Density Functional Theory in the Prediction of Mutagenicity: A Perspective

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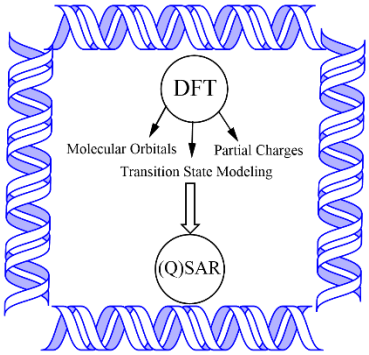
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Abstract
As a field, computational toxicology is concerned with using *in silico* models to predict and understand the origins of toxicity. It is fast, relatively inexpensive and avoids the ethical conundrum of using animals in scientific experimentation. In this perspective, we discuss the importance of computational models in toxicology, with a specific focus on the different model types that can be used in predictive toxicological approaches (SARs and QSARs). We then focus on how quantum chemical methods, such as density functional theory (DFT), have previously been used in the prediction of mutagenicity. It is then discussed how DFT allows for the development of new chemical descriptors that focus on capturing the steric and energetic effects that influence toxicological reactions. We hope to demonstrate the role that DFT plays in understanding the fundamental, intrinsic chemistry of toxicological reactions in predictive toxicology.

1. Introduction

In the last few decades, the ethical conundrum of *in vivo* animal testing has plagued toxicology; the use of animals in science has been under intense scrutiny for many years, and finding fast, sustainable, alternative ways to reduce animal testing is of huge interest to both scientists and non-scientists across the globe. Currently, there are many well established *in vitro* and *in vivo* methods in toxicology, each having advantages and disadvantages. Computational methods in toxicology, however, are not so well established, and they will play an important role in finding solutions to the complex ethical issue of animal testing. Computational toxicology has seen a surge in popularity throughout the last two decades – this is due to an increase in the accessibility of toxicological databases, pressure from industries to provide low-cost methods to test the safety of compounds, and reducing the need for animal testing.[1] Assuming that sufficient accuracy can be achieved, *in silico* methods in toxicology are often inexpensive, relatively fast and allow circumvention of the associated ethical issues attached to animal testing. For this reason, legislative programs are increasingly keen to explore the use of computational methods. Furthermore, computational chemistry has an important role to play in the development of *in silico* approaches in toxicology. Current computational approaches are not typically built on the fundamental chemical origins of toxicity, and quantum mechanical methods such as density functional theory (DFT) can be used to explore the intrinsic chemistry behind a toxicological reaction. This perspective will explore a few topics: the benefits and problems associated with *in vivo* animal testing (section 1), current and widely used methodologies for *in silico* mutagenicity prediction (section 2), and lastly, how methods, such as quantum chemical methods *e.g.* DFT, can be used to explore the chemistry of mutagenicity (section 3).

1.1 Animal Testing in Toxicology

Throughout the course of history, animals have been utilised by humans for many different uses: food supply, transport, and domestication to name a few. However, the most contentious use of animals since the dawn of the scientific era is their use in scientific research *e.g.* testing new pharmaceutical products and toxicological screenings.[2] A formal definition for animal testing (or animal experimentation) is given by the German Animal Welfare act – "the use or manipulation of animals that involves the inflicting of suffering, pain or injury to them".[3] This applies to any procedure involving an animal that subjects them to "stress equivalent to, or higher than that caused by the introduction of a needle in accordance with good veterinary practice" as defined in a 2010 European directive on the protection of animals used for scientific purposes.[4] Animal testing is a contentious issue due to the purposeful inflicting of pain for the acquisition of knowledge - proposing the question, is it ethically justifiable to kill for the advancement of knowledge? To understand the extent of animal testing in the modern era, it is important to apply it to our everyday lives. A colossal number of domestic products such as food, utilities and pharmaceuticals have likely used animal testing in their development cycle at one stage or another. Despite the widespread criticism of animal testing, it plays a critical role in ensuring that substances are safe for human use and consumption. Animal testing directly allows scientists to empirically observe the emergent properties of chemical exposure to organisms; without its widespread use, many adverse toxicological properties would remain misunderstood and unexplored. It is thus important to acknowledge the vital role that animal testing has played and continues to play in the development of *in vitro* and *in silico* approaches in toxicology.[5] For toxicologists, a clear challenge lies in developing *in vitro* assays and cheminformatic tools that are accurate and reliable testing methods, without of missing key information that could lead to human harm or death. It has been

argued that experiments involving animals can often be poorly predictive and wasteful by design.[6] For this reason, combined with the ethical considerations, it is imperative that science tries to reduce animal testing where possible, and finds alternative solutions. However, it is more realistic to acknowledge that animal testing and alternative approaches (*in silico, in vitro, in chemico* methods*)* could exist as complementary methods, as opposed to permanent and direct replacements. A good starting point for this movement was proposed by Russell and Burch in 1959 - three R's were defined: replacement, reduction and refinement.[7] The three R's should be applied to any experimental design or methodology that involves animals. Can you replace the process that involves *in vivo* testing with an alternative method? Can you reduce the number of animals used in experiments? And lastly, if you must use animals, can you refine the process such that husbandry and care is refined? A recent study highlighted how the three R's have become an integral part of scientific research in the United Kingdom, whilst simultaneously becoming a "transnational gold standard" in laboratory ethics.[8] Despite widespread acceptance of the three R's, a large number of animals are still involved in scientific experimentation. A recent report published by the U.K. government suggests that around 3.80 million experiments involving animals were performed in 2017.[9] This is undoubtedly one of the largest drivers for the development of computational methods within toxicology. As a field it absolutely meets the three R's, and at its core, aims to drastically reduce the use of animals in safety testing and pharmaceutical drug design.

1.2 Legislation in Toxicology

To ensure widespread consistency, and to minimize the risk of harm to society and public health, legislation plays an important role in chemical toxicology. There are many boards that regulate toxicology, such as the Organization for Economic Co-operation and Development (OECD) and the International Council for Harmonization (ICH). However, one of the most important and prominent legislative programs is a European Union regulation called REACH (Registration, Evaluation, Authorisation and restriction of Chemicals). This regulation aims to protect human health and keep the environment safe whilst simultaneously promoting innovation in the EU chemicals industry. Its aims also prove to meet a few principles of green chemistry as proposed by Anastas and Warner.[10] In particular, REACH aligns with principle 4 'designing safer chemicals' and principle 12 'safer chemistry for accident prevention'. The principles of green chemistry should be key considerations when designing any new, modern chemical process or technology. One of REACH's main goals is to support the use and development of alternative methods for the assessment of chemical safety; methods such as quantitative structure activity relationships (QSARs, see section 2). This is indicative of the vital role that computational toxicology is set to play in REACH's vision of the future. It has become increasingly common for alternative non-testing methods to be cited as possible ways of meeting data requirements within a regulatory context. For example, Annex VII of REACH regulation requires *in vitro/in chemico* tests as a first step in addressing the skin sensitization risk of a chemical.[11] Traditionally, *in vivo* and *in vitro* chemical safety assessment has been performed according to test guidelines (TG) as put forward by the OECD, ensuring that consistency and reliability are core to the test procedures and outcomes. Although documentation does exist for guidance on how to utilize and report data obtained from computational approaches (e.g. QSAR), no formal test guidelines have been put forward for *in silico* approaches.[12] Evaluating *in silico* approaches for the assessment of mutagenicity and other endpoints of concern remains an area of active interest for computational toxicologists. This perspective aims to introduce and appraise how density functional theory (DFT)

can and has previously been used as a tool for the assessment of mutagenic risk in pharmaceutically relevant organic molecules.

2. Computational Models for the Prediction of Mutagenicity

As a biological concept, mutagenicity refers to "the permanent and transmissible changes in the amount or structure of the genetic materials of cells and organisms". These changes can be focused towards a single gene, clusters of genes or entire chromosomes.[13] The chemical causing changes to DNA is itself termed a "mutagen", and mutagens can cause direct (or indirect) damage to DNA, resulting in different types of mutation to the genome. A variety of experimental approaches exist for assessing mutagenic risk, but these will not be discussed in this review. Please see Hasselgrin *et al* for a thorough, in depth analysis of *in silico* genotoxicity assessment and associated experimental protocols.[12] Before broadly discussing *in silico* approaches for mutagenicity prediction, it is first important to highlight the role that *in vivo* and *in vitro* data plays in constructing computational models. Without large, high-quality experimental datasets, there would be no method of anchoring chemical structures to their associated adverse toxicological outcomes. However, computational methods are amongst the most dynamic, flexible tools for the assessment of chemical safety. Predictions are relatively inexpensive and fast when compared to *in vivo* and *in vitro* methods, and this continues to drive the development of *in silico* approaches. Since 1991, when Ashby and Tennant published a study that successfully correlated chemical structure with genotoxicity and DNA reactivity[14], *in silico* approaches to predict mutagenicity have been at the forefront of toxicological research. Models for the prediction of mutagenicity typically fall into one of two categories. This section will explore these categories and the approaches they take towards mutagenicity prediction.

2.1 Structure Activity Relationships (SARs)

As a concept, Structure-Activity Relationships (SARs) underpin all fundamental investigations in toxicology. SARs are computational models that attempt to link qualitative chemical structure with qualitative biological activity. The central idea of SARs is that molecular structure implicitly determines physical and chemical properties. These properties then directly influence the biological interactions and therefore the toxicological mode of action.[15] In 1991, Ashby and Tennant published ground-breaking work that introduced the role of SARs and computational

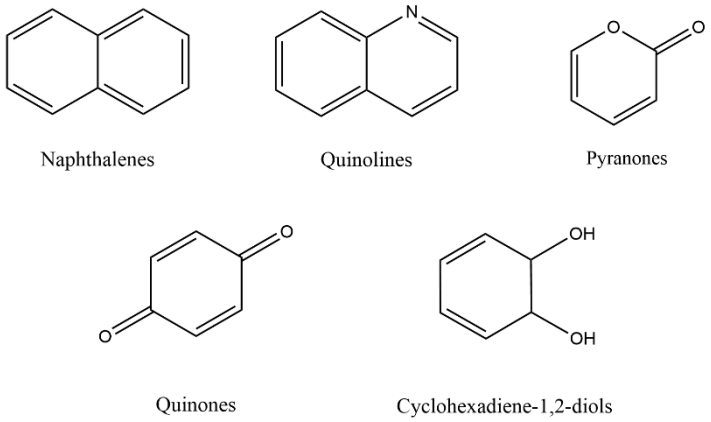

Figure 1. A diagram showing 5 structural alerts associated with DNA reactivity. These compounds sit within the mechanistic domain of 'Michael addition'. These were developed more recently by Enoch and Cronin. However, Ashby and Tenant laid the foundation for this type of work.

modeling in the prediction of mutagenicity.[16] They chose 301 chemicals and categorised them according to pre-existing chemical 'structural alerts' that indicate a propensity towards DNA reactivity (see Figure 1). The chemicals were split into 154 'alerting' chemicals and 147 'non-alerting' chemicals. The alerting chemicals were further sub-categorised into aromatic amino/nitro types, DNA alkylating agents and an 'assorted' structurally alerting group. The results of this study showed that most structurally alerting chemicals were mutagenic, whilst approximately 95 % of the non-alerting chemicals were not mutagenic. These results showed that using so-called 'structural alerts' can give a good level of confidence in predicting mutagenicity. This work by Ashby and Tennant laid the foundation for further work in attempting to correlate structural features with mutagenicity, and their work still plays an important role in modern predictive techniques. The idea of 'chemical category' formation is fundamental in the development of SARs, and it is has previously been proposed that chemicals should be categorised according to their initial mode of action – the so-called "molecular initiating event" (MIE).[17] Category formation and the MIE are built around the concept that chemicals with similar profiles will exhibit similar toxicological responses. The first discussion of the MIE can be traced back to 2006, where Schultz et al. showed that a framework for reactive toxicity can be constructed according to the initial covalent reaction of biological nucleophiles (such as DNA) with soft electrophiles.[18] It is worth noting, however, the applicability of mechanistic organic chemistry in toxicology does have limitations due to wide-ranging conditions in which reactions are carried out. Typically, in organic chemistry, reactions are carried out in different solvents and at a range of different temperatures. This differs greatly from an aqueous, well-controlled biological or cellular environment. Thus, reactions conditions can be highly relevant to reactivity and extent of reaction. Despite these limitations, understanding the initial MIE, and the fundamental chemistry associated with a toxicological endpoint is of paramount importance in predictive toxicology. The approach of category formation according to the MIE intrinsically focuses on the mechanistic chemistry as opposed to previously obtained toxicological datasets that only rely on empirical evidence (e.g. Ames test data).[19] For mutagenicity, the most important MIE for category formation is chemicals which can react to form covalent DNA adducts. Although the chemistry of DNA adducts will not be discussed in this work, Benigni and Bossa present a large number of chemical categories that show evidence of mutagenicity and carcinogenicity, and acts as a great starting point for understanding the mechanisms behind DNA reactivity.[20] Returning to the structural alerts developed by Ashby and Tennant[17], a number of 'expert systems' exist that utilise structural alerts for toxicity prediction – systems such as Derek and Toxtree.[21,22] An expert system is one of the earliest forms of artificial intelligence which uses rules and knowledge to make 'if-then' decisions. It takes advantage of information gathered from human experts and makes decisions according to a set of rules. Derek and Toxtree are widely used SAR softwares, and both use chemical categories and structural alerts to make predictions about the mutagenic risk of chemicals. However, despite their widespread use, one problem that frequently occurs relates to the 'applicability domain' of the models. Computer models can be constructed and trained with a limited dataset. A recent study by Bossuyt et al. showed that the applicability domain is important in assessing the confidence of a predictive model. The study showed that the predictive potential is moderately low for chemicals that are new to a model and not included in the initial training dataset.[23] This therefore leads to difficulties in evaluating test compounds that are structurally different to those in the initial dataset. To evaluate the predictive performance of SAR models, sensitivity, accuracy, specificity, positive predictivity and negative predictivity are all parameters that should be evaluated according to the OECD guidance document.[24] In particular, model sensitivity and accuracy are two of the most

important metrics when developing SAR models. Overall, studies show that SARs are widely used and well developed in the field of toxicology. However, due to the size of their wide-ranging applicability domains, there can exist issues with model performance.[25] Begging the question, can large robust models with a large applicability domain be developed, allowing for universal models that allow the accurate and sensitive prediction of mutagenicity? Alternatively, does the key lie in the construction of individual small models for each chemical category – although these models may have a limited applicability domain, can their targeted focus ensure they remain highly sensitive and highly predictive?

2.2 Quantitative Structure Activity Relationships (QSARs)

Quantitative structure activity relationships (QSARs) are models built from biological, chemical and statistical data to better understand toxicological events (see Figure 2). The fundamental principle of a QSAR is to establish links between a chemical descriptor and the biological activity. In this section, the use of QSAR models in mutagenicity is examined, with particular emphasis on how computational chemistry can play a powerful role in underpinning the data used in QSAR models. Molecules are represented as numerical models and their properties can be calculated using classical and quantum equations – these properties, alternatively called descriptors (e.g. Number of bonds, HOMO/LUMO energies), can then

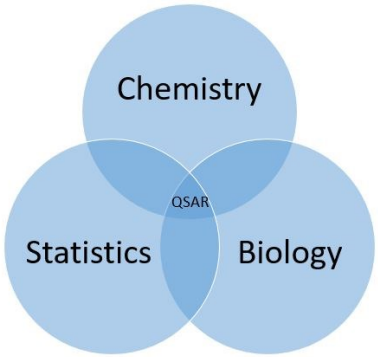

Figure 2. (Q)SARs are computational models that rely on statistics, chemistry and biology to make predictions in toxicology.

be analysed for variation and coupled to their associated biological activity. This allows development of a model that contains 'rules' for predicting the activity A_B of any chemical structure.[26] Generally, QSARs adopt the form of a linear equation as below:

$$A_B = c_o + \sum_{i=1}^{N} c_i P_i \qquad (1)$$

Where c_i are coefficients, P_i are parameters derived from molecular structure (e.g. hydrophobicity) and N is the number of parameters included within the model. The descriptors are computed for each molecule in the chosen dataset, followed by calculating the coefficient for each parameter; this is done by fitting variation in both parameters and the biological activity. QSAR models can be one of two types: global or local. Global QSAR models take large datasets of chemicals (which are both structurally comparable and non-comparable) and attempt to refine the predictive potential of the model. Alternatively, local QSAR models take congeneric groups of chemicals and refine the models predictivity – e.g. Gramatica et al. successfully developed a local (Q)SAR model to predict the toxicological response of phenylureas and s-triazines.[27] They arrived at an important conclusion; although the same toxicological endpoint was considered for the two different groups of chemicals, the descriptors showing highest predictivity were different between

groups. This highlights the variability in chemical structure, and how not all adverse outcomes arise from the same chemical 'origin'. Despite the widespread use of local models, global models come with the advantage of being able to offer predictions on any chemical entity, accompanied by a numerical level of confidence in the prediction. Two of the most popular global (Q)SARs are Sarah Nexus[28] and CAESAR[29], both of which have shown to be successful models. A recent study by Honma et al. compared the performance of global (Q)SAR models for the prediction of Ames test results in three different phases over the course of three years.[30] In 2014 (phase I), the models respectively showed a sensitivity of 51.2 % and 69.5 %, whilst three years later in 2017 (phase III) the models showed a sensitivity of 44 % and 67.5 %. This study demonstrates that the prediction of Ames test results using global (Q)SAR models has room for improvement. Despite the success of global (Q)SAR models such as Sarah Nexus and CAESAR, the confidence in prediction for large datasets of congeneric compounds can suffer; this is due to global models being built around chemicals with largely varying structures and physicochemical properties. Due to the commercial success of global models, (Q)SARs built specifically for congeneric groups of chemicals can often be left underutilised. This is despite the fact that improved levels of confidence and predictivity may be achieved through use of a local model as opposed to large global (Q)SAR model.[31] The development of QSARs to predict mutagenicity has been an active area of research for many years, with an increasing focus on using them as part of evidence-based regulatory submissions. The OECD proposed a set of guidelines for the validation of (Q)SARs when used for a regulatory purpose.[24] It is suggested that any (Q)SAR should be constructed with the following characteristics: (i) a clearly defined endpoint, (ii) an unambiguous algorithm, (iii) A defined applicability domain, (iv) appropriate measures of predictivity and robustness and lastly, (v) if possible, a mechanistic interpretation. These guidelines are heavily appropriate for the development of a useful, highly applicable (Q)SAR. The authors encourage particular emphasis on guideline 2 – often, commercially available software can be difficult to interpret due to the ambiguity in its algorithm. This often means that the reliable use of (Q)SAR models is restricted to experts in programming and computer science. Ensuring that transparent, easily interpretable algorithms are available to accompany regulatory submissions is vital in ensuring that models can be independently assessed.

3. Density Functional Theory in Predictive Toxicology

One of the fundamental steps in developing a (Q)SAR is the selection of relevant toxicological descriptors. Chemical descriptors are at the core of any (Q)SAR model, and many types of descriptor have been proposed that represent different levels of chemical structure (e.g. atom counts (0D), substructures (1D), topological (2D), geometrical (3D) descriptors). Many of these descriptors can be calculated using quantum mechanical methods. This chapter will explore the basics of DFT, the quantum chemical descriptors that can be calculated (such as HOMO/LUMO energies), and discuss why DFT transition-state modeling could have an important role to play in the prediction of mutagenicity.

3.1. Introducing Density Functional Theory (DFT)

It is well established that chemicals, when seen as atomic scale constructs, obey the laws of quantum mechanics. Thus, to gain a detailed energetic understanding of chemicals, it is necessary to use the mathematical toolset provided by quantum chemistry. As a field, quantum chemistry is defined by the application of quantum mechanical models to study chemical reactions. For the last forty years, DFT has been one of the primary methods in physics and chemistry for probing the

electronic structure of periodic systems such as crystals.[32] However, more recently, the uses of DFT have become more widespread. As a quantum chemical method, DFT has the best trade-off between accuracy and speed. Thus, in the last twenty years, DFT has become widely used for the calculation of molecular properties in toxicologically relevant organic and inorganic species. DFT is a quantum mechanical method used in computational chemistry for calculating potential energy surfaces (PES) of chemical systems; a PES provides information about the energy of a chemical at a multitude of geometries and degrees of freedom.

Quantum chemistry is primarily concerned with solving the time-independent, non-relativistic electronic Schrödinger equation as below:

$$\hat{H}\Psi(r_1, r_2, \ldots r_N) = E\Psi(r_1, r_2, \ldots r_N) \qquad (2)$$

Where $\hat{H}$ is the Hamiltonian operator, E is the energy, Ψ is the wavefunction, and r_i are the coordinates of each electron. In quantum chemical methods, the Born-Oppenheimer approximation is invoked meaning that electronic (electrons) and nuclear motion (nuclei) has been decoupled and separated. The electronic Schrödinger equation can be solved through the construction of approximate many-electron wavefunctions, for example in Hartree-Fock (HF) theory[33]. The central object in DFT, as proposed by Hohenberg and Kohn, is the electron density distribution ρ(r) rather than the wavefunction. The electronic ground state energy of a molecule can be calculated as a functional of its density, $E[\rho(r)]$. To understand the principles of DFT from an intuitive, non-mathematical point of view, E. B. Wilson proposed three fundamental ideas about the electron density (see Figure 3): (i) So-called 'cusps' in the electron density correspond to the position of nuclei, (ii) the heights of these cusps are directly linked to nuclear charge and (iii) numerical integration of the electron density gives the total number of electrons in the system e.g. the electron density in benzene would integrate to 42.[34] These core ideas are what allow us to understand the direct relationship between the electron density and the energy of a system under study. To use DFT in computational chemistry (and therefore toxicology), an orbital approach

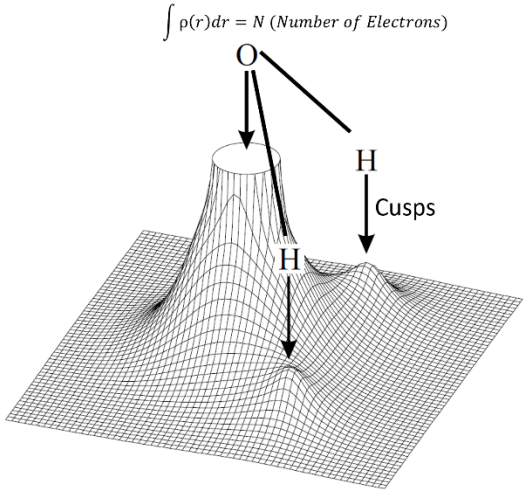

Figure 3. Graphical representation of the electron density surface for a water molecule. Cusps are observed at the position of nuclei, and the total electron density must integrate to the total number of electrons. Diagram adapted from Koch and Holthausen.[34]

needs to be adopted as put forward by Kohn and Sham.[35] The Kohn-Sham (KS-DFT) approach defines the total electron density as a sum over Kohn-Sham orbital densities as seen in the equation below:

$$\rho(r) = \sum_{i}^{n_{elec}} \varphi_i^2 (r) \qquad (3)$$

A problematic term in the overall energy expression is the unknown exchange-correlation energy functional. Many, many approximate functional forms of this term have been developed over the years and so it is not always clear which one to choose for a given chemical problem. One of the most commonly used functionals is B3LYP but benchmarking studies should be consulted to determine which functional performs best for the chemical system of interest.[36–38]

The advantages of DFT are best described by comparing it to wavefunction based approaches. Speed of calculation is an important consideration when working with large datasets of chemical structures. Although calculation length will differ for each functional, generalizations can be made for different quantum chemical methods. The simplest wavefunction based method, HF theory, can show N^4 scaling, where N is a relative measure of the system size. Higher level wavefunction methods such as MP2 can show N^5 scaling, whilst coupled-cluster singles, doubles and perturbative triples (CCSD(T)) can show very expensive N^7 scaling.[39] DFT can show a substantial reduction in computational cost, with N^3 scaling.[40] Further, a research field of wide-ranging interest, linear-scaling DFT, aims to further reduce the scaling to N for very large systems.[39] Although a reduced scaling can be attached to DFT, many popular functionals show improved performance when compared to HF.[36] Some functionals have also been shown to outperform MP2.[38] It is this fine balance between accuracy and computational efficiency that makes Kohn-Sham DFT so desirable for the calculation of molecular properties. This is paramount in predictive toxicology and (Q)SAR, where accurate geometries and molecular properties are vital in building consistent, reliable models quickly.

3.2 DFT-Derived Chemical Descriptors in Mutagenicity Prediction

Molecular structures are complex entities. Much research has been concerned with trying to capture and utilise the theoretical information embedded within chemical structures for the construction of (Q)SARs. Evidence of scientific focus on molecular descriptors is shown by many (> 5000) proposed descriptors for use in fields such as toxicology and environmental protection. Molecular descriptors are described as “the final result of a logic and mathematical procedure which transforms chemical information encoded within a symbolic representation of a molecule into a useful number or the result of some standardized experiment”, and have an important role to play in predictive toxicology (see Figure 4).[41] Many mutagenic events are initiated by the reaction between exogenous electrophiles with nucleophilic atoms in DNA nucleobases such as nitrogen and sulphur.[42,43] A number of mechanisms and reaction types can occur, such as the formation of cyclic adducts, frameshift mutations, and strand breaks.[44] Many of these mechanisms will be fundamentally governed by electrophilicity, nucleophilicity, and regioselectivity, and building models that utilise the descriptors that control this behaviour can prove powerful in predictive potential.[45] Quantum mechanical methods such as DFT can be used to calculate and develop such descriptors for use in predictive toxicological models. These descriptors can be simple zero-dimensional parameters such as molecular weight or higher-dimensional descriptors such as free energy of activation. This chapter will detail and examine some of the most commonly used descriptors in the prediction of mutagenicity.

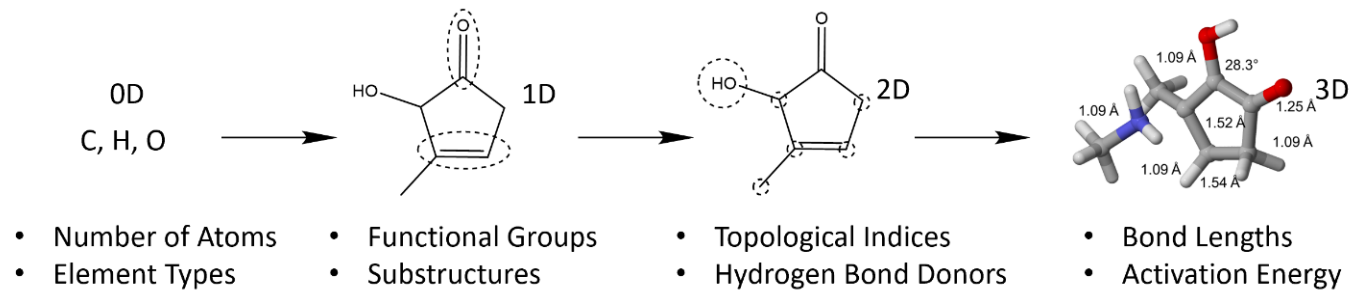

Figure 4. Chemical descriptors can vary from simple 0D parameters (e.g. number of atoms) up to complex 3D descriptors such as free energy of activation. The graphic on the right is a typical output from quantum chemical DFT calculations.

HOMO/LUMO Energies. The highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and difference between them, can be key determinants in the likelihood of reaction between two chemical species. These descriptors are easily and routinely calculated using quantum chemical methods such as DFT. They find their origins in Frontier Molecular Orbital (FMO) theory as proposed by Fukui, who proposed there is better orbital overlap when the nucleophile HOMO and the electrophile LUMO are closer in energy.[46] The HOMO-LUMO energy gap can be used in predictive (Q)SAR models; however, it is not uncommon for studies to examine only toxicant energies e.g. individual LUMO energies for a range of congeneric toxicants. A recent study by Kuhnke et al. used the DFT-derived HOMO-LUMO energy gap as a descriptor to predict Ames mutagenicity data for primary aromatic amines.[47] Their results showed that the HOMO-LUMO gap was an effective descriptor, particularly when combined with a quantum mechanical stability term, when applied to the prediction of Ames mutagenicity. HOMO/LUMO energies can also be utilised for calculation of chemical hardness η and chemical softness S according to Hard and Soft, Acids and Bases (HSAB) theory and the equations below:

$$\eta = \frac{\varepsilon_{LUMO} - \varepsilon_{HOMO}}{2} \qquad (10)$$

$$S = \frac{1}{\eta} = \frac{2}{\varepsilon_{LUMO} - \varepsilon_{HOMO}} \qquad (11)$$

LoPachin et al. used DFT to show that hardness and softness as chemical descriptors can be instrumental in understanding irreversible, covalent toxicant-target interactions.[48] They showed that soft-soft and hard-hard interactions are favourable, and nucleophile-electrophile selectivity is significant when examining toxicological phenomena. The authors believe this paper highlights the importance of developing parameters that relate to the molecular initiating event. Building QSAR models that utilise DFT-derived chemical descriptors associated with regioselectivity, could be key to predicting the most prevalent molecular sites that control covalent toxicological phenomena. We also consider that many descriptors focus exclusively on a single molecule of interest, and more research should be pursued to examine the fundamental chemistry between toxicant and target.

Molecular Size and Shape. The size and shape of a molecule plays an important role in its degree of bioavailability. Once a structure has been geometrically optimised using DFT, the shape can be graphically visualised. The relevant metrics are both molecular weight and molecular

volume, e.g. oral bioavailability is not significant with a molecular weight > 1000 Da.[49] These descriptors are amongst the simplest descriptors to calculate yet can often be vital building blocks when constructing multi-variate QSAR models.

Partial Charges. Partial charges are extremely useful for understanding inter- and intra-molecular electrostatic interactions. In chemistry, a partial charge is typically considered to be a non-integer charge on atoms in molecules, brought about by the asymmetrical distribution of electrons between chemical bonds. These charges play a vital role in steering where reactivity is likely to occur, and therefore which molecular regions will likely be involved in mutagenicity. Although many different methods exist for the calculation of partial charges, accurate atomic charges are generally obtained only through quantum mechanical calculations such as DFT. For example, a study by Korchowiec *et al* showed the strength of DFT in studying the relative reactivity of different sites in purine bases.[50] By examining charge distribution in guanine, regions that would likely be involved in electrophilic attack were ascertained; many toxic chemicals are known electrophiles that cause genetic damage, and this type of model allows better prediction of where and why these reactions occur. There are different types of charge that may be calculated for use in QSAR. Class I charges are obtained by matching to experimental data or using non-quantum models that involve methods employed from classical physics. The advantage of using class I charges relates to the speed of acquiring data - they can be very useful for investigating large datasets.[51] Class II charges are obtained by using wavefunction or electron density-based approaches, such as HF or DFT, with the charges being partitioned into individual atomic contributions. An example of a class II approach is Mulliken Population Analysis (MPA). This method has been used previously in toxicology, where Kim et al. used DFT to perform MPA for examination of partial charges on exocyclic nitrogen atoms in aryl amines.[52] Their results showed that nitrenium ions formed from known mutagenic aryl nitro drug candidates show greater partial charges on their exocyclic nitrogen, when compared to other similar drug classes. This work directly shows that partial charges can control the extent of mutagenic activity and has an important role to play in understanding mutagenesis, allowing them to be utilised as chemical descriptors where possible. Class III charges are obtained through the direct analysis of physical observables that are predicted from the molecular wavefunction. However, for an understanding of intermolecular interactions, Class III charges appear to have limited accuracy and applicability.[51] Class IV charges show remarkable accuracy for fast, low-cost calculations, and can be considered semi-empirical in nature. They typically utilise pre-determined values from Class II charges that are mapped onto the atom types, both of which can be calculated using DFT.[53]

Hydrogen Bonding. As a phenomenon, hydrogen bonding is one of the most important concepts in the field of biochemistry. Proteins, DNA, RNA and many reactive biological nucleophiles have a variety of residues that can accept and donate hydrogen bonds. Hydrogen bonding as a descriptor can be approached by understanding the energetics behind hydrogen bond formation using quantum chemical methods such as DFT. Numerous studies have used DFT to investigate hydrogen bonding and the associated interaction energies between toxicological phenomena.[54–56] However, in predictive toxicology, detailed energetic studies are limited, and the number of hydrogen bond donors/acceptors is typically chosen as a simple descriptor. It has been shown that when probing these energetics, wavefunction based approaches can show improved performance when compared to DFT. Boese tested over 50 DFT functionals and their performance for assessing hydrogen bonding, and showed that large errors are omnipresent when compared to higher level wavefunction-based methods such as Møller-Plesset (MP2, MP3) perturbation theory and Coupled-Cluster (CC) methods.[57] It should be made clear to the reader, that when working with

quantum mechanical methods, a fine balance exists between accuracy and computational feasibility. Many high-level wavefunction based methods, such as Coupled-Cluster, can take unrealistic lengths of computation time, ranging from hours to days for large, individual molecules. Naturally, a cheminformatic setting will often consider thousands of molecules, ensuring that high-level wavefunction methods are difficult to consider.[58]

As described above, there are many types of chemical descriptor that can be included in (Q)SAR models for mutagenicity prediction. However, many of these descriptors are solely obtained from the potential toxicant itself and neglects any target-toxicant interaction. As discussed in section 2.1, the molecular initiating event is an important step in toxicological reactions, and more broadly, adverse outcome pathways (AOP's).[59] To fully probe this step, and gain a detailed understanding of the MIE, methods that investigate the steric and energetic interactions between toxicant and target could reveal a hidden layer of information when attempting to build and develop new descriptors and models.

3.3 DFT Transition-State Modeling for Mutagenicity Prediction

DFT transition state modeling is a quantum mechanical method for exploring complex organic reaction mechanisms (see Figure 5). Many mutagenic events arise due to reaction between a biological nucleophile and an organic molecule, and thus, transition state modeling can be an invaluable tool for probing these reactions. According to IUPAC nomenclature, a transition state is defined as a specific geometric assembly of atoms, which when randomly placed at the saddle point, would have an equal probability of forming the reactants or of forming the products.[60] Reaction activation barriers can be calculated by using quantum chemical methods to calculate the energy of the reactants (toxicant and target) and transition states (toxicant-target). The magnitude of the activation barrier gives an indication to the likelihood of reaction between a biological nucleophile and an exogenous electrophile. If this methodology can be successfully implemented into predictive computational toxicology, new insights into the energetic and mechanistic details of mutagenic events may be possible. Transition state modeling can give the user insight into competing reaction pathways and will often reveal the lowest energy pathway.[61–63] Few attempts at using this methodology for the prediction of mutagenicity have previously been performed but

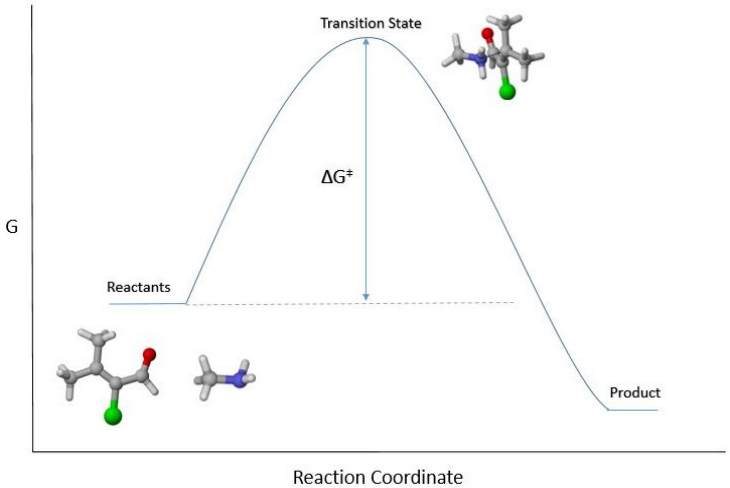

Figure 5. Diagram of a reaction coordinate showing reactants, products and a transition state. Transition state modeling involves calculating reactant and transition-state energies with quantum mechanical methods e.g. DFT, HF.

will be highlighted below. In 2011, Cronin et al. used DFT to show that transition state modeling can be used to predict the reactivity of α,β-unsaturated carbonyl compounds with glutathione.[64] They showed that steric hindrance plays a key role in the reactivity profiles, and that mechanistic information is an invaluable tool in predicting electrophilic toxicity. Although this study was not targeted directly at mutagenicity, it showed that transition-state modeling can be successfully used to group compounds according to their intrinsic reactivity. In the same year, Mulliner *et al* studied a dataset of 35 electrophilic 1,4-Michael acceptors using DFT transition-state modeling. Although this study focused on correlating transition-state barriers to experimental rate constants, with a targeted endpoint of aquatic toxicity, it proved that modeling transition-states can be vital for the *in silico* study of the MIE.[65] In 2012, Kostal et al. used transition state modeling to examine an S_N2 reaction between 15 epoxides and a chloride anion.[66] Their results showed that free energy of activation ($\Delta G^{\ddagger}$) could not be used effectively to examine the mutagenic potential of their epoxide dataset. However, in this case, a chloride anion was chosen as the nucleophile because of "comparable nucleophilic strength to DNA nucleotides in aqueous solution". This could be an oversimplification of the true situation, due to DNA nucleotides having multiple nucleophilic sites with different relative strengths. An unsuitable choice of nucleophile for examination in transition-states could drastically affect the predictive performance of a model. Following this in 2013, Leach *et al* carried out a set of Ames test procedures on a virtual array of aminopyrazoles, and in parallel, used DFT to predict the associated probability of being positive in the Ames test.[67] The dissociation energy ΔE was calculated for a variety of activated aminopyrazole conjugates at the B3LYP/6-31G* level of theory. The probabilistic results generated from DFT calculations showed excellent promise for predicting the risk of mutagenic activity in the Ames test. This work directly highlights the pivotal role that DFT can play in making predictions related to mutagenicity. In 2018, Goodman et al. published a study investigating whether DFT transition-state modeling can be used to predict the Ames test result, and thus the mutagenic potential, of 19 1,4 Michael acceptor-type compounds.[61] Their chosen nucleophile was methylamine, and the results demonstrated that free energy of activation shows good predictivity for the mutagenic potential of Michael acceptors. This study has importance in showing that transition state modeling may be widely applicable for studying the mutagenicity of different groups of electrophilic chemicals. We have since published work that builds upon this model, where improvements were made to the previously published transition-state barriers, and LUMO energies were proven to show significant predictivity towards Ames Test results, and thus, their mutagenic risk.[68] The study showed that a dataset of 29 1,4 Michael acceptors could be separated by their Ames test result, with 100% of compounds being correctly predicted and categorised. The work showed that compounds with reaction barriers less than 20.7 kcal/mol and LUMO energies less than -1.85 eV should be Ames positive, whilst those with reaction barriers greater than 22 kcal/mol and LUMO energies greater than -1.83 eV should be Ames negative. We believe that transition state modeling has an important role to play in the future of predictive toxicology. We further propose that free energy of activation (with a relevant biological nucleophile) should be more commonly examined as a chemical descriptor when building future (Q)SAR models for mutagenicity. In previous years, quantum chemical calculations could take considerable time and expertise to perform. However, with the continued increase in computational processing power and automation, DFT transition state calculations are more readily performed than ever, thus unlocking the potential for toxicologists to incorporate them into chemical risk assessment.

4. Conclusion

This perspective has provided a broad insight into the current status of how *in silico* methods can identify genotoxicants, specifically mutagens, with particular emphasis on how DFT can aid in the computational prediction of mutagenicity. We first discussed the importance of developing predictive *in silico* methods in toxicology, along with the increasing desire to reduce animal testing where possible. Different *in silico* approaches (SARs and QSARs) for examining mutagenic potential were discussed, followed by rationalising how DFT and transition-state modeling are both powerful tools for calculating molecular descriptors in predictive toxicology. Despite the broad approach in this perspective, we have discussed and highlighted why the computational sciences have an important role to play in the prediction of mutagenicity. We further ask the research community to consider transition-state modeling as a fundamental method for assessing the mutagenic potential of electrophilic toxicants. We thank you for your audience and hope that reading this perspective has been a fruitful endeavour.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Matthew N. Grayson is a Lecturer (Assistant Professor) in Drug Discovery at the University of Bath. After graduating with 1st Class Honours in Natural Sciences at the University of Cambridge, Matt obtained his PhD at Cambridge in 2014 with Jonathan M. Goodman. In 2014, he took up an independent Girton College Research Fellowship at Cambridge. As a recipient of a Lindemann Trust Fellowship, Matt conducted postdoctoral research in the group of Kendall N. Houk at the University of California, Los Angeles during the full calendar year of 2015 before returning to Cambridge to continue his fellowship. In 2018, Matt joined the faculty at Bath.