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REACH and *in silico* methods: an attractive opportunity for medicinal chemists

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REACH, the most ambitious chemical legislation in the world, provides unprecedented opportunities for medicinal chemists. Companies must report (eco)toxicological information about their chemicals, disseminated to the public domain by the European Chemicals Agency after their registration. The availability of this wealth of new toxicological data, together with the promotion of REACH of *in silico* methods, appoints medicinal chemists to a leading role in the regulatory hazard assessment process. In fact, Quantitative Structure–Activity Relation ship (QSAR) models and predictive toxicology have been applied in drug design and development for decades. Here, we discuss toxicological endpoints and areas where further development is needed to provide an enlightened appraisal of this attractive new opportunity.

Introduction

Currently, approximately 30 million chemical substances are estimated to be on the market [1]. They include food coloring and preservatives, varnishes, pesticides, and, of course, drugs. In December 2006, the European Community (EC) issued a new regulation concerning the Registration, Evaluation, Authorization and restriction of Chemicals [2]. Known as REACH, this legislation brought about a revolution in the chemical regulatory world. For the first time, the responsibility of proving the safety of chemicals was moved from public authorities to industry. The main aim of REACH is: '[...] to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances, as well as the free circulation of substances on the internal market while enhancing competitiveness and innovation. [...]' [2]. REACH is now the paradigm of an intentional shift toward a more responsible, sustainable, and green use of chemicals. These objectives are inspired by the principle 'No data – no market' and are pursued through the authorization and restriction processes in place for the most hazardous substances.

Each chemical, manufactured or imported in Europe in a quantity above 1 ton per year, has to be registered to the European Chemicals Agency (ECHA) by submitting a dossier describing physicochemical, biological, and toxicological properties (all together called endpoints; Fig. 1). Chemicals whose uses are covered by other EU legislation (e.g. food additives or medicines) are partially exempted from REACH. However, precursors in the synthesis of the drugs or medicines with additional uses need to be registered. The information requirements and the deadline for registration depend on the level of concern raised by the substance. For carcinogens, mutagens, and reproductive toxicants (CMRs), substances highly toxic to aquatic organisms, and large production volume chemicals, the requirements are stricter.

Not surprisingly, international mass media have welcomed REACH as 'the most important legislation in European Union in 20 years' (BBC News, 28 November 2005) and 'the strictest law to date regulating chemical substances' (*San Francisco Chronicle*, 14 December 2006). Other countries, such as China, Turkey, Japan, and, very recently, South Korea, are adopting REACH-type regulations, proving its success. The USA is also working in this direction [3].

By contrast, REACH has also been (and still is) subject to strong criticism from different stakeholders. In particular, industry

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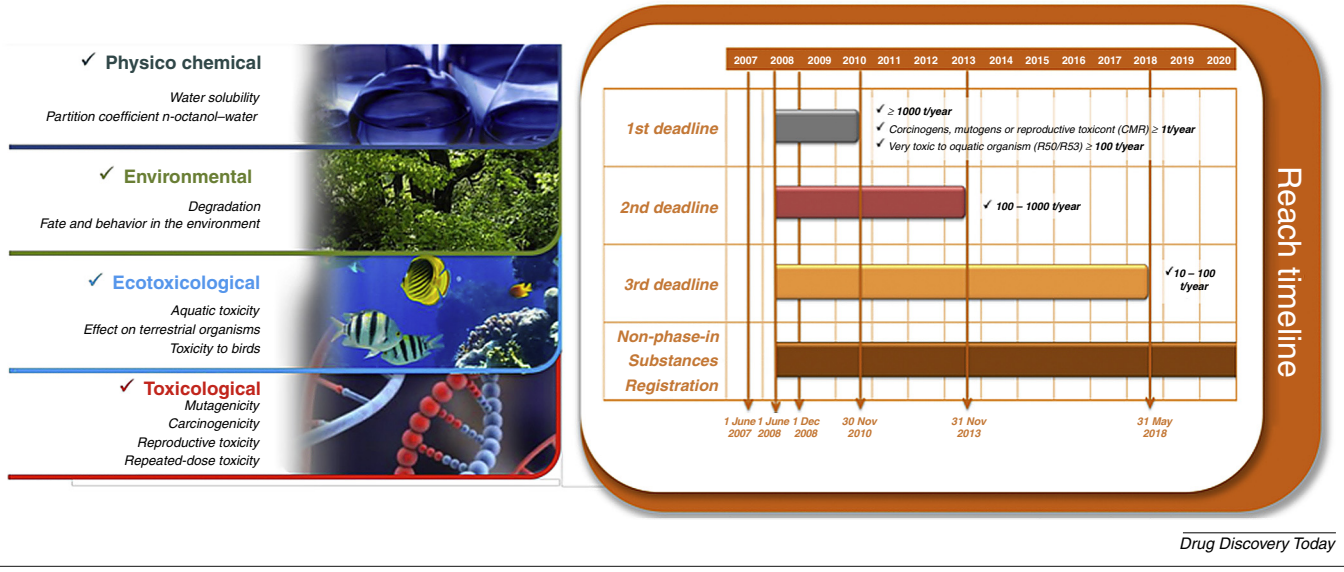

FIGURE 1

(a) Main REACH endpoint areas. The first is relative to physicochemical properties (e.g. melting/freezing point; boiling point; relative density; vapor pressure; surface tension; water solubility; partition coefficient; flash point; dissociation constants; viscosity). The second is the toxicological group (i.e. skin irritation or corrosion; eye irritation; skin sensitization; mutagenicity; acute toxicity; repeated dose toxicity; reproductive toxicity; toxicokinetics; carcinogenicity). The third is known as the ecotoxicological group (i.e. aquatic toxicity; or effects on terrestrial organisms; toxicity to birds). The fourth comprises environmental properties (i.e. degradation; fate and behavior in the environment). **(b)** Gantt chart for REACH implementation. The first registration deadline, which expired in November 2010, was related to substances in quantities exceeding 1000 ton per year, to carcinogens, mutagens, and reproductive toxicant (CMR) substances (≥1 ton/year) (i.e. cause reproductive and developmental toxicity), and to substances that are toxic to the aquatic environment (≥100 ton/year). The second registration deadline was in May 2013 and was relevant for chemicals in the tonnage band 100–1000 ton/year, and for the new substances placed on the European market for the first time (that needed to be registered immediately). The third and last deadline (May 2018) relates to the registration of substances totaling ≥1 ton/year.

criticizes the costs of the registration process because of the burden of the administrative and experimental work needed, which could undermine their competitiveness on the world market. The business of small and medium enterprises is even more exposed to this risk, to the point that they might cease manufacturing some of their products. In addition, other interested parties, such as nongovernmental organizations (NGOs), pose the ethical problem of the huge number of animal tests necessary to fulfill the information requirements [4]. Thus, it is clear why the use of nontesting methods in place of animal tests is supported by REACH. An appropriate use of these techniques could partially solve the concerns of both industry and NGOs.

Although drugs do not represent the frontline mission of REACH, we think that great opportunities for medicinal chemists are just around the corner. Their background in *in silico* methods and exploratory toxicology gives them the right perspective to investigate REACH endpoints, especially those in the toxicological (or human health) group. Medicinal chemists can have a firm role both from the side of the registrants (industry) and the evaluators [i.e. ECHA and Member States REACH Competent Authorities (MSCAs)]. In fact, they can lend their skills to the broad spectrum of expertise necessary to ensure the successful implementation of REACH. Nevertheless, the final deadline, in 2018, is expected to involve the most substances and registrations that have occurred so far, and experts will be strongly needed to face it.

In silico approaches: QSAR and read-across

Predicting the effects of a substance is far from being a safe bet. The interplay of xenobiotics with living organisms, such as the activation of an enzyme cascade or the opening of an ion channel responsible for definite biological and/or toxicological effects, is largely unknown. In 2012, the Organisation for Economic Cooperation and Development (OECD) launched a program to develop new adverse outcome pathways (AOPs), defined as 'an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect. AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning' (http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm).

The biological and/or toxicological potential of a molecule can be assessed at diverse levels: (i) *in vivo* experiments based on direct animal testing; (ii) *in vitro* experiments making use of cell cultures; or (iii) *in silico* simulations adopting computer models. Given REACH, the need for wider application of computational methods to reduce or replace animal experimentation is stronger than ever. However, replacing animal testing is a double-edged sword. On the one hand, it is well known that *in vivo* experiments are time consuming, expensive and ethically questionable, whereas the use of *in vitro* and *in silico* approaches, such as QSARs, can lead to significant savings [5]. On the other hand, the reliability of *in silico* predictions is often not well documented enough to make safe decisions and justify waiving animal tests. Thus, new models and a more critical use of QSAR are required to obtain more robust predictions that are acceptable also from a regulatory perspective.

The EC has issued, in Annex XI of REACH [2] and in Annex IV of the Biocidal Products Regulations [6], four conditions for the

regulatory acceptance of QSAR: (i) results have to be derived from a QSAR model whose scientific validity has been well established; (ii) the substances are expected to fall within the applicability domain of the QSAR model; (iii) results need to be adequate for the purpose of classification and labeling and/or risk assessment; and (iv) adequate and reliable documentation of the applied method has to be provided.

Notably, even the best QSAR models developed for drug discovery might not fulfill these conditions (i.e. regulatory acceptance of QSAR). In fact, the primary goal of drug discovery is that of deriving predictive models relegating the transparency and adequacy of the results to a secondary role. Moreover, the validation could be based on in-house data, which can be unavailable to REACH regulators. In this case, the predictions need to be supported by other valuable pieces of information in a weight-of-evidence approach. However, in recent years, a critical rethinking of the approach to assess impartially the reliability of the results has started. A proof of this shift toward a more responsible use of QSAR is given by several recent papers challenging overinterpreted or not properly validated models. Applying QSARs to substances whose effects are still unknown is sustainable only when a model has a solid basis and is not the result of theoretical speculations. A blacklist of simply decorative and colorful QSAR models has been openly deplored in severe self-critical retrospective studies [7]. We believe that full awareness of the regulatory context will give to medicinal chemists the right assets to meet the scientific (i.e. robust studies for risk assessment) and regulatory (i.e. registration of a substance) REACH requirements. In this respect, the approval of REACH represents a chance for a QSAR renaissance [8].

QSAR can also count on the support of another *in silico* technique available for the prediction of toxicity, the read-across [9]. As shown in Fig. 2, read-across covers the information requirement for a chemical with a data gap (called the target substance) with the experimental data available for substances (called the source substances) that are chemically and/or mechanistically related to the target. Read-across can predict both qualitative and quantitative endpoints with a 'one-to-one' or a 'category' approach.

The prediction can be derived by: (i) projecting the property of the source compound(s) to the target chemical (e.g. the source chemical is mutagen; thus, the target chemical is mutagen); or (ii) by identifying a trend within a category of chemicals (e.g. if an increase of the partition coefficient determines an increase of toxicity, it might be possible to derive an equation quantitatively linking toxicity and partition coefficient of category members. Then, the toxicity of the target chemical can be estimated by linear regression). The first approach is preferred for qualitative endpoints or one-to-one read-across, whereas the second is useful when predicting quantitatively outcomes within a category. The cornerstone of the read-across approach is the correct identification of the most appropriate source chemical(s). In this regard, many aspects need to be simultaneously considered. Chemical similarity, a common mode of action, and similar metabolic pathways are crucial to consider the approach meaningful. To the best of our knowledge, the only publicly available tool to select analogs, build categories on mechanistic bases, derive trends, and perform read-across or trend analysis predictions is the OECD QSAR Toolbox, which is part of the OECD QSAR Project. (http://www.oecd.org/chemicalsafety/testing/oecdquantitativestructure-activityrelationshipsprojectqsars.htm). The tool is co-developed with ECHA and aims to increase the regulatory acceptance of *in silico* predictions. Further guides on the use of the Toolbox to make predictions for different endpoints (skin sensitization and aquatic toxicity) are available on the website of the ECHA as illustrative examples (http://www.echa.europa.eu/documents/10162/21655633/illustrative_example_qsar_part2_en.pdf). Other platforms, such as VEGA [10], EPISuite [11], T.E.S.T. [12], or QSPR-Thesaurus [13], have also been developed in a regulatory context. A synoptic view of softwares relevant for REACH is provided in Table 1.

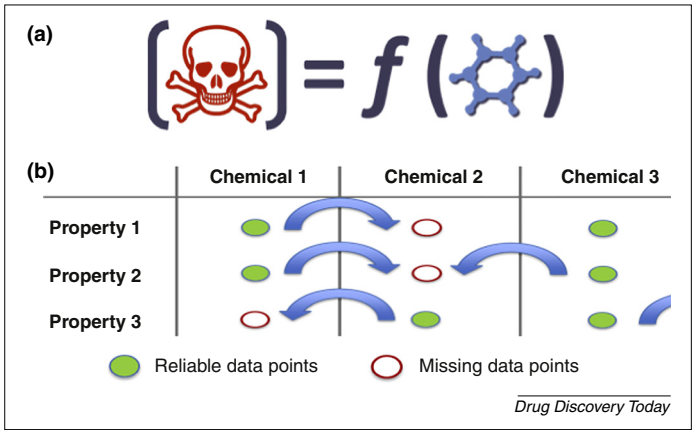

FIGURE 2

Schematic view of **(a)** Quantitative Structure–Activity Relation ship (QSAR) and **(b)** read-across strategies.

By using these tools developed specifically for regulatory purposes, *in silico* practitioners have the possibility of extending the use of computational approaches, often viewed with skepticism, and of satisfying the high REACH regulatory standards. Otherwise, the risk is to lose a great chance and keep *in silico* methods relegated to a second-line role. Confidence in the predictions is often dependent on the interpretability of the results. Some seminal papers [14–16] have clearly demonstrated that the acceptability of QSAR models is mostly dependent on the quality of chemical descriptors and chemical data rather than on the complexity of the optimization techniques. It is undeniable that pharmacokinetics and toxicology go hand-in-hand with the physicochemical properties of compounds. Our belief is that the use of absorption, distribution, metabolism, excretion and toxicity (ADMET) descriptors increases the interpretability of QSAR models and ensures adequate transparency of the results. ADMET properties have always been considered important for the study of the fate and disposition of drugs [17] and the monitoring of their behavior in the body at therapeutic doses (i.e. pharmacokinetic properties). A sound example comes from the popular Lipinski's rule of five [18], which predicts whether a substance is likely to be orally available in humans by linking bioavailability with physicochemical parameters (molecular weight, octanol–water partition coefficient, and hydrogen-bond capabilities). ADMET properties can be predicted by virtual methods, but nowadays properties such as solubility, stability, and permeability through cellular membranes are assessed in high-throughput screening plate format and rapidly monitored using liquid chromatography–mass spectrometry (LC–MS)/MS or fluorescent-based quantitation [19] using a typical

TABLE 1

List of free (F) and commercial (C) applications relevant for regulatory purposes

Physico-chemical endpoints		
Melting/freezing point		
EPI Suite™ (US EPA) – module MPBPWIN v1.43	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
ChemOffice (CambridgeSoft)	http://www.cambridgesoft.com/	C
ProPred (Technical University of Denmark)	http://www.capec.kt.dtu.dk	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Boiling point		
EPI Suite™ (US EPA) – module MPBPWIN v1.43	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
SPARC (University of Georgia)	http://archemcalc.com/sparc	F
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
Advanced Chemistry Development (ACD) program	http://www.acdlabs.com	C
ChemOffice (CambridgeSoft)	http://www.cambridgesoft.com	C
Molecular Modeling Pro	http://www.chemsw.com	C
ProPred (Technical University of Denmark)	http://www.capec.kt.dtu.dk	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Relative density		
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
Vapor pressure		
EPI Suite™ (US EPA) – module MPBPWIN v1.43	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
SPARC (University of Georgia)	http://archemcalc.com/sparc	F
Advanced Chemistry Development (ACD) program	http://www.acdlabs.com	C
Molecular Modeling Pro	http://www.chemsw.com	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
Surface tension		
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
Water solubility		
EPI Suite™ (US EPA) – modules WSKOW v1.42 and WATERNT v1.01	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
OSIRIS property explorer	http://www.organic-chemistry.org/prog/peo/	F
SPARC (University of Georgia)	http://archemcalc.com/sparc	F
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
VCCLAB (Virtual Computational Chemistry Lab)	http://www.vcclab.org/	F
ACD/PhysChem Suite and ADME Suite with AbSolv module (ACD Labs)	http://www.acdlabs.com	C
ADMET Predictor and GastroPlus (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
ADMEWORKS including Predictor and ModelBuilder (Fujitsu)	http://www.fqs.pl/	C
ChemDBsoft with MOLPRO Package including SLIPPER (ChemDBsoft)	http://www.chemdbsoft.com/	C
ChemProp (Helmholtz Centre for Environmental Research, UFZ)	http://www.ufz.de/	F
ChemSilico Predictors, i.e. CS LogWS/D/P, CS BBB/PB/HIA (ChemSilico)	http://www.chemsilico.com	C
DISCOVERY STUDIO including Cerius2 (Accelrys)	http://www.accelerys.com/products/discovery-studio/	C
KnowItAll ADME/Tox (Bio-Rad Laboratories)	http://www.bio-rad.com/	C
MetaDrug™ (Genego)	http://www.genego.com/	C
NorayMet ADME (Noray Bioinformatics)	http://www.noraybio.com/	C
Pipeline Pilot (Accelrys Scitegic)	http://accelrys.com/	C
ProPred (CAPEC)	http://www.capec.kt.dtu.dk/	C
PreADME (Bioinformatics and Molecular Design Research Centre) PreADMET web-based application (BMDRC)	http://www.bmdrc.org/	C
QikProp (Schrödinger)	http://www.schrodinger.com/	C
StarDrop (BioFocus DPI)	http://www.scientific-computing.com/	C
VolSurf/VolSurf+ (molecular discovery and tripos)	http://www.moldiscovery.com/	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Partition coefficient *n*-octanol/water		
MLR model for Exp LogKow (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
XLogP (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
EPI Suite™ (US EPA) – module KOWWIN v1.68	http://www.epa.gov/opptintr/exposure/pubs/episuite.htm	F
SPARC (US EPA)	http://archemcalc.com/sparc	F
VCCLAB (Virtual Computational Chemistry Lab)	http://www.vcclab.org/	F
ACD/ADME Suite with AbSolv module and PhysChem Suite (ACD Labs)	http://www.acdlabs.com/	C

TABLE 1 (*Continued*)

ADMETox/Pallas including MetabolExpert, MEXAlert, pKalc, PrologD, TPSA, RetroMEX, RuleOf5, PrologP, ToxAlert, Cytotoxicity (CompuDrug)	http://www.compudrug.com/	C
ADMET Predictor (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
ADMEWORKS including Predictor and ModelBuilder (Fujitsu)	http://www.fqs.pl/	C
Bio-Loom (BioByte)	http://www.biobyte.com/bb/prod/bioloom.html	C
ChemOffice (CambridgeSoft)	http://www.cambridgesoft.com/	C
ChemProp (Helmholtz Centre for Environmental Research, UFZ)	http://www.ufz.de/	C
ClogP (DAYLIGHT)	http://www.daylight.com/	C
ChemDBsoft with MOLPRO Package including SLIPPER (ChemDBsoft)	http://www.chemdbsoft.com/	C
ChemSilico Predictors, i.e. CS LogWS/D/P, CS BBB/PB/HIA (ChemSilico)	http://chemsilico.com/	C
Jchem with Calculator Plugins/Marvin (ChemAxon)	http://www.chemaxon.com/	C
KnowItAll ADME/Tox (Bio-Rad Laboratories)	http://www.bio-rad.com/	C
MetaDrugTM (Genego)	http://www.genego.com/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
NorayMet ADME (Noray Bioinformatics)	http://www.noraybio.com/	C
OSIRIS Property Explorer	http://www.organic-chemistry.org/prog/peo/	C
Pipeline Pilot (Accelrys Scitegic)	http://accelrys.com/	C
PreADME (Bioinformatics and Molecular Design Research Centre) PreADMET web-based application (BMDRC)	http://www.bmdrc.org/	C
ProPred (CAPEC)	http://www.capec.kt.dtu.dk/	C
TerraQSAR™	http://www.terrabase-inc.com/	C
TSAR (Accelrys)	http://accelrys.com/	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Flash point		
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
CODESSA PRO software	http://www.codessa-pro.com/	C
Dissociation constant		
pKa (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/toxpredict	F
EPISUITE (US EPA)	http://www.epa.gov/opptintr/exposure/pubs/episuite.htm	F
SPARC (University of Georgia)	http://archemcalc.com/sparc	F
VCCLAB (Virtual Computational Chemistry Lab)	http://www.vcclab.org/	F
ACD/ADME Suite with AbSolv module and PhysChem Suite (ACD Labs)	http://www.acdlabs.com/	C
ADMETox/Pallas including MetabolExpert, MEXAlert, pKalc, PrologD, TPSA, RetroMEX, RuleOf5, PrologP, ToxAlert, Cytotoxicity (CompuDrug)	http://www.compudrug.com/	C
ADMET Predictor and GastroPlus (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
ChemDBsoft with MOLPRO Package including SLIPPER (ChemDBsoft)	http://www.chemdbsoft.com/	C
JAGUAR (Schrödinger)	http://www.schrodinger.com/	C
Jchem with Calculator Plugins (ChemAxon)	http://www.chemaxon.com/	C
KnowItAll ADME/Tox (Bio-Rad Laboratories)	http://www.bio-rad.com/	C
MoKa (Molecular Discovery)	http://www.moldiscovery.com/	C
NorayMet ADME (Noray Bioinformatics)	http://www.noraybio.com/	C
Pipeline Pilot (Accelrys Scitegic)	http://accelrys.com/	C
StarDrop (BioFocus DPI)	http://www.scientific-computing.com/	C
Viscosity		
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
Environmental endpoints		
Degradation		
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
START biodegradation and persistence plug-in (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
CRAFT (JRC)	http://www.molecular-networks.com/products/craft	F
EPI Suite™ (US EPA) – modules BIOWIN v4.10 (abiotic degradation); BIOHCWIN v1.01 (ready biodegradability); KOCWIN v2.00 (soil simulation testing); HYDROWIN v2.00 (hydrolysis as a function of pH)	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
CATABOL (Catalogic Pty Ltd)	http://oasis-lmc.org	C
META (MultiCASE Inc)	http://www.multicase.com/products/prod05.htm	C

TABLE 1 (*Continued*)

MolCode Toolbox (MolCode)	http://molcode.com/	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
OASIS CATALOGIC	http://oasis-lmc.org/?section=software&swid=1	C
SPARC (University of Georgia)	http://archemcalc.com/sparc	F
BiotS (Cefic LRI)	http://www.cefic-lri.org/lri-toolbox/biots	F
UM-BBD Pathway Prediction System (University Of Minnesota)	http://www.umbbd.ethz.ch/predict/	F
MetabolExpert (CompuDrug)	http://www.compudrug.com/	C
MetaSite (Molecular Discovery)	http://www.moldiscovery.com/soft_metasite.php	C
METEOR (Lhasa Ltd.)	http://www.lhasalimited.org/	C
VolSurf+ (molecular discovery and tripos)	http://www.moldiscovery.com/	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Fate and behavior in the environment		
EPI Suite™ (US EPA) – module KOCWIN v2.00 (absorption/desorption screening); BCFBAF v3.01 (bioaccumulation in aquatic species)	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
BASL4 (Canadian Centre for Environmental Modelling and Chemistry)	http://www.trentu.ca/academic/aminss/envmodel/models/BASL4110.html	F
MolCode Toolbox (MolCode)	http://molcode.com/	C
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
CAESAR Project models (CAESAR Consortium)	http://www.caesar-project.eu/	F
Fish model (Canadian Centre for Environmental Modelling and Chemistry)	http://www.trentu.ca/academic/aminss/envmodel/models/Fish2.html	F
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
TAOBAC model (Canadian Centre for Environmental Modelling and Chemistry)	http://www.trentu.ca/academic/aminss/envmodel/models/TAOv101.html	F
ACD/LogDSuite (ACD Labs)	http://www.acdlabs.com/products/pc_admet/physchem/physchemsuite/	C
MultiCASE (MultiCASE Inc)	http://multicase.com/	C
OASIS CATABOL	http://oasis-lmc.org/?section=software&swid=1	C
QSPR-Thesaurus	http://qspr-thesaurus.eu/model/select.do	F
OCHEM	http://ochem.eu/model/select.do	F
Ecotoxicological endpoints		
Aquatic toxicity		
Demetra (Demetra consortium)	http://www.demetra-tox.net/	F
EPI Suite™ (US EPA) – module ECOSAR v1.00	http://www.epa.gov/opptintr/exposure/pubs/episuite.htm	F
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
ADMET Predictor and GastroPlus (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
TerraQSAR™	http://www.terrabase-inc.com/	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
EPAFM LC50 fish OpenTox model created with SCR regression model (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
Lazar	http://lazar.in-silico.de	F
AQTESOLV	http://www.rockware.com/product/overview.php?id=204	C
AquiferWin32	http://www.rockware.com/product/overview.php?id=71	C
GPS-X	http://www.hydromantis.com/GPS-X.html	C
OPTImaster	http://www.srtcontrol.com/optimaster.htm	C
PetWin+ 3.1	http://www.envirosim.com/	C
SRTmaster (SRT)	http://www.srtcontrol.com/srtmaster.htm	C
Visual Water Designer	http://www.innovativehydraulics.net/	C
Effects on terrestrial organisms		
Demetra (Demetra consortium)	http://www.demetra-tox.net/	F
EPI Suite v.4.1 (US EPA)	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	F
MolCode Toolbox (MolCode)	http://molcode.com/	C
Toxicological endpoints		
Skin irritation or skin corrosion		
ToxTree: skin irritation	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
BfR-DSS (German Federal Institute for Risk Assessment – BfR) (it has been implemented within Toxtree)	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
TerraQSAR™	http://www.terrabase-inc.com/	C
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
ACD/Tox Suite (formerly ToxBoxes)	http://www.acdlabs.com/products/pc_admet/tox/tox/	C
Eye irritation		

TABLE 1 (*Continued*)

ToxTree: eye irritation	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
BfR-DSS (German Federal Institute for Risk Assessment – BfR) (it has been implemented within Toxtree)	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
ACD/Irritation	http://www.acdlabs.com	C
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
Skin sensitization		
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
CAESAR Project models (CAESAR Consortium)	http://www.caesar-project.eu/	F
SA for skin sensitization MoA (implemented within Toxtree)	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
HazardExpert	http://www.compudrug.com	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
OASIS-TIMES (Laboratory of Mathematical Chemistry, Bourgas University)	http://oasis-lmc.org/?section=software&swid=4	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
Mutagenicity		
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
CAESAR Project models (CAESAR Consortium)	http://www.caesar-project.eu/	F
OSIRIS property explorer	http://www.organic-chemistry.org/prog/peo/	F
Lazar	http://lazar.in-silico.de	F
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
Toxtree (JRC)	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
ACD/Tox Suite (formerly ToxBoxes)	http://www.acdlabs.com/products/pc_admet/tox/tox/	C
Bio-Loom (BioByte)	http://www.biobyte.com/bb/prod/cqsarad.html	C
Multicase MCASE/MC4PC (MultiCASE Inc)	http://www.multicase.com/	C
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
HazardExpert	http://www.compudrug.com/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
OASIS-TIMES (Laboratory of Mathematical Chemistry, Bourgas University)	http://oasis-lmc.org/?section=software&swid=4	C
PASS (Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences)	http://www.genexplain.com/pass	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
OpenTox model created with TUM's J48 model learning web service for Micronucleus Data (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
OCHEM	http://ochem.eu/model/select.do	F
Acute toxicity		
T.E.S.T. (US EPA)	http://www.epa.gov/nrmrl/std/qsar/qsar.html	F
ACD/Tox Suite (formerly ToxBoxes)	http://www.acdlabs.com/products/pc_admet/tox/tox/	F
ADMET Predictor (Simulations Plus Inc.)	http://www.simulations-plus.com/	F
Multicase MCASE/MC4PC (MultiCASE Inc)	http://www.multicase.com/products/prod01.htm	F
TerraQSAR™	http://www.terrabase-inc.com/	F
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	F
Repeated dose toxicity		
Lazar (human MRTD)	http://lazar.in-silico.de	F
ADMET Predictor (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
Reproductive toxicity		
IST development testing model (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
VEGA – F software for QSAR	http://www.vega-qsar.eu/	F
OSIRIS property explorer	http://www.organic-chemistry.org/prog/peo/	F
CAESAR Project models (CAESAR Consortium)	http://www.caesar-project.eu/	F
ADMET Predictor (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
Bio-Loom (BioByte)	http://www.biobyte.com/bb/prod/cqsarad.html	C
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
HazardExpert	http://www.compudrug.com	C
Multicase MCASE/MC4PC (MultiCASE Inc)	http://www.multicase.com/products/prod01.htm	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
PASS (Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences)	http://www.genexplain.com/pass	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
Toxicokinetics		
Althotas Virtual Laboratory (prediction of binding to albumin)	http://albumin.althotas.com/	F

TABLE 1 (*Continued*)

SmartCYP: Cytochrome P450-Mediated Drug Metabolism (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
IndusChemFate (Cefic LRI)	http://www.cefic-lri.org/lri-toolbox/induschemfate	F
PBPK/MEGen (Cefic LRI)	http://www.cefic-lri.org/lri-toolbox/megen	F
ACD/ADME Suite with AbSolv module (ACD Labs)	http://www.acdlabs.com	C
Accord for Excel with ADME/Tox Add-on (Accelrys)	http://accelrys.com/	C
DISCOVERY STUDIO including Cerius2 (Accelrys)	http://accelrys.com/	C
ADMENSA1 (Inpharmatica)	http://www.inpharmatica.co.uk./admensa.html	C
ADMET Predictor (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
ADMETox/Pallas (CompuDrug)	http://www.compudrug.com/	C
ADMEWORKS including Predictor and ModelBuilder (Fujitsu)	http://www.fqs.pl/	C
ChemDBsoft with MOLPRO Package including SLIPPER (ChemDBsoft)	http://www.chemdbsoft.com/	C
ChemSilico Predictors, i.e. CS LogWS/D/P, CS BBB/PB/HIA (ChemSilico)	http://www.chemsilico.com/	C
Cloe including Cloe PK, Cloe PredictHIA (Cyprotex)	http://www.cyprotex.com/	C
KnowItAll ADME/Tox (Bio-Rad Laboratories)	http://www.bio-rad.com/	C
META/METAPC/MCASE ADME Module (MultiCASE Inc)	http://www.multicase.com/	C
MetaDrug (Genego)	http://www.genego.com/	C
MetaSite (Molecular Discovery)	http://www.moldiscovery.com/	C
METEOR (Lhasa Ltd.)	http://www.lhasalimited.org/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
NorayMet ADME (Noray Bioinformatics)	http://www.noraybio.com/	C
PK SiM (Bayer Technology Services)	http://www.systems-biology.com/	C
PreADME (Bioinformatics and Molecular Design Research Centre)	http://www.bmdrc.org/	C
QikProp (Schrödinger)	http://www.schrodinger.com/	C
StarDrop (BioFocus DPI)	http://www.scientific-computing.com/	C
Simcyp Simulator (SimCYP)	http://www.simcyp.com/ProductServices/Simulator/	C
OASIS-TIMES (Laboratory of Mathematical Chemistry, Bourgas University)	http://oasis-lmc.org/?section=software&swid=4	C
VolSurf/VolSurf+ (molecular discovery and tripos)	http://www.moldiscovery.com/	C
Carcinogenicity		
OpenTox model created with TUM's kNNregression model learning web service for carcinogenicity (through OpenTox platform: ToxPredict)	http://apps.ideaconsult.net:8080/ToxPredict	F
VEGA – free software for QSAR	http://www.vega-qsar.eu/	F
CAESAR Project models (CAESAR Consortium)	http://www.caesar-project.eu/	F
Lazar	http://lazar.in-silico.de	F
OncoLogic (US EPA)	http://www.epa.gov/oppt/sf/pubs/oncologic.htm	F
OSIRIS property explorer	http://www.organic-chemistry.org/prog/peo/	F
Toxtree	http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree	F
ADMET Predictor and GastroPlus (Simulations Plus Inc.)	http://www.simulations-plus.com/	C
Bio-Loom (BioByte)	http://www.biobyte.com/bb/prod/cqsarad.html	C
DEREK (Lhasa Ltd)	http://www.lhasalimited.org/derek_nexus/	C
HazardExpert (CompDrug) (addresses oncogenicity)	http://www.compudrug.com	C
Leadscope (Leadscope)	http://www.leadscope.com/	C
Multicase MCASE/MC4PC (MultiCASE Inc)	http://www.multicase.com/	C
MolCode Toolbox (MolCode)	http://molcode.com/	C
PASS (Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences)	http://www.genexplain.com/pass	C
TOPKAT (Accelrys)	http://accelrys.com/solutions/scientific-need/predictive-toxicology.html	C
Other software		
ADMET		
PaDEL-DDPredictor	http://padel.nus.edu.sg/software/padelddpredictor	F
OECD QSAR Toolbox v. 3.2	http://www.qsartoolbox.org	F

traffic light spreadsheet to report *in vitro* physicochemical and/or ADME data [20]. Thus, the confidence in the reliability of these descriptors is increasing and the same could be said for their application.

Modeling human health endpoints

Human health endpoints represent a large part of the hazard assessment of chemicals. Some of these endpoints, because of their link to regulatory risk management measures, can be considered more important than others and are described below. REACH regulation imposes the authorization process to substances of high concern (SVHCs) [2]. The aim is to ensure that the risks posed by these chemicals are properly controlled and that suitable alternative substances are considered. There are different groups of substance meeting the SVHC criteria. Among these, CMR substances, and those of equivalent concern

(e.g. endocrine-disrupting chemicals) are defined on the basis of human health endpoints and are of particular interest for medicinal chemists. In fact, these properties are well known in the drug discovery world [21] and substantial work has already been done to enable their prediction using *in silico* techniques.

Mutagenicity and carcinogenicity

Mutagens are substances that can cause genetic mutations. Carcinogens are substances causing cancer or promoting its onset and propagation. In particular, carcinogens are classified into: (i) genotoxic carcinogens, which are (or can be metabolically activated to) electrophilic reactive species determining direct damage to DNA via covalent binding (several known mutagens belong to this class because mutations are often an early hallmark of cancer) [22]; and (ii) nongenotoxic (or epigenetic) carcinogens acting by a variety of mechanisms that do not involve covalent binding to DNA. In this respect, an unprecedented breakthrough in assessing genotoxic effects was provided by the Ames test [23], a cheap *in vitro* assay based on bacterial strains of genetically engineered *Salmonella typhimurium* that are sensitive to specific carcinogen classes. Despite its implementation being harshly criticized by regulators, the Ames test currently represents the *in vitro* short-term alternative to a rodent bioassay and is a valuable support for carcinogenicity studies. Notably, assessment of mutagenicity potential is explicitly required by REACH for all the tonnage bands [2]: in some cases, a negative result of *in vitro* mutagenicity can be considered sufficient evidence for non-mutagenic potential, whereas positive results must be confirmed *in vivo*.

The availability of data sets reporting several experimental outcomes for mutagenicity has allowed many researchers to develop *in silico* models to predict this endpoint. Given that the mutagenicity outcome is qualitative (positive or negative), the resulting models are mostly SAR models, namely based on structural alerts (SAs) rather than QSAR equations. We believe that the best approach to predict mutagenicity reliably comes from the case-by-case selection of the most appropriate models (i.e. those models where the target chemical fits well in the applicability domain), and the integrated use of their prediction. All the evidence has to be considered together and eventual inconsistencies and disagreements between predictions critically analyzed.

More recent studies have mined SAs for the early recognition of nongenotoxic carcinogenicity of chemicals. Enoch [24] developed tools implemented as a DNA-binding profiler in the OECD QSAR Toolbox (http://www.qsartoolbox.org) based on 26 new SAs to predict covalent and noncovalent binding to DNA. Benigni [25] proposed a new set of nongenotoxic SAs, included in the publicly available ToxAlert platform [26]. Despite the existence of many models mostly based on electrophilicity as the key feature, only a few fine-tuned mutagenicity models are known [27–28] to achieve the same reproducibility level (i.e. 85%) as that of the Ames test.

The study of the mutagenic and carcinogenic potential of substances is decisive in medicinal chemistry. Addressing adverse effects is of utmost importance when designing compounds during the early stages of drug discovery [29]. As reported, mutagenicity is one of the numerous adverse properties of a compound that hampers its potential to become a marketable drug [30]. As an example, the development of anticancer drugs has to deal with the intrinsic high carcinogenicity potential of the medicine, which can unintentionally affect normal cells. For instance, many anticancer drugs (e.g. cyclophosphamide, chlormethine, uramustine, melphalan, chlorambucil, ifosfamide, and bendamustine) in clinical use for the treatment of a variety of cancers (i.e. brain cancer, leukemia, prostate cancer, non-Hodgkin's lymphoma, multiple myeloma, and ovarian cancer) act as DNA-alkylating agents. As shown in Fig. 3a, these drugs belong to the class of nitrogen mustards, which are potential toxicophores encoded as SAs in rule-based models.

Reproductive toxicity and endocrine disruptors

An endpoint intimately linked to mutagenicity and/or carcinogenicity is toxicity to reproduction, also named reprotoxicity. Predictive reprotoxic studies are important to address the adverse effects that chemicals might have on sexual function and fertility, and the induction of nonheritable harmful effects on offspring. Unlike other toxicological endpoints, few examples of QSAR are known for reprotoxicity. The difficulty comes from the presence of multiple pathways that are able to disrupt the endocrine system and from the paucity of data for model development, an element that strongly limits their applicability domain. As explained elsewhere [31], reprotoxicity has been assessed by using: (i) local models of congeneric panel of compounds; (ii) global models comprising diverse chemotypes; (iii) ADME-based models; and (iv) chemical categories and read-across. More models [32] for developmental toxicity have recently been published that use statistical methods or a suitably large collection of chemical cores associated with different pathways responsible for the effect. The former methods are based on a training set of approximately 200 compounds. The latter method started from a larger pool of molecules. However, results could be biased by the fact that all of them are toxic.

Models related to endocrine active substances, better known as endocrine disruptors (EDs), are also worth mentioning. EDs are substances causing adverse health effects in an intact organism, or in its progeny, because of changes in the endocrine system functions. The US Environmental Protection Agency has a leading role in this topic with the ongoing endocrine disruptor-screening program (EDSP, http://www.epa.gov/endo/) that, among others, makes use of computational simulation techniques. In fact, EDs also represent an appealing area for medicinal chemists, because EDs can interact with nuclear steroid hormone receptors (e.g. estrogen, androgen, glucocorticoid, and mineralcorticoid receptors) as well as enzymes controlling steroid hormone synthesis and metabolism (i.e. hydroxysteroid dehydrogenases). Alongside with reprotoxicity, the exposure to EDs is considered responsible for typical Western world diseases, such as sex hormone-dependent cancers, obesity, diabetes, cardiovascular complications, and immune disorders. In this respect, advanced computational methods (i.e. shape-based screening, pharmacophore mapping, and docking simulations) [33–34], aiming to identify bioactive compounds from millions of available substances, can be profitably applied to screen potential ED chemicals [35]. An example (Fig. 3b) is the recent successful study based solely on virtual screening strategies that enabled the identification of the first industrial chemical (i.e. AB110873) disrupting corticosteroid action [36]. Its identification was made possible by using computational approaches taken from drug discovery programs.

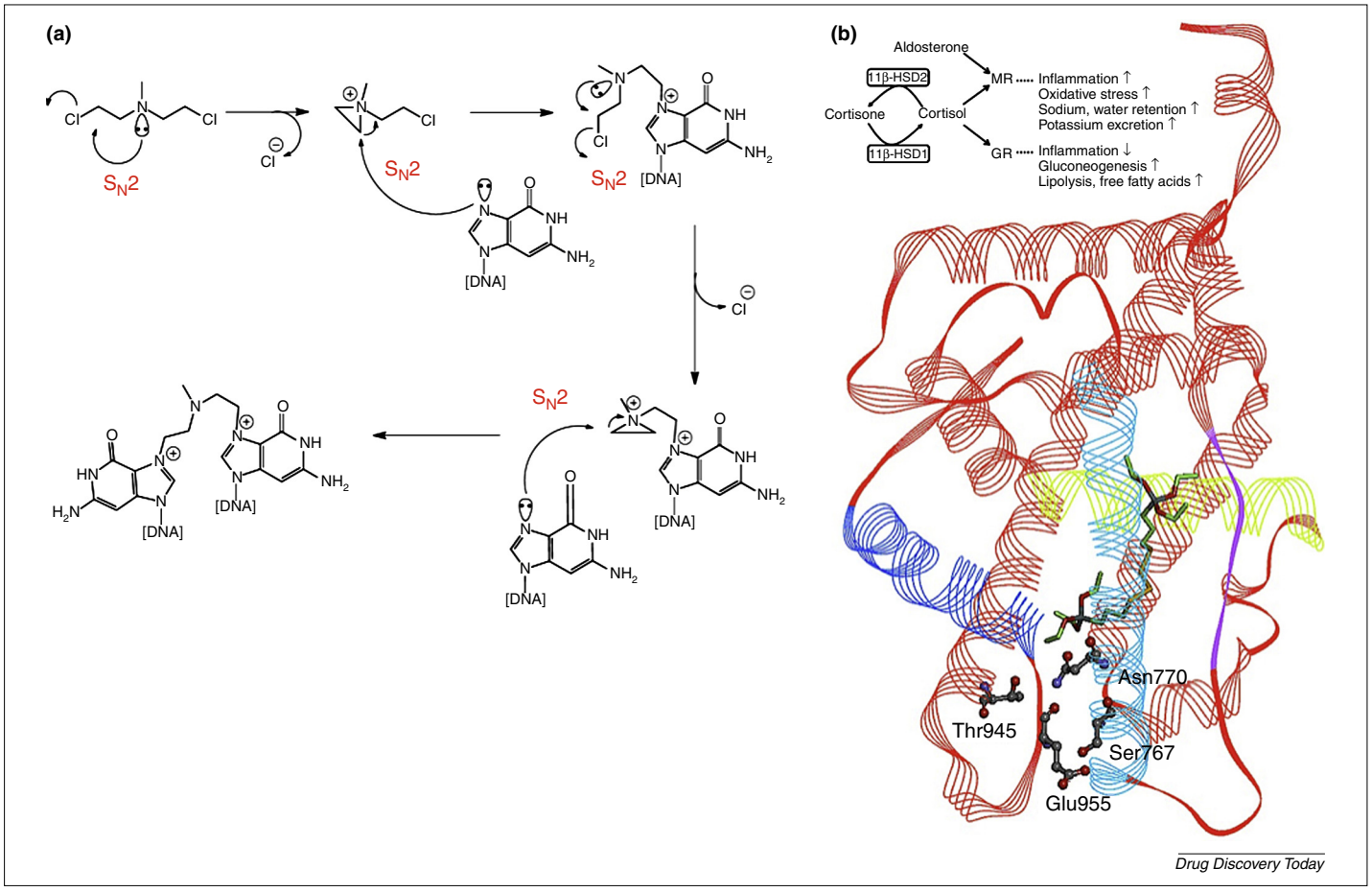

FIGURE 3

(a) Genotoxic mechanism of action of nitrogen mustards. Nitrogen mustards can be transformed in reactive electrophilic aziridines, which can react with DNA nucleophilic sites to form adducts cross-linking DNA; **(b)** schematic overview of corticosteroid receptor regulation by 11β-hydroxysteroid dehydrogenase (11-HSD) enzymes and binding mode of silane AB110873 to mineralocorticoid receptor (MR). The amino acids that form the hydrogen bond network with steroidal agonists are highlighted using a ball and stick style. AF-2 (helix 12) is in dark blue, helix 5 in green, helix 3 in light blue, and the β-sheet in lilac. The buried hydrophobic pocket additionally occupied by AB110873 is formed by amino acids from helices 3 (Val780) and 5 (Leu809, Ala813), and from the β-sheet (Val750). The model of AB110873 docked to MR is from [34].

Repeated dose toxicity

Another relevant endpoint is represented by repeated dose toxicity (RDT). RDT experiments are needed to determine crucial values for the risk assessment, that is, the thresholds for the no observed effect level (NOEL), the no observed adverse effect level (NOAEL), the lowest observed effect level (LOEL), and the lowest observed adverse effect level (LOAEL). The differences in the accepted protocols (e.g. differences in administration routes and dose spacing), along with their large experimental variability (because of events associated with whole-body assessment), make the derivation of sound and mechanistically transparent SAR models difficult. To date, the few available models are mostly based on read-across strategies. However, intensive work is required to consolidate current knowledge [37–39].

Nanotoxicology

Recent years are characterized by the global technological revolution brought by nanomaterials, and this will continue over years to come. Their uses are widely spreading and, with them, the concerns about their safety. REACH is being adapted to account for them properly, and nanotoxicology certainly represents a fascinating challenge for modern medicinal chemists [40]. At present, a large knowledge gap exists in the comprehension of toxic effects of nanomaterials, which already have widespread real-life applications [41]. Unlike bulk materials, novel engineered nanomaterials are made of nanoparticles with unique properties, such as the high surface-volume ratio or the quantum tunneling effects, which are observed in matter at a nanoscopic level. Solubility is also strongly affected (often enhanced) by nano particle size. Furthermore, having a size comparable with that of macromolecules, the nanomaterials might be involved in interactions not normally observed in bulk materials, with effects largely unknown, but potentially toxic, to the cells. Thus, QSAR models adapted to predict the physicochemical properties and toxicity of newly designed nanomaterials would be of great help [42]. This is a new intriguing and unexplored area for predictive toxicology. Indeed, systematic physicochemical, geometrical, structural, and biological studies of nanomaterials are rare. Consequently, the development and validation of statistically reliable computational models is difficult. To date, only a few nano-QSAR models exist. Thanks to REACH, new and valuable data on toxicity of

nanomaterials will be soon available to start facing the issue of nanosafety properly.

As an example, it has been reported that metal oxide nanoparticles can be extremely toxic [43] despite their bulk counterparts being substantially safe. Metal oxides represent an important pool of engineered nanoparticles used in numerous real-life applications [44]. On the basis of a nano-QSAR approach, a predictive model has been proposed. It indicates a causative relation between solely one descriptor (the enthalpy of formation of a gaseous cation having the same oxidation state as that in the metal oxide structure) and the cytotoxicity toward bacterial *Escherichia coli* cells [45]. Interestingly, other recent applications demonstrated the utility of molecular dynamics (MD) simulations, an investigative computational technique largely used in drug discovery. For instance, MD can be used for studying the structural changes of cellular membranes after insertion of carbon nanotubes, as well as for assessing the affinity of drugs to carbon nanotubes in an aqueous environment [46,47]. We believe that predictive nanotoxicology is only in an early stage and that its success will require the harmonic integration of different computational techniques (most of which are already largely used in medicinal chemistry programs, such as MD simulations, chemoinformatics, and quantum mechanics) for applications on large data sets.

Outlook and concluding remarks

After the long journey started with the first step of Hansch and the accumulated evidence of the importance of QSAR in drug discovery, medicinal chemists interested in REACH have the chance to have a key role. However, they need to adjust their aims more specifically toward predictive toxicology. In addition, a shift in the way of thinking about QSAR is needed. In fact, the acceptance of QSAR under REACH has to fulfill more restrictive conditions [48] that are necessary to ensure the adequacy of the results for regulatory purposes (i.e. avoiding unnecessary risks for human health and environment). A critical case-by-case assessment and discussion of the reliability of the results is fundamental to give credibility to the approach. Medicinal chemists can share their experience in data modeling, QSAR, and toxicology to make proper risk assessments, especially when referring to endpoints covering the protection of human health. In this respect, medicinal chemists and REACH can benefit from each other. On one side, experts in drug design and metabolism can be extremely useful to REACH, by taking quick steps forward in predictive toxicology. On the other side, REACH can open important opportunities both for young medicinal chemists starting their career and interested in the regulatory world, and for experienced scientists looking for new challenges [49]. Worthy of mention is the emphasis given to the ecotoxicity safety of chemicals by the pharmaceutical industry. Initiatives (http://www.imi.europa.eu/content/11th-call-2013-8) have been taken to establish an ecotoxicological database for pharmaceuticals with the aim of developing harmonized predictive strategies to assess and prioritize environmental hazards and risks of pharmaceuticals. We believe that the final end of REACH (protecting human health and environment from risk posed by chemicals) can be achieved with a holistic approach, where the scientific community puts in place a heterogeneous network. Gathering competencies from all the complementary fields related to REACH is the only solution to make proper hazard assessments. Medicinal chemists can use their background to have the central role of connecting environmental, toxicological, and computational competencies for successful REACH implementation.

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References

1 Benfenati, E., ed. (2012) *The e-Book on QSAR and REACH: Theory, Guidance and Applications*,
2 European Commission (2006) Regulation (EC) No 1907/2006 of The European Parliament and of The Council of 18 December 2006 concerning the Registration, Evaluation. Authorization and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Off. J. Eur. Union Lett.* 396, 1–849
3 Van Heerden, S. (2012) Recent developments in global regulatory framework in the chemical industry. *Popul. Plast. Packag.* 57, 46–50
4 Rovida, C. and Hartung, T. (2009) Re-evaluation of animal numbers and costs for in vivo tests to accomplish REACH legislation requirements for chemicals: a report by the Transatlantic Think Tank for Toxicology (T(4)). *ALTEX* 26, 187–208
5 Hornberg, J.J. *et al.* (2013) Exploratory toxicology as an integrated part of drug discovery. Part I: why and how. *Drug Discov. Today* http://dx.doi.org/10.1016/j.drudis.2013.12.008
6 European Union (2012) Regulation (EU) No 528/2012 of The European Parliament and of The Council of 22 May 2012 concerning the making available on the market and use of biocidal products. *Off. J. Eur. Union Lett.* 167, 1–123
7 Tropsha, A. *et al.* (2003) The importance of being earnest: validation is the absolute essential for successful application and interpretation of QSPR models. *QSAR Comb. Sci.* 22, 69–77
8 Cramer, R.D. (2012) The inevitable QSAR renaissance. *J. Comput. Aided Mol. Des.* 26, 35–38
9 Patlewicz, G. *et al.* (2013) Use of category approaches, read-across and (Q)SAR: general considerations. *Regul. Toxicol. Pharmacol.* 67, 1–12
10 Benfenati, E. *et al.* (2013) Using toxicological evidence from QSAR models in practice. *ALTEX* 30, 19–40
11 US EPA (2014) *Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11.* United States Environmental Protection Agency
12 Martin, T.M. *et al.* (2008) A hierarchical clustering methodology for the estimation of toxicity. *Toxicol. Mech. Methods* 18, 251–266
13 Brandmaier, S. (2014) The QSPR-THESAURUS: the online platform of the CADASTER project. *Altern. Lab. Anim.* 42, 13–24
14 Young, D. *et al.* (2008) Are the chemical structures in your QSAR correct? *QSAR Comb. Sci.* 27, 1337–1345
15 Zhu, H. *et al.* (2008) Combinatorial QSAR modeling of chemical toxicants tested against *Tetrahymena pyriformis*. *J. Chem. Inf. Model.* 48, 766–784
16 Tetko, I.V. *et al.* (2008) Critical assessment of QSAR models of environmental toxicity against *Tetrahymena pyriformis*: focusing on applicability domain and overfitting by variable selection. *J. Chem. Inf. Model.* 48, 1733–1746
17 van de Waterbeemd, H. and Gifford, E. (2003) ADMET in silico modelling: towards prediction paradise? *Nat. Rev. Drug Discov.* 2, 192–204
18 Lipinski, P.A. *et al.* (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Res.* 23, 3–25
19 Gleeson, M.P. *et al.* (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. *Nat. Rev. Drug Discov.* 10, 197–208
20 Abou-Gharbia, M. and Childers, W.E. (2014) Discovery of Innovative therapeutics: today's realities and tomorrow's vision. 2. Pharma's challenges and their commitment to innovation. *J. Med. Chem.* http://dx.doi.org/10.1021/jm401564r
21 Bickerton, G.R. *et al.* (2012) Quantifying the chemical beauty of drugs. *Nat. Chem.* 4, 90–98

22 Arcos, J.C. and Argus, M.F. (1995) Multifactor interaction network of carcinogenesis – a ''Tour Guide''. In *Chemical Induction of Cancer. Modulation and Combination Effects* (Arcos, J.C. *et al.* eds), pp. 1–20, Birkhäuser

23 Ames, B.N. (1984) The detection of environmental mutagens and potential carcinogens. *Cancer* 53, 2030–2040

24 Enoch, S.J. and Cronin, M.T. (2010) A review of the electrophilic reaction chemistry involved in covalent DNA binding. *Crit. Rev. Toxicol.* 40, 728–748

25 Benigni, R. *et al.* (2013) Nongenotoxic carcinogenicity of chemicals: mechanisms of action and early recognition through a new set of structural alerts. *Chem. Rev.* 113, 2940–2957

26 Sushko, I. *et al.* (2012) ToxAlerts: a Web server of structural alerts for toxic chemicals and compounds with potential adverse reactions. *J. Chem. Inf. Model.* 52, 2310–2316

27 Ferrari, T. and Gini, G. (2010) An open source multistep model to predict mutagenicity from statistical analysis and relevant structural alerts. *Chem. Cent. J.* 29 (Suppl 1), S2

28 Novotarskyi, S. *et al.* (2011) A comparison of different QSAR approaches to modeling CYP450 1A2 inhibition. *J. Chem. Inf. Model.* 51, 1271–1280

29 Segall, M.D. and Barber, C. (2014) Addressing toxicity risk when designing and selecting compounds in early drug discovery. *Drug Discov. Today* http://dx.doi.org/10.1016/j.drudis.2014.01.006

30 Kazius, J. (2005) Derivation and validation of toxicophores for mutagenicity prediction. *J. Med. Chem.* 48, 312–320

31 Cronin, M.T. and Worth, A.P. (2008) (Q)SARs for predicting effects relating to reproductive toxicity. *QSAR Comb. Sci.* 27, 91–100

32 Wu, S. *et al.* (2013) Framework for identifying chemicals with structural features associated with the potential to act as developmental or reproductive toxicants. *Chem. Res. Toxicol.* 26, 1840–1861

33 Nicolotti, O. *et al.* (2009) Improving quantitative structure–activity relationships through multiobjective optimization. *J. Chem. Inf. Model.* 49, 2290–2302

34 Vuorinen, A. *et al.* (2013) In silico methods in the discovery of endocrine disrupting chemicals. *J. Steroid Biochem. Mol. Biol.* 137, 18–26

35 Tanrikulu, Y. *et al.* (2013) The holistic integration of virtual screening in drug discovery. *Drug Discov. Today* 18, 358–364

36 Nashev, L.G. *et al.* (2012) Virtual screening as a strategy for the identification of xenobiotics disrupting corticosteroid action. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0046958

37 Sakuratani, Y. *et al.* (2013) Hazard Evaluation Support System (HESS) for predicting repeated dose toxicity using toxicological categories. *SAR QSAR Environ. Res.* 24, 351–363

38 Mazzatorta, P. *et al.* (2008) Modeling oral rat chronic toxicity. *J. Chem. Inf. Model.* 48, 1949–1954

39 Gadaleta, D. et al. A k-NN algorithm for predicting the oral sub-chronic toxicity in the rat. ALTEX (in press), http://dx.doi.org/10.14573/altex.1405091.

40 Collins, F.S. *et al.* (2008) Transforming environmental health protection. *Science* 319, 906–907

41 Fourches, D. *et al.* (2010) Quantitative nanostructure–activity relationship modeling. *ACS Nano* 4, 5703–5712

42 Lubinski, P. *et al.* (2013) Evaluation criteria for the quality of published experimental data on nanomaterials and their usefulness for QSAR modelling. *SAR QSAR Environ. Res.* 24, 995–1008

43 Dreher, K.L. (2004) Environmental impact of nanotechnology: toxicological assessment of manufactured nanoparticles. *Toxicol. Sci.* 77, 3–5

44 Puzyn, T. *et al.* (2009) Toward the development of 'nano-QSARs': advances and challenges. *Small* 5, 2494–2509

45 Puzyn, T. *et al.* (2011) Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles. *Nat. Nanotechnol.* 6, 175–178

46 Liu, J. *et al.* (2008) Identification of possible sources of nanotoxicity from carbon nanotubes inserted into membrane bilayers using membrane interaction quantitative structure–activity relationship analysis. *Chem. Res. Toxicol.* 21, 459–466

47 Liu, J. *et al.* (2009) Affinity of drugs and small biologically active molecules to carbon nanotubes: a pharmacodynamics and nanotoxicity factor? *Mol. Pharm.* 6, 873–882

48 Gissi, A. *et al.* (2014) An alternative QSAR-based approach for predicting the bioconcentration factor for regulatory purposes. *ALTEX* 31, 23–36

49 Tetko, I.V. *et al.* (2014) Experimental and theoretical studies in the EU FP7 Marie Curie Initial Training Network Project, Environmental ChemOinformatics (ECO). *Altern. Lab. Anim.* 42, 7–11