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Review Article

Genotoxicity of Drugs: Introduction, Prediction and Evaluation

Sagar Kishor Savale *

*Department of Pharmaceutics, R. C. Patel Institute of Pharmaceutical Education and Research Shirpur, Dist. Dhule, Maharashtra, India.

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ABSTRACT

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens; they can cause mutations. Genotoxins include both radiation and chemical genotoxins. A substance that has the property of genotoxicity is known as a genotoxin. There are three primary effects that genotoxins can have on organisms by affecting their genetic information. Genotoxins can be carcinogens, or cancer-causing agents, mutagens, or mutation-causing agents, or teratogens, birth defect-causing agents. In most cases, genotoxicity leads to mutations in various cells and other bodily systems. Mutations can lead to a host of other problems, from cancer to a wide variety of different diseases. Mutations can come in many different forms; genetic information can be duplicated, deleted, or inserted. The present review focus on need of genotoxicity, importance of genotoxicity, Mechanisam of genotoxicity, genotoxicity testing, prediction (in silico software) and Evaluation of genotoxicity.

KEY WORDS: Genotoxicity, Genotoxins, mutagens, Ames test, in silico Software, carcinogens.

INTRODUCTION

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens; they can cause mutations. Genotoxins include both radiation and chemical genotoxins. A substance that has the property of genotoxicity is known as a genotoxin. There are three primary effects that genotoxins can have on organisms by affecting their genetic information. Genotoxins can be carcinogens, or cancer-causing agents, mutagens, or mutation-causing agents, or teratogens, birth defect-causing agents. In most cases, genotoxicity leads to mutations in various cells and other bodily systems. Mutations can lead to a host of other problems, from cancer to a wide variety of different diseases. Mutations can come in many different forms; genetic information can be duplicated, deleted, or inserted. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic; however, not all genotoxic substances are mutagenic [1].

The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations. The permanent, heritable changes can affect either somatic cells of the organism or germ cells to be passed on to future generations. Cells prevent expression of the genotoxic mutation by either DNA repair or apoptosis; however, the

***Corresponding Author,**
Mr. Sagar Kishor Savale,
Department of Pharmaceutics,
R. C. Patel Institute of Pharmaceutical Education and Research Shirpur,
Dist. Dhule, Maharashtra, India.
Email: avengersagar16@gmail.com
Mob. No. 9960885333.

damage may not always be fixed leading to mutagenesis. Genotoxicity refers to interaction with, or damage to, DNA and/or other cellular components which regulate the fidelity of the genome. It is a broad term that, as well as mutation includes damage to DNA such as the production of DNA adducts by the chemical itself or its metabolites. Cells have the capacity to protect themselves from such potentially lethal or mutagenic genotoxic effects by many repair processes and therefore many genotoxic events do not become evident as mutations. However, the capacity to damage the genome (genotoxicity) is an indicator of potential mutagenicity. Thus, some methods that measure genotoxicity may not provide direct evidence of heritable mutation. Genotoxicity and mutagenicity testing are an important part of the hazard assessment of chemicals for regulatory purposes. To assess genotoxicity and/or mutagenicity, different endpoints must be taken into considerations: beside point mutations induction, a compound can induce changes in chromosomal number (polyploidy or aneuploidy) or in chromosome structure (breaks, deletions, rearrangements). However, aneuploidy can arise as a result of both genotoxic and non-genotoxic events, since loss of chromosomes can be caused either by direct effects on the chromosome to produce an acentric fragment, or by interference with the site of attachment of the chromosome on the spindle. Due to the diversity of the endpoints, it is then clear that the potential genotoxicity and/or mutagenicity of a compound cannot be assessed by a single assay system. For this reason, the group of experts has attempted to suggest a strategy to better investigate the mutagenic and/or genotoxic potential of the cosmetic products taking into consideration the needs of the cosmetic industry.

NEED OF GENOTOXICITY TESTING

Approval and registration of drugs requires a comprehensive assessment of their genotoxic potential. Genotoxicity testing is an integral component of regulatory toxicity evaluation in most countries. Since no single test is capable of detecting all relevant genotoxic end-points, a battery of in vitro and in vivo tests for genotoxicity is recommended by regulatory agencies. The recommended standard test battery includes in vitro tests for gene mutation in bacteria (Ames test) and in mammalian cells (mouse lymphoma assay) or an in vitro test for chromosomal damage in mammalian cells (in vitro micronucleus test or in vitro metaphase chromosomal aberration assay). Eventually, an in vivo test for chromosomal damage (in vivo micronucleus or in vivo chromosomal aberration assay) is required by regulatory agencies as part of an IND application. In the recent years, assessment of genotoxicity testing has evolved towards earlier stages of drug discovery in order to identify genotoxic liabilities as soon as possible. Assays such as the Ames fluctuation test and the in vitro micronucleus assay (for the assessment of the in vitro chromosomal aberration) are routinely used for screening drug discovery compounds.

IMPORTANCE OF GENOTOXICITY TESTING

Regulatory authorities all over the world require data on the genotoxic potential of new drugs, as part of the safety evaluation process. The pre-clinical studies are generally conducted to obtain the basic toxicological profile of new chemical entities (NCE). The toxicological data are used to evaluate the safety and efficacy of NCE, which will help in predicting the drug's likely risk/benefit assessment in New Drug Application (NDA) process. Genotoxicity assays have become an integral component of regulatory requirement [2]. In addition to it, many people in India are not aware of genotoxicity that it has now become mandatory to include it in drug master file required by European and United States regulatory authorities. Genotoxicity testing of new chemical entities (NCE) is generally used for hazard identification with respect to DNA damage and its fixation. These damages can be manifested in the form of gene mutation, structural chromosomal aberration,

recombination and numerical changes. These changes are responsible for heritable effects documented that somatic mutations can also play an important role in malignancy These tests have been used mainly for the prediction of carcinogenicity and genotoxicity because compounds, which are positive in these tests, have the potential to be human carcinogens and/or mutagens.

MECHANISMS FOR GENOTOXICITY

The genotoxic substances induce damage to the genetic material in the cells through interactions with the DNA sequence and structure. For example, the transition metal chromium interacts with DNA in its high-valent oxidation state so to incur DNA lesions leading to carcinogenesis (Fig. 1). The metastable oxidation state Cr(V) is achieved through reductive activation. The researchers performed an experiment to study the interaction between DNA with the carcinogenic chromium by using a Cr(V)-Salen complex at the specific oxidation state. The interaction was specific to the guanine nucleotide in the genetic sequence. In order to narrow the interaction between the Cr(V)-Salen complex with the guanine base, the researchers modified the bases to 8-oxo-G so to have site specific oxidation. The reaction between the two molecules caused DNA lesions; the two lesions observed at the modified base site were guanidinohydantoin and spiroiminodihydantoin. To further analyze the site of lesion, it was observed that polymerase stopped at the site and adeninewas inappropriately incorporated into the DNA sequence opposite of the 8-oxo-G base. Therefore, These lesions predominately contain G>Transversions. High-valent chromium is seen to act as a carcinogen as researchers found that "the mechanism of damage and base oxidation products for the interaction between high-valent chromium and DNA. are relevant to in vivo formation of DNA damage leading to cancer in chromate-exposed human populations. Consequently, it shows how high-valent chromium can act as a carcinogen with 8-oxo-G forming xenobiotics.Example of a genotoxic substance causing DNA damage is pyrrolizidine alkaloids (PAs). These substances are found mainly in plant species and are poisonous to animals, including humans; about half of them have been identified as genotoxic and many as tumorigenic. The researchers concluded from testing that when metabolically activated, "PAs produce DNA adducts, DNA cross-linking, DNA breaks, sister chromatid exchange, micronuclei, chromosomal aberrations, gene mutations, and chromosome mutations in vivo and in vitro (Fig. 1).

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Figure 1:

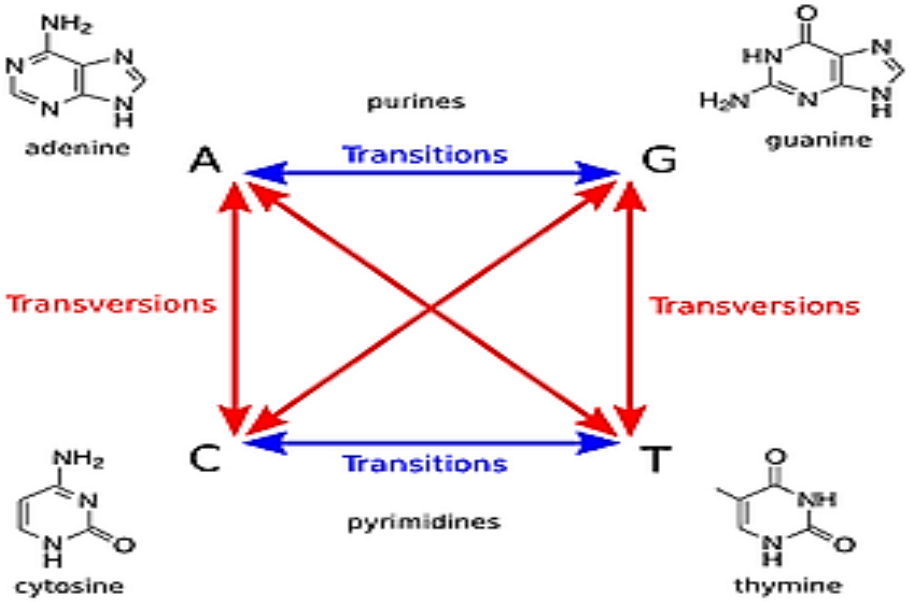

Figure 1: Mechanisms for Genotoxicity

TESTING OF GENOTOXICITY

The purpose of genotoxicity testing is to determine if a substrate will influence genetic material or may cause cancer. They can be performed in bacterial, yeast, and mammalian cells with the knowledge from the tests, one can control early development of vulnerable organisms to genotoxic substances.

BACTERIAL REVERSE MUTATION ASSAY

1. Ames test

The Bacterial Reverse Mutation Assay, also known as the Ames Assay, is used in laboratories to test for gene mutation. The technique uses many different bacterial strains in order to compare the different changes in the genetic material. The result of the test detects the majority of genotoxic carcinogens and genetic changes; the types of mutations detected are frame shiftsand base substitutions (Fig. 2).

Figure 2:

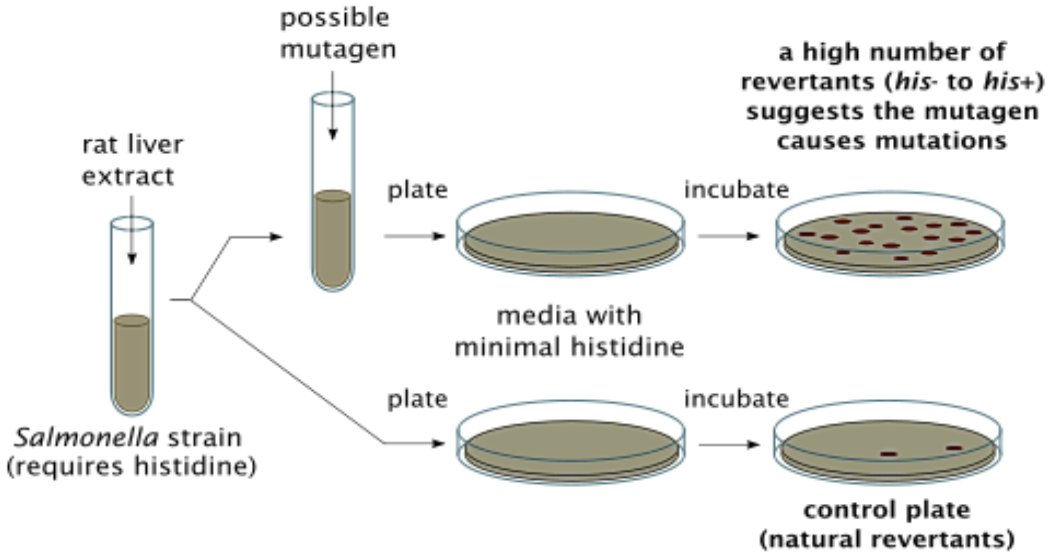

Figure 2: Ames test procedure to test for gene mutations present in the various bacterial strain

2. In vitro Toxicology Testing

The purpose of in vitro testing is to determine whether a substrate, product, or environmental factor induces genetic damage. One technique entails cytogenetic assays using different mammalian cells. The types of aberrations detected in cells affected by a genotoxic substance are chromatid and chromosome gaps, chromosome breaks, chromatid deletions, fragmentation, translocation, complex rearrangements, and many more. The clastogenic or aneugenic effects from the genotoxic damage will cause an increase in frequency of structural or numerical aberrations of the genetic material. This is similar to the micronucleus test and chromosome aberration assay, which detect structural and numerical chromosomal aberrations in mammalian cells. In a specific mammalian tissue, one can perform a mouse lymphoma TK+/- assay to test for changes in the genetic material. Gene mutations are commonly point mutations, altering only one base within the genetic sequence to alter the ensuing transcript and amino acid sequence; these point mutations include base substitutions, deletions, frame-shifts, and rearrangements. Also, chromosomes' integrity may be altered through chromosome loss and clastogenic lesions causing multiple gene and multilocus deletions. The specific type of damage is determined by the size of the colonies, distinguishing between genetic mutations (mutagens) and chromosomal aberrations (clastogens). The SOS/umu assay test evaluates the ability of a substance to induce DNA damage; it is based on the alterations in the induction of the SOS response due to DNA damage [3]. The benefits of this technique are that it is a fast and simple method and convenient for numerous substances. These techniques are performed on water and wastewater in the environment (Fig. 3).

Figure 3:

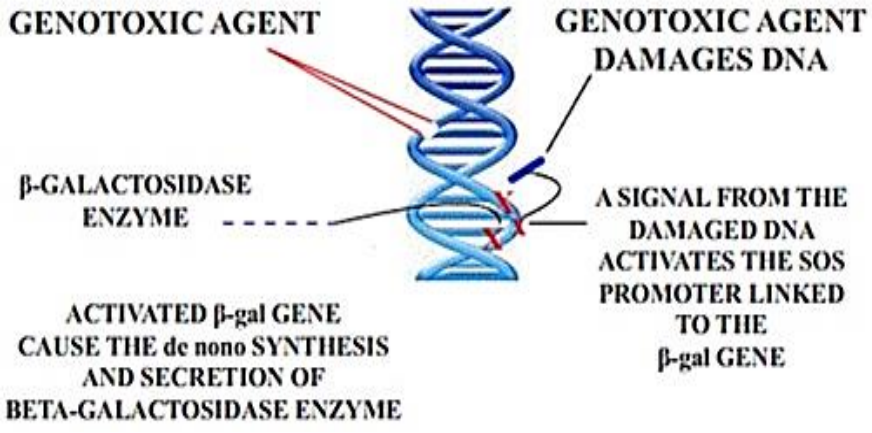

Figure 3: Overview of the use of SOS response for genotoxicity

3. In vivo Testing

The purpose for in vivo testing is to determine the potential of DNA damage that can affect chromosomal structure or disturb the mitotic apparatus that changes chromosome number; the factors that could influence the genotoxicity are ADME and DNA repair. It can also detect genotoxic agents missed in in vitro tests. The positive result of induced chromosomal damage is an increase in frequency of micronucelated PCE. A micronucleus is a small structure separate from the nucleus containing nuclear DNA arisen from DNA fragments or whole chromosomes that were not incorporated in the daughter cell during mitosis. Causes for this structure are mitotic loss of acentric chromosomal fragments (clastogenicity), mechanical problems from chromosomal breakage and exchange, mitotic loss of chromosomes (aneugenicity), and apoptosis. The micronucleus test in vivo is similar to the in vitro one because it tests for structural and numerical chromosomal aberrations in mammalian cells, especially in rats' blood cells.

PREDICTION OF GENOTOXICITY

1. ADMET Predictor

This commercial program is designed to estimate certain ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) properties of a drug-like chemical from its molecular structure. It includes a qualitative assessment of oestrogen receptor toxicity in rats (TOX_ER_filter), together with a quantitative measure of oestrogen receptor toxicity in rats (TOX_ER (IC50 (estrogen)) that is applied only for compounds classified as 'Toxic' by the previous model.

2. ACD/Tox Suite

The ACD/Tox Suite (formerly called Tox Boxes), provided by ACD/Labs and Pharma Algorithms, provides predictions of various toxicity endpoints including ER binding affinity The predictions are associated with confidence intervals and probabilities, thereby providing a numerical expression of prediction reliability. The software incorporates the ability to identify and visualize specific structural toxi-cophores, giving insight as to which parts of the molecule are responsible for the toxic effect. It also identifies analogues from its training set, which can also increase confidence in the prediction. The algorithms and datasets not disclosed.

3. Caesar

A series of statistically-based models, developed within EU-funded CAESAR project have been implemented into open-source software and made available for online use via the web. The freely accessible CAESAR model for developmental toxicity was built using 292 compounds. Two models were developed, one using WEKA (Waikato Environment for Knowledge Analysis) and Random Forest, and the other using the Adaptive Fuzzy Partition (AFP) classification model.

4. Derek

Predictions for the reproductive toxicity effects of 60 substances were compared with experimental data. The authors concluded that reprotoxicity is poorly predicted by this software. A further study by the RIVM reached the same conclusion. The study examined the ability to correctly predict the developmental toxicities of 108 industrial chemicals by using Derek and by applying the chemical categories developed by the USEPA to support the implementation of the Toxic Substances Control Act (TSCA; The conclusion was based on the observation that Derek only recognised 10% of substances which may cause impaired fertility, and only 19% of chemicals which may harm the foetus (on the basis of the harmonised EU classifications of chemicals in Annex I of the Dangerous Substances Directive). However, this conclusion is unfair to the extent that it ignores the fact that Derek is only designed to identify positives and does not make negative predictions – the absence of a prediction simply means there are no rules identifying chemical features of toxicological concern, and does not necessarily reflect the absence of toxicity. For the same reason, use of the ten TSCA categories also revealed low sensitivities (percentage of correctly predicted positive substances) – 19% and 18% for fertility and teratogenicity effects, respectively. The authors also noted that Derek and TSCA had one structural alert in common for the studied chemicals and thus the applicability domain is different for the two predictive approaches. For this reason, it would be worthwhile to build on the RIVM study by investigating the combined use of prediction based on the use of TSCA categories and Derek [4].

5. Endocrine Disruptor Knowledge Base (EDKB)

This online database, developed and made publicly available by the US FDA's National Center for Toxicological Research (NCTR), contains computer based predictive models to predict the binding affinity of compounds to the oestrogen and androgen nuclear receptor proteins.

6. Leadscope

The Leadscope software has a module containing QSAR models for predicting the developmental toxicity of the rodent foetus, including dysmorphogenesis (structural and visceral birth defects), developmental toxicity (foetal growth retardation and weight decrease), and foetal survival (foetal death, post-implantation loss, and preimplantation loss). The Leadscope QSAR models for reproductive toxicity include rodent male reproductive, rodent male sperm, female reproductive.

7. Molcode Toolbox

This is a commercial tool developed and marketed by Molcode Ltd It has a range of modules for predicting toxicological endpoints and ADME properties between them endocrine activity. The models are well documented and the underlying experimental data is made available with references and structure files (MDL molfile).

8. Multicase

The program automatically generates predictive models from datasets provided by the user. It is based on a fragment-based technology sometimes referred to as the CASE approach. The program performs a hierarchical statistical analysis of a database to discover substructures that appear

mostly in active molecules thus being with high probability responsible for the observed activity. Initially, it identifies the statistically most significant substructure within the training set. This fragment, labeled the top biophore, is considered responsible for the activity of the largest possible number of active molecules. The active molecules containing this biophore are then removed from the database, and the remaining ones are submitted to a new analysis for identification of the next biophore. The procedure 3 is repeated until either the activity of all the molecules in the training set has been accounted for or no additional statistically significant substructure can be found. Then for each set of molecules containing a specific biophore, the program identifies additional parameters called modulators, which can be used to derive QSAR within the reduced set of congeneric molecules. The modulators consist of certain substructures or physicochemical parameters that significantly enhance or diminish the activity attributable to the biophore. QSARs are then derived by incorporating the biophores and the modulators into the model. For the endpoints prediction, the software uses its own toxicity scale, from 0 to 100 CASE units, to cover the range from inactive, marginally active and active. In many cases, it is difficult to relate these CASE units to traditional measures of toxicity.

9. OECD QSAR Application Toolbox

The OECD QSAR Application Toolbox is a standalone software application for data gaps for assessing the hazards of chemicals. Data gaps are filled by following a flexible workflow in which chemical categories are built and missing data are estimated by read-across or by applying local QSARs (trends within the category). The Toolbox also includes a range of profilers to quickly evaluate chemicals for common mechanisms or modes of action. In order to support read-across and trend analysis, the Toolbox contains numerous databases with results from experimental studies. It is freely available from the OECD[5].

10. PASSPredictor

The PASS (Prediction of Activity Spectra for Substances) is developed and marketed by the Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences. Chemicals structures are presented in mol format and used to generate Multilevel Neigh-bourhood of Atoms (MNA) descriptors. The system predicts the probability (Pa) of a biological activity for a new compound, by estimating the similarity/dissimilarity of the new substance to substances with well-known biological activities present in the training set. The tool also gives a cross reference between biological activities on the basis of the knowledgebase of mechanism-effect relationships. A Bayesian algorithm is used to predict various biological activities in terms of the probabilities of presence (Pa) and absence (Pi) of each particular activity. It predicts several specific toxicities among them teratogenicity and embryotoxicity.

11. T.E.S.T.Predictor

The Toxicity Estimation Software Tool is an open-source application developed by the US EPA. It estimates the toxicity of a compound by applying several QSAR methodologies thus allowing the user to have greater confidence in predicted toxicities. Among other toxicities it predicts developmental toxicity.

12. TIMESPredictor

Tissue Metabolism Simulator is a heuristic algorithm to generate metabolic maps from a library of biotransformation's and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. The TIMES platform is also used to predict different endpoints including receptor mediated endpoints for oestrogen, androgen and aryl hydrocarbon binding affinity. They are based on the Common Reactivity Pattern (COREPA) approach developed by the Laboratory

of Mathematical Chemistry at the Bourges University Bulgaria. The COREPA approach is a probabilistic classification method which assesses the impact of molecular flexibility on stereo electronic properties of chemicals. Similarity between chemicals is analyzed by comparing their conformational distributions, and the system automatically identifies the parameter that best discriminate chemicals in groups. A Bayesian decision tree is then developed for classifying untested chemicals.

13. TopkatPredictor

This QSAR-based system, developed by Accelrys Inc., makes predictions of a range of toxicological endpoints, including developmental toxicity. The Developmental Toxicity Potential (DTP) module of the TOPKAT software was developed from experimental studies selected after review of literature citations on rat oral data. TOPKAT comprises three QSAR models, each applicable to a specific class of chemicals. The output is the probability of a submitted chemical structure being a developmental toxicant in the rat. A probability below 0.3 indicates no potential for developmental toxicity (NEG), whereas a probability above 0.7 signifies developmental toxicity potential (POS). The probability range between 0.3 and 0.7 refers to the "indeterminate" zone (IND). The TOPKAT model automatically determines whether the submitted structure belongs to the Optimum Prediction Space (OPS) of the model in order to evaluate the reliability of prediction. The OPS is TOPKAT's formulation of the model applicability domain – a unique multivariate descriptor space in which a given model is considered to applicable. Any prediction generated for a query structure outside of the OPS space is considered unreliable.

14. Toxmatch

This freely available software does not in itself generate predictions of reprotoxicity endpoints, but it can be used to develop categories and support read-across assessments. This study illustrates the use of 2D similarity indices within Toxmatch to form categories for 57 query chemicals. The underlying hypothesis is that chemicals selected as being similar should act via a single mechanism of action, even if that mechanism is unknown. Read-across predictions were performed for the 17 query chemicals for which a category could be formed. The authors concluded that 2D similarity methods offer a useful method for building chemical categories for reproductive toxicity in which a priori mechanistic knowledge is limited. Although the categories proposed are limited in terms of their applicability (40 query chemicals were not allocated to categories), the results form a good basis for further investigations.

15. Virtual Tox Lab

This is a commercial tool for predicting endocrine disrupting potential by simulating and quantifying the interactions with aryl hydrocarbon, oestrogen alpha/beta, androgen, thyroid alpha/beta, glucocorticoid, liver X, mineralocorticoid and peroxisome proliferator-activated receptor gamma. It also includes metabolic considerations by simulating interactions with the enzymes CYP450 3A4 and 2A13. The tool is based on the combined use of automated flexible docking with multi-dimensional QSAR (mQSAR).

16. Derek Nexus

The Derek model has been described in the literature. Version 2.0.0 of Derek Nexus was used in this study, which looks for substructures in the test compound that are known to have adverse toxicological properties. These are then shown to the user as a graphic display and summarized in tabular form for hardcopy output. Potentially toxic substructures (toxicophores or structural alerts) are identified by matching patterns of atoms and bonds in the rule base to the target molecule. These substructures are based on empirical observations backed up either by an understanding of the

mechanism of toxicity or by rigorous vetting. Each possible toxicophores is then further evaluated by "reading" from the rule base of a list of scope and limitation questions. Derek uses the answers to these questions to decide whether the toxicophores should be shown to the user or disregarded

17. Vega Caesar

The open source algorithm is described in detail by Ferrari and Gini. VEGA CAESAR automatically calculates chemical descriptors and contains subset of Toxtree rules. The integrated model utilizes two complementary techniques in series: a machine-learning algorithm (support vector machine), to build an early model with the best statistical accuracy, combined with two sets of rules for the removal of false negatives based on known SAs from Toxtree, to refine its predictions. The first set of rules give as output mutagenic or not. If the chemical is predicted to be nonmutagen, the second set of rules is applied. However, the number of false positives using this set of rules may be high, thus the output is given as suspicious or nonmutagenic. In the present study, the conservative approach was adopted, and thus the chemicals labeled as suspicious were considered mutagenic [6].

18. Vega Sarpy

SARpy (SAR in python) is a QSAR approach aimed at finding relevant fragments in a transparent way, and extracting a set of rules directly from data without any a priori knowledge. The algorithm generates substructures of arbitrary complexity; the fragment candidates that become SAs are automatically selected on the basis of their prediction performance for a training set. In particular the SARpy standalone program was used to extract 112 rules from the dataset of the CAESAR model; and then these rules were used for predicting mutagenicity through the VEGA platform. The implementation is a Python script employing the open source OpenBabel 2.2.3 library via a set of bindings to the C++ code. The application is available through a graphic interface or through the VEGA platform (as used here). SARpy handles the molecular data converted into SMILES disregarding chirality information, by using a set of convenience Python functions and classes that simplifies accessto the OpenBabel module (Pybel).

19. Demetra

It is an EU-funded project. This project aim has been to develop predictive models and software which give a quantitative prediction of the toxicity of a molecule, in particular molecules of pesticides, candidate pesticides, and their derivatives. The input is the chemical structure of the compound, and the software algorithms use "Quantitative Structure-Activity Relationships" (QSARs). The DEMETRA software tool can be used for toxicity prediction of molecules of pesticides and related compounds. The DEMETRA models are freely available. Five models have been developed to predict toxicity against trout, daphnia, quail (oral and dietary exposure) and bee. The software is based on the integration of the knowledge acquired in the DEMETRA EU project in a homogeneous manner using the best algorithms obtained as the basis for hybrid combinative models to be used for predictive purposes.

20. OCHEMPredictor

The OCHEM is an online database of experimental measurements intergrated with the modeling environment. Submit your experimental data or use the data uploaded by other users to build predictive QSAR models for physical-chemical or biological properties.

21. Chemistry Development Kit (CDK)

The Chemistry Development Kit (CDK) is a Java library for structural chemo- and bioinformatics. It is now developed by more than 50 developers all over the world and used in more than 10 different academic as well as industrial projects worldwide.

EVALUATION TEST

The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) has a remit to provide UK Government Departments and Agencies with advice on the most suitable approaches to testing chemical substances for genotoxicity. The COM published guidance in 1981, 1989 and again in 2000. This document, incorporating some significant changes, reports on the COM views regarding the most appropriate strategy for genotoxicity testing reached in 2011.

The COM recommends a staged approach to testing

Stage 0: consists of preliminary considerations which include physico-chemical properties of the test chemical substance, Structure Activity Relationships (SAR), and information from screening tests. However, data from SAR and screening tests should not overrule test data from adequately designed and conducted genotoxicity tests [7].

Stage 1:consists of in vitro genotoxicity tests. The COM recommends a core-test battery of the Ames test combined with the in vitro micronucleus test. This combination provides information on three types of genetic damage for which data are required (namely, gene mutation, chromosomal damage and aneuploidy) and gives appropriate sensitivity to detect chemical mutagens. There is no need to independently replicate adequately designed and conducted core in vitro tests which are either clearly negative or clearly positive. The strategy document also considers the value which can be attributed to a number of non-core in vitro tests.

Stage 2:consists of in vivo genotoxicity tests. A case-by-case strategy should be developed to answer one or more of the following specific queries; Investigation of mutagenic end point(s) identified in Stage 1, Investigation of genotoxicity in tumor target tissue(s), Investigation of potential for germ cell genotoxicity, Investigation of in vivo mutagenicity for chemicals, which were negative in Stage 1 but where there is high or moderate and prolonged exposure, Investigation of genotoxicity in site of contact tissues.The core tests in Stage 2 are the rodent micronucleus/chromosome aberration assays for aneuploidy and clastogenicity, the transgenic rodent gene mutation assay and the rodent Comet assay for DNA damage. Usually negative results obtained in a carefully selected in vivo test (possibly studying more than one endpoint and tissue) will be sufficient to address positive results found in vitro. However, a further test(s) may be needed if some of the genotoxic effects seen in Stage 1 in vitro tests had not been adequately studied in vivo (e.g. the chemical affects multiple mutagenic end-points), or other aspects of the genotoxic potential of the chemical had not been fully resolved (e.g. in the case where an investigation of heritable effects was required). The strategy document also considers the value which can be attributed to a number of non-core in vivo tests. In most instances information from core in vivo tests is sufficient to evaluate the in vivo mutagenicity of chemical substances. A supplementary in vivo test strategy can provide additional information on a case-by-case basis, to investigate aspects such as further characterization of germ cell genotoxicity, and DNA adduct data which can provide information to elucidate the mode of genotoxic action of carcinogenic chemicals.It is acknowledged that the field of genotoxicology and genotoxicity testing is rapidly developing. A short overview of possible future developments and techniques such as toxicogenomics is provided [8].

Significance of Chemical-Induced Mutation for Human Health

A mutation in the germ cells of sexually-reproducing organisms may be transmitted to the offspring, whereas a mutation that occurs in somatic cells may be transferred only to descendant daughter cells. Mutagenic chemicals may present a

hazard to health since exposure to a mutagen carries the risk of inducing germ-line mutations, with the possibility of inherited disorders, and the risk of somatic mutations including those leading to cancer .A separate statement discussing the significance of chemical-induced mutation to human health is in preparation.

GENERAL PRINCIPLES OF TESTING STRATEGY

The COM recommends a two-stage genotoxicity testing strategy (Stages 1 and 2) for the detection of the mutagenic hazard of chemicals which can be supported by appropriate preliminary screening tests and/or in silico data (Stage 0). Initial testing for mutagenic potential in Stage 1 is based upon two core in vitro tests that are chosen to provide information on gene mutation, clastogenicity and aneuploidy, with case-by-case additional testing and investigation depending on the results of these initial genotoxicity tests. All in vitro tests should be designed to provide the best chance of detecting potential activity, with respect to (a) the exogenous metabolic activation system; (b) the ability of the compound or its metabolite(s) to reach the target DNA and/or targets such as the cell division apparatus, and; (c) the ability of the genetic test system to detect the given type of genotoxic event. Where international guidance is available, the assays should be carried out to conform to those internationally recognised protocols e.g. as published by the OECD, the IWGT and in the EU test methods Regulation The same approach to testing can be used for chemical substances where in vivo genotoxicity testing is not permitted (e.g. cosmetics). Investigations regarding MoGA are important to derive conclusions on biological relevance of genotoxicity test results, to aid in overall risk assessment, and to inform on the strategy for in vivo tests. This is of particular importance for those chemicals where no in vivo genotoxicitytesting is permitted. For most chemicals, results from the two Stage 1 core tests should besufficient to reach a conclusion on the presence or absence of mutagenic potential. However, in some instances, even when Stage 1 tests are negative, regulatory authorities may require consideration of the need for in vivo Stage 2 testing particularly where exposure is considered to be high, or moderate and prolonged (e.g. most human medicines), or where there is achemical class precedent of positive in vivo genotoxicity data. Guidance on the level of exposure which equates to high, moderate or prolonged is beyond the remit of the COM.Stage 2consists of a number of in vivo tests designed to investigate whether in vitro genotoxic activity including specific mutagenic end-points identified by in vitro tests can be expressed in the whole animal. This may also include assays for specific target organs (e.g. rodent tumors detected in carcinogenicity bioassays) or in germ cells. Few chemicals are active only invivo and in such cases this may be due to a number of factors such as metabolic differences, the influence of gut flora, higher exposures in vivo compared to in vitro and pharmacological effects (e.g. folate depletion or receptor kinase inhibition). There is currently no single in vivo test which can assay all three types of genetic damage and thus a strategy for Stage 2 has to be designed based on the nature of genotoxic effects identified in Stage 1 and the possibility that genotoxic activity will only be expressed in vivo as discussed above. However consideration should be given to the possibility of evaluating different genotoxicity endpoints in a single set of test animals. There should be a clear strategy for planning tests within each stage and for progressing from Stage 1 to Stage 2. Clear statements can be made regarding the initial in vitro tests to be used in Stage 1 as these methods have been well studied, whereas the strategy for Stage 2 is more complex and, if not a specific regulatory requirement, needs to be developed on a case-

bycase basis. Under the strategy recommended by COM, the use of animals in mutagenicity testing is primarily required when it is necessary to investigate whether genotoxic activity detected in Stage 1 in vitro is reproduced in vivo, to study target organ genotoxicity (for example involvement of genotoxicity in rodent tumors and to evaluate the potential for heritable mutagenic effects. Genotoxicity testing using animals should be carried out when there is no suitable alternative, and the minimum number of animals should be used, consistent with obtaining valid results. If feasible, studies can be conducted as an adjunct to single or repeat dose toxicity studies. The COM supports current and future developments to replace, refine or reduce the need for in vivo genotoxicity testing [9].

GENOTOXICITY TESTING STRATEGY

The COM guidance provides a strategy for testing chemical substances where no genotoxicity data are available. Test substances may also contain impurities at varying levels which may exhibit genotoxic activity. Separate guidance on the genotoxicity assessment of impurities has been identified as a priority project during the COM horizon scanning exercise in 2010 and is currently the subject of an ICH expert working group. The strategy recommended in the following sections is concerned with testing for genotoxic activity of chemical substances and does not specifically address complex mixtures of chemicals. Since the publication of the COM guidance in 2000, assessments of the performance of QSAR approaches, screening tests and genotoxicity assays regarding the prediction of rodent carcinogenicity have been published. Reference to these publications can provide an insight into the performance of the in vitro genotoxicity assays specifically in relation to the particular data sets analyzed and the end points considered, predominantly rodent carcinogenicity but also in vivo genotoxicity. Relevant sensitivity and specificity data and assay performance assessments have been summarized in Annex 1, and are discussed further in Annex 3, for information and are cited where appropriate in the text below. Overall the older available data suggest that mammalian cell assays did not perform well at discriminating between rodent carcinogens and non-carcinogens. However, recent experience suggests that mammalian cell tests conducted and interpreted according to current recommendations perform more robustly [10].

Stage 0: Preliminary Considerations Prior to Genotoxicity Testing

The intrinsic chemical and toxicological properties of the test substance must be considered before devising the genotoxicity testing programme.

Physico-chemical and Toxicological Properties

The physico-chemical properties of the test substance (for example, pKa, partition coefficient, solubility, volatility and stability in, and potential reactions with and solvents/vehicles) and its purity can affect the ease of conduct and results of in vitro tests. For example, the tolerance of cells to acidic chemicals can be enhanced by neutralization but this may affect the inherent reactivity of substances to DNA Potential reactions of the test substance with solvent /vehicle should also be considered (e.g cisplatin reacts with DMSO) Alternatively, low solubility may limit the feasibility of undertaking some or all of the in vitro mutagenicity tests recommended in this strategy. The potential for auto-oxidation of the test chemical in the culture medium can also affect the outcome of in vitro genotoxicity tests It is noteworthy that the toxic properties of test substances, such as target organ effects, or irritancy/corrosively in contact with skin or mucous membranes and their toxico-kinetic and metabolism will influence the choice of route of administration and the highest dose level achievable in Stage 2 in vivo mutagenicity tests [11-13].

STRUCTURE ACTIVITY RELATIONSHIP

Whether the test substance would be expected to have mutagenic potential may be assessed from its chemical structure, which may provide structural alerts for mutagenicity. A composite model structure was originally devised by Ashby and Tennant indicating substituent chemical groups or moieties associated with DNA-reactivity A number of freely available and commercial systems to investigate structure activity relationships (SAR) for mutagenicity have been developed and evaluated since 2000 Further information on various models is provided in Annex 1. The OECD and the European Commission have published principles for the validation of (Q) SAR ((Quantitative) Structure Activity Relationships) (Q)SAR assessment of the in vitro mutagenicity in bacteria has been attained by two types of approach; statistical analyses of structure and mutagenic activity Whether the test substance would be expected to have mutagenic potential may be assessed from its chemical structure, which may provide structural alerts for mutagenicity. A composite model structure was originally devised by Ashby and Tennant indicating substituent chemical groups or moieties associated with DNA-reactivity A number of freely available and commercial systems to investigate structure activity relationships (SAR) for mutagenicity have been developed and evaluated since 2000 Further information on various models is provided in Annex 1. The OECD (OECD, 2004) and the European Commission have published principles for the validation of (Q)SAR ((Quantitative) Structure Activity Relationships) (Q)SAR assessment of the in vitro mutagenicity in bacteria has been attained by two types of approach; statistical analyses of structure and mutagenic activity the derived rules can be used for preliminary identification of in vivo mutagens Overall, (Q)SAR approaches for the prediction of genotoxic activity can be a valuable tool to aid in the high throughput screening of compounds, the provision of assessments for chemicals for which no genotoxicity test data are available and also prioritisation for genotoxicity testing. (Q)SAR can also aid in the interpretation of genetic toxicology tests, although such predictions cannot replace the need to undertake the in vitro and in vivo genotoxicity tests required to derive conclusions on mutagenic hazard. However, expert judgement is needed when reaching conclusions on mutagenic hazard on the basis of (Q) SAR information alone. In reaching conclusions, data from well conducted in vitro or in vivo genotoxicity tests should be attributed a much higher weight of evidence than (Q) SAR predictions, although all information should be assessed on a case-by-case basis [14].

Screening test

There are a number of current initiatives which attempt to combine data mining in silico approaches with high throughput tests to develop approaches to screening large numbers of novel chemicals In this guidance, genotoxicity screening tests refers to high throughput tests which have been designed to be rapid, economical, reproducible, require only small amounts of test substances and have a high concordance with comparator genotoxicity end points in genotoxicity tests. High throughput bacterial tests have been developed using combinations of Salmonella tester strains primary DNA damage (umu assay), mutations in ampicillinase gene (Muta-Gen assay), bioluminescence or 5- fluorouracil resistance Other screening systems cited in the literature include DNA repair activity in yeast cells One research group has proposed a combination of two commercial screening assays for rapid screening of compounds. A number of genotoxicity screening tests using in vitro systems have been proposed, including alkaline elution using rat hepatocytes the detection of DNA damage in cell lines and

differential survival in DNA repair proficient and deficient cell lines A screening test for genotoxicity using HepG2 cells based on four different luciferase-reporter assays has been published. The authors claim, based on a small dataset, a high sensitivity for identification of genotoxicity when used in combination with the commercially available systems none of these genotoxicity screening tests have reached the stage of development where they could routinely be used to replace data generated from in vitro genotoxicity testing. The predominant use of high throughput screening tests is as an aid in prioritisation of compounds for developmentundertaken by industry. The COM reviewed the GADD45a-GFP assay and it was agreed that currently, it is most suited as part of a battery of high throughput screening High throughput genotoxicity screening tests can be used in a tiered approach with in vitro genotoxicity tests to aid in the selection of chemicals for development. It has been suggested that greater validation and acceptance by regulatory authorities of these tests could lead to the replacement of existing genotoxicity testing strategies with a combination of high throughput screening tests [15].

Stage 1: In Vitro Genotoxicity Testing

Overview of strategy The COM concluded in 1989 and 2000 that it was appropriate to concentrate on a relatively small number of assays, using validated, sensitive methods particularly chosen to avoid misleading negative results. Two important parts of the revised Stage 1 strategy include using appropriate tests to gain an insight into the nature of the genotoxic effects of a test substance and also to avoid misleading positive results. Misleading positive results have been reported for certain mammalian cell assays particularly when multiple test systems were used. As outlined above in paragraphs 13 and 14, Stage 1 involves tests for genotoxic activity using in vitro methods and comprises a two test core system (namely an Ames test and in vitro micronucleus test (MNvit)) with the objective of assessing mutagenic potential by investigating three different end points (gene mutation, structural chromosomal damage and changes in chromosome number). The rationale for this test strategy is given in Annex 3. A clear positive result in either of these two core tests is sufficient to define the chemical as an in vitro mutagen, although further in vitro and/or in vivo testing may be undertaken to understand the relevance of the positive results. The Committee considers that this revised strategy allows for efficient identification of all mutagenic-end points but, by reducing the number of mammalian cell tests from that recommended by COM in 2000, and following improved methodologies, the risk of misleading positive results is decreased. Additional investigations of chemicals which give positive or repeated equivocal results in Stage 1 tests can include an assessment of mode(s) of in vitro genotoxic action. There are a number of reasons why positive results in in vitro genotoxicity tests might occur by mode(s) of action not relevant to human health hazard assessment Such MoGA evaluation in vitro is particularly relevant for those chemicals (e.g. cosmetics) where there is a regulatory constraint which precludes the use of in vivo genotoxicity assays in the testing strategy. The COM does not recommend the use of in vitro genotoxicity assays that have not been considered in detail in this guidance such as assays for sister chromatid exchange, the in vitro UDS assay or tests using fungi. A table of mutagenic endpoints detected by each genotoxicity assay cited in Stage 1. For chemicals which give equivocal results or repeated small positive effects, it is important to consider evidence of reproducibility in the same assay or in different assays detecting similar effects, and the magnitude of the induced genotoxic effect in relation to historical negative control data, and then consider whether further in vitro genotoxicity

testing is needed. Further consideration of SAR data for these chemicals may also give valuable information if clear negative results are obtained in both core in vitro tests undertaken, it can generally be concluded that the chemical has no mutagenic activity. However, there are some occasions when additional in vitro and/or in vivo genotoxicity testing may be undertaken for chemicals giving a negative response in the two in vitro core genotoxicity tests. For example, situations where tumours are found in rodents, where the in vitro metabolic activation systems are not optimal or where there are human-specific metabolites, may need to be subject to further genotoxicity assessment. A further testing strategy would have to be designed on a case-by-case basis An IWGT working group has published guidance on this topic An important part of any additional in vitro strategy should be consideration of the appropriate exogenous metabolic activation system Further information on in vivo genotoxicity testing of such test substances is provided in Stage 2 of this strategy. Information from other combinations of genotoxicity tests which may include one or more non-core tests outlined below in paragraphs 54-59 may also give adequate data on all three end-points on a case-by-case basis. In vitro genotoxicity tests using human reconstructed skin may provide useful information on in vitro mutagenic hazard in circumstances where in vivo testing is not permitted, or when extensive dermal exposure is anticipated (e.g. cosmetic ingredients). The full Stage 1 strategy should be performed and the results of studies evaluated before a decision is made on whether to proceed to Stage 2 testing or whether a conclusion on mutagenic hazard can be derived for test substances where no in vivo genotoxicity testing is permitted. An outline of Stage 0 and Stage 1 (in vitro genotoxicity testing) and a description of the assays recommended is provided in the following paragraphs [16-18].

Discussion Stage 1: Non-Core Tests

In Vitro Chromosomal Aberration Assay in Mammalian Cells (Metaphase Analysis) for Clastogenicity and Aneuploidy

The in vitro chromosome aberration assay in mammalian cells has been widely used in genotoxicity testing for many decades and provides information on genetic damage that may be associated with adverse health outcomes. Only limited information can be obtained on potential aneugenicity by recording the incidence of polyploidy and/or modification of mitotic index The COM notes that polyploidy may not be a reliable indicator for aneugenicity and may result from a number of different genetic changes It is possible to adapt the chromosome aberration assay to include the use of chromosome specific centromere probes with FISH to assess the potential for aneuploidy An IWGT working group has agreed that the preferred measure of cytotoxicity in the chromosomal aberration test should be one based on cell proliferation (e.g. relative population doubling or relative increase in cell counts) compared to negative control cultures rather than simple cell counts. The available data indicate that the in vitro metaphase analysis and the in vitro micronucleus assay have similar overall performance for determination of clastogenicity. On balance it is considered preferable to use the in vitro micronucleus test for the initial assessment of clastogenic and aneugenic potential

In Vitro Mouse Lymphoma Assay for Gene Mutation and Clastogenicity

The COM reaffirms the view stated in the 1989 and 2000 guidance, that the most appropriate in vitro mammalian cell gene mutation test is the mouse lymphoma assay. Since 2000, there has been considerable development of suitable protocols, negative solvent control data, criteria to define an acceptable positive control response and the use of the Global Evaluation Factor and statistical analysis

of test results Many of the published studies were undertaken by the US National Toxicology Program and a recent re-evaluation of these results shows many of the studies to be uninterpretable or the outcomes to be equivocal Some authors have reported that the mouse lymphoma assay can detect, in addition to gene mutations and clastogenicity, information on recombination, deletion and aneuploidy It is possible that aneuploidy in these cells could be a secondary effect of chromosomal rearrangement. However, the COM considers that this assay is not appropriate for the routine assessment of aneuploidy.In Vitro HPRT assays for Gene Mutation An in vitro cell mutation assay which uses forward mutation in the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene to assessmutations has been developed in several cell lines, principally Chinese hamster ovary cells (CHO) cells it is described in the OECD 476 guideline. The Committee have previously considered the sensitivity of this assay and it was concluded that 107 surviving cells are required for a valid test. Thus, certain mammalian cell gene mutation protocols that have been widely used, particularly some involving CHO cells, are considered to be insufficiently sensitive for the identification of mutagens, predominantly on statistical grounds [19].

In Vitro Assays using Human Reconstructed Skin

A number of research groups have developed genotoxicity assays based on micronuclei measurement using commercial sources of human reconstructed skin or a co-culture technique involving reconstructed skin and mouse lymphoma L5178Y cells Proposals for the measurement of DNA damage using theComet assay in reconstructed skin have also been made The primary purpose in developing genotoxicity tests using reconstructed skin has been to supplement genotoxicity data-packages for cosmetic chemicals where no in vivo genotoxicity tests are permitted. A tiered approach to testing cosmetic ingredients for genotoxicity has recently been published.

In Vitro Alkaline Comet Assay for DNA Damage

The in vitro alkaline Comet assay for DNA damage has been proposed as an alternative to clastogenicity assessment in mammalian cells since cell proliferation is not needed, therefore any cell type can be used, and the assay is reported to result in fewer misleading positive results due to cytotoxicity or precipitation than chromosomal aberration tests The alkaline Comet assay detects a wide range of genetic damage including single and double strand breaks, repair induced breaks, alkali labile lesions and abasic sites. There is evidence that the in vitro Comet assay can be used to detect DNA cross-linking agents The Comet-FISH assay has been developed to provide information on site specific DNA strand breaks There is evidence that the in vivo Comet assay can detect substances that induce gene mutations in vitro Extrapolation from this suggests that the in vitro Comet assay can also detect substances that induce gene mutations and this capability has been demonstrated However, it is not recommended as a routine replacement for gene mutation tests in vitro. Thus, the Comet assay measures DNA damage irrespective of genotoxic end-point, with the exception of aneuploidy. A positive Comet assay result may be due to repairable DNA damage or lesions which lead to cell death and not necessarily mutations or micronuclei. Negative results from an Ames test and MNvit would reduce the level of concern associated with positive results from an in vitro Comet assay. Thus, the in vitro Comet assay can serve as a very useful adjunct to the recommended core-tests, especially in instances where in vivo testing is not permitted such as in cosmetic testing. However, since the Comet assay does not detect aneuploidy, and may report repairable DNA

damage, it is not recommended as a core in vitro test.

Stage 2: In Vivo Genotoxicity Tests

Overview of Strategy Stage 2 of the testing strategy involves an assessment of genotoxic activity in vivo in somatic tissues and in germ cells The in vivo genotoxicity testing strategy has to be designed on a case-by-case basis and can be used to address aspects of in vivo mutagenicity, for example;Investigation of mutagenic end point(s) identified in Stage 1,Investigation of genotoxicity in tumor target tissue(s),Investigation of potential for germ cell genotoxicity,Investigation of in vivo mutagenicity for chemicals which were negative in Stage 1 but where there is high or moderate and prolonged exposure.Investigation of genotoxicity in site of contact tissues.

It is thus possible for there to be one or more separate Stage 2 strategies designed to assess points 1)-5) for a particular test substance. This rationale leads to different approaches from those advocated by the COM in 2000 where the weight of available evidence suggested that the in vivo bone marrow micronucleus assay or bone marrow clastogenicity assay in rodents was the preferred first test in almost all cases. The exception was for direct acting DNA reactive mutagens where a site of contact test was the preferred first test. There was a preference in the 2000 COM guidance for the rat liver UDS assay as a second tissue in vivo test, which was selected primarily to provide reassurance of absence of in vivo genotoxicity when positive results had been obtained in vitro but negative results were obtained in an in vivo bone marrow micronucleus or chromosomal aberration assay. The selection of rat liver UDS was based largely on experience in use and the availability of an OECD guideline (DOH, 2000). The revised in vivo Stage 2 strategy based on the selection of tests to provide information on one or more specific aspects such as species and/or tissue genotoxicity combined with investigation of particular genotoxic end points and modes of genotoxic action does not necessarily lead to the selection of the rodent bone marrow micronucleus test as the first assay or the rat liver UDS assay as a second tissue assay. A table of in vivo genotoxicity tests and end-points is provided in Annex 2. Genotoxicity testing strategy include whether the testing strategy can be integrated into other regulatory toxicity tests (such as sub-acute or sub-chronic toxicity studies). Consideration needs to be given to the nature of the chemical (including physico-chemical properties), the results obtained from in vitro genotoxicity tests and the available information on the toxicokinetic and metabolic profile of the chemical (for example when selecting most appropriate species, tissue and end point). The routes of exposure in animal studies should be appropriate to ensure that the substance reaches the target tissue. Routes unlikely to give rise to significant absorption in the test animal should therefore be avoided. Unless systemic exposure can be confirmed from other toxicological studies, or evident toxicity in the target organ is seen, confirmatory toxicokinetic studies to measure blood or tissue exposure as appropriate should be undertaken to accompany all in vivo genotoxicity studies to assess the adequacy of any negative results obtained. The design of in vivo genotoxicity tests should incorporate appropriate approaches to reduce the number of animals used in tests, such as the integration of genotoxicity endpoints into repeat-dose studies. Options for reduction in animal usage include: use of one sex only (if supported by metabolism data or other data indicating equivalence), reduced numbers of sampling times for micronucleus and chromosomal aberration assays when repeat dosing is performed, integration of micronucleus and comet end points into repeat-dose toxicity (including transgenic mutation) studies, combining micronucleus and Comet assays into a single acute test employing a few

administrations of test chemical. Stage 2 in vivo genotoxicity tests should be undertaken for test substances that are positive in any of the in vitro Stage 1 genotoxicity tests where there is a need to ascertain whether genotoxic activity can be expressed in vivo. There are many reasons why activity shown in vitro may not be observed in vivo (for example, lack of absorption, inability of the active metabolite to reach DNA, rapid detoxication and elimination). Data from in vivo genotoxicity tests are, therefore, essential before any definite conclusions can be drawn regarding the potential mutagenic hazard to humans from test substances which have given positive results in one or more in vitro genotoxicity tests. However, conclusions on mutagenic hazard and MoGA may have to be derived from in vitro genotoxicity data for test substances when no in vivo genotoxicity testing is permitted. In addition, an in vivo genotoxicity test may give positive results for chemical substances which only act in vivo; experience though, has shown that such chemicals are rare [20].

CONCLUSION

To assay for genotoxic molecules, researchers assay for DNA damage in cells exposed to the toxic substrates. This DNA damage can be in the form of single and double-strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations, and structural and numerical chromosomal aberrations. The compromised integrity of the genetic material has been known to cause cancer. As a consequence, many sophisticated techniques including Ames Assay, *in vitro* and *in vivo* Toxicology Tests, and Comet Assay have been developed to assess the chemicals potential to cause DNA damage that may lead to cancer. For prediction of genotoxicity various I silico software was employed.

Table 1: In Silico software used for prediction of Genotoxicity

Name of software	Method	Prediction	Applicability	Availability
TOPKAT	QSAR statistical method on 2D descriptors (e-state, topological)	Toxicolical end point,include mutagenicity,devlopmental toxicitiy rodent,carcinogenicity	Optimum predictive space	No Freely available
VEGA Caesar	QSAR statistical model based on Neural Network + rule-based model	Mutagenicity(ames),carcinogenicity,developmental toxicity,skin sensitization,bioconcentration factor	Quantitative AD Measurement	Freely available
+Toxtree	Collection of knowledge rule Based	Carcinogenicity, mutagenicity, aromatic amine, alpha, beta, unsaturated aldehyde.	Not applicable	Freely available
ACD Tox Suite	Statistical model employing binominal PLS, predefined set of	Ames genotoxicity	Reliability index	No Freely available

	fragmental descriptors, local correction to baseline, using experimental data for similar compound			
VEGA SARpy	Statistical model based on structural alerts automatically selected on the basis of their occurrence in toxic/nontoxic compounds	Ames mutagenicity	Quantitative AD Measurement	Freely available
Derek Nexus	Collection of knowledge—rule based	developmental toxicity (3 alerts), teratogenicity (5 alerts), testicular toxicity (1 alert) and oestrogenicity (4 alerts)	Not applicable	No Freely available
Leadscope	QSAR methodologies	The Leadscope software has a module containing QSAR models for predicting the developmental toxicity of the rodent foetus, including dysmorphogenesis (structural and visceral birth defects), developmental toxicity (foetal growth retardation and weight decrease), and foetal survival (Foetal death, post-implantation loss, and preimplantation loss). The Leadscope QSAR models for reproductive toxicity include rodent male reproductive, rodent male	Classification models for developmental toxicity in the rodent fetus dysmorphogenesis (structural and visceral birth defects), developmental toxicity (fetal growth retardation and weight decrease), and fetal survival (fetal death, post-implantation loss, Preimplantation loss). Models of reproductive	Commercial

		sperm, female reproductive	toxicity: rodent male reproductive, rodent male sperm, female reproductive	
Endocrine Disruptor Knowledge Base (EDKB) database (US FDA)	*In vitro* or *in vivo* tests. *in silico* models for endocrine disruption	Computer based predictive models to predict the binding affinity of compounds to the oestrogen and androgen nuclear receptor proteins	Quantitative models to predict the binding affinity of compounds to the estrogen and androgen nuclear receptor proteins	Commercial
Multicase (MC4PC)	based on a fragment-based technology sometimes referred to as the CASE approach	quantitative prediction of mutagenicity which is then further refined through taking into consideration physico-chemical properties as well as the existence of potential 'deactivating fragments' or biophobes	Classification models for developmental toxicity associated with a variety of Datasets, mainly drugs. The marketed software includes modules for predicting mammal sperm toxicity, developmental toxicity, developmental fetal growth retardation, development fetal weight decrease and survival fetal death	Commercial
ADMET Predictor	Neural network ensemble	This commercial program is designed to estimate certain ADMET (Absorption, Distribution,	Qualitative and quantitative prediction of oestrogen	Commercial

		Metabolism, Elimination, and Toxicity) properties of a drug-like chemical from its molecular structure. It includes a qualitative assessment of oestrogen receptor toxicity in rats (TOX_ER_filter), together with a quantitative measure of oestrogen receptor toxicity in rats (TOX_ER (IC50(estrogen)) that is applied only for compounds classified as 'Toxic' by the previous model	receptor toxicity in rats. Based on two models: a qualitative model and, if toxic, the quantitative ratio of IC50 estradiol/IC50 compound). Moleculardescript or space	
MolCode Toolbox	QSAR methodologies	predicting toxicological endpoints and ADME properties between them endocrine activity	Quantitative prediction of rat ER binding affinity and AhR binding affinity	Commercial
OSIRIS property explorer	QSAR methodologies		Classification model which predicts "undesirable" effects (mutagenicity, tumorigenicity, irritating effects and reproductive effects), mainly based on the RTECS database of >3500 compounds	Freely available
PASS	QSAR methodologies	Prediction of Activity Spectra for Substances The system predicts the probability (Pa) of a biological activity	Classification models giving probability of reprotoxic effects. The	Commercial

		for a new compound, by estimating the similarity/dissimilarity of the new substance to substances with well-known biological activities present in the training set	embryotoxicity model predicts the probability that a substance crosses the placental membrane and causes any toxic effect (e.g. fetal bradycardia, low Birth weight) or death of an embryo. The teratogenicity model predicts the probability that a substance crosses the placental membrane and causes abnormal development of one or more body systems in the embryo	
T.E.S.T.: The Toxicity Estimation Software Tool	QSAR methodologies Consensus method: average of the predicted toxicity values by 3 models: neural network, Food and Drug Administration, and hierarchical clustering	It estimates the toxicity of a compound by applying several QSAR methodologies thus allowing the user to have greater confidence in predicted toxicities. Among other toxicities it predicts developmental toxicity	Quantitative AD Measurement Developmental toxicity estimation. The prediction is done by applying several QSAR methodologies resulting in a greater confidence of the results	Freely available
TIMES	QSAR statistical	The TIMES platform is	Classification	Commercial

(COREPA) Laboratory of Mathematical	model based	also used to predict different endpoints including receptor mediated endpoints for oestrogen, androgen and aryl hydrocarbon binding affinity. predict oestrogenicity	models for the prediction of estrogen, androgen and aryl Hydrocarbon binding. The chemical is predicted to fall in one of several activity bins (ranges of binding affinity)	
VirtualToxLab	multi-dimensional QSAR (mQSAR)	predicting endocrine disrupting potential by simulating and quantifying the interactions with aryl hydrocarbon, oestrogen alpha/beta, androgen, thyroidalpha/beta, glucocorticoid, liver X, mineralocorticoid and peroxisome proliferator-activated receptor gamma	Classification model for endocrinedisrupti ong potential based on simulations of the interactions towards aryl hydrocarbon, estrogen, androgen, thyroid, glucocorticoid, liver X, mineralocorticoid, peroxisome proliferator-activated receptor •, as well as the enzymes CYP450 3A4 and 2A13	Commercial
OECD QSAR Application Toolbox	(Q)SAR Toolbox	The Toolbox also includes a range of profilers to quickly evaluate chemicals for common mechanisms or modes of action	Altough primarily a tool for chemical categories and read-acros, it also includes several databases, including	Freely Available

			reprotoxicity data: 166,072 ER binding data from Danish EPA (pre-generated predictions, not experimental values) as well as 1606 experimental ER binding affinity values from the OASIS commercial database	
Toxmatch	2D similarity methods	Read-across predictions were performed for the 17 query chemicals for which a category could be formed	This study illustrates the use of 2D similarity indices within Toxmatch to form categories for 57 query chemicals. The underlying hypothesis is that chemicals selected as being similar should act via a single mechanism of action, even if that mechanism is unknown	freely available
CASE(Computer Automated Structure Evaluation)	QSAR models	to predict Ames test mutagenicity using 520 proprietary drug candidate (Test set 1) and 94 commercial (Test set 2) compounds	Improve current predictive toxicology capabilities for mutagenicity and carcinogenicity through	Commercial

			customizing and augmenting current predictive software. Prioritization of synthesis & testing candidates. Identification of substructures responsible for an observed mutagenic liability and suggested synthetic alternatives. Regulatory and due diligence support	
Oncologic	QSAR models	Rule based expert system for the prediction of carcinogenicity	Knowledge based expert system , mimicking the decision logic of human experts	Commercial
Computer-Aided Molecular Modeling (CAMM)	3D-receptor modelling , Virtual screening , Fragment-based screening	Optimization of chemical series (quality of leads)All activities of promising compound classes should focus on multiple ADME–Tox-related parameters in parallel to activity and selectivityResults of commercially available tools for calculating physicochemical properties and ADME-related parameters have to be interpreted with great care, The use of generic models can only be recommended	assessment of impurities, degradation products, side products, metabolites,...e.g. structural evaluation of synthesis schemes	Commercial

		if they have been validated for a particular project; results of new compounds outside of the training sets can be misleading (ionization constants, lipophilicity and solubility) Shift in optimization strategy, use of measured values calls for high quality, fast and standardized assays (100–500 compounds per week)		
VEGA Caesar	QSAR statistical model based on Neural Network + rule-based model	The integrated model utilizes two complementary techniques in series: a Machine-learning algorithm (support vector machine), In the present study, the conservative approach was adopted, and thus the chemicals labelled as suspicious were considered mutagenic. The list of structures, toxicity data, and chemical descriptors is available online	Quantitative AD Measurement	Freely Available
LAZAR	It is based on the use of statistical algorithms for classification (k-nearest neighbours and kernel models) and regression (multi-linear regression and kernel models)	toxicological endpoints (currently, mutagenicity, human liver toxicity, rodent and hamster carcinogenicity	performs automatic applicability domain 14 estimation and provides a confidence index for each prediction, and is usable without expert knowledge	Freely Available

REFERENCE

[1] Contrera JF, Matthews EJ, Kruhlak NL, Benz RD. In silico screening of chemicals for bacterial mutagenicity using electrotopological E-state indices and MDL QSAR software. Regul Toxicol Pharmacol 2005; 43:313-323.

[2] Hansen K, Mika S, Schroeter T, Sutter A, ter Laak A, Steger-Hartmann T,Heinrich N, M¨ uller KR. Benchmark data set for in silico prediction of Ames mutagenicity.J Chem Inf Model2009; 49:2077-2081.

[3] Mazzatorta P, Tran LA, Schilter B, Grigorov M. Integration of structure-activityrelationship and artificial intelligence systems to improve in silico prediction of Amestest mutagenicity. J Chem Inf Model2007; 47:34-38.

[4] Sushko I, Novotarskyi S, K¨orner R, Pandey AK, Kovalishyn VV, Prokopenko VV,Tetko IV. Applicability domain for in silico models to achieve accuracy of experimentalmeasurements. J Chemometrics2010; 24:202-208.

[5] Helma C, Cramer T, Kramer S, Raedt LD. Data mining and machine learningtechniques for the identification of mutagenicity inducing substructures and structureactivity relationships of noncongeneric compounds. J Chem Inf Comput Sci2004; 44:1402-1411.

[6] Kazius J, McGuire R, Bursi R. Derivation and validation of toxicophores for mutagenicitypredictionJ Med Chem 2005; 48:312-320.

[7] Feng J, Lurati L, Ouyang H, Robinson T, Wang Y, Yuan S, Young SS. Predictivetoxicology: benchmarking molecular descriptors and statistical methods. J Chem InfComput Sci2003; 43:1463-1470.

[8] Judson PN, Cooke PA, Doerrer NG, Greene N, Hanzlik RP, Hardy C, et al. Towards the creation of an international toxicology information centre. Toxicology2005; 213:117-128.

[9] Steinbeck C, Han Y, Kuhn S, Horlacher O, Luttmann E, Willighagen EL. TheChemistry Development Kit (CDK): an open-source java library for chemo- and bioinformatics.J Chem Inf Comput Sci2003; 43:493-500.

[10] Benigni R, Bossa C, Netzeva T, Rodomonte A, Tsakovska I. Mechanistic QSAR ofaromatic amines: new models for discriminating between mutagens and nonmutagens,and validation of models for carcinogens. Environ Mol Mutag2007; 48:754-771.

[11] Benigni R, Bossa C. Mechanisms of chemical carcinogenicity and mutagenicity: areview with implications for predictive toxicology. Chem Rev 2011; 111:2507-2536.

[12] Piegorsch WW, Zeiger E. Measuring intra-assay agreement for the Amessalmonella assay. In: L Hotorn, ed. Statistical Methods in Toxicology. Lecture Notes inMedical Informatics. Berlin: Springer-Verlag 1991; 43:35-41.

[13] Kamber M, Fl ¨ uckiger-Isler S, Engelhardt G, Jaeckh R, Zeiger E. Comparison ofthe Ames II and traditional Ames test responses with respect to mutagenicity, strainspecificities, need for metabolism and correlation with rodent carcinogenicity. Mutagen2009; 24:359-366.

[14] Milan C, Schifanella O, Roncaglioni A, Benfenati E. Comparison and possible useof in silico tools for carcinogenicity

within REACH legislation. J Environ Sci Health CEnviron Carcinog Ecotoxicol Rev2011; 4:300-323.

[15] Piegorsch WW, Zeiger E. Measuring intra-assay agreement for the Ames salmonella assay. In: L Hotorn, ed. Statistical Methods in Toxicology.Lecture Notes inMedical Informatics. Berlin: Springer-Verlag 1991; 43:35-41.

[16] Kamber M, Fl ¨ uckiger-Isler S, Engelhardt G, Jaeckh R, Zeiger E. Comparison ofthe Ames II and traditional Ames test responses with respect to mutagenicity, strainspecificities, need for metabolism and correlation with rodent carcinogenicity. Mutagen2009; 24:359-366.

[17] Dobo KL, Greene N, Fred C, Glowienke S, Harvey JS, Hasselgren C, et al. In silicomethods combined with expert knowledge rule out mutagenic potential of pharmaceuticalimpurities: an industry survey. Regul Toxicol Pharmacol 2012; 62:449-455.

[18] Naven RT, Louise-May S, Greene N. The computational prediction of genotoxicity.Expert Opin Drug Metab Toxicol2010; 6:797-807.

[19] Hillebrecht A, Muster W, Brigo A, KansyM,Weiser T, Singer T. Comparative evaluationof in silico systems for Ames test mutagenicity prediction: scope and limitations.Chem Res Toxicol2011; 24:843-854.

[20] Naven RT, Greene N,Williams RV. Latest advances in computational genotoxicityprediction. Expert Opin Drug Metab Toxicol2012; 8:1579-1587.