S9 activation) and yielded a number of structural features associated with mutagenicity and nonmutagenicity. This work was extended by Zhang et al.[47] who studied 61 heterocyclic amines formed during food preparation. In both studies, the major feature leading to mutagenic activity was the aromatic amino group. Electronic parameters were also calculated, and the LUMO energy was found to correlate negatively with the mutagenic potency of the molecules. A model based on a number of fragments (the amino group in different combinations of atoms), together with LUMO attained $r^2 = 0.857$.

Within a research program aimed at highlighting the structural determinants that make the chemicals good substrates for cytochrome P-4501 (CYP1) metabolism, Lewis et al.[48] studied a noncongeneric set of food mutagens, the majority being heterocyclic amines ($n = 17$). For the TA98 strain (frame shift mutations) of *S. typhimurium*, the best correlation of mutagenicity was with molecular diameter ($r = 0.91$), hence with planarity. For the TA100 strain (base pair mutations), the best correlation was with the difference between the LUMO and HOMO energies: high mutagenicity was related to low values of the difference, hence to high chemical reactivity.

Basak and Grunwald[49] considered a set of 73 aromatic and heteroaromatic amines—previously studied by Debnath et al.[42]—and calculated a wide range ($n = 90$) of topological indices. They constructed five similarity spaces, based on (a) counts of atom pairs; (b) principal components (PC) from the topological indices; (c) PCs from topological indices plus physicochemical parameters; (d) PCs from physicochemical parameters; (e) physicochemical parameters. In each of the five similarity spaces, the mutagenic potency of every chemical was estimated by averaging the potency of its K-nearest neighbors ($k = 1-5$). The easily computable method based on atom pairs was almost as reliable ($r = 0.77$) as the similarity method based on physicochemical properties ($r = 0.83$).

In another paper, Basak et al.[50] considered a set of 127 aromatic and heteroaromatic amines (from the Debnath et al. compilation[42]) to discriminate between mutagens and nonmutagens. They computed a large range of topological indices: (a) topostructural indices (which encode information about the adjacency and distances of atoms irrespective of the chemical properties of the atoms involved); and (b) topochemical indices (which quantify information regarding the topology as well as specific chemical properties of the atoms). The combinations of topochemical and topostructural parameters had an unbalanced performance, missing many of the nonmutagens (accuracy: 42.9% nonmutagens; 93.4% mutagens). Log *P* and the quantum chemical parameters did not contribute to improve the discrimination.

Hatch et al.[51] studied the mutagenic potency (frameshift mutations in TA98 or TA1538 *S. typhimurium* strains) of a series of heteroaromatic amines formed during the cooking of the food, from two classes: aminoimidazo-azaarene (AIA) ($n = 38$) and aminocarboline (AC) ($n = 23$). For the AIA compounds, the features relevant for the mutagenic activity were number of fused rings, number of heteroatoms in rings 2 and 3, methyl substitution on imidazo ring nitrogen atoms, and methyl substitution on ring carbon atoms ($r^2 = 0.78$). The relevant features for the AC compounds were position of the pyridine-type nitrogen atom in ring 1, position of the exocyclic amino group in ring 1, and methyl substitution at ring carbon atoms ($r^2 = 0.80$).

In a further analysis, Hatch et al.[52] considered several molecular orbital properties calculated at different approximations, together with structural factors, for 16 AIA mutagens and their nitrenium ion metabolites. The major findings were (1) the potency increased with the size of the aromatic ring system; (2) potency was enhanced by the presence of an *N*-methyl group; (3) introduction of additional nitrogen atoms in pyridine, quinoline, and quinoxaline rings supported potency; (4) potency was inversely related to the LUMO energy; (5) potency was directly (although weakly) related to the LUMO energy of the derived nitrenium ions; (6) the calculated thermodynamic stability of the nitrenium ions was directly correlated with nitrenium LUMO energy and with the negative charge on the exocyclic nitrogen atom. The authors did not find a clear explanation for the role of LUMO energy. Hatch and Colvin[53] reconfirmed the above results in a wider set of 95 aromatic and heteroaromatic amines, together with the puzzling role of LUMO energy.

Further studies on the mutagenicity of the heterocyclic amines formed during meat cooking were performed by Felton et al.[54] They studied a set of 10 isomeric imidazopyridines showing a wide range of mutagenic potency in *S. typhimurium* strains TA98 and YG1024. Ab initio and semiempirical computational quantum chemical methods were used to predict the structures, energies, and electronic properties of the parent amines of these mutagens, as well as their nitrenium, imine tautomer, and imidazole ring protonated form. No explicit QSAR equation was built. Correlations were found between the dipole moments predicted for the parent amines and the logarithm of the potencies ($r = 0.86-0.91$), whereas there was only a borderline correlation between mutagenic potency and the LUMO energy of the parent amine (at odds with the strong correlation seen in other QSAR studies of heterocyclic amine mutagens). No attempt was made to measure the correlation of the mutagenic potency with hydrophobicity.[54]

Hatch et al.[55] extended their previous studies[54] by considering the mutagenic potency (TA98 or TA1538 *S. typhimurium* strains) of a set of 80 aromatic and heterocyclic amines, for which a wider range of

descriptors was calculated. The most significant structural variable was the similarity to 3,4-methylimidazoquinoline, with an adjusted $r^2 = 0.64$ nearly equal to the adjusted r^2 achievable by any multivariate model studied. The mutagenic potency was also highly correlated with the ring number, molecular weight, and volume. The calculated π-electron energy from Huckel theory and the ab initio LUMO energy yielded the highest correlations for single quantum chemical variables ($r = 0.757$ and 0.755, respectively). It should be noticed that the π-electron energy was highly collinear with the ring number and molecular weight. Log P did not show significant correlation with mutagenic potency.

Another reevaluation of the Debnath et al.[42] dataset was performed by Maran et al.,[56] which calculated a very large set of descriptors ($n = 619$) (including various constitutional, geometrical, topological, electrostatic, and quantum chemical descriptors). A final model with six descriptors was established ($r^2 = 0.8344$). The most important descriptor was the number of aromatic rings, followed by (in decreasing order of importance): polarizability (second-order hyperpolarizability), hydrogen acceptor surface area, hydrogen donors surface area, maximum total interaction energy for the C−C bond, and maximum total interaction energy for a C−N bond. The authors observed that the leading descriptor in their model (number of rings) was approximately proportional to the area of the hydrophobic aromatic hydrocarbon part of these molecules and was thus directly related to the hydrophobicity of polycyclic and condensed aromatic compounds (correlation coefficient between number of rings and log P, $r^2 = 0.3715$). This correlation is only weak, and the authors stressed that they could not add log P to their model. There is probably a high multiple correlation between the entirety of their variables and log P, which was not investigated. However, the number of rings was preferred by the authors to log P based on the argument that it is not an empirical parameter. The HOMO ad LUMO energies did not appear in the model.

2.1.2. QSARs for the Carcinogenic Activity of the Aromatic Amines

Yuta and Jurs[57] applied the automatic data analysis using pattern-recognition techniques (ADAPT) software to a set of 157 aromatic amines, divided into carcinogens and noncarcinogens (including five structural classes: biphenol, stilbene, azo-compounds, fluorene, methylene). Topological and geometrical descriptors were used, and the analyses repeated with several pattern-recognition methods. The chemicals were divided into 11 possible subsets, according to organs and route of administration. The iterative least-squares program enjoyed the most success (classification rates around 90%). Overall, the number of rings (related to molecular volume or bulk) was the descriptor most suitable to discriminate between carcinogens and noncarcinogens. Other important descriptors were related to size and shape (e.g., smallest principal moment). Several subsets of descriptors supported linear discriminant functions that could separate carcinogens from noncarcinogens.

Loew et al.[58] studied four pairs of isomeric amines. One of each pair was an active carcinogen, while the other was inactive or of doubtful activity. Mutagenic potency data paralleled the carcinogenic activity; the weak mutagens were the inactive or more marginally active carcinogens. These pairs of isomers were selected as ideal tests of the ability of calculated electronic parameters alone to predict relative biological activity since effects such as transport and elimination should be more nearly the same for both isomers of a given pair than for the group as a whole. Electronic reactivity parameters relevant to the relative ease of metabolic transformation of each parent compound to hydroxylamine by cytochrome P-450, as well as to other competing metabolic products involving ring epoxidation and hydroxylation, were calculated. In each pair, the N atom superdelocalizability—chosen as an indicator of the extent of formation of hydroxylamine from parent compounds—was larger for the more potent mutagen/carcinogen. Moreover, the less potent isomer in each pair had the ring carbon that was most reactive (i.e., larger values of ring carbon superdelocalizability) to direct phenol formation, which appeared to be an effective detoxification pathway. Ring epoxidation (as measured by π-bond reactivity) appeared to be more activating than detoxifying. In addition, two measures of covalent adduct formation ability of the hypothesized intermediate reactive species (arylnitrenium ion) paralleled the biological activity within each pair (electron density on N and C_6 atoms in lowest energy empty molecular orbital of the arylnitrenium ion).

The carcinogenicity of nonheterocyclic aromatic amines was been studied in two related papers. In the first analysis, only the carcinogenic chemicals were considered, and the structural factors that influence the gradation of carcinogenic potency in rodents were investigated.[59] The second analysis discriminated between carcinogenic and noncarcinogenic amines.[60]

In[59] the QSAR models for the carcinogenic potency in rodents (BRM = carcinogenic potency in mice; BRR = carcinogenic potency in rats) were

$$\begin{aligned}\mathrm{BRM} = {} & 0.88(\pm 0.27)\log P^{*}I(\mathrm{monoNH_2}) + \\ & 0.29(\pm 0.20)\log P^{*}I(\mathrm{diNH_2}) + \\ & 1.38(\pm 0.76)\mathrm{HOMO} - 1.28(\pm 0.54)\mathrm{LUMO} - \\ & 1.06(\pm 0.34)\mathrm{EMR_{2,6}} - 1.10(\pm 0.80)\mathrm{MR_3} - \\ & 0.20(\pm 0.16)E_{\mathrm{S}}(\mathrm{R}) + 0.75(\pm 0.75)I(\mathrm{diNH_2}) + \\ & 11.16(\pm 6.68)\end{aligned} \tag{8}$$

$$n = 37,\ r = 0.907,\ r^2 = 0.823,\ s = 0.381,\ F = 16.3,\ P < 0.001$$

$$\begin{aligned}\mathrm{BRR} = {} & 0.35(\pm\forall 0.18)\log P + 1.93(\pm\forall 0.48)I(\mathrm{Bi}) + \\ & 1.15(\pm 0.60)I(\mathrm{F}) - 1.06(\pm 0.53)I(\mathrm{BiBr}) + \\ & 2.75(\pm 0.64)I(\mathrm{RNNO}) - 0.48(\pm 0.30)\end{aligned} \tag{9}$$

$$n = 41 \quad r = 0.933 \quad r^2 = 0.871 \quad s = 0.398 \quad F = 47.4 \quad P < 0.001$$

where $\mathrm{BRM} = \log(\mathrm{MW}/\mathrm{TD_{50}})_{\mathrm{mouse}}$ and $\mathrm{BRR} = \log(\mathrm{MW}/\mathrm{TD_{50}})_{\mathrm{rat}}$.

TD50 is the daily dose required to halve the probability for an experimental animal of remaining tumorless to the end of its standard life span.[61] Besides log *P*, HOMO, and LUMO, the chemical parameters in the equations are $EMR_{2,6}$, sum of MR of substituents in the ortho positions of the aniline ring; MR_3, MR of substituents in the meta position of the aniline ring; E_S(R), Charton's substituent constant for substituents at the functional amino group; $I(\text{monoNH}_2) = 1$ for compounds with only one amino group; $I(\text{diNH}_2) = 1$ for compounds with more than one amino group; $I(\text{Bi}) = 1$ for biphenyls; $I(I(\text{BiBr}) = 1$ for biphenyls with a bridge between the phenyl rings; $I(\text{RNNO}) = 1$ for compounds with the group N(Me)NO; $I(\text{F}) = 1$ for aminofluorenes. The multiplicative terms "log $P^*I(\text{monoNH}_2)$" and "log $P^*I(\text{diNH}_2)$" are aimed at giving different weights (equation coefficients) to the log *P*'s of amines with one or two NH_2 groups, respectively.

The key factor for carcinogenic potency is hydrophobicity (log *P*). Both BRM and BRR increase with increasing hydrophobicity. In the case of BRM (mouse), the influence of hydrophobicity is stronger for compounds with one amino group (characterized by the indicator variable $I(\text{mono NH}_2)$) in comparison with compounds with more than one amino group (characterized by the indicator variable $I(\text{di NH}_2)$) (see the different coefficients 0.88 and 0.29). For BRM, electronic factors also play a role: potency increases with increasing energy of HOMO and with decreasing energy of LUMO. Such effects seem to be less important for BRR (rat): no electronic terms occur in eq 9. Carcinogenic potency also depends on the type of the ring system: aminobiphenyls (indicator variable *I*(Bi)) and, in the case of BRR, also fluorenamines (indicator variable *I*(F)) are intrinsically more active than anilines or naphthylamines. A bridge between the rings of the biphenyls decreases potency (*I*(BiBr)). Steric factors are involved in the case of BRM but cannot be detected in the case of BRR. BRM strongly decreases with bulk in the positions adjacent to the functional amino group, and bulky substituents at the nitrogen and in position 3 also decrease potency. The latter effects are, however, not so important. In the case of BRR, R = (Me)NO strongly enhances potency (compounds with this substituent have no measured value for BRM).

Equations 8 and 9 were derived from the analysis of the carcinogenic aromatic amines only. When applied to the noncarcinogenic amines, the equations did not predict well their lack of carcinogenic effects (the noncarcinogens were predicted as having a certain, even though low, degree of activity). This means that the molecular determinants that rule the gradation of carcinogenic potency are not the same determinants that make the difference between carcinogens and noncarcinogens. In a subsequent work, the differences in molecular properties between the two classes of carcinogenic and noncarcinogenic aromatic amines were specifically studied.[60] Four equations were derived, one for each of the experimental groups (rat and mouse, male and female). The two classes of chemicals were coded as 1 = inactive; 2 = active compounds.

The following discriminant equation achieves a highly significant separation of classes for female rat carcinogenicity:

$$w = 0.65\,L(\text{R}) + 0.79\,\text{HOMO} - 1.54\,\text{LUMO} + 0.76\,\text{MR}_2 - 0.50\,\text{MR}_5 + 1.32\,I(\text{An}) - 0.53\,I(o\text{-NH}_2) + 0.99\,I(\text{BiBr}) + 0.99\,I(\text{diNH}_2) - 1.08\,\log P^*I(\text{diNH}_2) \quad (10)$$

$$w_{(\text{mean,class1})} = 1.05,\ \text{N1} = 30$$

$$w_{(\text{mean,class2})} = -1.21,\ \text{N2} = 26$$

where *L*(R) is the length of the substituent at the amino group; *I*(An) = 1 for anilines; $I(o\text{-NH}_2) = 1$ if nonsubstituted amino group occurs in the ortho position to the functional amino group. The correct reclassification rate of discriminant function (10) amounts to 91.1% (Class 1: 93.3%; Class 2 88.5%) with a fairly stable cross-validation (all compounds: 80.4%; Class 1: 93.3; Class 2: 84.6%).

For male rat carcinogenicity a good separation of classes is achieved by the discriminant function:

$$w = 0.48L(\text{R}) + 0.90\text{HOMO} - 1.43\text{LUMO} + 0.72\text{MR}_2 + 1.13I(\text{An}) - 0.54I(o\text{-NH}_2) - 0.45\text{MR}_5 + 0.70I(\text{diNH}_2) - 0.80\,\log P^*I(\text{diNH}_2) + 0.65I(\text{BiBr}) \quad (11)$$

$$w_{(\text{mean,class1})} = 1.15,\ \text{N1} = 28$$

$$w_{(\text{mean,class2})} = -1.01,\ \text{N2} = 32$$

The correct reclassification rate amounts to 91.7% (Class 1: 92.9; Class 2: 90.6%) with a good result for cross-validation (all compounds: 83.3%; Class 1: 82.1; Class 2: 84.4%).

The results for male and female resemble each other. Of key importance for class separation are electronic properties as expressed by HOMO and LUMO, the type of ring system, and substitution in the ortho position as well as at the amino nitrogen. The probability of a compound to be assigned to the active class increases with increasing values of LUMO, decreasing values of HOMO, decreasing bulk of substituents in position 2 (ortho), decreasing length (or bulk) of substituents at the amino nitrogen, and increasing number of aromatic rings (anilines have a distinctly lower probability of being active than biphenyls, fluorenes, or naphthalenes). An important feature promoting carcinogenic potency also is the occurrence of an amino group in the ortho position to the functional amino group. Of lesser importance are the other variables.

For female mouse carcinogenicity, the following discriminant function reclassifies 85.7% of the compounds correctly (Class 1: 87.9%; Class 2: 83.3%) and is of acceptable stability in cross-validation (all compounds: 81.0%; Class 1: 84.8%; Class 2: 76.7%):

$$w = -0.47I(\mathrm{NR}) + 1.38 \log P^{*}I(\mathrm{monoNH_2}) + 1.68 \log P^{*}I(\mathrm{diNH_2}) - 0.37I(\mathrm{An}) + 0.33I(o\text{-}\mathrm{NH_2}) - 0.55\mathrm{MR_5} - 0.45I(\mathrm{BiBr}) \quad (12)$$

$$w(\mathrm{mean,class1}) = -0.92,\ \mathrm{N1} = 33$$

$$w(\mathrm{mean,class2}) = 1.01,\ \mathrm{N2} = 30$$

where $I(\mathrm{NR}) = 1$ if the amino nitrogen is substituted.

For male mouse, the discriminant function obtained is

$$w = -1.96L(\mathrm{R}) + 1.69\mathrm{B_5}(\mathrm{R}) - 0.83\mathrm{HOMO} + 0.97\mathrm{LUMO} - 1.22I(\mathrm{An}) + 0.73I(o\text{-}\mathrm{NH_2}) + 0.59\mathrm{MR_3} + 0.69\mathrm{MR_5} + 0.77\mathrm{MR_6} - 0.76I(\mathrm{diNH_2}) + 1.09 \log P^{*}I(\mathrm{diNH_2}) - 0.79I(\mathrm{BiBr}) \quad (13)$$

$$w(\mathrm{mean,class1}) = -1.11,\ \mathrm{N1} = 25$$

$$w(\mathrm{mean,class2}) = 1.16,\ \mathrm{N2} = 24$$

where the Sterimol parameter $\mathrm{B_5}$[14] is the maximal width of the substituent at the amino group.

Discriminant function (13) shows a good reclassification rate (all compounds: 89.8%; Class 1: 96.0%; Class 2: 83.3%) and stability in cross-validation (all compounds: 83.7%; Class 1: 96.0%; Class 2: 70.8%).

The results for the mouse resemble that for the rat for the key importance of the substitution at the amino nitrogen and at the ortho position, as well as the type of ring system. The male mouse model (eq 13) also contains HOMO and LUMO, like the two models for the rat (eqs 10 and 11). Only eq 12 is different for the absence of electronic properties.

The comparison of the models for the carcinogenic potency (eqs 8 and 9) with the models for the discrimination between carcinogens and noncarcinogens (eqs 10−13) shows that the key factors differentiating between active and inactive compounds on one hand and governing potency within the group of active compounds are different. The most pronounced differences are with respect to the importance of hydrophobicity (crucial for potency and minor for yes/no) and the directionality of electronic effects (while the degree of carcinogenic potency in mice increases with increasing values of HOMO and decreasing values of LUMO, the reverse is true for the probability of a compound of belonging to the class of carcinogens).[60] Interestingly, the mutagenic properties of the aromatic amines also pointed to a similar picture: the patterns of molecular determinants for the potency and the yes/no activity were different and were analogous to those found for the rodent carcinogenicity.[44]

Aromatic amines were included in two datasets of noncongeneric chemicals, which were studied with theoretical descriptors using artificial neural networks. The models devised by Vracko[62] were able to describe the training set, but their prediction ability of carcinogenic potency ($\mathrm{TD_{50}}$) was limited. Gini et al.[63] performed a retrospective study on 104 N-containing benzene derivatives that resulted in quite a good correlation after removal of several outliers.

2.1.3. Conclusions on the Aromatic Amines

In the most mechanistically oriented QSAR analyses, the toxic activity of the amines was demonstrated to correlate with the ease of formation of the N-hydroxylamine,[58] with the stability of the nitrenium ion,[41,52] and with the ease of formation of epoxides on the aromatic ring.[58] Loew et al. also found that the ease of formation of phenols (a detoxifying pathway) is actually negatively correlated with the carcinogenic activity.[58]

Various studies[39,42,59] pointed to the central role of hydrophobicity in the modulation of the mutagenic and carcinogenic potency of the aromatic amines. An exception is represented by the results of Hatch et al.[55] on a specific set of aromatic and heterocyclic amines.

Regarding the electronic descriptors, HOMO and LUMO energies were found to have a role both for the mutagenicity in *S. typhimurium*[42,44,47,48,52] and the carcinogenicity in rodents.[59;60] The role of HOMO energy can be rationalized in terms of propensity of the toxic amines to form the intermediate metabolite hydroxylamine. The role of the LUMO energy is quite puzzling. The two terms LUMO and HOMO may be linked together through the concept of "hardness" [η = (LUMO−HOMO)/2] as a measure of chemical reactivity; or LUMO energy may account for the reduction of the nitro group present, together with the amino group, in a number of amines.[42] However, a LUMO term appears also in datasets without nitroarenes.[47,52] King at al.[64] found a new enzymatic mechanism of carcinogen detoxification: a microsomal NADH-dependent reductase that rapidly converts the N-hydroxy arylamine back to the parent compound. Within this perspective, a low LUMO energy could favor the detoxification. However, the LUMO energy of the metabolite is not necessarily coincident with that of the parent amine; thus the entire matter needs further clarification.

Regarding steric effects, bulky substituents at the nitrogen of the amino group generally inhibit bioactivation (see the $E_s(\mathrm{R})$ contribution in eq 8[59] and the inhibiting effect of the acetylation of the amino group found by Trieff et al.[39]). Moreover, in all four equations (eqs 10−13) that model the separation between carcinogenic and noncarcinogenic amines the first term indicates that the probability of the amines of being noncarcinogenic increases with increasing length of the substituent ($L(\mathrm{R})$) or simply with the presence of a substituent ($I(\mathrm{NR})$) at the amino nitrogen.[60] A general finding is that the activity decreases with steric bulk in ortho to the amino function. This is consistent with the decrease in mutagenic potency found by Trieff et al.,[39] the decrease in carcinogenic potency in mouse (eq 8[59]), the decreased probability of the amines of being carcinogenic in rat (eqs 10 and 11[60]), and the decreased probability of the subclass of diphenyl methanes of being mutagenic in *S. typhimurium*.[44] These findings are in line with the observation of Glende et al.[45] that bulky alkyl substituents ortho to the amino group decreased the mutagenicity of the arylamines. The mechanistic rationale for these

observations is that steric bulk prevents enzymatic access to the nitrogen and formation of the reactive intermediate.

Several authors used topological or substructural parameters, as well as indicator variables. A finding common to many of them is the correlation between activity and/or potency, and number of (fused) aromatic rings.[42,48,51,52,55−57,60] This can be interpreted in different ways: (a) indicator for the planar systems apt to induce frameshift mutations in TA98 *S. typhimurium* strain; (b) indicator for the hydrophobicity of polycyclic and condensed aromatic rings; (c) indicator for the presence of extended conjugated systems that favor the formation of reactive intermediates. Debnath et al.[42] showed that, beside log *P*, an additional contribution to the mutagenic potency in TA98 was given by the presence of three or more fused rings. This effect was absent in TA100 strain and was related to the specificity of TA98 for frameshift mutations.

Carcinogenic potency also depends on the type of the ring system: aminobiphenyls (and, in the case of the rat, also fluorenamines) are intrinsically more active than anilines or naphthylamines. A bridge between the rings of the biphenyls decreases carcinogenic potency,[59] as well as the probability of being carcinogenic.[60]

An important finding is that the models and molecular determinants for the mutagenic and carcinogenic potency of the aromatic amines are different from those relevant to the separation between active and inactive compounds.[43,60] In other words, the Hansch equations permit the recognition of strong carcinogens and the estimation of the gradation of potency within active compounds but cannot separate weak carcinogens from inactive compounds. A similar concept applies to the mutagenic activity.

2.2. Nitroaromatic Compounds

The concern for the mutagenicity and carcinogenicity shown by many nitroaromatic compounds derives from the fact that they have long been of importance as intermediates in the synthesis of several kinds of industrial chemicals, as well as present in matrices of environmental importance (e.g., automobile exhaust fumes).[65] Nitroaromatics are also used for their antibacterial activity and in chemotherapy.[66] The nitroarenes appear to induce mutations and cancer through a mechanism similar to that of the aromatic amines. Both compounds are believed to be biochemically transformed to a common hydroxylamine intermediate, which is then activated to give an electrophilic nitrogen species. The difference is that the amines are oxidized by P-450 enzymes, whereas the nitroarenes are reduced to the critical hydroxylamine by cytosolic reductases. The reductases are present also in bacteria, whereas, in the case of bacterial tests of aromatic amines, the P-450 enzymes have to be added to the experimental mixture (S9 fraction).[67]

2.2.1. QSARs for the Nitroarenes

Because of the role of nitroheterocyclic compounds in chemotherapy, Biagi et al.[66] studied the relationship between the mutagenic activity of 17 nitroimidazo(2,1-b) thiazoles and their lipophilic character. The mutagenic activity was measured by means of the Ames test with *S. typhimurium* TA100 strain.

Lipophilicity was expressed in two ways: by chromatographic retention time (R_m) (eq 14) and sum of logP contributions ($\sum\pi$) (eq 15). The alternative equations obtained were

$$\log 1/C = +10.955(\pm1395)\,R_m - 3.721(\pm0.481)\,R_m^2 - 8.140(\pm0.889)$$
$$n = 17,\ r = 0.903,\ s = 0.464,\ F = 30.85 \quad (14)$$

$$\log 1/C = +3.507(\pm1.278)\sum\pi - 1.236(\pm0.479)(\sum\pi)^2 - 3.012(\pm0.656)$$
$$n = 17,\ r = 0.598,\ s = 864,\ F = 3.90 \quad (15)$$

The mutagenic potency was expressed as micromolar concentration increasing revertants by five times (C).

Equation 14 had a much better fit than eq 15. The authors argued that the experimental R_m contributed with both lipophilic (cell permeation) and polar (drug receptor binding) information, and both were necessary for describing the action mechanism.

Walsh and Claxton[68] studied a large set of nitroarenes (114 nitrogenous cyclic compounds with different basic structures) tested for their mutagenic activity in *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, TA1538) with the ADAPT software (using pattern-recognition techniques).[69]

The analysis was aimed at modeling the yes/no response (instead of the mutagenic potency of the active chemicals): out of the 114 substances, 64 were mutagenic and 50 were nonmutagenic.

Out of more than 100 molecular descriptors (topological, electronic, geometrical, and physicochemical), 19 were left after statistical feature selection.

The obtained QSAR classified correctly 96% of compounds. In a subsequent analysis, 109 compounds were utilized for the learning set, and 5 for the test set, obtaining 89% of correct classification. As a further validation test, 10 external similar compounds were selected from the literature: the QSAR model obtained 100% correct prediction.

The parameters included in the QSAR model were related to size and branching of the molecules.

Maynard et al.[70] studied the relationship of the mutagenicity of 20 nitrated PAHs (in *S. typhimurium* TA98, TA100, TA1537, TA1538 strains) with their electron affinities (LUMO energies calculated by the STO-3G method).

The equations relative to the strains were

$$\mathrm{TA98} = -64.892\mathrm{LUMO} + 11.559$$
$$n = 20,\ r = -0.82,\ F = 35.9 \quad (16)$$

$$\mathrm{TA100} = -46.342\mathrm{LUMO} + 8.583$$
$$n = 20,\ r = -0.75,\ F = 20.5 \quad (17)$$

$$\mathrm{TA1537} = -73.702\mathrm{LUMO} + 11.689$$
$$n = 20,\ r = -0.88,\ F = 38.1 \quad (18)$$

$$\text{TA1538} = -62.662\text{LUMO} + 11.008$$
$$n = 20,\ r = -0.86,\ F = 39.6 \quad (19)$$

The mutagenic potency was expressed as number of revertants/nmol of mutagen.

The authors remarked that the studied nitroarenes exhibit high assay variability under identical experimental conditions and significant interlaboratory and intralaboratory mutagenic variability, thus explaining the limited r values obtained (especially for TA100). This may be related to the concomitant antibacterial activity of nitroarenes.

The authors concluded that their results indicated that the dominant metabolic pathway of the mutagenicity involves a reduction of the nitro groups to give hydroxylamines and an ultimate conversion to electrophilic intermediates (arylnitrenium ions) which interact with the key tissue macromolecules. Moreover, the existence of a positive electrostatic region above and below the $C-NO_2$ bond is confirmed by the increasing LUMO energies with nitro rotation relative to the aromatic ring system. This situation facilitates the reduction process, i.e., it is a site for nucleophilic reception.

The data considered by Maynard et al.[70] were reexamined by Compadre et al.,[71] which included also lipophilicity (logP) in the analysis. This was the first of a series of analyses performed in the laboratory of Hansch on the nitroarenes.

Adding the hydrophobic parameter, they obtained the equations:

$$\log \text{TA100} = -1.91\text{LUMO} + 1.30 \log P - 6.59$$
$$n = 18,\ r = 0.874,\ s = 0.783 \quad (20)$$

$$\log \text{TA98} = -2.82\text{LUMO} + 1.48 \log P - 8.59$$
$$n = 20,\ r = 0.926,\ s = 0.737 \quad (21)$$

TA100 and TA98 are revertants/nmol of mutagen. LUMO, expressed in electronvolts, is calculated using the MNDO method.

It appears that a lipophilicity parameter improved remarkably the quality of the QSARs.

The demonstration, provided with the above work, that lipophilicity was essential to model the mutagenicity of nitroarenes stimulated Compadre et al.[65] to study the mutagenicity in TA100 and TA98 *S. typhimurium* strains for a wider set of fluorenes derivatives and other nitroarenes.

The models for the two strains were

$$\log \text{TA100} = 1.36(\pm 0.20) \log P - 1.98(\pm 0.39)\text{LUMO} - 7.01(\pm 1.2)$$
$$n = 47,\ r = 0.911,\ s = 0.737,\ F_{1,44} = 99.9 \quad (22)$$

$$\log \text{TA98} = -2.29(\pm 0.41)\text{LUMO} + 1.62(\pm 0.28) \log P - 4.21\ (\pm 0.80) \log(\beta\ 10^{\log P} + 1) - 7.74(\pm 1.4)$$
$$n = 66,\ r = 0.886,\ s = 0.750,\ F_{2,61} = 54.3,\ \text{optimum } \log P = 4.86,\ \log \beta = -5.06\ (4.60-5.06) \quad (23)$$

TA100 and TA98 are revertants/nmol of mutagen.

Equations 22 and 23 confirmed that the mutagenicity of the nitro aromatic compounds is significantly influenced by their hydrophobic character, in addition to their electronic properties. Higher mutagenic activity is associated with lower values of LUMO, i.e., better electron acceptors, which is consistent with an increasing ease of nitroreduction.

Interestingly, the authors compared the above models with the QSARs for the reduction of nitrobenzenes to hydroxylamines by xanthine oxidase. The hydrophobic and steric effects of substituents were absent in the reduction of the substances. Thus, it could be that the logP term mainly reflects the interaction of the nitroarenes and its metabolites with lipophilic components of the cell and/or interaction with other enzymes.

After the above paper,[65] in the Hansch laboratory the databases for TA98 and TA100 strains of *S. typhimurium* were expanded, and new QSAR analyses were developed on larger numbers of chemicals, belonging to wider ranges of basic structures. The results are in the two following papers.

Debnath et al.[72] derived a QSAR for 188 aromatic and heteroaromatic nitro compounds tested in *S. typhimurium* strain TA98.

The model developed was

$$\log \text{TA98} = 0.65(\pm 0.16) \log P - 2.90(\pm 0.59)\log(\beta\ 10^{\log P} + 1) - 1.38(\pm 0.25)\text{LUMO} + 1.88(\pm 0.39)I_1 - 2.89(\pm 0.81)I_a - 4.15(\pm 0.58)$$
$$n = 188,\ r = 0.900,\ s = 0.886,\ \log P_0 = 4.93,\ \log \beta = 5.48,\ F_{1,181} = 48.6 \quad (24)$$

TA98 is the mutagenic activity in revertants/nmol. I_1 is an indicator variable, set equal to 1 for compounds with three or more fused rings and to 0 when two or fewer rings are present. I_a is set equal to 1 for five substances of the set that are much less active than expected. LUMO was calculated with the AM1 method.

Hydrophobicity is a major factor in mutagenic activity of aromatic nitro compounds: in this dataset, logP follows a bilinear relationship. The negative coefficient of LUMO is explained by the initial reduction of the nitro group as a rate-limiting step in nitroarene activaction: substances with lower LUMO energies are supposed to be reduced more easily by cytosolic nitroreductases. Electron-attracting elements conjugated with the nitro group enhance mutagenicity. The positive coefficient of I_1 means that large-ring compounds are more active than expected from logP and LUMO alone.

A new QSAR for the mutagenicity of 117 nitroarenes in *S. typhimurium* TA100 strain, without metabolic activation, was developed as well.[67]

The following relationship was obtained:

$$\log \text{TA100} = 1.20(\pm 0.15)\log P - 3.40(\pm 0.74)\log(\beta\, 10^{\log P} + 1) - 2.05(\pm 0.32)\text{LUMO} - 3.50(\pm 0.82)I_a + 1.86(\pm 0.74)I_{ind} - 6.39(\pm 0.73) \quad (25)$$
$$n = 117,\ r = 0.886,\ s = 0.835,\ \log P_0 = 5.44(\pm 0.24),\ \log \beta = -5.7,\ F_{1,110} = 24.7$$

I_a is an indicator variable, set 1 for compounds where the acenthrylene ring is present; I_{ind} is another indicator variable, set 1 for the 1- and 2-methylindazole derivatives (six compounds).

Hydrophobicity plays a crucial role in determining the relative mutagenicity in most systems; hence, hydrophobicity alone can make the difference between an inactive and a highly mutagenic compound. As shown by the bilinear dependence of the activity on log P, the nitroarene mutagenicity increases slowly at low log P and then decreases more rapidly at high log P, probably because of a combination of adverse hydrophobic and steric effects. In addition, the activity increases as −LUMO (for more easily reduced compounds). The mutagenic activity in TA100 does not depend on the size of the aromatic ring systems, whereas it does in TA98 (see eq 24).

Benigni et al.[43] considered the ability of eqs 24 and 25 to model the lack of activity of the nonmutagenic nitroarenes. The above equations, in fact, were derived from the mutagenic potency values of only active nitroarenes. It appeared that only a few nonmutagenic nitroarenes were predicted to have very low potency, whereas the predicted potency for most of them spanned a large range of values, up to very high potency values. The subsequent analysis showed that this depended on the fact that log P, which was the major determinant of the mutagenic potency, had no discriminant power for separating the active from the inactive nitroarenes. On the contrary, LUMO and MR had discriminant power, even though the models obtained were far from being satisfactory. The general lesson was that the rate-limiting steps for determining yes/no activity and potency may be different and require different descriptors to be modeled.

Debnath and Hansch[73] expanded the modeling of the mutagenicity of the nitroarenes by considering data obtained with the SOS chromotest in *E. coli* PQ37, relative to 23 polycyclic aromatic nitro compounds.

The following relationships were found:

$$\log \text{SOSIP} = 1.07(\pm 0.36)\log P - 1.57(\pm 0.57)\text{LUMO} - 6.41(\pm 1.8) \quad (26)$$
$$n = 15,\ r = 0.922,\ s = 0.534,\ F_{1,12} = 36.21$$

Genotoxicity was expressed as SOS induction potential values (SOSIP), obtained without activation by S9, from a plot of the induction factor (IF) vs nmol of compound.

Equation 26 indicates that the mutagenicity of these chemicals is related to the hydrophobicity (main determinant) and to LUMO, like in *S. typhimurium*. Since there is no indicator variable for congeners with three fused rings and more, the SOS system does resemble the TA100 *S. typhimurium* strain and not the TA98 strain.

A special class of nitroaromatic compounds are the nitrofurans. These are used as antimicrobial agents in human and veterinary medicine. Although eq 24 (QSAR for the mutagenicity in TA98) applies to a large array of heterocycles, it did not fit the 2-nitroarenes.[74] Debnath et al.[74] further investigated the applicability of traditional QSAR and comparative molecular field analysis (CoMFA) to predict the genotoxicity of nitrofuran derivatives and to propose a possible mechanism for their unusual genotoxic behavior.

The best model for the genotoxic activity in the SOS chromotest was

$$\log \text{SOSIP} = -33.1(\pm 11.9)q_{c2} + 1.00(\pm 0.26)\log P - 1.50(\pm 0.49)I_{sat} - 1.19(\pm 0.49)\text{MR} - 0.76(\pm 0.49)I_{5,6} - 3.76(\pm 1.56) \quad (27)$$
$$n = 40,\ r = 0.900,\ s = 0.475,\ F_{1,34} = 9.76$$

SOSIP is the SOS induction potential in *E. coli* PQ37. The electronic descriptor q_{c2} is the partial atomic charge on the carbon attached to the nitro group (calculated by AM1 method). MR is molar refractivity. I_{sat} is an indicator variable equal to 1 for saturated ring compounds. $I_{5,6}$ is also an indicator variable equal to 1 for compounds with substituents at the 5- or 6- position of 2-nitronaphthofurans and pyrenofurans. The two latter variables account for steric effects.

The partial atomic charge on the carbon attached to the nitro group is the single most important variable; the second one is log P whose coefficient near 1 is in line with the results obtained for a variety of compounds in the mutagenicity assays. MR, a measure of substituent bulk, applies only to substituents adjacent to the nitro group: its negative coefficient points to the detrimental effect of such substituents.

Subsets of chemicals were also tested in *S. typhimurium* TA100 and TA98 strains, for which the following equations were found:

$$\log \text{TA100} = 1.15(\pm 0.65)\log P - 57.6(\pm 22)q_{c2} - 1.46(\pm 0.86)I_{sat} - 1.57(\pm 0.98)I_{5,6} - 6.32(\pm 3.1) \quad (28)$$
$$n = 20,\ r = 0.881,\ s = 0.626,\ F_{4,15} = 51.81$$

$$\log \text{TA98} = -18.1(\pm 15.6)q_{c2} - 1.20(\pm 0.52)I_{sat} + 2.15(\pm 0.84)I_L + 0.29(\pm 1.7) \quad (29)$$
$$n = 22,\ r = 0.894,\ s = 0.490,\ F_{3,22} = 71.74$$

Equation 29 is different from eq 28, and it seems that log P does not affect the mutagenicity of chemicals on the TA98 strain, in contrast to previous results. However, there is a strong collinearity between several variables in the TA98 dataset, so that

it is impossible to obtain a clear understanding of the QSAR for TA98.

In the same paper,[74] the SOSIP data were further analyzed with CoMFA, a procedure that provides means to assess specifically steric factors.[75] For the CoMFA analysis, the CH_3 probe in combination with H^+ probe was chosen, and the genotoxicity was predicted for a final set of 44 compounds (including 6 compounds initially omitted) by means of the following relationship:

$$\begin{aligned}\log \text{SOSIP} = {} & 0.080(\pm 0.003)Z1_{CH3,H+} + \\ & 0.084(\pm 0.005)Z2_{CH3,H+} + \\ & 0.056(\pm 0.006)Z3_{CH3,H+} + \\ & 0.045(\pm 0.007)Z4_{CH3,H+} + \\ & 0.020(\pm 0.006)Z5_{CH3,H+} + 3.572(\pm 0.030)\end{aligned} \quad (30)$$

$$n = 44,\ r = 0.981,\ s = 0.202,\ F = 195,\ P = 0.0001,\ \text{press } s = 0.413$$

Press is the standard deviation from the leave-one-out jacknife cross-validation. The parameters in eq 30 are latent variables obtained from the mathematical reduction of the interaction energies between the probes and the molecule, at the different grid points (for more technical details, see ref 75).

The CoMFA analysis reveals that combination of steric and electrostatic probes explains a majority of the variance in the data.

Overall, (a) the SOS *E. coli* system resembles the TA100 and not the TA98 system; (b) a high electron density on the carbon in position 2 promotes mutagenicity, which seems counterintuitive. This "aberrant" behavior of the nitrofurans may be related to the unusual ring opening which these compounds are susceptible to upon reduction; (c) a coefficient of about 1 for log *P* is common to a variety of classes regarding mutagenicity; (d) three types of steric effects were uncovered. The negative I_{sat} for ring saturation suggests that planar rings are important. The MR term shows that a bulky group adjacent to the nitro group has a deleterious effect. The negative $I_{5,6}$ shows that bulk in this region decreases potency; (e) CoMFA confirms the importance of an electronic factor, although not in a way that enables mechanistic discussion.

The CoMFA model was used by other investigators to further elucidate the steric factors in the mutagenicity of nitroaromatic compounds in *S. typhimurium*. Caliendo et al.[76] provided details on the steric probe−ligand ineraction energies and found evidence of the importance of the overall lipophilicity and of LUMO energy in the mutagenic acitvity in *S. typhimurium* TA98 strain. Fan et al.[77] compared the CoMFA models for the *S. typhimurium* strains TA98 and TA100 (see also a review in ref 78). The molecular areas of nitroaromatic chemicals found to be associated with mutagenicity were the high electronic density regions equivalent to C4−C5 in the pyrene ring and an electron-deficient site equivalent to C6. The authors argued that the electron-deficient areas may be associated with the nitroreductive bioactivation of nitroaromatics, whereas the electron-rich sites may be involved with oxidative mechanisms, also present in the bioactivation pathway of PAHs. Steric factors were more important for TA98 than for TA100 strain: increasing bulk perpendicular to the aromatic plane would decrease mutagenicity, and increasing the aromatic ring system along the C6−C7 region in I-nitropyrene would increase mutagenicity.

The structural basis of the genotoxicity of nitrofurans was also investigated with the CASE approach by Mersch-Sundermann et al.[79] Genotoxicity was examined with the SOS chromotest that measures the potency of a compound to induce the expression of the sfiA gene in *E. coli* PQ37 (DNA damage).

The model found was

$$\text{activity (logarithmic CASE units, LCU)} = -12.8 + n_1B_1 - n_2B_2 + 4.539 \log P \quad (31)$$

where n_1B_1 is the QSAR activity value of each biophore B_1 of the molecule multiplied by the number of occurrences; and n_2B_2 is the QSAR activity value of each biophobe B_2 (inactivating fragment) of the molecule multiplied by the number of occurrences.

Out of nine major activating structural fragments (biophores), the most important was the nitro group at position 2 of the furan ring. In addition, an increase in genotoxicity can be expected as a result of the addition of one, two, or more aromatic rings to the 2-nitrofuran structure, and log *P* is an important descriptor for genotoxic potency in *E. coli* PQ37.

CASE correctly predicted the probability of genotoxicity of all active and inactive compounds (94% of all predicted results were within ± 1 order of magnitude of the experimental value) and correctly predicted the probability of sfiA induction of 95.8% of 24 unknown nitroarenofurans ($r = 0.88-0.97$) used as validation set.

2.2.2. Conclusions on the QSARs for the Nitroarenes

Overall, the QSAR studies on the mutagenicity of the nitroaromatics provide a coherent picture. A common pattern is the presence of hydrophobicity and LUMO energy in most equations. The LUMO energy term indicates that the lower the energy of the lowest unoccupied molecular orbital (i.e., the more readily it can accept electrons), the more potent the mutagen. This suggests that the electronic effect is associated with the reduction step commonly accepted as crucial in the biotransformation of these compounds by the nitroreductases. Interestingly, the QSARs for the reduction of nitrobenzenes to hydroxylamines by xanthine oxidase[65] did not include terms for the hydrophobic (and steric) effects. Thus, it could be that the log *P* term mainly reflects the interaction of the nitroarenes and its metabolites with lipophilic components of the cell and/or interaction with other enzymes.

The steric effects were also subjected to extended investigations. Generally speaking, a major difference between the TA98 and TA100 strains of *S. typhimurium* was that steric effects were more effective for TA98. It also appeared that the SOS *E. coli* system resembles the TA100 and not the TA98 system.[74] As discussed in ref 43, this difference can be related to

the influence that the size of mutagens have in the induction of frame shift mutations (specific of TA98, and not of TA100).

Regarding the steric effects, interesting results were found in the context of investigations aimed at reducing the mutagenicity of nitroaromatics.[80,81] Despite the large range of structures used to develop eqs 24 and 25, essentially variations of the aromatic ring system were covered. Substituent effects were poorly represented in the dataset, and the authors stated that "it is important to investigate the hydrophobic effect of aliphatic side chains on the mutagenicity to see if it parallels that of the flat aromatic systems."[72] In a first paper, Klein et al.[80] studied the effect of bulky alkyl substitutents ortho to the nitro group and in the 2′-position on the mutagenic activity of 4-nitrobiphenyl in TA98 and TA100 strains. The experimental mutagenicities were compared with those predicted by eqs 24 and 25 and results showed that the nitrocompounds most sterically hindered were remarkably less mutagenic than predicted. This reduction in mutagenicity was attributed to purely steric effects. In the case of substituents ortho to the nitro group, the reduction was correlated with deviations from the coplanar orientation of the nitro group relative to the aromatic plane. In the case of alkyl groups in 2′-position, the coplanarity of the nitro group was not affected, but the twisting of the two aromatic rings was associated with a less effective charge delocalization of the nitrenium ion. In a second paper,[81] the authors studied the influence of bulky alkyl substituents far from the nitro group (4′-position in nitrobiphenyls, 7-position in 2-nitrofluorenes). The mutagenicity of all compounds decreased with increasing steric demand of the attached alkyl groups, to an extent greater than predicted based on their contributions to log *P* or LUMO (see eqs 24 and 15). The authors hypothesized that changes of the molecular shape from planar to nonplanar may be responsible for this effect by interfering with an efficient intercalation into DNA.

The evidence from the two latter papers has practical importance since it provides a recipe for synthesizing nontoxic nitro compounds. In addition, its theoretical importance should be remarked as well. The discrepancy between actual mutagenicity potency and that predicted by the QSAR models points to the intrinsic difficulty of providing of an exhaustive definition of a chemical series (congeners). Even in cases in which the database consists of large numbers of well-selected compounds (between 100 and 200 chemicals for eqs 24 and 25), specific structural changes (e.g., the bulky alkyl substituents considered in refs 80 and 81) may radically change the underlying chemistry and require substantial modifications of the QSAR models. Whereas several papers point to statistics as the most important check of the validity of a QSAR models, the above evidence strongly reaffirms the predominance of the chemical knowledge in the process of generating valid models.

2.3. *N*-Nitroso Compounds

A very large number of *N*-nitrosamines are known to be carcinogenic in various animal species. Human exposure to preformed *N*-nitrosamines occurs through the diet, in certain occupational settings (e.g., in the rubber industry), and through the use of tobacco products, cosmetics, pharmaceuticals, and agricultural chemicals. In addition, *N*-nitrosamines are generated in the body by nitrosation of amines (via acid- or bacterial-catalyzed reaction with nitrite) or by reaction with products of nitric oxide generated during inflammation or infection. *N*-Nitrosamines are activated by cytochrome P-450 to the ultimate mutagens and carcinogens.[82]

2.3.1. QSARs for the Mutagenicity of N-Nitrosamines

Singer et al.[83] studied the mutagenicity of a set of *N*-nitroso-*N*-benzylmethylamines in *S. typhimurium* TA1535. The chemicals were metabolically activated with the S9 fraction. The physicochemical parameters screened were the Hammett σ constant, Hansch's π, MR, and two molecular connectivity indices. The resulting QSAR model was

$$\log(1/C) = 3.55(\pm 1.40)\sigma - 3.88(\pm 1.85)\sigma^2 + 1.62(\pm 0.71)^3\chi_{Pv} - 5.11 \quad (32)$$
$$n = 13,\ r^2 = 0.761,\ p = 0.004$$

where *C* is the molar concentration of nitrosamine inducing 50 revertants/plate.

The authors did not find any involvement of hydrophobicity (π) in the mutagenicity. The quadratic term of σ takes into account the fact that mutagenicity in this dataset first increases with increasing values of σ and then decreases. According to the authors, eq 32 indicates that substituents that are electron withdrawing and therefore tend to polarize and weaken the methylene C−H bond result in greater mutagenicity. The rupture of the C−H bond is considered to be a rate-limiting step in the metabolic process, which initiates with the hydroxylation at the α-carbon and subsequent conversion to more reactive species. The presence of the connectivity term $^3\chi_{Pv}$ in eq 32 indicates that activity is dependent on overall shape and volume.

Hansch and Leo[11] reconsidered the dataset studied by Singer et al.[83] and replaced the experimental σ values with ordinary σ values from the benzene system. The new equation was

$$\log(1/C) = 0.92(\pm 0.43)\pi + 2.08(\pm 0.88)\sigma - 3.26(\pm 0.44) \quad (33)$$
$$n = 12,\ r^2 = 0.794.\ s = 0.314$$

According to the authors, eq 33 is better than eq 32. One reason is that eq 33 has a slightly better fit than eq 32 and contains only two terms. More importantly, one normally finds hydrophobicity to be a parameter of importance in mutagenicity, when direct alkylation of DNA is not involved. Moreover, evidence is accumulating that the coefficient of hydrophobicity is near 1.0. All this information points to eq 33 as the best rationalization of the data. Overall, eq 33 says that hydrophobic, electron-releas-

ing substituents on the ring of *N*-nitroso-*N*-benzylmethylamines increase mutagenicity.

2.3.2. QSARs for the Carcinogenicity of N-Nitroso Compounds

The SIMCA pattern-recognition method was used by Dunn III and Wold[84] to classify 45 *N*-nitroso compounds as active or nonactive, with respect to rat carcinogenicity. The set included nitrosoamines, *N*-nitrosoureas, and *N*-nitrosourethans. The structural description of the substituents consisted of six parameters: the Rekker's lipohilicity constant, σ^* and Es Taft's parameters, MR, L and B_4 Verloop's steric constants.

Initially, all the active compounds were examined together as the training set and the inactive and untested compounds as the test set. No model was achieved. Then, the active chemicals were divided into subclasses and used as the training set.

The compounds requiring metabolic activation were split in two classes:

Class 1: dialkylnitrosoamines with electronically neutral and/or electron-donating substituents on the amine nitrogen (27 compounds);

Class 2: compounds that putatively do not require metabolic activation (9 compounds);

Class 3: dialkylnitrosoamines which have at least one rather strongly electron-withdrawing substituent on the amine nitrogen (14 compounds).

The SIMCA analysis procedure was applied separately to the three classes of chemicals, giving the following results:

Class 1: 23 out 27 chemicals were correctly classified with a two-components model;

Class 2: 7 out 9 with a one-component model;

Class 3: all 14 substances were correctly classified with a three-component model.

A total of 44 out 50 (88%) of the active compounds were correctly classified, with all 12 variables relevant for the description of the classes.

The test set consisted of three compounds (two nonactive, one active); two out three were correctly predicted with one false positive.

2.3.3. Carcinogenicity of N-Nitrosamines

Frecer and Miertus[85] performed a theoretical investigation of the putatively crucial steps in the activation process, namely, the initial enzymatic C_α-oxidation (C_α is the carbon atom adjacent to the aminic nitrogen, N_a), the amine nitrogen hydroxylation or the N-dealkylation. Also the transport properties of the studied compounds and their metabolites were considered.

The study of the molecular reactivity and transport properties of the parent compounds was modeled by

$$\log 1/D_{50} = 0.476\Delta G^{w,o}_{coul} - 0.228\Delta G^{w,o}_{d,r} + 0.155\Delta G^{w,o}_{cav} + 3.979$$
$$n = 12,\ r = 0.868,\ F = 6.140 \quad (34)$$

where D_{50} is the mean lethal dose (mol/Kg), used as measure of the experimental carcinogenic potency in rat; $\Delta G^{w,o}_{coul}$ is the difference in Coulombic contributions to the Gibbs solvation energies in water and octanol (kcal/mol); $\Delta G^{w,o}_{d,r}$ is the difference in dispersion−repulsion contributions; and $\Delta G^{w,o}_{cav}$ is the difference in cavitation contributions.

From eq 34, it appears that the initial transport of the parent molecules to the site of biodegradation influences significantly the carcinogenic activity. The negative value of the term $\Delta G^{w,o}_{coul}$ indicates that the metabolic activation could take place preferentially in a nonpolar lipophilic phase, i.e., in the liver. Therefore, higher metabolic activation, and then a stronger carcinogenicity, is expected for more lipophilic molecules.

Regarding the metabolic activation process, the authors tried to describe the probable initial steps: the radical C_α-hydroxylation and the amine N_a-hydroxylation. They calculated the theoretical reactivity indices of atoms and chemical bonds involved in these processes (net atomic charges, orbital energies, orbital electron densities, etc.). The best model for this step was

$$\log 1/D_{50} = 21.568Q_{C\alpha} - 1.825$$
$$n = 12,\ r = 0.669,\ F = 8.122 \quad (35)$$

where $Q_{C\alpha}$ is the net charge on the C_α atom of parent molecule.

Equation 35 supports the hypothesis that the initial biotransformation reactions occur at the C_α atom and not at the aminic nitrogen: the most probable metabolic activation mechanism starts at the C_α atom or the C_α−H_α bond.

A model including both reactivity and transport characteristics was also formulated:

$$\log 1/D_{50} = 0.456\Delta G^{w,o}_{coul} + 12.576Q_{C\alpha} + 1.001$$
$$n = 12,\ r = 0.813,\ F = 6.825 \quad (36)$$

In a next step, the authors found that the charge on the C_α-radicals was more correlated with the carcinogenic potency than that of the parent molecules; thus a final equation was formulated:

$$\log 1/D_{50} = 0.188\Delta G^{w,o}_{coul} + 16.285Q_{C\alpha} + 1.162$$
$$r = 0.903,\ F = 12.618 \quad (37)$$

where $Q_{C\alpha}$ refers to the first metabolite and not to the parent compound as in eq 35. Overall, this study indicates: (a) the role of the transport of the parent compound is quite dominant; (b) the combination of transport and reactivity indices does not improve the correlation; (c) for the most probable first metabolites (C_α-radicals of NA) the correlation of the biological activity with $Q_{C\alpha}$ is the most significant; (d) the combination of the transport properties of the parent molecule with the $Q_{C\alpha}$ of the first metabolite gives a meaningful QSAR equation for the carcinogenic properties of the nitrosamines.

2.4. Quinolines

Quinoline and its derivatives are environmental pollutants found in automobile exhausts, urban air

particulate, and tobacco smoke, and are used for the preparation of industrial chemicals and pharmaceutical drugs. Some are also food mutagens. A number of quinolines are recognized mutagens and carcinogens. In this paper Debnath and co-workers[86] studied the mutagenesity of 33 quinoline derivatives in *S. typhimurium* TA100 strain, with metabolic activation (S9 fraction).

A considerable amount of study was devoted to the consideration of electronic descriptors, including LUMO and HOMO energies, as well as electroinc density on various ring atoms. During this preliminary work, it was discovered that quinolines substituted in 2-, 3- and 4-positions did not fit the correlation equations and hence were omitted from the final formulation.

The resulting model was

$$\log \text{TA100} = 1.14(\pm 0.40) \log P - 45.76(\pm 27.83) q_2 - 5.39(\pm 1.70) \quad (38)$$
$$n = 21,\ r = 0.852,\ s = 0.565,\ F_{1,18} = 11.9$$

where q_2 is the net charge on carbon atom 2, i.e., adjacent to nitrogen atom. The mutagenic activity was experimentally measured and expressed as rate of mutation in revertants/nmol; log P values were experimentally determined.

As in other studies relative to the mutagenicity of compounds requiring S9 metabolic activation, log P is of overwhelming importance and has a coefficient close to 1.0.[18]

Using q_5 or q_7 in eq 38 results in much poorer correlation, but using q_4 or q_N yields almost as good results as q_2. The authors elected position 2 as being the most likely site of activation to form the ultimate mutagen and bind with DNA not just because of eq 38 but also because a similar suggestion derived from previous mechanistic studies.

Another approach, based on parameters of the physical organic chemistry, gave rise to the following equation:

$$\log \text{TA100} = 0.99(\pm 0.44) \log P - 1.48(\pm 1.19) R_8 - 2.68(\pm 2.32) R_6 - 3.19(\pm 0.98) \quad (39)$$
$$n = 21,\ r = 0.842,\ s = 0.599,\ F_{2,17} = 20.8$$

where R is the resonance parameter in different positions, whose negative coefficient means that an electron release to the ring increases the activity (this effect is more important for 6-substituents than for 8-substituents). The addition of a term in F (field inductive parameter) did not improve the equation.

The QSARs found provide a considerable insight to this structure−activity problem, even though they are not able to rationalize the substitutions in all positions. This difficulty is likely to derive from the existence of several different patterns of metabolic activation.

In a subsequent study, two different types of activity for the same set of quinolines were simultaneously considered. Fifteen 8-substituted quinolines were tested for both mutagenic and cytotoxic activity in Ames test, TA100 strain (with S9 activation).[87]

The mutagenicity of the chemicals was modeled by

$$\log \text{TA100} = 1.16(\pm 0.35) \log P - 0.51(\pm 0.26)\text{B5-8} - 1.56(\pm 0.98) \quad (40)$$
$$n = 13,\ r^2 = 0.870,\ q^2 = 0.784,\ s = 0.402$$

where: B5-8 is the intermolecular steric Verloop parameter (STERIMOL). Log P was experimentally measured by the shake-flask method; two substances were omitted.

Similarly to eq 38, log P is present with a coefficient close to 1.0. The negative coefficient of the Sterimol parameter denotes that bulky substituents inhibit the mutagenic process either in the metabolic activation step (i.e., interaction with P-450) or in the reaction of the final metabolite with DNA.

The toxicity against the bacterial cells was described by:

$$\log \text{Tox} = 1.16(\pm 0.42) \log P + 1.02(\pm 0.94)\sigma - 5.71(\pm 0.94) \quad (41)$$
$$n = 13,\ r^2 = 0.801,\ q^2 = 0.685,\ s = 0.371$$

where Tox is the toxic activity to the TA100 bacterial cells, i.e., the inhibition of revertant growth observed as a decrease from the initial slope value; σ is the Hammett parameter.

Thus, it appears that it is possible to develop QSAR of two distinctly different biological activities for the same set of congeners. For the mutagenicity of these quinolines, both hydrophobicity and steric interactions appear to be important. In contrast, the cytotoxicity is mainly affected by increasing hydrophobicity and by addition of withdrawing substitutents to the quinoline ring. The latter effect may be related to the protonation of the basic nitrogen in the quinoline ring.

2.5. Triazenes

Triazenes are chemotherapeutical drugs that, as many other compounds of the same category, also induce DNA damage and mutations themselves. Shusterman and co-workers[88] examined a set constituted of 21 phenyl- and heteroaromatic triazenes, active for mutagenicity in *S. typhimurium* strain TA92 containing the S9 fraction.

Two QSAR models were developed:

$$\log 1/C = 0.95(\pm 0.25) \log P + 2.22(\pm 0.88)\text{HOMO} + 22.69 \quad (42)$$
$$n = 21,\ r = 0.919,\ s = 0.631$$

$$\log 1/C = 0.97(\pm 0.24) \log P - 7.76(\pm 2.73) q_{\text{HOMO}} + 5.96 \quad (43)$$
$$n = 21,\ r = 0.931,\ s = 0.585$$

where C stands for the molar concentration of chemical that causes 30 mutations above background/10^8 TA92 bacteria, and q_{HOMO} is the HOMO electron density on the alkylated N1.

The two equations suggest that the mutagenicity of these compounds is ruled by the hydrophobicity

and electronic properties of the molecules. The positive coefficient of log P shows that the mutagenic activity increases with increasing lipophilicity. The positive coefficient of HOMO and the negative coefficient of q_{HOMO} indicate that an increase of mutagenicity is correlated with increased electron donation from the ring to the triazene moiety and, thereby, with an increased ease of triazene oxidation.

The generally accepted scheme for triazene activation involves an initial hydroxylation of the *N*-methyl group by cytochrome P-450, followed by spontaneous tautomerization and hydrolysis reactions, leading to the formation of a reactive carbocation. Even though the reactive carbocation actually reacts with DNA, eqs 42 and 43 do not reveal any correlation between the structure/stability of the carbocation and the triazene mutagenicity. Instead, hydrophobicity and electron-donating substituents (coded for by the equations) are the same factors involved in the cytochrome P-450 catalyzed hydroxylation of the triazenes. Thus the QSAR models suggest that the rate-limiting step in the mutagenic activity is determined by the rate of initial triazene activation and not by the rate of DNA alkylation by the reactive carbocation.

2.6. Polycyclic Aromatic Hydrocarbons

PAHs constitute a large class of ubiquitous environmental pollutants formed from the incomplete combustion of fossil fuels, tobacco products, food, and virtually any organic matter. PAHs are of high priority concern for human health because of significant human exposure and because both individuals PAHs and mixtures are associated with human and experimental carcinogenicity.[89] The mechanisms of action of the PAHs have been studied extensively. The most consistent and salient feature of the carcinogenic PAHs is the presence of a bay or fjord region in the molecule. The bay or fjord region diol epoxides are the ultimate carcinogens of these PAHs and are able to form stable DNA adducts. In addition depending on the type of PAH, the target organ, and the level of expression of the activation enzymes, at least two other metabolic pathways are believed to play important roles: (1) one-electron oxidation at the most electrophilic carbon to form radical cation; and (2) conversion of dihydrodiols of PAH to reactive and redox active *o*-quinones. PAHs are also known to have a variety of nongenotoxic activities that can play important contributory roles in the overall carcinogenic process.[6]

The PAHs have been the subject of several QSAR analyses; whereas a number were clearly mechanistically based (notably, ref 21), most of the papers were aimed at providing classification tools, with little mechanistic insight.

2.6.1. QSARs for the Carcinogenicity of PAHs

The theories of K region (e.g., 9,10-bond of phenanthrene) activation and bay (or L-) region (e.g., region between the 4- and 5-positions of phenanthrene) activation as responsible for carcinogenicity are at the basis of the work by Zhang and co-workers.[21]

The QSAR regarded the very specific endpoint of skin carcinogenicity in mice for a set of 161 compounds. The criteria for the selection of the chemicals was that compounds containing an unencumbered bay region as well as carcinogenic activity which could be expressed as Iball index (see below) should be considered as a class of congeners (and then included in this analysis). As a matter of fact, the authors list also a set of 30 compounds that have been tested for skin carcinogenicity but that do not contain a bay region or have an encumbered bay region (i.e., with one or more substituents placed in the positions presumed to be oxidized to the diol epoxide). As a support to the bay region theory of PAH carcinogenicity and to criteria used for the selection of chemicals the authors note that (a) none of the 14 compounds with encumbered bay region show activity; and (b) of the 16 compounds without bay region, only 5 have been found to be carcinogenic.

Regarding the selection of chemicals for the analysis, the authors also remark that a large range of basic structures (including chemicals with heteroatoms in the ring or in the substituents) was necessary to break the collinearity among descriptors (notably, log P and HOMO), which is a common plague for QSARs of PAHs.

The QSAR equation obtained was

$$\log \text{Iball} = 0.55(\pm 0.09) \log P - 1.17(\pm 0.14) \log(\beta\, 10^{\log P} + 1) + 0.39(\pm 0.11)\text{LK} + 0.47(\pm 0.26)\text{HOMO} + 1.93(\pm 2.4) \quad (44)$$

$$n = 161,\ r = 0.845,\ s = 0.350,\ \log P_0 = 6.67(\pm 0.217),\ \log \beta = -6.81,\ F_{1,155} = 12.8$$

The Iball index of carcinogenicity is defined as follows:

$$\text{Iball index} = (\text{tumor incidence})(100\%)/\text{mean latent period in days}$$

where tumor incidence = number of animal with tumors/number of animals alive when the first tumor appears. LK is an indicator variable set 1 for compounds with a substituent attached to a L or K region. A positive coefficient of LK means that a substitution in a L or K region leads to increase potency of these congeners, other factors being equal. The activity largely depends on hydrophobicity, with LK and HOMO being of relatively minor importance.

In eq 44, it is seen that carcinogenicity initially increases with the increase of log P, and then after reaching an optimum value of 6.8, declines linearly. Carcinogenicity begins to become apparent at log P of 4, peaks at 6.8, and disappears at about 9. Since many simple organic compounds with log P values lower than 2 penetrate skin readily, the high log P required for skin carcinogenicity cannot be charged to penetration. The authors argue that high log P is necessary to induce enough P-450 in the skin cells. In addition, tumor promoting activity is known to depend on hydrophobicity (in phorbol esters). These results support the conclusion that the threshold

value for log P is to be associated more with the activation and promotion process than with skin penetration. As further support, the authors also show that the hydrophobicity distribution of chemicals causing cancer via diet, water, gauge inhalation, or injection is totally different and peaks at much lower log P values.

Regarding the other parameters in eq 44, unprotected (without substituents) K- and L regions result in lower than expected potency. The authors argue that substituents at these points act by inhibiting deactivating metabolism. It has been assumed for many years that L regions, because of their well-known high chemical reactivity, are readily metabolized by P-450 to hydrophilic compounds resulting in carcinogenic activity reduction or loss. Equation 44 indicates that K regions behave so much like L regions in terms of overall activity.

The presence of HOMO in eq 44 is rationalized in terms of the first oxidation step in P-450 activation yielding an epoxide.

Other authors reported on QSARs for the carcinogenic activity of PAHs, characterized by minor emphasis on mechanistic considerations and major focus on the derivation of classification rules. A selection will be summarized below; if interested, the reader also can see refs 90−92.

Richard and Woo[89] used the CASE model to develop QSAR for the carcinogenicity of a large group of PAHs. The aim was to (a) develop a classification rule; (b) for a set of untested PAHs, compare the CASE predictions with those generated by human "expert judgment" based on mechanistic considerations; (c) evaluate the CASE methodology.

The CASE program is presented in more detail in Section 4.1. It is an automated, statistically based correlative program that starts from chemical structures and explores all possible chemical substructures to find those correlated with the activity under study.[93,94] In this case, activity/nonactivity carcinogenicity data were used and thus the qualitative CASE mode was employed.

The chemicals were divided into the following groups:

LEARN set: 78 compounds (31 active, 25 inactive, 22 marginal). These PAHs had known carcinogenic activity and were used to train the CASE program;

TEST set: 106 compounds with unknown activity, previously considered by the human experts;

VALIDATE set: 24 compounds with known activity, used to check the prediction ability of the CASE model developed on the LEARN set.

In the LEARN set, the procedure identified eight activating fragments and four inactivating ones. The most significant fragment identified by CASE represents an unsubstituted bay region benzo ring, with unoccupied *peri* position at C5. This is consistent with the current bay region theory of PAH carcinogenicity. The remaining activating fragments are basically modifications or extensions of the bay region or account for chemicals with no apparent bay region. These fragments classified correctly the activities of 94% of PAHs in the LEARN set.

The above CASE model was applied to the prediction of the chemicals in the TEST set. The agreement between CASE predictions and the predictions of the human experts was only 64%. This was attributed to limitations of the LEARN database in terms of inadequate representation of 2- and 3-ring PAHs. When these subclasses were not considered, the concordance improved to 90%.

The VALIDATE set contained 24 compounds with more than three rings, and their activity was predicted with a total prediction accuracy of 75%. It should be noted that only 3 out 24 of the VALIDATE structures were represented within the LEARN set; therefore, almost total extrapolation was necessary.

Yuan and Jurs[95] modeled the carcinogenicity of a set of 191 PAHs with the computer program ADAPT. The range of screened molecular descriptors was very large ($n = 56$) and included topological descriptors (e.g., fragments, sigma electronic distribution of substructures, connectivity), geometric descriptors, and whole molecule descriptors, like log P. Pattern-recognition methods were used to model the data.

A set of 28 descriptors was found to be able to separate the 191 PAHs into carcinogens and noncarcinogens. In a cross-validation test (leaving out 10 chemicals for 10 times), the predictive ability was 90.5%.

The results of the study neither confirmed nor disproved any particular theory of PAH carcinogenicity. Among the final 28 descriptors were substructure descriptors containing K regions, as well as bay regions. While several geometric descriptors were among the final set, the dataset could not be separated based on this information alone. Finally, while log P alone was capable of separating a comparatively large amount of the dataset, there were several descriptors that were more powerful.

2.6.2. QSARs for the Genotoxicity of PAHs

Recently, the above laboratory generated a QSAR model for the genotoxicity of 277 PAHs in the SOS Chromotest.[96]

On the basis of the extent of genotoxic response, the PAHs were divided into the classes of genotoxic ($n = 80$) and nongenotoxic ($n = 197$). Approximately 240 descriptors were generated, using the ADAPT routine: topological, geometric, electronic, and hybrid descriptors. Three mathematical classification methods were used, together with cross-validation procedures.

The k-nearest neighbor method, based on eight descriptors, provided the best separation, with a correct training set classification of 93-5%. A consensus model that incorporated the three mathematical classifiers correctly classified 81.2% of the compounds and provided a higher prediction rate on the genotoxic class than any other single model.

2.7. Halogenated Aliphatics

The toxic and aneuploidizing activities in *Aspergillus nidulans* of 55 halogenated aliphatic hydrocarbons were studied by Crebelli et al.[97] Aneuploidy is a kind of genetic mutation that involves changes in the correct number of chromosomes.

Halogenated aliphatic hydrocarbons are widely used as industrial and household solvents, as intermediates in chemical synthesis, and for a variety of other uses. Since several halogenated aliphatics are recognized to be genotoxic and/or carcinogenic, human exposure, which occurs both at workplaces and in outdoor and indoor environments, may pose a significant health hazard. They may act by several mechanisms of action. Short-chain monohalogenated (excluding fluorine) alkanes and alkenes are potential direct-acting alkylating agents. Dihalogenated alkanes are also potential alkylating or cross-linking agents (either directly or after GFH conjugation). Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms or undergo reductive dehalogenation to yield haloalkenes that in turn could be activated to epoxides.[9]

A first analysis of 35 chlorinated C1−C8 alkanes and alkenes led to the development of QSARs for the induction of aneuploidy and toxicity in *Aspergillus nidulans*; the QSARs correctly predicted the activity of further six halogenated methanes.[98] More chemicals were experimentally tested, and new QSAR models were developed.[97] These were very similar to the previous ones. For each compound, the following biological measures were performed:

(1) the dose able to block mitotic growth (ARR);

(2) the dose with 37% of survival (D_{37});

(3) the lowest efficient concentration in aneuploidy induction (LEC).

The chemical descriptors considered were log *P*, MR, HOMO, LUMO, their difference HOMO − LUMO (DIF).

The QSAR models for the extended set of chemicals were

$$\log(1/D_{37}) = 0.24 + 0.08\mathrm{MR} - 5.13\ \mathrm{DIF}$$
$$n = 55,\ r = 0.80,\ F\ \text{ratio} = 45.45 \tag{45}$$

$$\log(1/\mathrm{ARR}) = 0.16 + 0.09\mathrm{MR} - 5.28\mathrm{DIF}$$
$$n = 55,\ r = 0.86,\ F\ \text{ratio} = 75.68 \tag{46}$$

$$\log(1/\mathrm{LEC}) = 0.83 + 0.07\mathrm{MR} - 4.91\mathrm{LUMO} - 3.41\mathrm{DIF}$$
$$n = 24,\ r = 0.94,\ F\ \text{ratio} = 69.07 \tag{47}$$

In all the above equations, MR was far more important than the electronic parameters.

Equation 48 regards only 24 chemicals, which resulted to be positive for induction of aneuploidy. A QSAR model for discriminating between aneuploidizing (mutagenic) and inactive chemicals was formulated. The model was based on LUMO, MR, and Iz (inertia momentum along the *z*-axis) and had 86% accuracy.

The lack of log *P* in the model for aneuploidy seems to exclude that this mutational event is driven by aspecific disturbance at the cell membranes. At the same time, the available experimental evidence allows ruling out the induction of concurrent DNA damage, as expected for strong electrophilic compounds capable of a direct interaction with nucleophilic centers on DNA. The authors suggest the involvement of reductive metabolism and the generation of free radicals that would damage critical targets of the mitotic apparatus.

2.8. Direct-Acting Compounds

2.8.1. Mutagenicity of Platinum Amines

A set of 13 substituted (*o*-phenylenediamine)-platinum dichlorides, tested for their mutagenicity in *S. typhimurium* TA92 strain, was studied by Hansch et al.[99] These are chemotherapic agents, and are direct-acting mutagens, since they are active in *S. typhimurium* without activation by microsomes.

Mutagenicity with TA92 strain was modeled by

$$\log(1/C) = 2.23(\pm 0.32)\ \sigma^- + 5.78(\pm 0.18)$$
$$n = 13,\ r^2 = 0.956,\ s = 0.260 \tag{48}$$

where *C* is the molar concentration of mutagen producing 30 mutations above background.

The term σ^- electronic term indicates that electron withdrawal through resonance is an important determinant of the mutagenic activity, probably because the electron-withdrawal weakens the nitrogen−platinum bond. The author remarked the absence of a hydrophobic term in eq 49 and related this to the fact that these compounds do not need metabolic activation to attack the DNA.

2.8.2. Mutagenicity of Lactones

Lactones are reactive chemicals widely used as intermediates in chemical synthesis, solvents, constituents of paint removers, and antibacterial agents. They are mutagenic in test systems without metabolic activation.

The mutagenicity of lactones on *S. typhimurium* TA100 strain was modeled by LaLonde et al.:[100]

$$\log \mathrm{Mn} = -6.50\mathrm{LUMO} - 6.24$$
$$n = 58,\ r^2 = 0.925 \tag{49}$$

Mn is the mutagenic response of each compound in TA100; *n* does not represent 58 different compounds but only 10 compounds, each tested a number of times. LUMO was calculated with the MNDO-PM3 program. Electrophilicity (LUMO) was found to be correlated with the mutagenic activity; no hydrophobic term appears in the model.

The mutagenicity data for another set of lactones (24 halogenated furanones) was studied by Tuppurainen.[101] 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX, Mutagen-X) and its analogues are strong direct-acting bacterial mutagens. They can be formed from the chlorine bleaching of wood pulp and the chlorination of humic waters. Whereas unsubstituted lactones are generally weakly mutagenic, MX and its analogues are special cases with halogens and double bonds imparting electrophilicity. MX is one of the most potent mutagens ever tested in *S. typhimurium* TA100 strain. Specific features of these mutagens are that they exhibit strong mutagenic activity in only one tester strain (TA100), and there is no evidence of formation of stable adducts

with DNA. The QSAR model obtained was:

$$\ln \text{TA100} = -12.7(\pm 1.1)\text{LUMO} - 12.0(\pm 1.3)$$
$$n = 24,\ r = 0.930,\ s = 1.33,\ F = 141.0 \quad (50)$$

It appears that an electrophilic π electron system (low LUMO energy values) promotes mutagenic potency, as in eq 49. An hydrophobic term did not improve the QSAR model. The lack of the hydrophobic term is rather characteristic of direct-acting mutagens (that do not require metabolic activation).[11]

On the basis of the QSAR found and on a range of evidence, the author conjectures that the halogenated furanones can undergo one-electron transfer during nonbonding interaction with DNA. As a matter of fact, the GC sites (predominant target for TA100 mutagens) on DNA provide the most stable centers for a positive hole in DNA. This hypothesis also agrees with the lack of evidence for significant DNA adducts.

2.8.3. Mutagenicity of Epoxides

Aliphatic epoxides have found widespread use as valuable industrial and laboratory alkylating agents, which has led to much interest in their potential genotoxicity.

The mutagenicity of propylene oxides with *S. typhimurium* has been studied by Hooberman at al.[102] They found the following QSAR:

$$\log \text{TA100-LSA} = 1.28(\pm 0.37)\text{NICOT} - 0.56(\pm 0.21)$$
$$n = 14,\ r^2 = 0.823,\ s = 0.302 \quad (51)$$

TA100-LSA is a measure of mutagenicity in TA100 strain using the liquid suspension assay (without metabolic activation), expressed as revertants/nmol. NICOT is the rate constant for the reaction with nicotinamide, which is used as a model for the stereoelectronic effect of the chemical in the DNA reaction (alkylation of the model nucleophile nicotinamide). Experimentally determined hydrophobic values did not improve the correlation, neither did other calculated parameters [Taft σ^* values, MR, STERIMOL parameters (L,B1−B4), molecular volumes (υW)].

Another study on a very small dataset ($n = 6$) of 3- and 4-substituted styrene oxides tested on *E. coli* gave a similar QSAR (based on the electronic reactivity parameter σ, and with no hydrophobic term):[103]

$$\log \text{rev/nmol} = -1.93(\pm 0.57)\sigma^+ + 1.40(\pm 0.12)$$
$$n = 6,\ r^2 = 0.956,\ s = 0.101 \quad (52)$$

QSAR models of mutagenicity of 7 p-substituted styrene oxides were developed by Tamura et al.[104] The biological activity was measured by C, molar concentration required to induce a mutation frequency of 10^{-6} on *S. typhimurium* TA100 strain. In addition, both the mutation frequency at the dose of 1 mM [$MF_{(mM)}$] and the mutation frequency at the lethal dose LD_{50} [$MF_{(LD50)}$] were considered. The toxic activity was considered as well, as measured by LD50.

The QSAR for toxic activity was

$$\log(1/\text{LD50}) = 1.863(\pm 0.390)\pi + 2.421$$
$$n = 8,\ r = 0.979,\ s = 0.164,\ r^2 = 0.958 \quad (53)$$

For mutagenicity (excluding *p*-nitrostyrene oxide because of its particular metabolic pathway):

$$\log 1/C = -1.010(\pm 1.441)\sigma + 8.763(\pm 9.034)\log[V_{w(X)}/V_{w(H)}] - 0.505(\pm 2.102)\pi + 3.077$$
$$n = 7,\ r = 0.970,\ s = 0.238,\ r^2 = 0.940 \quad (54)$$

$$\log \text{MF}_{(mM)} = -1.201(\pm 1.555)\sigma + 10.432(\pm 9.747)\log[V_{w(X)}/V_{w(H)}] - 0.704(\pm 2.267)\pi - 5.875$$
$$n = 7,\ r = 0.973,\ s = 0.256,\ r^2 = 0.946 \quad (55)$$

$$\log \text{MF}(\text{LD}_{50}) = -1.718(\pm 0.840)\sigma + 12.943(\pm 5.252)\log[V_{w(X)}/V_{w(H)}] - 3.711(\pm 1.227) - 5.012$$
$$n = 7,\ r = 0.985,\ s = 0.139,\ r^2 = 0.970 \quad (56)$$

where $V_{W(X)}$ and $V_{W(H)}$ are the van der Waals volumes of X-subsituted and unsubstituted styrene oxides, respectively.

It appears that the lethal effects (LD50) depend only on hydrophobicity and not on chemical reactivity. This is a general effect for aspecific toxicity.[11] Mutagenicity (eqs 54−56) is correlated with epoxide reactivity (σ) and steric effects. In eqs 54 and 55 also a hydrophobic term appears.

3. Nongenotoxic or Epigenetic Carcinogens

Epigenetic carcinogens are agents that act through a secondary mechanism that does not involve direct DNA damage. They are inactive in the classical genotoxicity tests. In reality, the demarcation is seldom absolute: it would probably be more accurate to define carcinogens as predominantly genotoxic or epigenetic. In general, genotoxic carcinogens tend to induce tumors in more than one species (multispecies) or more than one target organ (multitarget), whereas epigenetic carcinogens tend to induce tumors in a single target organ/tissue/cell or a narrowly related set of target organs/tissue/cells. Whereas the action of genotoxic carcinogens has an underlying unity in the fact that they attack directly, or after metabolic activation, the genetic macromolecules, the epigenetic carcinogens act through a myriad of mechanisms; thus each class has to be described individually.

In the past several years, there has been an explosive growth of the scientific literature on epigenetic/nongenotoxic mechanisms of chemical carcinogenesis. A very detailed recent review on the argument is ref 6. Here I will summarize briefly the main points of this presentation, mainly emphasizing

the few examples in which quantitative modeling of SAR has been performed.

One important class of epigenetic carcinogens is peroxisome proliferators. Peroxisomes are single-membrane organelles found in mammalian cells and cells of some other organisms. A major role of peroxisomes in the liver is modulation of lipid homeostasis, including the metabolism of long-chain fatty acids and conversion of cholesterol to bile salts. Out of over 100 peroxisome proliferators identified to date, about 30 chemicals have been adequately tested and shown to be carcinogenic (primarily inducing tumors in the liver). They belong to different chemical classes, including: (a) phenoxy acid derivatives; (b) alkylcarboxylic acids and precursors; and (c) phthalate esters. One of the characteristic features of many, but not all, of these peroxisome proliferators is the presence of an acidic function. This acidic function is usually a carboxyl group either present in the parent structure or formed after metabolism. QSAR studies have pointed out to similarities in the three-dimensional structures for a number of peroxisome proliferators and (for a series of phthalate esters or clofibrate analogues) to a correlation between induction of peroxisomal enzyme activities and electronic structural parameters.[105,106]

Another nongenotoxic mechanism of carcinogenesis regards Ah receptor-mediated and other enzyme inducers. The aryl hydrocarbon receptor (AhR) mediates the broad spectrum of toxicity of the extremely potent environmental toxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Activation of AhR leads to induction of Phase 1 (e.g., CYP1A1, CYP1B2, CYP1B1) and Phase 2 (e.g., glutathione *S*-transferase) drug metabolizing enzymes, modulates the expression of many genes, and causes hepatotoxic response, immunotoxicity, developmental and reproductive toxicity, disruption of endocrine pathways, chloracne, tumor promotion, and carcinogenesis. From the chemical structural point of view, there are three major categories of ligands: (a) polyhalogenated hydrocarbons, (b) PAHs, and (c) indolecarbazole type phytochemicals. With some possible mechanistic overlap with AhR, the induction of certain cytochrome P-450 isozyme has long been regarded as a predictor of potential rodent hepatocarcinogenicity because of its cell proliferative activity and its ability to activate some classes of chemicals. The above notion underlies the development of a computerized system (computer optimized molecular parametric analysis for chemical toxicity or COMPACT) that models the ability of chemicals to become substrates for the P-450 enzymes family or to interact with the Ah receptor; the model is based on steric (planarity) and electronic parameters. The COMPACT assessment has been proposed as predictor of carcinogenicity and used in the framework of comparative exercises on the prediction of rodent carcinogenicity (see following sections).[107,108]

The above mechanistic classes of nongenotoxic carcinogens have been the subject of a limited number of QSAR analyses. It should be remarked that the literature contains more experimental data potentially suitable for further modeling. The same applies to the following classes, for which only qualitative structure−activity considerations have been presented.

One class of nongenotoxic carcinogens acts via inhibition of Gap junctional intercellular communication (GJIC). GJIC is a form of "local communication" between adjacent cells via a membrane-bound protein structure (the gap junction) which facilitates direct cytoplasm-to-cytoplasm exchange of small molecules (<900 Da) such as ions, growth factors, and hormonal second messenger. It is an important means to control cell proliferation and differentiation to ensure homeostasis. A wide spectrum of tumor promoters and carcinogens have been shown to inhibit GJIC. The inhibition appears to be reversible. Among others, this class includes perfluorinated fatty acids, dialkyl phthalates, and PAHs.

Another class is composed of agents that cause oxidative stress. Oxidative stress arises when the production of reactive oxygen species (ROS) or intracellular free radicals overrides the antioxidant capacity of the target cells. Free radicals and ROS can readily react with most biomolecules to initiate a chain reaction of free radical formation which can be terminated only after reaction with another free radical or free radical scavengers such as antioxidants. ROS can react with all the macromolecular machinery of a cell, particularly lipids, protein, and DNA. The major structural classes of chemical carcinogens that can induce oxidative stress are (a) peroxides; (b) polyhaloalkanes such as carbon tetrachloride; (c) quinones; (d) PAHs; (e) phenolics; (f) homocyclic and heterocyclic aromatic amines; (h) nitroaromatics; (i) hydrazines; (j) metals such as As, Cr, Cu, Fe; (k) mineral fibers such as asbestos; and (l) miscellaneous agents such as potassium bromate, nitroalkanes.

Alteration of the extent of methylation at the 5-position of cytosine in DNA is an important epigenetic mechanism that can regulate gene activity, which in turn can contribute to carcinogenesis. Differential methylation of DNA is a determinant of higher order chromatin structure, and the methyl group provides a chemical signal recognized by transacting factors that regulate transcription and expression of genes. Under normal conditions, the extent of DNA methylation in each target/organ/cell is maintained at a level consistent with its status of cell replication or its cell differentiation-programmed need for gene expression.

Some nongenotoxic carcinogens cause hormonal imbalance. The endocrine system is under delicate homeostatic control involving complex feedback regulatory networks: the central controlling unit is the hypothalamus−pituitary axis. Because of the negative feedback relationships, stoppage of secretion by a target gland or elimination of the secreted hormone leads to overproduction of the respective pituitary trophic hormone. If this is maintained for a long time, tumor will develop in either the overactive pituitary gland or the overstimulated target gland. The specific mechanisms for this are various and involve a large number of chemical classes (including many pesticides).

Compensatory regenerative cell proliferation, in response to cell necrosis caused by cytotoxic agents, is a common nongenotoxic mechanism associated with many carcinogens. Unlike receptor-mediated mechanisms, cytotoxicity-induced cell proliferation only occurs at high doses that overwhelm the cellular defense mechanisms. A particular mechanistic class provokes $\alpha_{2\mu}$-globulin nephropathy, through an excessive accumulation of $\alpha_{2\mu}$-globulin-containing Hyaline droplets in renal proximal tubules ensues. Despite the chemical structural diversity of the renal carcinogens which act via the $\alpha_{2\mu}$-nephropathy mechanism, molecular modeling showed that all active chemicals fit deeply into a hydrophobic pocket of the protein. Beyond the dimensional requirement, there is also a requirement for a degree of lipophilicity and a need for an electronegative atom in the molecule or its active metabolite.[109]

As remarked above, the nongenotoxic carcinogens act through a myriad of different mechanisms, and the rationalization of this area (in terms of SARs) is still poor. However, a number of very general conclusions on our present ability to assess their risk with QSAR approaches can be attempted.

In a study on the prediction of the carcinogenicity of pharmaceutical drugs via human expert considerations (i.e., largely based on the knowledge of the SAs),[110] up to 84% of the chemicals that were carcinogenic in the four rodent groups were correctly predicted, against 30% correct prediction for the chemicals carcinogenic only in one rodent group. Since the knowledge of the SAs of genotoxicity is more advanced than that for nongenotoxic carcinogens, this may indicate that the chemicals with a wider carcinogenic activity spectrum (hence probably more harmful) are more likely to act via genotoxic mechanisms than those acting through epigenetic mechanisms. At the same time, we are better equipped to recognize the carcinogens with a wider spectrum of potential targets.

The above optimistic evidence goes hand in hand with the fact that the epigenetic carcinogens are probably a minority in the general database of used chemicals[111] (except perhaps among the pharmaceutical drugs) and that some studies seem to indicate that the carcinogenic potency of epigenetic carcinogens is remarkably lower than that of genotoxic carcinogens.[112] If this is correct, the risk for the human health deriving from the difficulties in identifying the epigenetic carcinogens is less dramatic than that posed by lack of recognition of genotoxic carcinogens.

However, a word of caution should be spent here to counterbalance the above considerations. It should be remembered that the hazard posed by a carcinogen depends both on its intrinsic carcinogenic potency and on the extent of human exposure. Even very weak carcinogens, like benzene, can have disastrous effects if the population exposed is very large.[113] Thus, even if the nongenotoxic carcinogens pose a lower intrinsic threat to human health than the genotoxic carcinogens, they are still to be considered as a primary area of health concern.

4. QSAR Models For Noncongeneric Chemicals

The application of QSAR modeling to individual classes of chemicals, acting through distinct mechanisms of action, constitutes the area where the classical QSAR approaches give their best contribution, both in terms of understanding the biological activity (here, mutagenicity and carcinogenicity) and of predictive ability. The restriction of traditional QSAR approaches to congeneric chemicals represents both the key to its success and an inherent limitation to more general applications.[11] This is particularly evident in toxicology, where diverse chemical structures are studied for reasons that rarely take into consideration QSAR modeling concerns. In addition, the database of experimental results is not populated of enough representative chemicals to provide a sufficiently representative basis for modeling the carcinogenicity or mutagenicity of each chemical class of interest; this is also because the chemicals of practical interest change with the time since new compounds (pharmaceuticals, dyes, etc.) are produced and marketed every year. The main consequence is of practical order and regards the use of QSAR for risk assessment of chemicals devoid of experimental data: in the historical carcinogenicity and mutagenicity database, it is often impossible to retrieve chemicals congeneric to a query one, to derive QSAR models for the predictions. Despite the challenges, there is strong motivation to develop QSAR models for toxicity predictions for use in screening, for setting testing priorities, and for reducing reliance on animal testing. This need has motivated attempts to construct "general" QSAR models, not tailored to congeneric classes of chemicals. A scientific aspect of this is the interest in exploring nonapparent associations crossing traditional chemical class boundaries. Most of the general QSAR modeling efforts, to date, have focused on the challenge of predicting rodent carcinogenicity, with efforts to predict mutagenicity viewed as a component of this challenge.

Issues and difficulties related to the concept itself of "general" QSAR models have been addressed in a number of publications.[114−117] Since, typically, multiple mechanisms of action can lead to the same toxicity endpoint, this entails distinguishing islands of activity from within a sea of inactive chemicals, with each island representing a local chemical biological mechanism domain. In practice, a "general" QSAR model has to model, simultaneously, the various different mechanisms of action of the various types of chemicals present in the studied set; this task is obviously much more demanding than describing the variation of potency or the difference between active and inactive chemicals relative to a homogeneous class of chemicals, based on the variation of a limited number of chemical features. Thus, the concept of a general model is seemingly at odds with the inherently local nature of chemical-class based QSAR approaches; as a matter of fact, all "general" models consist of multiple local models, whose definition may or may not be readily apparent.[117]

General models for the prediction of mutagenicity and carcinogenicity have ranged from purely heuris-

tic human expert judgment, to those that require prior hypothesis and human intervention in choosing relevant parameters, to automated rule-based approaches derived from human expert knowledge, to automated statistical, machine learning and pattern-recognition approaches that independently derive algorithms for prediction from existing data. Many of these approaches are driven by the recognition of SAs in the molecules. SAs are chemical substructures or functional groups that have been mechanistically and/or statistically associated with the induction of mutations or cancer. At a more sophisticated level, the prediction methods contain information on both known SAs and modulating factors. Some approaches for the prediction of the long-term carcinogenicity bioassay also include the knowledge on short-term test results, like the *S. typhimurium*, as basis for the prediction. A generalized useful distinction is between (a) statistically based approaches, which attempt to analyze objectively noncongeneric datasets to extract empirical models (in analogy with the classical QSAR approach); and (b) knowledge-based approaches, which attempt to bring diverse types of a priori information, in the form of generalized rules. Some of these approaches have been implemented into commercial software programs. Detailed reviews are refs 118−121.

Here examples of the various categories of systems will be briefly presented, whereas applications and results will be given more in detail.

4.1. Statistically Based Approaches for Noncongeneric Datasets

Statistically based methods attempt to uncover general associations useful for hypothesis generation and prediction. These methods use, for example, statistical, multivariate, rule-induction, artificial intelligence, cluster analysis, or pattern-recognition techniques to analyze diverse, noncongeneric toxicity databases in unbiased ways, i.e., with limited or no prior chemical or biological classification according to mechanism. Examples include the commercial programs CASE, MULTICASE, TOPKAT, and ADAPT, as well as other methods that have not been implemented into commercial softwares.

CASE and MULTICASE are well-known representatives of a very characterized QSAR approach, which is distinguished from other approaches by its central reliance on computer-generated substructural fragments, which are its major type of molecular descriptors, and the completely automated and unbiased manner in which these descriptors are generated and chosen for inclusion in the QSAR model. In this the CASE technology relies on previous research, whose first examples can be found in refs 122 and 123. Along the same line of thought is the more recent PROGOL program.

In CASE, each of the molecular structures belonging to the training database is decomposed by the program into all possible constituent fragments of length 2−10 contiguous heavy atoms, with attached hydrogens and one possible side chain. The statistical analysis of the set of fragments generated by the decomposition of all molecules in the training set involves examination of the distribution of each unique fragment among active and inactive molecules and identification of fragments whose distribution deviates from an ideal symmetrical binomial distribution: each of the fragments significantly deviating from the reference distribution is labeled either a biophore (activating fragment) or a biophobe (inactivating fragment). Biophores and biophobes are the primary molecular descriptors of the CASE QSAR model: this may be expressed either as a (Bayesian) activity/inactivity probability or as a linear regression relating a potency to the substructural descriptors.

PROGOL, as CASE, is open-ended: it examines the chemical structures on its own and does not depend on predefined lists of chemical substructures. However, the implementations are different. PROGOL looks at atoms, their bond connectivities, and higher order molecular structures and forms fully relational descriptions of chemical structures: these descriptions are manipulated through the inductive logic programming (ILP) algorithm. PROGOL finds rules in form of relational patterns.

MULTICASE (MULTIple Computer Automated Structure Evaluation) is a development of the CASE program, and it is interesting since it evolved from the recognition of problems found in the course of CASE analyses. In particular, MULTICASE responds to the problem of distinguishing between fragments that provoke the activity and fragments that modulate the activity. In more general terms, it attempts to face the presence of hierarchy and nonlinearity within SAR models as applied to noncongeneric sets of chemicals. As CASE, MULTICASE starts by creating its own dictionary of descriptors directly from the database. At this point, and in contrast to CASE, MULTICASE selects the statistically most important of these fragments as a biophore, believed to be responsible for the observed activity of those molecules that contain it, and separates out all the molecules containing this biophore from the remaining database. This process is repeated on the remaining database with the next most significant biophore, and so on, until the database is segmented into major biophore-containing chemical classes. CASE analysis is then applied to each biophore class separately to determine substructural modifiers to the biophore activity.

References to CASE and MULTICASE are 94 and 124−131. An implementation of MULTICASE, tailored specifically on the issue of predicting the carcinogenicity of pharmaceutical drugs, is in ref 132. Independent software implementations of the CASE technology were developed by Parodi and collaborators.[133−135] References to PROGOL are 136−139.

Another type of approach are methods that look for statistical associations starting from predefined lists of molecular descriptors. A commercial implementation is exemplified by Toxicity Prediction by Komputer Assisted Technology (TOPKAT). It is an automated computer system consisting of different modules for the prediction of a variety of acute and chronic toxic endpoints (rodent carcinogenicity and *S. typhimurium* mutagenicity, but also developmental toxicity, skin and eye irritation, acute oral toxicity

LD50, etc.). Each model has been derived from a specific database.

Whereas earlier versions of the TOPKAT equations depended, to a substantial extent, on the presence or absence of substructural fragments, the recent versions use only continuous-valued descriptors. These include topological shape and symmetry indices, and electrotopological state (E-state) on one- and two-atom fragments. The latter indices permit the transformation of information relative to the presence or absence of fragments (selected from a predefined list of about 3000 substructures) into quasi-continuous-valued structural descriptors. Unlike fragment substructures, the latter take into approximate account the steric/electronic environment of the fragment and conform more easily to the various statistical analyses. TOPKAT performs the assessment relative to the query chemical in four steps. In the first step, TOPKAT determines if the chemical is "covered", on a univariate basis, by the structural fragments in the training set. This step identifies fragments in the query chemical not contained in the training set compounds. In most cases, the presence of such a fragment is sufficient to cause the toxicity assessment to be rejected as unreliable. The second step checks whether the chemical fits within the optimum prediction space (OPS) of the estimating equation. This enables the user to determine whether the test structure is contained in the descriptor space of the model: a chemical that is out of the OPS will have little confidence associated with its toxicity prediction. The third step assesses the toxicity of the chemical. In the fourth step, TOPKAT allows the user to perform a further independent test on the reliability of the assessment through a similarity search in the database. The training database is scanned for molecules "similar" to the query molecule, to independently assess the possible chemical or biological significance of model associations. In practice, the user can check both the actual toxicity of "similar" chemicals and the accuracy of the TOPKAT prediction generated for the similar chemicals to assess model reliability in that portion of the chemical biological activity space.

Selected references for the TOPKAT methodology and its applications are refs 140−143.

Another example of statistically based approaches for noncongeneric datasets is provided by Automated Data Analysis and Pattern Recognition Toolkit (ADAPT). Each compound is represented by calculated molecular structure descriptors encoding the topological, electronic, geometrical, or polar surface area aspects of the structure. Topological descriptors use only the connection table of a molecule and therefore do not require accurate three-dimensional (3-D) optimized structures. These descriptors encode simple counts of atom types, bond types, connectivity indices, and interatomic distances: they correlate with molecular size, shape, and degree of branching. Geometric descriptors, which encode information on the overall size and shape of a molecule and require 3-D geometries, including length-to-breadth ratios, two-dimensional (2-D) shadow projection areas, and solvent accessible surface areas. Examples of electronic descriptors are atomic partial charges, dipole moment, electron−core repulsion energies, and charged partial surface areas. Hundreds of descriptors can be calculated for each compound. To control redundancy, very small subsets of descriptors, usually 3−10 descriptors per model, are identified in a stepwise manner. Finally, the QSAR model for the biological activity of interest is found by applying separately different pattern-recognition methods (in recent papers, they include k-nearest neighbor, linear discriminant analysis, probabilistic neural networks, and support vector machines) and selecting the preferred model(s). Selected references for ADAPT are refs 114, 144, and 145.

Within the framework of an analysis of both congeneric and noncongeneric sets of mutagens, extensive use of topological (topochemical and topostructural), geometrical, and quantum chemical descriptors was made in Basak's laboratory.[146] The above paper also contains an interesting theoretical discussion on chemical parameters.

As a last example of a statistically based approach for noncongeneric chemicals, I will quote an application in which substructural fragments were analyzed with back-propagation neural networks. The initial set of data comprised 1280 fragments, relative to a database of 607 chemicals tested for *S. typhimurium* mutagenicity. The paper also shows how the analysis of the hidden layer permitted understanding of how the neural network performed its classification.[147]

4.2. Knowledge-Based Approaches for Noncongeneric Datasets

The other large area of systems used for modeling noncongeneric datasets includes expert system approaches that attempt to codify existing knowledge, derived from human expert judgment, bioassay data, or any of the previous modeling approaches, into generalized rules for use in prediction. Examples include the DEREK system, applicable to prediction of multiple toxicity endpoints, and the OncoLogic system, restricted to chemical carcinogenicity. Each of these expert systems serves as a repository for existing knowledge, and each rule conveys an explicit hypothesis at the local SAR level that can be refined and modified as further information becomes available. Hence, expert systems do not discover new associations but rather can be considered the end-stage of the model development process.

OncoLogic was developed for the express purpose of capturing, and making available for outside use, expertise in the field of structure-based mechanisms of chemical carcinogenesis routinely being applied in regulatory setting by the U.S. Environmental Protection Agency. Mechanism-based SAR analysis has been effectively used by the EPA for many years to assess the potential carcinogenic hazard of new chemicals, for which there are no or scanty data, under the Premarketing/Premanufacturing Notification program of the Toxic Substance Control Act. Essentially, mechanism-based SAR analysis involves comparison of an untested chemical with structurally related compounds for which carcinogenic activity is known. The entire process is described with great

clarity in ref 9: "All available knowledge and data relevant to evaluation of carcinogenic potential of the untested chemical are considered. These include a) SAR knowledge base of the related chemicals; b) toxicokinetics and toxicodynamics parameters (including physicochemical properties, route of potential exposure, and mode of activation or detoxification) that affect the delivery of biologically active intermediates to target tissue(s) for interaction with cellular macromolecules or receptors; c) supportive noncancer screening or predictive data known to correlate to carcinogenic activity."

OncoLogic is a computer program consisting of four independent subsystems for estimating the carcinogenicity of fibers, metals or metal-containing compounds, polymers, and organics. Each subsystem has a hierarchical, decision-tree construction consisting of "IF-THEN-ELSE" rules that attempt to mimic the reasoning of the human experts. This reasoning goes beyond the recognition of specific SAs to consider general reactivity properties of the chemical class, structural modulators to activity, metabolic activation, and mechanisms of chemical carcinogenesis. An enhancement allows for consideration of functional, noncancer toxicity data (e.g., genotoxicity, oncogene activation, P-450 induction, etc.) in the overall decision tree to improve the chemical carcinogenicity evaluation capabilities. The organics subsystem in OncoLogic is by far the largest and most well developed of the four subsystems, with separate and distinct modules for nearly 50 chemical classes (examples include acrylates, aldehydes, and aromatic amines), although these modules vary considerably in coverage and information content.

OncoLogic differs from other prediction systems in that a query molecule is not entered at the start of the analysis. Rather, a carcinogenicity evaluation of an organic chemical begins with user assignment of the chemical to one of the predefined chemical classes and proceeds through selection of structural templates or user-drawn entry of structures within the constraints of the chosen class. Finally, the program produces, as its primary output, a detailed justification report in which the discreet program rules are converted into a dialogue that intelligibly conveys the mechanism-based expert reasoning underlying the semiquantitative evaluation. OncoLogic rules are all based on qualitative associations with chemical structure, i.e., the program has no capabilities for computing physical chemical properties. References to OncoLogic are 148 and 149.

Another rule-based expert system is Deductive Estimation of Risk from Existing Knowledge (DEREK), which is the result of a nonprofit collaboration among the University of Leeds, and various other educational and commercial institutions, who contribute to the review and evolution of the toxicity rule bases. Also confidential in-house information from industries is used. In DEREK, rules (of the type "IF-THEN-ELSE") associate particular chemical functional groups, or SAs, with various forms of toxicity. The rules are not chemical-specific; rather they are generalizations with respect to chemical structure (e.g., halogen-containing, acid, or alkylating agent). The resulting generalized structural features used in prediction are termed toxophores.

The toxicological end points currently covered by the DEREK system include carcinogenicity, mutagenicity, skin sensitization, irritancy, teratogenicity, and neurotoxicity. Each toxicity endpoint consults a different rule base and a set of toxophores. To interrogate the system, the query structure is entered, and the rulebase is searched by comparing structural features in the target compound with the toxophores described in its knowledge base. Any SA located within the query structure is highlighted, and a message indicating the nature of the toxicological hazard is provided. References to DEREK are 150 and 151.

4.3. The Predictive Ability of the QSARs for Noncongeneric Chemicals

The classical QSARs for congeneric classes of chemicals have a wide scope. In fact, they provide information on the action mechanisms, by pointing to the crucial properties/features of the chemicals. They point to outliers, thus helping to identify possible subsets of chemicals that either have different mechanisms of action or need more careful experimental testing. Finally, they permit prediction of the activity of chemicals, of the same class, which are devoid of experimental data. On the other hand, the "general" approaches for noncongeneric chemicals are able to provide little or no mechanistic information, and their value mainly resides in their ability to generate correct predictions of activity for untested chemicals. Thus, the assessment of the predictive ability is crucial to their use for risk assessment purposes. Usually, the authors report some accuracy measure of the system performance, which varies according to the type of method. A popular statistical approach is to split the available database into a training and a test set of chemicals: the model is built using only the training set, and then the predictive ability is checked by applying the model to the test set. The procedure is applied several times (internal validation, cross-validation).[15,152] A more stringent criterion is external validation: the predictions are performed by applying the model to compounds whose experimental results did not exist or were unknown to the investigators when the model was generated.

Several external validation exercises relative to QSARs for noncongeneric chemicals are available in the literature, and all together they contribute to assess the real value of the different approaches, and, most importantly, to point to the crucial issues in this field. Some of the external validation studies considered one (or a few) system(s). A number of studies were prediction exercises, in which a range of systems were challenged to predict the toxicity of a common set of chemicals, thus permitting one to compare the systems directly to each other. Particularly important were three comparative exercises held under the aegis of the U.S. National Toxicology Program (NTP). These were prospective prediction exercises: the exercises invited the modeling community to submit predictions on the rodent carcinogenicity (two stud-

ies) or *S. typhimurium* mutagenicity (one study) of chemicals that were in the process of being assayed by the NTP. It should be remarked that the experimental results were not known at the time of the prediction, thus contributing to the unbiased character of the assessment. I will present a summary of the various validation studies, and, because of their importance, I will expand more on the NTP studies.

In the prediction exercises that will be presented in this paper, the systems generated dichotomous predictions of the toxicity of the chemicals in the form yes/no, positive/negative. A comprehensive description of the predictive ability of a model that produces such dichotomous results is obtained through the calculation of several performance indices, namely: (a) accuracy, or concordance (percentage of chemicals correctly predicted); (b) sensitivity (percentage of positives correctly predicted); (c) specificity (percentage of negatives correctly predicted); (d) positive predictivity (percentage of chemicals correctly predicted as positive, out of all the chemicals predicted as positive); (e) negative predictivity (percentage of chemicals correctly predicted as negative, out of all the chemicals predicted as negative). Here, positive and negative indicate the chemicals that resulted as, respectively, toxic or nontoxic in the appropriate experiments. The above indices are very useful for a detailed analysis of a system; however, they do not permit an easy and direct comparison of a range of systems. For this reason, I will use mainly the receiver operating characteristic (ROC) space graphs, which have the advantage of comparing simultaneously the different aspects of the performance of several systems. For example, the accuracy index alone does not distinguish between positive and negative predictions and is influenced by the performance on the most numerous class, whereas the axes of the ROC graphs display independently the information relative to the prediction of positive and negative chemicals. In a ROC plot, true positive rate (or sensitivity) is plotted against the false positive rate (1 − specificity). According to the ROC curve theory, the diagonal line represents random responses, whereas the top left corner is obviously the ideal performance. Thus, the most finely tuned systems are those in the left upper triangle, as close as possible to the corner.[153]

4.3.1. Mutagenicity Prediction Models: External Validation Studies

The results of several external validation studies are summarized in the ROC graph of Figure 1. All the studies refer to applications to bacterial mutagenicity in the *S. typhimurium* assay.

The predictive ability of the expert system DEREK and of the statistically based MULTICASE and TOPKAT systems were compared in ref 154. They selected two databases of chemicals tested for *S. typhimurium* mutagenicity; the data were retrieved from open literature and U.S. government toxicity databases. A first database included 123 pharmaceutical drugs (codes DEREK1, MCASE1 and TOPKAT1 in Figure 1); the second one consisted of 516 nondrug chemicals (codes DEREK2, MCASE2, and TOPKAT2).

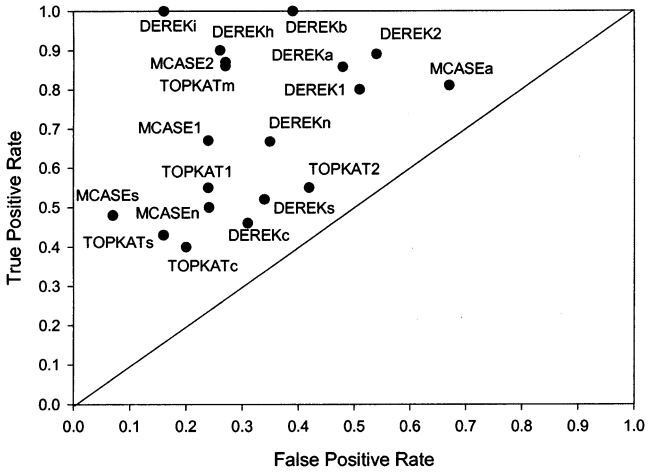

Figure 1. The ROC graph displays the performance of a number of prediction approaches for *S. typhimurium* mutagenicity, as resulted from different validation exercises on external sets of chemicals. The details of the different studies and the codes of the systems are in the text.

In another study, TOPKAT and DEREK were studied in ref 155. The results of over 400 mutagenicity tests conducted at Glaxo Wellcome during a period of 15 years were compared with the mutagenicity predictions of both computer programs. DEREK predicted the mutagenicity of 409 compounds (code DEREKc); TOPKAT predicted the mutagenicty of 303 compounds (code TOPKATc), since many of the Glaxo Wellcome chemicals were either out of the optimum prediction space of TOPKAT, or the prediction was in the indeterminate region.

A number of other validation studies were reported in ref 156. In a study, 169 new proprietary Novartis pharmaceutical candidate compounds were predicted with DEREK (code DEREKn) and MULTICASE (code MCASEn). A second study regarded 44 simple aromatic amines, whose bacterial mutagenicity was predicted with DEREK (code DEREKa) and MULTICASE (code MCASEa). A third study regarded the prediction of 27 mutagenicity tests, performed in Bayer Toxicology, with DEREK (code DEREKb). Another study regarded the application of DEREK (code DEREKi) to the prediction of 54 mutagenicity studies retrieved from the IUCLID database.

The evaluation of 83 chemicals from the open literature with TOPKAT (code TOPKATm) was performed in ref 157.

DEREK (code DEREKh) was evaluated for the prediction of the *S. typhimurium* mutagenicity of 44 chemicals in ref 158.

Finally, the ability to predict the Salmonella mutagenicity of 394 marketed pharmaceuticals through DEREK, TOPKAT and MCASE (codes: DEREKs, TOPKATs, MCASEs) was assessed in ref 159.

4.3.2. Carcinogenicity Prediction Models: External Validation Studies

The results of this series of external validation studies are reported in Figure 2.

The rodent carcinogenicity of a set of 142 pharmaceutical drugs was predicted with different systems in ref 154. The systems were (a) DEREK (code

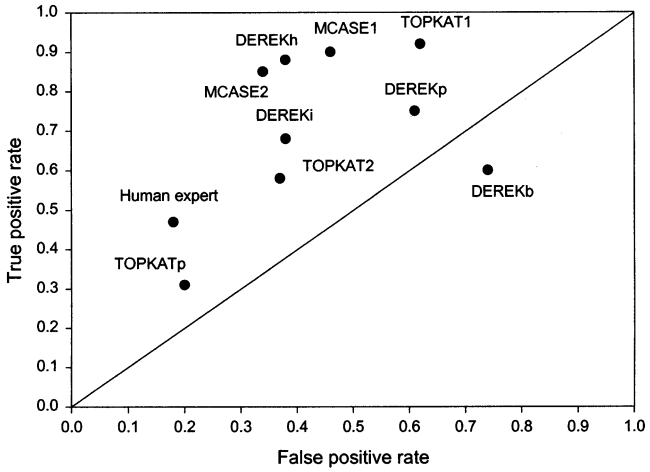

Figure 2. The ROC graph displays the performance of a number of prediction approaches for the rodent bioassay (carcinogenesis), as resulted from different validation exercises on external sets of chemicals. The details of the different studies and the codes of the systems are in the text.

DEREKp); (b) MULTICASE in the version specifically adapted by FDA to the prediction of drug carcinogenicity (codes: MCASE1, if a drug was considered carcinogenic when one rodent experimental group was positive; MCASE2, if a drug was considered carcinogenic when at least two rodent experimental groups were positive); (c) TOPKAT (codes: TOPKAT1, if a drug was considered carcinogenic when one rodent experimental group was positive; TOPKAT2, if a drug was considered carcinogenic when at least two rodent experimental groups were positive).

TOPKAT was tested also in ref 160 on a set of 117 chemicals bioassayed by NTP (code: TOPKATp).

Three external evaluation studies regarded the predictivity of DEREK. In the first study (code: DEREKb), 30 chronic rat studies performed by Bayer Toxicology were evaluated.[156] In another study, DEREK (code: DEREKi) predicted the rodent carcinogenicity of 61 compounds whose data were extracted from the IUCLID database.[156] A third study regarded 29 compounds from the IPCS database (code: DEREKh).[158]

Finally, a set of 273 pharmaceutical drugs was examined with the human expert approach in ref 110: they inspected the chemical formulas and reasoned in terms of chemical analogy with known carcinogens and noncarcinogens (code: human expert).

4.3.3. Considerations on the External Validation Exercises

The inspection of Figures 1 and 2 provides useful insights into the issue of how much the various proposed "general" prediction systems can predict the mutagenicity and carcinogenicity of chemicals. It appears that the number of studies available are in sufficient number to draw some conclusions. Moreover, the studies were essentially concentrated on the use of the three most popular commercial systems, namely, MULTICASE, DEREK, and TOPKAT.

Recalling that in a ROC graph the diagonal line represents random results and that the ideal performance is the top left corner, it also appears that, as a general trend, almost all results are in the "good" side of the diagonal line. Moreover, the cloud of mutagenicity validation studies (Figure 1) is distributed more closely to the ideal performance than is the cloud of carcinogenicity validation studies (Figure 2); this indicates that, as a trend, the prediction of mutagenic activity was more successful than was the prediction of carcinogenic activity.

The deceiving evidence is that the prediction performance of the systems is highly dependent on the set of chemicals used for the exercise. This can be easily recognized by following, in each figure, the performance of the individual systems. For example, in Figure 1 each system spans virtually the entire space in the left corner of the ROC graph, ranging from quite limited to quite good performance. This high variability is a seriously negative result since it indicates that the user can have very little confidence in the degree of reliability of a prediction.

Some of the prediction exercises in Figures 1 and 2 compared directly two or three systems, using the same set of chemicals to be predicted. For example, in ref 154, TOPKAT, MULTICASE and DEREK were challenged to predict the mutagenicity of two separate sets of chemicals (Figure 1: TOPKAT1, MCASE1, and DEREK1; TOPKAT2, MCASE2, and DEREK2) and the carcinogenicity of one set of chemicals (Figure 2: DEREKp, MCASE1, TOPKAT1, MCASE2, TOPKAT2). The other example is ref 155, where TOPKAT and DEREK were compared (Figure 1: TOPKATc, DEREKc). In the study in ref 154, it appears that MULTICASE performed constantly better than the other systems; however, the interest of this evidence is weakened by the general result of the highly variable character of the performance of all systems.

4.3.4. NTP Prospective Prediction Exercises

The US NTP sponsored comparative exercises on the prediction of (a) *S. typhimurium* (Ames test) mutagenicity (one exercise); and (b) rodent carcinogenicity (two exercises). The chemicals selected for these exercises were those assayed by NTP. The NTP exercises were characterized by the fact that (a) all the chemicals were tested with the same standardized protocol, thus contributing to the quality of the biological data; (b) the experimental results were unknown when the predictions were formulated by the various investigators. For example, several rodent bioassay results were obtained several years after the publication of the predictions. This aspect (prospective prediction) contributed strongly to the unbiased character and to the special interest of the evidence produced by the NTP exercises.

4.3.4.1. NTP Prediction Exercise on *S. typhimurium* Mutagenicity. The predictivity for *S. typhimurium* mutagenesis of two computer-based systems (TOPKAT and CASE), one physicochemical screening test (Ke), and one human expert (noncomputer-based) system were compared using 100 chemicals.[161] The chemicals were tested by the NTP, and their results had not yet been published at the time of the predictions. The results of the exercise are summarized in Table 1 and Figure 3.

Table 1. Concordance (Accuracy) of the QSAR Predictions and *S. typhimurium* Mutagenicity Results[a]

system	concordance
SA	0.72
SA+	0.76
Ke	0.60
Keb	0.61
TOPKAT	0.74
CASE/n	0.76
CASE/e	0.71

[a] In a prospective prediction exercise held under the aegis of the NTP (see details in the text).

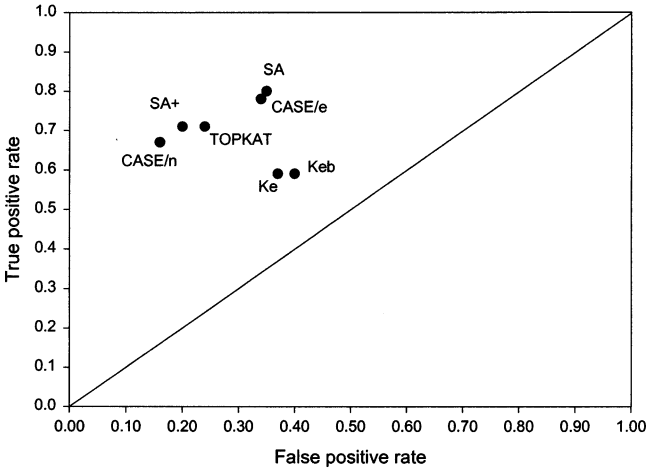

Figure 3. The ROC graph shows the performance of different prediction approaches for *S. typhimurium* mutagenicity, based on a prospective comparative exercise held under the aegis of NTP. The details of the study and the codes of the systems are in the text.

TOPKAT and CASE were described above in this paper. In this exercise, CASE was applied in two versions: (1) CASE was trained on a compilation of 820 chemicals tested for mutagenicity by the NTP (code: CASE/n); (2) CASE was trained on 808 mutagenicity results evaluated by the US-EPA Gen-Tox Program (code: CASE/e). There was partial overlapping between the two databases. The physiochemical measurement of Ke (the electron rate attachment constant) describes the potential electrophilicity of a chemical and was performed by Bakale and McCreary as described in ref 162 (code: Ke). The code Keb indicates a set of predictions in which borderline or indeterminate results were considered as negative. The human expert assessment was performed by Ashby, by identifying the chemical SAs as described in refs 8 and 163. Ashby produced two sets of predictions: (1) chemicals were checked against the list of SAs (code: SA); (2) in a secondary expert judgment, the identification of SAs was corrected with mechanistic inferences about the probability the potential electrophilicity of the SAs was actually expressed in *S. typhimurium* (code: SA+).

The inspection of Table 1 and Figure 3 indicates that the human expert approach and the two computer-based systems produced equivalent results (71−76% concordance with *S. typhimurium* results), whereas the physicochemical system (Ke) produced a lower concordance (60−61%).

Table 2. Concordance (Accuracy) of the QSAR Predictions and Rodent Bioassay Results[a]

system	concordance
Tennant/Ashby	0.75
RASH	0.68
Weisburger	0.65
Ke	0.65
DEREK	0.59
TOPKAT	0.57
Benigni	0.57
S. typhimurium	0.57
DEREKh	0.56
Lijinsky	0.55
COMPACT	0.53
CASE/MCASE	0.49

[a] In the first prospective prediction exercise held under the aegis of the NTP (see details in the text).

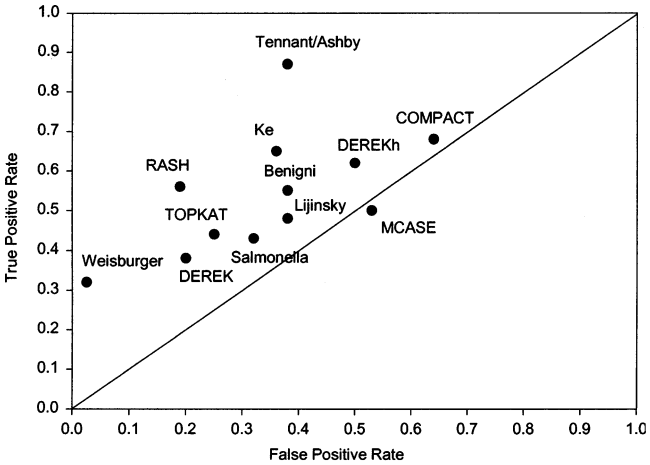

Figure 4. The ROC graph shows the performance of different prediction approaches for the rodent bioassay (carcinogenesis), based on the first prospective comparative exercise held under the aegis of NTP. The details of the study and the codes of the systems are in the text.

4.3.4.2. The First NTP Prediction Exercise on Rodent Carcinogenicity. For this exercise, 44 compounds of different chemical classes were selected.[164] The predictions presented were based on different approaches: QSAR, human experts, and experimental (see Table 2 and Figure 4).

The computerized approaches TOPKAT,[141] MULTICASE,[165] and DEREK[150] have been presented above. Other QSAR approaches were COMPACT and Benigni's approach. The computer-optimized molecular parametric analysis of chemical toxicity (COMPACT) system tried to evaluate the ability of chemicals either to be a substrate for the cytochrome P-450 I family metabolic enzymes or to be able to interact with the Ah receptor, based on the analysis of spatial and electronic properties.[107] Benigni presented predictions based on the combination of two types of information: (a) the Bakale's electrophilicity index (Ke) of the chemicals, theoretically estimated; (b) the presence of SAs in the molecules.[166] The experimental systems were Ke[167] and *S. typhimurium* mutagenicity.[168] Sets of predictions were generated by human experts as well. The human experts Tennant and Ashby AAR combined the information on SAs with other types of information: general and target organ toxicity in rodents, *Salmonella* mutagenicity, and

previous carcinogenicity data, when available in the literature.[164] The RASH (RApid Screening of Hazard) approach consisted of a modification of the Tennant approach through alternative means for ranking toxic potencies.[169] Weisburger[168] and Lijinsky[168] provided two sets of predictions based on expert judgment, using as their initial information the chemical structure of the NTP chemicals.

Results and analyses are in refs 22 and 170−172. It appeared that most of the prediction systems were concordant in the identification of the powerful carcinogens, whereas several noncarcinogens were predicted to be positive by different systems. This excessive sensitivity was attributed to the presence of alerting substructures in the noncarcinogens which were erroneously predicted. The various approaches essentially acted as gross "class-identifiers": they pointed to the presence or absence of alerting chemical functionalities but were not able to make gradations within each potentially harmful class.[22]

The overall accuracy of the predictions is reported in Table 2. For approaches that relied solely on information derived from the chemical structure, the overall accuracy in terms of positive or negative predictions was in the range 50−65%, whereas the Tennant and Ashby approach attained 75% accuracy.

4.3.4.3. The Second NTP Prediction Exercise on Rodent Carcinogenicity. A second comparative exercise on the prediction of rodent carcinogenicity was devised by the NTP, regarding 30 chemicals in the progress of being bioassayed.[173] The participating systems were very different. Several were human experts. The OncoLogic team at the U.S. EPA used the computerized expert system OncoLogic, in conjunction with human expertise; in fact OncoLogic (see description above) does not have rules for all chemical classes.[174] Huff et al. considered the presence of SAs and analogy with known carcinogens and noncarcinogens, previous bioassays when available, subchronic toxicity and relative toxicity, and reported or predicted metabolites.[175] Benigni et al. relied mostly on chemical analogy reasoning.[176] The expert judgment of Tennant and Spalding[177] and of Ashby[178] used largely biological evidence relative to the chemicals, together with SAs. Purdy[179] employed a combination of human expert rules and structure−activity information, as did the COMPACT/HAZARDEXPERT approach.[108] Human experts predictions were formulated also by Bootman[180] and the RASH approach.[181] A QSAR approach was FALS, involving the generation of eight submodels method, the universe of chemicals (from the point of view of carcinogenicity modeling) being divided into eight chemical classes.[182] Two computerized models based their predictions on organ-specific toxicity of the chemicals (R1, R2).[183] Predictions were also elaborated with the artificial intelligence systems MULTICASE[184] and PROGOL[136] and with the rule-based expert system DEREK.[185] Finally, experimental data were produced with two short-term screening tests: (1) transformation assay in Syrian hamster embryo [SHE] cells;[186] and (2) *S. typhimurium* mutagenicity assay.[173]

Results and analyses are in ref 187; they are summarized in Table 3 and Figure 5. As in the first comparative exercise, several noncarcinogens were predicted as carcinogens: these chemicals either had SAs or a clear analogy with known carcinogens. Thus, it appears that the prediction approaches were often unable to make gradations between potential and actual carcinogenicity. Table 3 and Figure 5 show that the human expert-based predictions performed best overall, especially those methods that incorporated the most information. In addition, the SHE assay, an experimental system specifically designed to incorporate key elements of the transformation process that a cell can undergo in becoming malignant, was among the best performing methods. The highest overall accuracy in this second exercise was in the range 65−70%.

Table 3. Concordance (Accuracy) of the QSAR Predictions and Rodent Bioassay Results[a]

system	concordance
OncoLogic	0.65
SHE	0.65
R1	0.64
Huff et al.	0.62
R2	0.61
Benigni et al.	0.61
Tennant et al.	0.60
Ashby	0.57
Bootman	0.53
FALS	0.50
RASH	0.45
COMPACT	0.43
DEREK	0.43
S. typhimurium	0.33
Purdy	0.32
Progol	0.29
MCASE	0.25

[a] In the second prospective prediction exercise held under the aegis of the NTP (see details in the text).

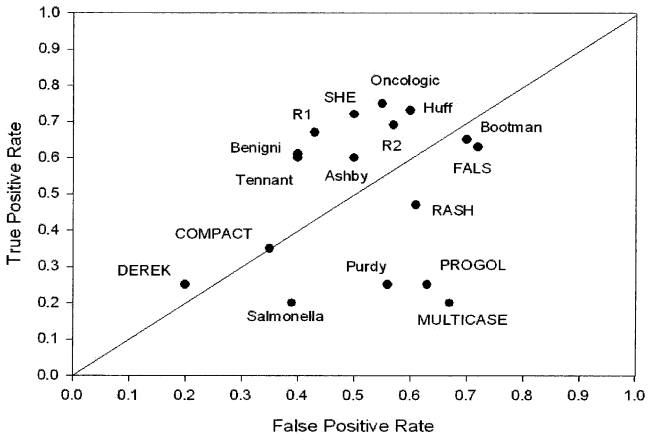

Figure 5. The ROC graph shows the performance of different prediction approaches for the rodent bioassay (carcinogenesis), based on the second prospective comparative exercise held under the aegis of NTP. The details of the study and the codes of the systems are in the text.

4.3.5. The Predictive Toxicology Challenge 2000–2001

This exercise on the prediction of rodent carcinogenicity was characterized by the fact that all methods applied machine learning (or artificial intelligence) methods. In addition, a same training set of molecules was provided to the participants: these were about 500 compounds from the NTP bioassay

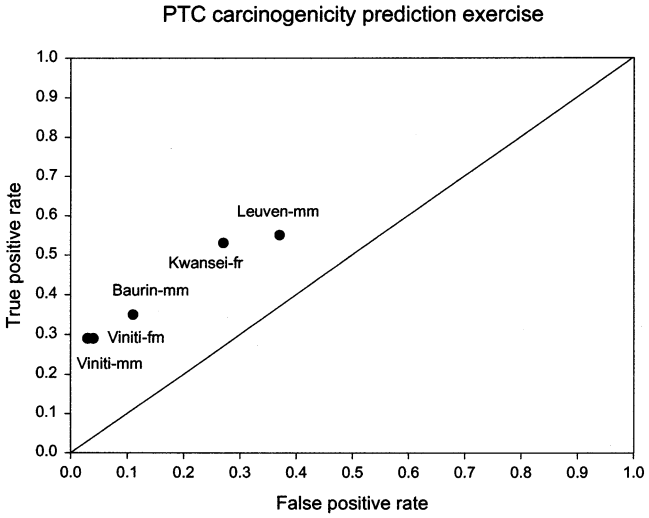

Figure 6. The ROC graph shows the performance of a number of machine learning-based systems in the Predictive Toxicology Challenge 2000−2001 (rodent carcinogenesis). The details of the study are in the text. The names in the codes (Leuven, Kwansei, Baurin, Viniti) refer to the individual scientists that submitted the predictions; their systems are fully explained in the site: http://www.predictive-toxicology.org/ptc/. The second part of the codes indicate mm = mouse, male, fr = rat, female, fm = mouse, female.

experimentation. Carcinogenicity classifications for male and female rats and mice were provided separately to account for sex and species-specific differences. Chemical structures were available as SMILES strings and MDL Molfiles. The test set consisted of 185 chemicals from the U.S. Food and Drug Administration (FDA). The participating groups were 14, which provided in a first phase sets of chemical descriptors and models for the training set, and, in a second phase, the sets of predictions. The descriptors included molecular properties, information about the presence/absence of substructures and functional groups (predefined and automatically generated from the data), graph theoretical indices, and 3-D parameters. Summaries and data can be found at http://www.predictive-toxicology.org/ptc/.

The 14 groups generated 111 models for the 4 rodent experimental groups. Five models performed better than random guessing (at a significance level p of 0.05), and they are shown in Figure 6. Statistical evaluations are cited in ref 188 according to which the three best predictive models included a small but significant amount of empirically learned toxicological knowledge. For further comments, see also ref 189.

5. Conclusions

It appears that the evidence on SARs relative to the mutagenic and carcinogenic properties of the chemicals is extremely rich. A large number of functional groups and/or chemical substructures able to elicit toxic effects have been recognized. Whereas these SAs define only the potential for the chemicals to be carcinogenic or mutagenic, the actual modulation of this potential needs to be modeled within the individual chemical classes, for each experimental system. As a matter of fact, a considerable number of QSARs for individual chemical classes are now available. Many focus on in vitro mutagenicity; however, a number of QSAR models for the animal carcinogenicity exist as well. Overall, they provide a consistent picture of the genotoxic mechanisms of toxicity of the chemical mutagens and carcinogens. On the contrary, QSAR research on nongenotoxic carcinogens is still in its infancy. The most important conclusion to be made from the studies on genotoxic carcinogens is the great importance of hydrophobicity in the modulation of the potential for mutagenicity and carcinogenicity. Hansch and colleagues have shown that for the compounds that require S9 activation to become mutagenic in bacteria, all have log P terms with coefficients near 1.0.[18] Other QSARs show that where a direct chemical reaction with DNA appears to be occurring, without metabolic activation, there is not a positive hydrophobic term in the equation (see, for example, the QSARs relative to the mutagenicity of aniloacridines, cis-platinum analogues, lactones, and epoxides).[190]

It should be remarked that QSAR results do not represent causal relationships, so that very careful evaluation and interpretation are absolutely necessary. However, they are very powerful tools to extract and systematize information from data to obtain hypotheses that can be put to experimental test. At the same time, the QSAR results should be viewed as a precious complement to the mechanistic information already existing. In this respect, interpretability and the systematic comparison of QSARs (lateral validation) are of the utmost importance.[15,190,191]

An exercise of mechanistic comparative QSAR has been performed relative to the aromatic amines. These chemicals have a great environmental and industrial importance, so a large database of experimental results and different QSARs are available. The comparison of QSARs showed that (1) for both bacterial mutagenicity and rodent carcinogenicity, the potency gradation of the active aromatic amines depends first on hydrophobicity and second on electronic and steric properties. This confirms the existence of a common first step in the mutagenicity and carcinogenicity of these compounds; (2) the QSARs for the potency of the active aromatic amines were not suitable for differentiating the inactives from the actives; (3) the QSARs for the aspecific toxicity of the aromatic amines in a variety of experimental systems were much simpler than those for bacterial mutagenicity and rodent carcinogenicity and usually relied only on hydrophobicity.[192]

The QSAR approaches for the evaluation of the toxic potential of noncongeneric sets of chemicals deserve a discussion on their own. The amount of results from exercises on the prediction of external datasets is now large enough to permit a number of conclusions.

A deceiving aspect of it is the extremely large variability of the accuracy of the predictions: Figures 1 and 2 show that one system can produce very good or very bad predictions, depending on the specific set of query chemicals to which it is applied. This is a

serious drawback, especially if one thinks that several of the prediction systems have built-in alerts, aimed at informing the user to what extent a query compound "resembles" the chemicals used for training the system itself. The correct definition of the system applicability domain is crucial in this respect. However, this issue has no easy solution. It should be emphasized that a QSAR model has a purely empirical character: it is relative to the chemical space from which it has been derived and is not the expression of "universal" scientific laws (such as, for example, the gravitational or electromagnetic interactions). There is no possibility, a priori, of defining in a exact way a chemical series; only the combination of chemical information and of biological data can lead to its definition.[193] On the contrary, the possibility of establishing a QSAR for a group of molecules is a further a posteriori support to the idea that they belong to the same chemical series. Since the foundation of the QSAR science, it has been clearly recognized that a QSAR, if carefully constructed, can be used to make predictions within the range of parameters values spanned by the training set, whereas nothing certain can be said about how far outside that space it will continue to be valid.[194] In addition, experience shows that sometimes it is possible to devise new chemicals, which, while still being within the parameters space of the training set, have substituents that change radically the action mechanisms, so the new chemicals are not predicted correctly by the QSAR model. Let us recall again an example already reported in this paper. Glende et al.[45] synthesized alkyl-substituted (ortho to the amino function) aromatic amines not included in original database used by Hansch and colleagues[42] to develop a QSAR for the bacterial mutagenicity. Most of the new chemicals had descriptors values in the range of original chemicals. The bulky alkyl substituents decreased the mutagenicity of the arylamines, probably because of the steric hindrance of the metabolic oxidation of the amino group by the enzymes; thus the predicted and experimental values differed considerably. The quoted example shows that the definition of the applicability domain may involve not only the range of parameters values but also extra information of mechanistic/structural nature.

In general, the models for noncongeneric chemicals are quite inferior, as quality, to the classical QSARs for congeneric series. This is not surprising since a QSAR for congeners is aimed at modeling only one phenomenon, whereas the general models for the noncongeneric sets try to model at the same time several mechanisms of action, each relative to a class of carcinogens. This is a much more difficult task.[117] In addition, successful modeling of noncongeners requires the availability of an adequate number of representatives for each chemical class. Whereas for some classes (e.g., aromatic amines) enough chemicals have been bioassayed, for most of the chemical classes only sparse data exist. A few years ago, we collected a database of about 800 chemicals tested for rodent carcinogenicity: the relative numerosity of the most represented classes was (1) aromatic amines $n = 197$; (2) nitroaromatics $n = 32$; (3) halogenated alkanes $n = 27$; (4) halogenated alkenes $n = 9$; halogenated alcohols $n = 8$, thus pointing to a remarkable under-representation of many important classes (our unpublished observation). No matter how sophisticated are the modeling approaches, they cannot be expected to overcome inadequate learning sets.

It should be remarked that an additional source of difficulty for the prediction systems is the evolving pattern of chemicals in use. Chemicals that play a practical role in our life (e.g., pharmaceutical drugs, dyes) change over the years to follow the evolution of research or fashion. In addition, there is a deliberate effort to design "safer" chemicals: the new knowledge of toxicity mechanisms is applied early on in chemical development, and its effect is to filter known and predictable toxicity mechanisms out of the new chemical pool and shift the composition of new chemicals to structures with unanticipated or less obvious modes of toxicity. For example, in the first NTP exercise on the prediction of carcinogens, many chemicals were mutagenic in *S. typhimurium*, whereas only a minority of the second NTP exercise chemicals were mutagenic. Thus, the mechanistic and chemical characteristics of the historical carcinogenicity database used as learning set by the various prediction approaches is somehow "backward" in respect to the need of predicting new types of chemicals.

Regarding the accuracy of the predictions obtained for noncongeneric sets of chemicals, the results of the NTP comparative exercises showed that, in all cases, the best performance was attained by approaches that relied largely on the human expert judgment. In the case of rodent carcinogenicity, a reasonable upper limit for accuracy of the available technologies is around 65%.[187] A further confirmation of the difficulty of crystallizing the structure−activity notions into automatic prediction tools comes from the experience of the Predictive Toxicology Challenge 2000−2001 (see above): the application of sophisticated artificial intelligence methods to grasp the information contained implicitly in the carcinogenicity databases generated only 5 nonrandom sets of predictions out of the 111 originally elaborated.[188] The human experts are likely to be more free and flexible in the use of different sources of information, including the general principles of chemistry and biochemistry, and adapt better to issues that require the simultaneous use of knowledge at different hierarchical levels. Obviously, the opposite is true in the case of the individual chemical classes, where the potency of the QSAR methods can model subtle gradations in a much more efficient way than the eye of the human expert.

Both in the first and second NTP carcinogenicity exercises, several noncarcinogens were predicted as carcinogens. Most often, these false-positive chemicals either had known SAs or showed a clear analogy with known carcinogens.[187] Thus, it appears that the weak side of the prediction approaches was their tendency to be unable to make gradations between potential and actual carcinogenicity. This evidence goes hand in hand with and finds a partial explana-

tion in the selection strategy for bioassays by NTP. Because of obvious practical limitations, the selection process for chemicals tested in the rodent bioassay has always been biased toward chemicals suspected of potential carcinogenicity, with the consequence that also the chemicals considered in the comparative exercises were not random samples from the universe of chemicals but were particularly "difficult" test sets, including several suspect structures (e.g., noncarcinogens with SAs). The recognition of this fact may mitigate the limited performance of the predictions and may suggest that the general level of our knowledge is more satisfactory than that expressed by the accuracy figures.

What is the role today of the QSAR research in the field of mutagens and carcinogens? The answer cannot be as simple as yes or no. The pessimistic side of it is that the predictions for the individual chemicals cannot be taken at face value and cannot replace the experiments, when necessary. However, a simplistic view of QSAR should be rejected. QSAR is not an automatic device for generating predictions. Here applies the same reflection made by Franke regarding the search for new drugs: "As the drug discovery process is of a very complex nature, effective drug design requires an entire spectrum of techniques in which QSAR methods still play an important role. ...The real power of drug design methods is to extract and synthesize information from data to obtain hypotheses that can be put to experimental test. No dramatic overnight discoveries of wonder drug will result, but an increase in the chance of success due to indications of promising directions is a realistic expectation...."[15] Thus, the qualitative knowledge of SARs, together with the generation of QSARs (when applicable), constitutes a large body of evidence that contributes to both the scientific research on mechanisms and the practice of risk assessment. In recent years, this has been proven brilliantly by the experience of the NTP in their process of setting priorities for the rodent experimentation. Two-thirds of the chemicals were selected based on "suspicions" about their toxicity (mainly mutagenicity evidence and structure−activity reasoning) and one-third were based on production/exposure considerations. It has been found that the proportion of carcinogens among the "suspected" chemicals was almost 10 times higher than that relative to the chemicals selected only on production/exposure considerations.[195] This is surely a great success obtained largely with the use of structure−activity concepts: thus, whereas assessments relative to individual chemicals should not be taken at face value, at the level of large numbers of chemicals the careful use of QSAR can provide substantial support. Within this context, the commercially available "all purpose" prediction software, together with "intelligent" databases, can be a useful support for the expert judgment as well, provided that they offer "transparent" predictions and not only black box responses. Transparency includes declaring the SAs and rules used for formulating the predictions, as well as the list of chemicals, with known activity, similar to the query chemical (possibly together with a similarity measure).

6. References

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CR030049Y