[Frontiers in Bioscience, Elite, 5, 375-398, January 1, 2013]

Regulatory issues in management of chemicals in OECD member countries

Valeria V. Kleandrova[1], Alejandro Speck-Planche[2]

[1]Faculty of Technology and Production Management, Moscow State University of Food Production, Volokolamskoe shosse 11, 125080 Moscow, Russia, [2]Department of Chemistry, Faculty of Natural Sciences, University of Oriente, Patricio Lumumba s/n, 90500 Santiago de Cuba, Cuba

TABLE OF CONTENTS

1. ABSTRACT

The chemical risk assessment is determinant for the approval of any kind of chemical. Each aspect of chemical is taken into consideration for the new chemical legislation registration, evaluation, and authorization of chemicals (REACH). However, some improvements can be made in order to select and authorize a chemical. QSAR techniques have been used for the study of several kind of toxicological properties in order to realize a deeper study concerning to risk assessment. For this reason, this work is focused into present a review of chemical legislation policies in the European Union (EU) and in Russia, and changes in chemicals regulations to meet the requirement

Table 1. Risk assessment framework (as adopted by DG SANCO).

Stage	Definition
Hazard identification	The identification of a risk source(s) capable of causing adverse effect(s)/event(s) to humans or the environment, together with a qualitative description of the nature of these effect(s)/event(s).
Hazard characterization	The quantitative or semi-quantitative evaluation of the nature of the adverse health effects to humans and/or the environment following exposure to a risk source(s). This must, where possible, include a dose response assessment.
Exposure assessment	The quantitative or semi-quantitative evaluation of the likely exposure of man and/or the environment to risk sources from one or more media.
Risk characterization	The quantitative or semi-quantitative estimate, including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s)/event(s) in a given population under defined exposure conditions based on hazard identification, hazard characterization and exposure assessment.

of REACH. Also, we reported the used of several approaches and chemo- bioinformatics tools applied to QSAR methodologies for the several parameters relative to toxicity and how they can be used for regulatory purposes in risk assessment.

2. INTRODUCTION

The regulation of chemicals constitutes a legislative intent of a variety of national laws or global initiatives such as agreements, strategies or conventions. These international initiatives define the rules of further regulations to be implemented locally as well as exposure or emission limits. Generally, regulatory agencies oversee the enforcement of these laws. Approximately 30,000 industrial chemicals used in Europe require additional safety testing to meet requirements of the new chemical regulation REACH (registration, evaluation and authorization of chemicals) (1). Since 2008, the REACH began to impact several companies across the world. Today, efficient and effective implementation of REACH continues to depend on the inter-action of Member State regulators during Indecision-making (2). This is the result of past political debates surrounding the legislation being narrowly focused on mechanisms for conducting hazard assessments. The main task of chemical legislations and risk assessment at the international level is harmonization with the globally harmonized system of classification and labeling of chemicals (GHS). For establishment of common criteria at the world level, it is essential to exchange the experience and knowledge about evaluation of toxicity of chemicals in different countries. Special attention should be paid to the excessive and irrational use of chemicals, such as drugs, agrochemicals (including pesticides) and food additives. In this sense, if we take into consideration the number of chemicals mentioned above, and they are tested on animals, this testing would require the use of an extra 10–20 millions animals' experiments.

Quantitative Structure-Activity Relationships (QSAR) modeling is one major prospect between alternative testing methods to be used in a regulatory context. Briefly, QSAR-like techniques predicts the Activity (QSAR), Toxicity (QSTR), or Properties in general (QSPR) of drugs, hazard compounds, mixtures, polymers, proteins, RNAs, and other molecular entities using as input numerical parameters (called molecular descriptors) that describe quantitatively the structure of these molecular entities. The activity in the field is very active in terms of publications of research papers. In this sense, many recent papers published by different group have appeared that review the State-of-Art on QSAR. See for instance, several special issues guest-edited by González-Díaz *et al.* (3-49). In the context of REACH and the Cosmetics Directive (Council Directive 2003/15/EC), it is anticipated that QSARs will be used more extensively, in the interests of time- and cost-effectiveness and animal welfare. Many different QSAR models for prediction of properties relevant for chemical management exist and have been published in the literature (50). However, works related to the field of risk assessment approaches used in Russia have been poorly reported in scientific journals published in English. Therefore, this review is focused on presentation of risk assessment criteria and hazardous substances classifications used within the Organization for Economic Co-operation and Development (OECD) member countries, in EU and in Russia. Also we expose the important role of chemo-bioinformatics tools applied to QSAR techniques toward the study of toxicological profile of chemical and the main aspects of QSAR modeling for regulatory purposes.

3. RISK ASSESSMENT

There are many definitions of the related terms 'hazard' and 'risk' to be found in the risk literature. Simply stated, hazard is the potential for harm and risk is the probability (or likelihood) that the hazard is realized (51). For the purpose of simplicity of this section, the terminology adopted by the European Commission's Directorate General for Health and Consumer Protection (DG SANCO) in a comprehensive report has been used:

- *Hazard* – the potential of a risk source to cause an adverse effect(s)/event(s)
- *Risk* – the probability and severity of an adverse effect/event occurring to man or the environment following exposure, under defined conditions, to a risk source(s).
- *Risk assessment* – a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s) (Table 1). Risk assessment can be considered as the determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (also called hazard).

Quantitative risk assessment requires calculations of two components of risk: R, the magnitude of the potential loss L, and the probability p, that the loss will occur. Risk assessment consists in an objective evaluation

Figure 1. Stages related to risk management.

of risk in which assumptions and uncertainties are clearly considered and presented. Part of the difficulty of risk management is that measurement of both of the quantities in which risk assessment is concerned - potential loss and probability of occurrence - can be very difficult to measure. The chance of error in the measurement of these two concepts is large. A risk with a large potential loss and a low probability of occurring is often treated differently from one with a low potential loss and a high likelihood of occurring. In theory, both are of nearly equal priority in dealing with first, but in practice it can be very difficult to manage when faced with the scarcity of resources, especially time, in which to conduct the risk management process. For this reason, the risk management will be formed by four stages (Figure 1). These are assess, evaluation, management and measure.

3.1. Types of risks

Although risks are defined in terms of probability and severity of effect, there are a range of characteristics, which can influence their acceptability or otherwise as outlined below:

- *Nature of hazard* – hazards, which are man-made, are generally regarded as being of more concern than those that are 'natural'.

- *Nature of effect* – certain effects, with particular reference to cancers, are often perceived with dread (hence the terms 'dread risks').

- *Acute vs. chronic* – in parallel with the above, acute (short-term) effects as a result of acute exposure are often of less concern than chronic (long-term) or delayed effects as a result of chronic exposure.

- *Reversible vs. irreversible effects* – similarly, irreversible effects are generally of more concern than reversible effects.

- *Risks and benefits* – for those taking the risks also receive the benefits (for example, workers), the tolerability of risks is higher than for those who take the risks without the benefits.

- *Known vs. unknown* – as would be expected, people are more concerned about risks that are difficult to understand or are very uncertain than where the risks are clearly understood.

Historically, the focal point for data and the assessment procedure on dangerous chemicals in EU is the European Chemicals Bureau (ECB). The ECB provides scientific and technical support for the conception, development, implementation and monitoring of EU policies related to dangerous chemicals. It co-ordinates the EU risk assessment programs covering the risks posed by existing substances and new substances to workers, consumers and the environment. It supports the legal classification and labelling, the notification of new substances, the information exchange on import and export of dangerous substances, the development and harmonization of testing methods and the authorization of biocides. Thus, biocides work area provides Technical and Scientific support to Member States' Competent Authorities and the Commission with respect to the implementation of the Biocidal Products Directive (BPD) 98/8/EC on the placing on the market of biocidal products, which entered into force on 14 May 2000 (52). The Directive defines biocidal products and sets out a frame for their evaluation in a two step procedure where the first step is the entry of the active substances onto Annex I (or IA or IB) and the second step is the authorization of the products in which the active substances are used. Active substances are divided into:

- New active substances that cannot be placed on the market for biocidal purposes unless they are included onto Annex I.

- Existing active substances evaluated in the Review Programme, according to Article 16 of the BPD. The Review Program was established via several Regulations.

The latest is regulation (EC) No 1451/2007, which repeals regulation (EC) No 2032/2003, and entered into force on 31 December 2007. Today, the REACH legislation defines the functions of the European Chemical Agency (EChA), established in Helsinki, which is dedicated to the management of the REACH legislation at the community level (Table 2). The proposal for the REACH was approved by the European Parliament on 13th December 2006 and by the Council of Ministers on 18th December 2006. The regulation came into force in April 2007. In the EU, risk assessment of chemical substances is driven by European Commission (EC) policies and regulations.

3.2. Risk assessment in public health

In the context of public health, risk assessment is the process of quantifying the probability of a harmful effect to individuals or populations from certain human activities. In most countries, the use of specific chemicals, or the operations of specific facilities (e.g. power plants, manufacturing plants) is not allowed unless it can be shown that they do not increase the risk of death or illness above a

Table 2. Policies and regulations considered by EC.

Regulations	Short description and Last changes
European Union **White Paper** on the Strategy for a future Chemicals 2001.	Strategy for a future Chemicals Policy ''new'' and **''existing'' substances (2003 published), proposed** REACH.
TGD-technical guidance Document (European Chemicals Bureau (2002))	Supports the Directive on New Substances and the Regulation on Existing Substances. Includes a chapter providing guidance on the use of QSARs in the Environmental Risk Assessment.
Directive on Dangerous Substances 67/548/EEC Classification and Labelling of Dangerous Substances	The Directive specifies the hazard classification, packaging and labelling requirements for dangerous substances supplied in the European Union. In view of the adoption of Regulation (EC) No. 1907/2006 concerning the REACH Directive 67/548/EEC should be adapted and its rules on the notification and risk assessment of chemicals deleted.
Commission Directive 93/67/EEC on risk assessment, notified in accordance with Council Directive 67/548/EEC	Defining the principles for risk assessment for substances subject for registration.
EC Council Regulation on Existing Substances **(EEC) 793/93** Evaluation and control of the risks of existing substances should be carried out in four steps: data **collection, priority setting; risk assessment and risk reduction.**	Distinction between "new" and "existing" substances. Evaluation and control of the risks posed by approximately 100,000 existing substances. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 and Directive 2006/121/EC repealing Council Regulation (EEC) No. 793/93.
Commission Regulation **(EC) No.1488/94**	Implementation provisions for risk assessment of existing substances. Regulation (EC) No. 1907/2006 and Directive 2006/121/EC repealing Commission Regulation (EC) No.1488/94.
Directive 76/769/EEC Restrictions on the marketing and use of certain dangerous substances and preparations.	REACH is replacing most of the existing chemicals legislation, with the aim of streamlining and updating it. Regulation (EC) No. 1907/2006 and Directive 2006/121/EC repealing Council Directive 76/769/EEC.

specific threshold (53). In the estimation of the risks, three or more steps are involved, requiring the inputs of different disciplines.

- *Hazard identification*: aims to determine the qualitative nature of the potential adverse consequences of the contaminant (chemical, radiation, noise, etc.) and the strength of the evidence it can have that effect. This is done, for chemical hazards, by drawing from the results of the sciences of toxicology and epidemiology. For other kinds of hazard, engineering or other disciplines are involved.

- *Dose-response analysis*: focused on determining the relationship between dose and the probability or the incidence of effect (dose-response assessment). The complexity of this step in many contexts derives mainly from the need to extrapolate results from experimental animals (e.g. mouse, rat) to humans, and/or from high to lower doses. In addition, the differences between individuals due to genetics or other factors mean that the hazard may be higher for particular groups, called susceptible populations. An alternative to dose-response estimation is to determine an effect unlikely to yield observable effects, that is, a no effect concentration. In developing such a dose, to account for the largely unknown effects of animal to human extrapolations, increased variability in humans, or missing data, a prudent approach is often adopted by including safety factors in the estimate of the "safe" dose, typically a factor of 10 for each unknown step.

- *Exposure quantification*: permits to determine the amount of a contaminant (dose) that individuals and populations will receive. This is done by examining the results of the discipline of exposure assessment. As different location, lifestyles and other factors likely influence the amount of contaminant that is received, a range or distribution of possible values is generated in this step. Particular care is taken to determine the exposure of the susceptible populations.

The safety of a chemical, in terms of human health, is related with the maximum quantity to which a chemical can be used for the intended purpose, with a minimum risk of adverse health (side) effects. It can also be defined as a "socially acceptable" level of risk. The aim of regulatory toxicology is to determine "safe" levels of human exposure to toxicants which are present in the environment. Determining "safe" levels of exposure connected with *safety factors* (uncertainty factors) approach (1).

3.2.1. The importance of dose

The most famous saying in toxicology is 'Dosage alone makes the poison', written by Paracelsus, the 'father of toxicology', in the 16th century. Any substance is potentially toxic if the dose and duration of exposure are high enough. People die from drinking too much water and from eating too much salt. There are many ways in which a chemical might affect the health of an organism. These include corrosive or irritant effects, acute and chronic toxicity, effects on the nervous system, impairment of the reproduction of cells or the organism (carcinogenicity, mutagenicity – *i.e*. causing genetic mutations – and reproductive toxicity), and damage to the hormone system (endocrine disruption). For each type of toxic effect, there are specific tests designed to determine whether the effect is evident at different levels of concentration or dose (54). The key question relating to low levels of chemicals in the environment is how organisms react to doses much lower than those normally shown by such tests to cause harm. The answer comes from a dose-response (or, more precisely, a concentration–response) curve. This illustrates the relation between the amount of a chemical administered to an animal and the degree of response it produces. This response is measured by the percentage of the exposed population that shows the defined effect (Figure 2). If that effect is death, such a curve may be used to estimate an LD_{50} (Lethal Dose 50) value. LD_{50} is the dose of a

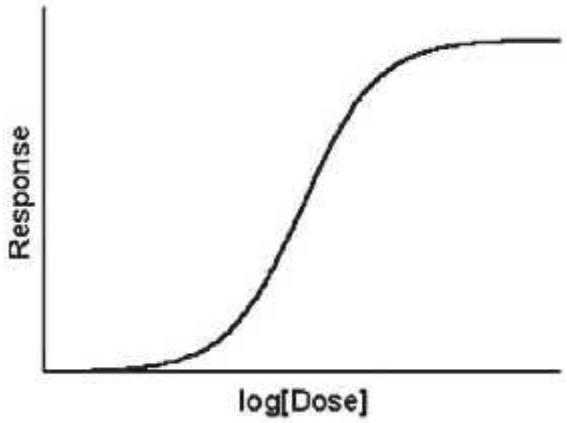

Figure 2. Shape of typical dose–response curve

chemical which kills 50% of a sample population. Such values are widely used as an effective measure of the potential toxicity of chemicals.

Most regulatory agencies assume that dose-response curves are all broadly of the shape shown in above figure in that there is a dose below which there are no significant biological effects. This is in agreement with observations, and also with expectations of how organisms should react when a response is due to binding at a receptor site. For this reason, many biologists believe evolutionary pressures have caused organisms to develop mechanisms to deal with low levels of insult. Finally, there is the issue of possible synergistic effects of mixtures of chemicals. Is it legitimate to consider a separate dose–response curve for each chemical in a mixture to which an organism is exposed? Or might the chemicals interact to reinforce each other's harmful effects, or even to negate each other's effects? We do know that pharmaceuticals can interact with each other in some cases and produce harmful or enhanced side-effects. In its comments to the Royal Commission on Environmental Pollution during the scoping of its chemicals study (55), the Natural Environment Research Council said 'Despite much discussion our actual knowledge of the synergistic effects of exposure to various groups of chemicals remains poor'.

3.3. Exposure standards, guidelines used in different countries

The establishment of exposure limits (variously referred to as standards, guidelines, quality criteria, etc.) includes consideration of the health-based scientific data and establishment of regulatory limits, taking into account the health-based recommendation along with other factors. Examples of *health-based exposure guidelines* include the acceptable daily intake (ADI), tolerable daily intake (TDI), provisional tolerable weekly intake (PTWI), and health-based maximum allowable concentrations (MAC). Acceptable/tolerable intakes are the amounts of a food additive, contaminant, pesticide or veterinary drug residue expressed on a body weight basis that can be ingested for a lifetime without appreciable risk to health (56). The term ADI is commonly used for additives to food since they impart some beneficial characteristic (and hence are considered "acceptable"), while a TDI commonly refers to environmental contaminants that are undesirable. Maximum allowable concentrations are either a time-weighted average concentration of a substance in a medium of exposure that does not present appreciable hazard for continuing exposure or an upper limit (ceiling value) which, if exceeded, will have adverse consequences for health. Often, health-based guidelines are considered, along with other factors (i.e., technological, socioeconomic, feasibility, enforcement), to develop operational regulatory limits such as the maximum residue level (MRL) for pesticides or veterinary drugs, MAC in exposure media and workplaces, occupational threshold limit values (TLV), maximum workplace concentrations (MAK), occupational exposure limits (OEL), air quality standards (AQS), water quality standards (WQS) or maximum use levels. In the case of the risk assessment to human health posed by chemicals can be done in different situations (Table 3), for example; exposures arising at work, from use in consumer products, from food or from exposures arising from environmental pollution in air, water and soil (57).

At the international level, there are different chemical databases that include risk assessment information. International toxicity estimates for risk database (ITER) is one of the free Internet databases on human health risk values from multiple organizations worldwide. ITER contains risk values and/or cancer classifications from six organizations: U.S. Agency for Toxic Substances and Disease Registry (ATSDR), Health Canada, International Agency for Research on Cancer (IARC), NSF International (NSF Intl), The National Institute of Public Health & Environmental Protection (RIVM) (the Netherlands) and, U.S. Environmental Protection Agency (US EPA). Risk values derived by independent groups will be accepted for inclusion on ITER after undergoing independent peer review and after approval by Toxicology Excellence for Risk Assessment (TERA). Risk value is determined like a dose in mg of chemical per kg of body weight per day (expressed as mg/kg-day), or concentration of chemical in mg of chemical per cubic meter of air (expressed as mg/m3) that for non-cancer toxicity is generally considered to be without adverse effects in populations of humans (including sensitive subpopulations) for the duration of exposure specified. Examples of non-cancer risk values (exposure limits) include: ADI, MRL, R_fD, R_fC, TC, TDI and etc (Table 4). For cancer toxicity, this dose or concentration is usually associated with a specified lifetime cancer risk from exposure to the chemical.

3.3.1. Non-occupational exposure limits for non-carcinogenic effects

Non-carcinogenic effects (e.g., neurotoxicity) are considered to have dose thresholds below which the effect does not occur. The lowest dose with an effect in animal or human studies is divided by safety factors (uncertainty factor [UF] and modifying factor [MF]) to provide a margin of safety. Exposure limits for non-carcinogenic effects: ADI, MRL, R_fD, R_fC, TC, TDI and etc. are used as permissible chronic exposure to human in living environment (see Table 4).

Table 3. Examples of exposure limits, standards, guidelines or quality criteria.

Media	Exposure limits or standards, guidelines, quality criteria, etc.
Food	Limits for food additives, contaminants, pesticide residues, veterinary drug residues. Limits for certain chemicals in food packaging materials. Limits for additives and contaminants in animal feed.
Cosmetics and other consumer products	Limits for additives and contaminants in cosmetic products (these include soap and toothpaste). Limits for other consumer products such as children's toys, paints and solvents.
Water	Drinking-water quality standards. Water quality standards for surface water. Water quality standards for fresh water used for fishing. Water quality standards for estuarine and marine waters. Aqueous effluent standards for industrial effluents and sewage treatment outfall. Guideline limits for the use of waste water in agriculture and aquaculture.
Air	Air quality (ambient or indoor) limits for gases, vapors, fibers, particulates Air quality standards for gaseous or smoke emissions from Industries.
Occupational exposure	Occupational exposure limits for gases, vapors, dusts, aerosol in workplace air and substances absorbed through the skin, mucous membranes or alimentary tract. Regulatory limits for exposure can be based on appropriate biomarkers.
Soil	Limits for certain chemicals in soil.
Agricultural chemicals	Limits for certain contaminants in agrochemicals (fertilizers). Limits for application rates of pesticides.
Chemical waste	Limits for disposal of chemicals as waste products (including liquid and solid). Chemical (including mixed industrial), dumps, surface water and deep well injection. Municipal surface and groundwater contamination, use of sludge in agriculture. Atmospheric effluents and residual ash from incineration.

Table 4. ITER non-cancer risk values.

Non-occupational exposure limits for non-carcinogenic effects. Acceptable daily intake (ADI) Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk. It is permissible chronic exposure levels for humans based on non-carcinogenic effects. ADI(human dose) = (NOAEL or LOAEL)(experimental dose) /UF, Where NOAEL no observed adverse effect level, LOAEL lowest observed adverse effect level, UF Uncertainty factor. Related terms: Reference dose (R_fD), tolerable daily intake (TDI). *Used by WHO*
Reference dose (R_fD) An estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime. R_fDs are based on non-carcinogenic effects and are usually calculated by applying uncertainty factors to a NOAEL or LOAEL. Expressed as mg/kg-day. *Used by the U.S. EPA and NSF International.*
Reference concentration (R_fC) An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime. R_fCs are based on non-carcinogenic effects and are usually calculated by applying uncertainty factors to a NOAEL or LOAEL. Expressed in units of mg/m^3. *Used by the U.S. EPA.*
Tolerable daily intake (TDI) The TDIs (or Tolerable Intakes (TIs)) expressed on a body weight basis (e.g., mg/kg b.w./day) are the total intakes by ingestion, to which it is believed that a person can be exposed daily over a lifetime without deleterious effect. The TDIs (or TIs) are based on non-carcinogenic effects and are usually calculated by applying uncertainty factors to a NOAEL or LOAEL. TDI = (NOAEL or LOAEL)/UF The term tolerable daily intake has been coined by the European Commission Scientific Committee on Food as a regulatory equivalent for acceptable daily *intake. TDI is expressed, unlike the ADI, in mg/person, assuming a body weight* of 60 kg. The term is in essence synonymous to acceptable daily intake for European Commission regulatory purposes. It tends to be used for contaminants rather than substances that might be deliberately added. *Used by OSHA - Occupational Safety & Health Administration, FAO, WHO, Health Canada and RIVM.*
Minimal risk level (MRL) The MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects over a specified duration of exposure. For inhalation or oral routes, MRLs are derived for acute *(14 days or less), intermediate (15-364 days), and chronic (365 days or more)* durations of exposures. *Used by ATSDR.*
Maximum contaminant levels (MCLs) The acceptable exposure level which, if exceeded, requires immediate water treatment to reduce the contaminant level. *Used by U.S. EPA for drinking water control.*
Tolerable concentration (TC) The TC (or TC in Air), generally expressed in mg/m3,isan airborne concentration to which it is believed that a person can be exposed continuously over a lifetime without deleterious effect. The TCs (or TCAs) are based on non-carcinogenic effects and are usually calculated by applying uncertainty factors to a NOAEL or LOAEL. *Used by Health Canada and RIVM.*

3.3.2. Occupational exposure limits

Occupational risk values in other terms are occupational exposure limits. The European Union has developed a program for protection of workers against risks from dangerous substances. Its objectives are: to prevent or limit the exposure of workers to dangerous substances at workplaces; and, to protect the workers that are likely to be exposed to these substances. Setting occupational exposure limits is an essential part of this strategy, which is endorsed under the following directives:

- Council Framework Directive 89/391/EEC on the introduction of measures to encourage improvements in the safety and health of workers at work.

Council Directive 98/24/EC on the protection of the health and safety of the workers from the risks relating

Table 5. Some of the most common non-carcinogenic occupational risk values.

Risk values/description
Maximale arbeitsplatzkonzentrationen Maximum concentration values (MAK) in the workplace. *Used in Germany*.
Technische richtkonzentrationen Technical exposure limits (TRK) . *Used in Germany*.
Maximale aanvaarde concentratie Maximale aanvaarde concentratie are dose levels that will not produce adverse health effects from repeated daily exposures in the workplace. *Used in the Netherlands*.
Maximum exposure limits (MEL) **MEL** is a recommendation by the ACGIH for the highest level of exposure to a chemical that is safe. The concentration in air to which it is believed that most workers can be exposed daily without an adverse effect. MELs are reserved for those substances for which a threshold cannot be identified or assumed (e.g. genotoxic carcinogens) or, where a threshold is considered to apply. *Used in United Kingdom*.
Occupational exposure limits (OEL) OELs are limits for concentrations of hazardous compounds in workplace air and are set by competent national authorities or other relevant national institutions. OESs are set for substances for which it is considered possible to identify, with reasonable certainty, an exposure concentration at which there is no significant risk to health. OELs are applied to those substances where there is believed to be a threshold for the critical effect. *Used in EU Member States and in some others countries*.

to chemical agents at work (the "Chemical Agents Directive").

- Commission Directive 2000/39/EC establishing a first list of indicative OELs (for 63 agents).

Threshold limit value (TLV) is one of the earliest quantitative criteria for evaluation occupational exposure levels developed in the 1940's by the American Conference of Governmental Industrial Hygienists (ACGIH). The TLV is defined as the concentration in air to which it is believed that most workers can be exposed daily without an adverse effect (i.e., effectively the threshold between safe and dangerous concentrations) (Table 5). This concept has developed steadily, and is now present in the legislation of most developed countries. In the United States there is the National Institute for Occupational Safety and Health (NIOSH)/Occupational Safety and Health Administration (OSHA) system of permissible exposure limits (PEL) originally based on the ACGIH TLV values. OSHA is responsible for promulgating and enforcing these limits. In Germany there are maximale arbeitsplatzkonzentrationen (MAK) maximum concentration values in the workplace) and technische richtkonzentrationen (TRK), or technical exposure limits. The United Kingdom has a system of occupational exposure standards (OES) and maximum exposure limits (MEL), and the European Union is developing a system of occupational exposure limits (OEL), which will apply to the whole Union. When estimating what is likely to be a human exposure that will not produce any adverse health effects (i.e., a safe level), uncertainty factors are used to make allowance for a lack of full information on the chemical being assessed. The factors selected are numbers such as 10 or 100, and they are applied to the most relevant dose level in safety evaluation studies in animals on the chemical in question, usually the highest level producing no adverse effects in the most sensitive species (1).

3.3.3. Evaluation of carcinogenic effects

Carcinogenicity risk value in the international level, as well as in Russia is the cancer slope factor (SF) This is a toxicity value that quantitatively defines the relationship between dose and response. Exposure is provided as daily averages over a lifetime. The cancer slope factor is a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to a chemical for a lifetime of 70 years. The cancer slope factor is expressed as mg/kg/day. The slope factor is expressed on the basis of chemical weight (milligrams of substance per kilogram body weight per day (mg/kg/day)) and can be used to compare the relative potency of different chemical substances on the basis either of chemical weight (as above) or moles of chemical (mmoles/kg/day). An oral SF is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime exposure to a contaminant (Table 6). This estimate is generally reserved for use in the low-dose region of the dose-response relationship. If the model selected for extrapolation from dose-response data is the linearized multistage model, the SF value is also known as the carcinogenic potency factor (CPF) value. Mathematical models are used to extrapolate from animal bioassay or epidemiology data to predict low dose risk. Most assume linearity with a zero threshold dose.

4. REGULATORY ELEMENTS OF CHEMICAL LEGISLATION IN RUSSIA

The main strategic document for chemical legislation in Russia is "The essential principles of the state policy to ensure chemical and biological safety of the Russian Federation for the period up to 2010 and for a longer terms," approved by ex-President of Russian Federation V.V. Putin on December 4, 2003. This document determines objectives, fundamental principles, priorities, tasks and measures of state support in ensuring chemical and biological safety of the individuals, society and the state as well as mechanisms and stages of implementation of the state policy in this field (58). One of the major tasks in improvement of legislation framework is harmonization of legislation framework of the Russian Federation in ensuring chemical and biological safety with provisions of international law, international treaties and agreements, of which the Russian Federation is a part of, in ensuring chemical and biological safety (Table 7). The laws and regulations of Russia aimed to protect of the environment and human health from adverse impacts of hazardous chemicals incorporate the following key documents:

Table 6. Cancer risk values

Cancer risk values
Cancer risk from inhalation exposure (CR(inhal)) The CR(inhal) is the 1 in 10,000 (E-4) lifetime excess cancer risk following exposure by inhalation (expressed in microgram/m3), as derived by RIVM. For comparison purposes on ITER, this value has been converted toa1in 100,000 (E-5) risk level, and has also been converted to milligrams/m3 .
Cancer risk from oral exposure (CR(oral)) The CR(oral) is the 1 in 10,000 (E-4) lifetime excess cancer risk following oral exposure (expressed in microgram/kg bw-day), as derived by RIVM. For comparison purposes on ITER, this value has been converted toa1in 100,000 (E-5) risk level, and has also been converted to milligrams/kg-day.
Risk specific concentration (RSC) **RSC.** The risk value of a chemical in mg/m^3 that is associated with a specified excess lifetime cancer risk, usually an upper 95% confidence limit. In ITER, all RSCs are calculated by TERA from the organization's unit risk or TC05 and represent the risk at a 1 in 100,000 (E-5) level.
Risk specific dose (RSD) **RSD** is the risk value of a chemical in mg/kg-day that is associated with a specified excess lifetime cancer risk, usually an upper 95% confidence limit. In *ITER, the RSDs for the U.S. EPA and Health Canada are calculated by TERA* from the organization's slope factor or TD05, respectively, and represent the 1 in 100,000 (E-5) risk level. NSF International calculates a human equivalent dose at the 10-5 risk level that is then used to calculate the TAC in drinking water.
Tumorigenic dose (TD05) **TD05** is the total intake (often expressed in mg/kg b.w./day) associated with a 5% increase in incidence or mortality due to tumors. The TD05 is not based on the confidence limit but rather is computed directly from the curve. Health Canada calculates TD05s for compounds classified in Groups I and II basing these values on tumors observed in epidemiological studies (generally) in occupationally exposed human populations, or those considered relevant to humans as observed in bioassays in experimental animals. The estimates of potency are generally restricted to effects for which there has been a statistically significant increase in incidence and a dose-response relationship, characterized by appropriate mathematical models (e.g., multistage). The Health Canada TD05 can be divided by a suitable margin, to provide a benchmark against which the adequacy of intake can be judged, with respect to potential carcinogenicity.
Tumorigenic concentration (TC05) **TC05** is the concentration in air (expressed in mg/m3) associated with a 5% increase in incidence or mortality due to tumors. The TC05 is not based on the confidence limit but rather is computed directly from the curve. Health Canada calculates TC05s for compounds classified in Groups I and II basing these values on tumors observed in epidemiological studies (generally) in occupationally exposed human populations, or those considered relevant to humans as observed in bioassays in experimental animals. The estimates of potency are generally restricted to effects for which there has been a statistically significant increase in incidence and a dose-response relationship, characterized by appropriate mathematical models (e.g., multistage). The Health Canada TC05 can be divided by a suitable margin, to provide a benchmark against which the adequacy of intake can be judged, with respect to potential carcinogenicity.

Table 7. Russian federal laws for chemical legislation

Regulations, Law	**Short description**
Federal Law of July 21, 1997 No. 116-FZ **''Industrial Safety of High-Risk Industrial Facilities''**	Higher-level legal act dedicated solely to chemical safety. Issues declaration and appraisal of industrial safety at industrial enterprises handling hazardous chemical substances (manufacturing, storage, use and transportation) starting from certain amounts.
Federal Law of January 10, 2002 No. 7-FZ **''On Environmental Protection''**	Protection of environment in manufacturing and use of chemicals as pollutants - limits and regulations of allowable emissions and discharge of chemicals, maximum concentration limit values, environment pollution fee.
Federal law of March 30, 1999 No. 52-FZ **''On sanitary and epidemiological welfare of population''**	State registration of potentially hazardous chemical and biological substances, setting requirements on specific products, radioactive substances, industrial and household waste as well as specific types of products imported in the Russian Federation potentially hazardous for human health.
Labor Code of the Russian Federation of December 30, 2001 No 197-FZ	Occupational safety rules for use of new substances, etc. Restrictions on industrial use of hazardous substances, materials and products in case of absence of methods and metrological control procedures when toxicological (sanitary, hygienic, medical biological) tests were not performed.
Federal law on protection **of consumer rights of** February 7, 1992 No. 2300-1	Requirement on safety of consumer goods and services to human life and health, property and natural environment in case of normal handling.
Federal Law of July 19, 1997. no. 109-F ''On Safe **Handling of Pesticides and Agrochemicals''**	Requirement for safe handling of pesticides and agrochemicals.

- The Criminal Code of the Russian Federation (Chapter 26 "Environmental Crimes").
- The Administrative Code of the Russian Federation.
- Federal Law on Environmental Protection.
- Federal Law on Environmental Expert Assessment.
- Federal Law on Sanitary and Epidemiological Wellbeing of the Population.
- Federal Law on Protection of Consumers' Rights.
- Federal Law on Quality and Safety of Food Products.
- Federal Law on Safe Handling of Pesticides and Agricultural Chemicals.
- Federal Law on Production and Consumption Waste.
- Federal Law on Protection of Ambient Air.
- The Water Code of the Russian Federation.
- Federal Law on Technical Regulation.
- Federal on industrial safety of dangerous and industrial enterprises.
- The Lab Code.
- Bases of Russian legislation on health protection of citizens, etc.

Development of state standards of the Russian federation ("gosudartsvennye standarty" or GOSTy) in

Table 8. Examples of Russian state standards (GOSTs) related to the human health or environment risk assessment.

State standards (GOSTs)	Short description
GOST 12.01.007-76.SSBT "Hazardous substances. Classification and general safety requirements".	Classification of hazardous substances. In manufacturing environment.
GOST 17.4.1.02-83 "Nature protection. Soils. Classification of chemicals for pollution control"	Classification of anthropogenic substances by their hazardous for pollution control and soil's state forecast.
GOST 12.0.003-74. SSBT Occupational safety standards system. Dangerous and harmful production effects. Classification.	Classification of hazardous substances in manufacturing environment, establishment of dangerous and harmful factors in manufacturing.
GOST 19433-88 Dangerous goods. Classification and marking.	Classification of dangerous goods. Classification and labeling.
GOST 30775-2001 Resources saving. Waste treatment. Waste classification, identification and coding. Basic principles.	Classification of waste.
GOST 12.1.044-89 SSBT Occupational safety standards system. Fire and explosion hazard of substances and materials. Nomenclature of indices and methods of their determination.	Classification of hazardous substances by their fire and explosive properties.

Table 9. Non-carcinogenic risk values

Permanent exposure levels Maximum allowable concentrations (MACs) do not result adverse effect on human directly or indirectly and can be used as environmental or occupational exposure limits.	
Temporary exposure levels Tentative safety exposure level (TSEL)	Ambient air and workplace air
Tentative permissible level (TPL)	Water
Tentative permissible concentration (TPC)	Soil

Russia is based on the 1995 Law of the Russian Federation on Standardization. The State Committee of the Russian Federation on Standardization and Metrology is responsible for approval of standards. Russian GOSTs apply to all materials, production activity and services related to industries (Table 8). The classification of hazard chemicals has been made depending on their properties, origin or application. The Russian Chemical Union is working on the development of legislation document in the field of safety of chemical production (59). Analysis of the existing chemicals management system in Russian Federation was made in the scope of projects "HELCOM (Helsinki Commission) data collection strategy" and BACCON Rus1 in co-operation between Russia, Nordic Council of Ministers, Sweden & Finland (60, 61). It was pointed that the Russian chemicals legislation currently is in changing process. In this sense, the most important findings were:

- There was no common framework acts on handling of hazardous chemicals in the Russian Federation (in the future, there should be a single framework act on chemical safety).

- In general, there is no common classification system and criteria in Russia.

- There are differences in risk assessment terminology in EU and in Russia.

This fact clearly indicates that the current russian system of classification and labelling of chemicals differs considerably from the GHS concept, except the requirements for safety data sheets.

4.1. Exposure standards, guidelines used in Russia

The first issues related to development of standardization and methods for risk assessment of chemicals as pollutants in different media in Russia referred to 1920–1950. In the 1930s–1940s, prominent Russian toxicologists such as A.N. Sysin, N.S. Pravdin, N.V. Lazarev, S.N. Cherkinskiy, V.A. Ryazanov, V.M. Perelygin and others worked out scientific rules for development of chemical exposure standards. Nowadays I.V. Sanotzkiy, N.F. Izmerov, G.N. Krasovskiy, Z.I. Zholdakova, M.A. Pinigin, L.A. Tepikina and coworkers are the leading scientists in this field. Exposure standards and guidelines aimed to protect the public from harmful substances and activities that can cause serious health problems and provide numerical exposure levels for various media (such as food, consumer products, water and air) that cannot be exceeded. The terms hygienic standards or hygienic norms are also applied in Russia. Exposure or hygienic standards or norms and guidelines are the products of risk management decisions. Like in others countries, in Russia, exposure *standards* are legal acceptable exposure levels or control and are legally enforceable. Violators are subject to punishment, including fines and imprisonment. Environmental and occupational exposure levels (standards, norms) for chemicals in Russia are approved by Chief State Sanitary Doctor and are published as legislative rules in official documents named Hygienic Norms (*HN)* (Gigienicheskie normativi (GN)). There are two types of exposure levels (hygienic standards, norms) in Russia: permanent and temporary (Table 9). Permanent exposure levels are called maximum allowable concentrations (MACs) (Predelno dopustimie kontsentratsii (PDK)) and belong to non carcinogenic risk values.

MAC does not result in adverse effect on human directly or indirectly. The temporary norms are elaborated using toxicity prediction methods, and are established for not more than three years. They are applied only at the stage of designing and building of enterprises and plants (Table 10). The news about hygienic norms in Russia can be viewed at Bulletin of Russian register of potentially hazardous chemical and biological substances at the website: http://www.rpohv.ru/magazin/b/ (62). At present, Russian sanitary legislation number of maximum permissible concentrations, tentative exposure levels and tentative permissible levels of chemicals in various environmental media considerably exceeds that in all other countries (63).

5. RISK ASSESSMENT OF CHEMICALS IN RUSSIA

5.1. Toxicity parameters used in Russia

The following toxicity parameters are currently used in Russia for determination of diverse safe levels of chemicals (see Table 11):

Table 10. Hygienic norms valid in Russia

Hygienic norms in workplace air	
G N2.2.5.1313-03	Maximum allowable concentrations (MAK) of hazardous substances in workplace air.
G N2.2.5.1314-03	Tentative safe levels (TSL) in workplace air.
G N2.2.5.1827-03	Maximum allowable concentrations (MAC) of hazardous substances in workplace air. Amendments to **G N2.2.5.1313-03**.
G N2.2.5.1828-03	Tentative safe levels (TSL) for air of work zone. Amendments to **Г Н 2.2.5.1314-03**.
G N2.2.5.2100-06	Maximum allowable concentrations (MAC) of hazardous substances in workplace air.
G N2.2.5.2101-06	Tentative safe levels (TSL) in workplace air
Hygienic norms for ambient air in populated area	
G N2.1.6.1338-03	Maximum allowable concentrations (MAC) of hazardous substances for ambient air in populated area.
G N2.1.6.1339-03	Tentative safe levels (TSL) for ambient air in populated area.
G N2.1.6.1764-03	Tentative safe levels (TSL) for ambient air in populated area. Amendments to **G N2.1.6.1339-03**.
G N2.1.6.1765-03	Maximum allowable concentrations (MAC) of hazardous substances for ambient air in populated area. Amendments to **G N2.1.6.1338-03**.
G N2.1.6.1983-05	Maximum allowable concentrations (MAC) of hazardous substances for ambient air in populated area. Amendments to **G N2.1.6.1338-03**.
G N2.1.6.1984-05	Tentative safe levels (TSL) for ambient air in populated area. Amendments to **G N2.1.6.1339-03**.
G N2.1.6.1985-06	Maximum allowable concentrations (MAC) of hazardous substances for ambient air in populated area.
G N2.1.6.1986-06	Tentative safe levels (TSL) for ambient air in populated area.
Hygienic norms for drinking water and for water used for household and recreational needs	
G N2.1.5.1315-03	Maximum allowable concentrations (MAC) of substances **for drinking water and for water used for household and recreational needs**.
G N2.1.5.1316-03	Tentative safe levels (TSL) for drinking water and for water **used for household and recreational needs**.
G N2.1.5.1831-04	Tentative safe levels (TSL) for drinking water and for water **used for household and recreational needs.** Amendment to **G N2.1.5.1316-03**.
Hygienic norms for water bodies for fishery	
Document approved by Chairman of Fish Industry Committee of Russian Federation N 96. 28.04.1999.	List of norms for water bodies for fishery: Maximum allowable concentrations (MAC) and tentative safe levels (TSL) for hazardous substances in water objects for fishery.
Hygienic norms for soil	
G N2.1.7.2041-06	Maximum allowable concentrations (MAC) of substances in soil.
G N2.1.7.2042-06	Tentative safe levels (TSL) in soil.

LD_{50} - lethal dose, LC_{50} - lethal concentration, Lim_{ac} -threshold dose or concentration for acute effect and Lim_{ch} - threshold dose or concentration for chronic effect, MNED - maximum non effective dose and MNEC - maximum non effective concentration. The level of hazardous and accumulation properties of substances can be characterized by the following parameters: Zac - zone of acute effect (Zac = LD_{50} (LC_{50})/Limac), Zbiol - zone of biological effect (Zbiol = LD_{50} (LC_{50})/ Limch), Zch - zone of chronic effect (Zch = Limac/Limch). In Table 12, non-cancer risk values used in Russia (MNEDs and MNECs) are presented.

5.2. Accumulative properties and factors used for risk assessment of chemicals

Qualitative criteria expressed as half-life duration of substances are used by most of international agencies for determination of accumulative properties of substances. All chemicals excreted or completely transformed in the organism during 24 hours accepted as non-accumulative. In contrast, in Russia, additional criteria are applied to express accumulative properties of substances. Accumulation of hazardous substance is expressed as a zone of biological effect Z_{bio}l during the time of so called functional accumulation which is different from half-life duration term. Considerable quantities of factors are taken into account for risk assessment of chemicals in Russia (Table 13). All possible adverse effects related to pointed factors are determined. A minimal effective (threshold) or maximum non-effective doses (concentrations) are calculated for each adverse effect. Minimal dose or concentration is chosen for determination of safety level of chemical.

5.3. Uncertainty factor

Different approaches are used in Russia for the establishment of uncertainty factors (UF). These approaches take into account real, as well as potential hazard of chemicals at the condition of their production and use. In Table 14 are entered the values of uncertainty factors recommended for calculation of safety levels in different media. Some approaches concerned with risk assessment of chemicals in different media: air, water, soil for establishment of safe levels is discussed below. More detailed information is reported by Rahmanin and coworkers (64).

6. QSAR MODELING FOR REGULATORY USES IN OECD MEMBER COUNTRIES AND IN EU

Quantitative structure-activity relationship (QSAR) (sometimes QSPR: quantitative structure-property relationship) is the process by which chemical structure is quantitatively correlated with a well defined process, such as biological activity or chemical reactivity (65-67). QSARs represent predictive models derived from application of statistical tools correlating biological activity (including desirable therapeutic effect and undesirable side effects) of chemicals (drugs/toxicants/environmental pollutants) with descriptors representative of molecular structure and/or properties. QSARs are being applied in many disciplines for example risk assessment, toxicity prediction, and regulatory decisions (68-70) in addition to drug discovery and lead optimization (71). QSARs are all quantitative models yielding a continuous or categorical

Table 11. Toxicity parameters

Toxicity parameter	Description
LD_{50}	Lethal dose-(50% of population is expected to die) The quantity of material what will result in death of 50% of the test animals.
LC_{50}	Lethal concentration-The air concentration of chemical that causes the death of 50% of test animals.
Lim_{ac}	Threshold dose (concentration) for acute effect.
Lim_{ch}	Threshold dose (concentration) for chronic effect.
$Z_{ac} = LD_{50}$ (LC_{50})/Lim_{ac},	Zone of acute effect.
$Z_{ch} = Lim_{ac}/Lim_{ch}$	Zone of chronic effect.
$Z_{biol} = LD_{50}$ (LC_{50})/Lim_{ch},	Zone of biological effect.
$Z_{spec} = LD_{50}(LC_{50})/Lim_{spec}$,	Zone of specific effects.

Table 12. Non-cancer risk values used in Russia

Risk value name	Description	Application
Maximum Non-Effective Dose (MNED)	Analogue of reference dose (R_fD). Calculated from threshold dose of chronic experiment on laboratory animals using uncertainty factor which differ from 3 to 10 in dependence of the cumulative properties and the possibility of ultimate effect.	For contaminations in water and food.
Maximum Non-Effective Concentration (MNEC)	Analogue of reference concentration (R_fC).	For contamination in air.

Table 13. Factors used in risk assessment of chemicals.

Factors, effects	Description
Organoleptic	Changes relating to qualities such as taste, color, odor that stimulate the sense organs. It can be foam or film on the surface of water. Olfactory terms are also in use.
Reflex	Short term effect caused by odor which creates irritation of mucous membrane, breath-holding, irritation of eyes, etc.
Sanitary-toxicological	Resorptive effect on human. Long term effect caused by gonadial-embryotoxic effects, mutagenic, carcinogenic, chronic toxic effects and etc.
Specific	Allergenic, gonadial-embryotoxic and teratogenic effects. The dose of a chemical is below the effective dose of chronic non specific effect.
Long-term effects	Mutagenic and carcinogenic effects.
Sanitary	Characterizes effect of pollutant to self-purification of water, air, soil and biological active properties of soil.
Migration into water	Characterizes ability of substance to migrate into water from different media to reach harmful level.
Migration into air	Characterizes ability of substance to migrate into air from different media to reach harmful level.
Phytoaccumulation	Accumulation of the pollutant in plants (food).

Table 14. Uncertainty factor values recommended for calculation of safe levels in different media.

UF value	Media	Toxicity parameters correlated with UF
4, 5, 6, 8, 10, 12,. . . , 20	Working place air	LC_{50}, Lim_{ac}, Lim_{ch}, Z_{ch}, Z_{biol}, interspecies differences coefficient.
Variation from 1 to 10	Ambient air	LC_{50},LD_{50},Z_{ac},Z_{ch},Z_{biol},Z_{sp}, Lim, MNEC.
10, 5, 3	Water	Zbiol, Zspec (gonadotoxic and embryotoxic effects).

result. The most common techniques for developing QSARs are regression analysis, neural nets, and classification methods. SARs are qualitative relationships in the form of structural alerts that incorporate molecular substructures or fragments related to the presence or absence of activity. QSARs for human health endpoints and certain eco-toxicological endpoints can be regarded as alternative methods to animal experiments since they could be used to replace or reduce animal testing.

6.1. Scientific and regulatory uses of QSARs

From the scientific perspective, QSARs can be developed for prediction of the following types of physicochemical properties, toxic potential and potency, environmental distribution and fate, bio-kinetic processes (absorption, distribution, metabolism and excretion), etc. The use of QSARs for regulatory purposes includes:

- Supporting priority setting of chemicals.
- Guiding experimental design of regulatory tests or testing strategies.
- Providing mechanistic information.
- Grouping chemicals into categories based on similarity.
- Filling in a data gap needed for classification and labeling.
- Filling in a data gap needed for risk assessment.

It must be emphasized that principles and procedures for scientific validation of QSARs are separate from the considerations and procedures necessary for regulatory acceptance (72).

6.2. Guidelines and documents for developing and application QSARs for regulatory uses

Preliminary guidance "The Characterization of (Quantitative) Structure-Activity Relationships" has been published (73). The following *endpoints* associated with EU Test Methods and OECD test guidelines have been proposed:

- *Physicochemical properties* such as melting point, boiling point, vapor pressure, K octanol/water

partition coefficient, Koc organic carbon/water partition coefficient, water solubility.

- *Ecological effects* such as acute fish, long-term toxicity, acute Daphnid, algal, terrestrial toxicity.
- *Environmental fate* such as biodegradation, hydrolysis in water, atmospheric oxidation, bioaccumulation.
- *Human health effects* such as acute oral, acute inhalation, acute dermal, skin irritation, eye irritation, skin sensitization, repeated dose toxicity, genotoxicity (*in vitro*, bacterial cells), genotoxicity (*in vitro*, mammalian cells), genotoxicity (*in vivo*), reproductive toxicity, developmental toxicity, carcinogenicity.

Guidelines for developing and using QSARs with examples of models for prediction toxicity was published (74). Regulatory uses and applications of QSAR models in the assessment of new and existing chemicals in OECD member countries were reported (75). The general acceptability criteria or validation principles of QSARs for Human Health and Environmental Endpoints was developed at the workshop "Regulatory Acceptance of QSARs for Human Health and Environmental Endpoints," hosted by the European Centre for Ecotoxicology and Toxicology of Chemicals and organized by the International Council of Chemical Associations (ICCA) and the European Chemical Industry Council (CEFIC) held 4–6 March 2002 in Setubal. In November 2004, at the 37th Joint Meeting of Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology, the OECD Member Countries and the European Commission adopted *five principles for the validation* of QSARs intended for use in the regulatory assessment of chemicals. Accordant with these principles a QSAR model for regulatory use should be associated with the following information:

- A defined endpoint.
- An unambiguous and easily applicable algorithm.
- A defined domain of applicability.
- Appropriate measures of goodness-of–fit, robustness and predictive power.
- A mechanistic interpretation, if possible.

Recently, the EC funded project DEMETRA addressed the specific case of the QSAR models for the European legislation for pesticides (76). Several major points have been defined. The QSAR model should clearly mention the specific legislation involved. This point should be more extensively considered in all QSAR models for regulatory purposes because different regulations have different ways to address and express the phenomenon. Related to the legislation, in many cases there are specific guidelines that have to be considered. In some legislations, these guidelines are strict, while in others, a certain degree of freedom is given. The developer should be aware of this fact. DEMETRA addressed some other issues, not directly defined within the OECD principles, which can be used to evaluate QSAR models for regulatory purposes. For instance, DEMETRA dedicated efforts on the definition of the model utility for regulatory purposes, in order to identify the QSAR models which should be more useful for legislation. DEMETRA defined tools to address and reduce false negatives. False negatives are very important in case of QSAR models for regulatory purposes because regulators want to avoid predictions that predict safety chemicals which are toxic; the reverse case is not so critical. DEMETRA addressed the situation of model uncertainty in a detailed way. For regulatory purposes, it is not enough to have a predicted value: its uncertainty has to be characterized, and relation to its use and the uncertainty of the input values. All these points have been thoroughly addressed and discussed within DEMETRA (76).

6.3. QSARs for human health and environmental endpoint

In the mini-monograph, Cronin and coworkers reported the use of QSARs in international decision-making frameworks to predict ecological effects and environmental fate (77). QSARs for prediction health effects of chemical substances are presented in another mini-monograph (78). Most expert systems, SARs, and QSARs are based on chemical classes or on mode of action. More details on in silico methodologies with regard to their usage in REACH are provided in report on the "Review of the Status of the Development of Alternatives to using Animals in Chemical Safety Testing and Identification of New Areas for Development or Research in the Context of the Proposed REACH Regulation" (79). The comprehensive investigation of quantitative methods of hazard characterization used in food safety assessment and used for regulatory decision-making in Europe was reported in monograph (80).

6.4. Difficulties to validation of QSARs

It should be noted that there are many practical difficulties to the validation of QSARs, in particular obtaining data for a meaningful external validation, as well as obtaining transparent models for some methodologies (e.g., commercial expert systems, neural networks, etc.). There are three main reasons why QSARs and expert systems have not been used to their full potential:

- None have yet been formally validated.
- They need to be improved to cover a wider spectrum of toxic mechanisms of action, especially for endocrine disruption and non-genotoxic carcinogenesis (that are both based on receptor-binding).
- Their coordinated and combined use has not been explored sufficiently.

6.5. OECD's database on chemical risk assessment models

Models (computerized or capable of being computerized) that are used by OECD countries to predict health or environmental effects, exposure potential, and

Table 15. Information included in models of OECD's database.

Exposure/risk models for predicting human health or environmental exposure potential and potential environmental, worker or consumer risk	
Areas of assessment	**Human health, environment**
Human health Exposure covered	Indirect human exposure via the environment. Consumer product exposure. Worker exposure.
Routes of exposure covered	Inhalation. Ingestion. Dermal. Multi-media.
Environment Organisms covered	Freshwater organisms. Marine organisms. Sediment organisms. Terrestrial organisms. Micro-organisms in sewage treatment plant. Fish-and-worm eating predators.
Pathways of exposure covered	Air, water, sediment, soil, biota, sewage treatment plant, multi-media.
Type of information provided	Daily intake, potential dose, margin of safety, predicted environmental concentration, risk quotient (predicted environmental concentration/predicted no-effect concentration).

Table 16. Different parameters included in OECD's database.

Health or environmental effects models for predicting physical/chemical properties, chemical and fate properties, and human and aquatic hazard effects
Category of information provided
Physical/chemical properties
Melting point, boiling point, vapor pressure
Octanol-water partition coefficient (KOW)
Water solubility
Organic carbon adsorption coefficient (KOC)
Environmental fate properties
BCF (bio-concentration factor)
AOP (atmospheric oxidation potential)
Biodegradation
Hydrolysis
Percent removal in wastewater treatment
Hazard-human health (Hazard-environmental)
Mutagenicity (Aquatic biota)
Neurotoxicity (Terrestrial biota)
Reproductive toxicity
Developmental toxicity
Systemic toxicity
Skin/eye irritation
Oncogenicity

possible risks were organized into searchable database. But it should be taken into account that this database is created for developmental use and the methods described there have not been evaluated or validated by OECD; no endorsement of the methods by OECD should be inferred by the inclusion of certain methods in this database. This database is intended as an information resource only. The models are listed by countries and by property or effect which was assessed (Tables 15-17). Screening level methods described there are useful, when chemical-specific data are lacking, for establishing priorities for chemical evaluation and for identifying issues of potential concern (81).

6.6. QSARs based on metabolism and *in vitro* data

Several *in silico* systems for predicting metabolism are available, including QSAR models and expert systems, but none of these have been compared extensively for their relative performances, and none have been formally accepted for regulatory use, although some models can be used to provide supporting information in chemical risk assessments. There is currently no consensus on how *in silico* models for predicting biotransformation should be validated. Also, a variety of systems are in different stages of development, assessment and validation. If they are to be of more practical use outside the pharmaceutical industry for regulatory testing, then further research needs to be undertaken to make them more amenable for a wider range of chemicals. Problems with regard to the availability of good quality data for benchmarking purposes, apply to techniques for using *in silico* prediction systems and bio-kinetic models to assess the metabolic fate of chemicals after uptake by different routes of exposure in different species. Before these systems can be validated, more chemicals with good quality data need to be found, for use as test sets. Nevertheless, it was agreed that at least one bio-kinetic modeling system is, in principle, ready for more formal consideration for validation. Clearly, *in silico* systems for predicting toxicity should take account of the possibility that biotransformation could modulate toxicity. This could be achieved by modifying these systems, so that they can model the toxicity of the principal metabolites of chemicals, or by linking those with systems specifically designed to predict metabolite formation (82). It has been emphasized that QSAR analyses are used in conjunction with expert system and bio-kinetic modeling, and information on metabolism and identification of the principal metabolites in humans. Several recommendations are made, the most important of which is that the European Union (EU) should actively promote the improvement and validation of QSAR models and expert systems, and computer-based methods for bio-kinetic modeling. It was highlighted that if mechanistically-based toxicokinetic and toxicodynamic data are obtainable, risk characterization can be improved considerably. This is illustrated by physiologically-based toxicokinetic modeling (pBTK models), which can be used at various stages of risk assessment (83).

6.7. Perspectives in QSAR modeling for regulatory use

Thousands of predictive models have been published in recent years, but typically they are not suitable for regulatory purposes because they have not taken into account essential factors for validation or quality assurance and specific requirements for regulation. The project CAESAR (computer assisted evaluation of industrial chemical substances according to regulations) ongoing in the scope of FR6 Six Framework Programme will develop QSAR models as non-animal alternative tools for assessment of chemical toxicity under REACH. CAESAR will include the high quality factors that are needed to make the use of QSARs acceptable for regulatory purposes (such as the implementation of the REACH proposal) for the prediction of the toxicity of chemical substances in a transparent manner by applying new and unique modeling

Table 17. Different kind of information, endpoints and approaches included in OECD's database.

Type of Information Provided	**Endpoint(s)**
• Qualitative • Quantitative • Range • Point estimate	• Reproduction • Growth Mortality
Categorical information Species/compartment addressed by model	**Model approach**
• Air	• Deterministic or probabilistic • QSARs • SARs
• Water	
• Sediment	
• Soil	
• Multi-media	
• Aquatic biota	
• Terrestrial biota	

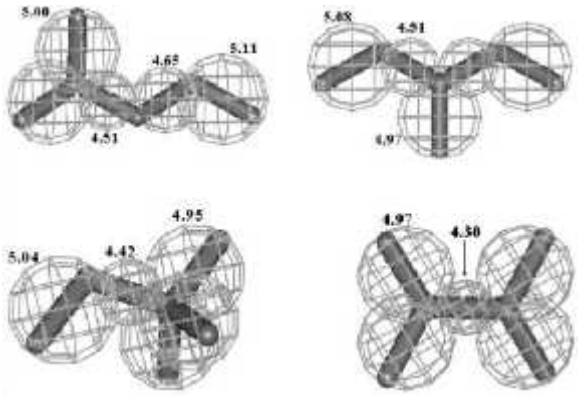

Figure 3. Quantitative contribution of some fragments to the molecular refractivity.

and validation methods. Five endpoints will be addressed within CAESAR, chosen on the basis of the animal use that is expected for the REACH legislation. In order to have high quality data sets, data have been selected from high quality sources, and structures checked independently by at least two groups in the consortium. Preliminary results on a model for the bio-concentration factor are superior to those previously published. The predictions of properties together with all modeling details can be easily used in chemical regulation. The CAESAR project goal is to design and develop a web site incorporating the models developed. This site will be freely accessible and QSAR models and protocols will be available for non-commercial use (84).

7. CURRENT PROMISING COMPUTATIONAL APPROACHES AND TOOLS FOR REGULATORY USES AND DIRECTIONS

7.1. TOPS-MODE approach

TOPS-MODE (Topological Sub-Structural Molecular Design) is a theoretical approach developed in the last ten years by Estrada and coworkers to be used in QSAR, QSPR, drug design, and knowledge generation. It is based on the method of moments developed in the 70's and applied in solid-state physics and chemistry. We have extended these concepts to the use of bond moments and we have included the use of bond weights, which have permitted the consideration of hydrophobic, electronic and steric molecular features (85-87). This method have been intensively studied and applied in both academia and industry in the last years. In all cases MODESLAB software version 1.5 (88), has served as the computational platform for making these studies. They have permitted the development of models for drug discovery of anti-cancer and anti-HIV compounds, for predicting eco-toxicity of chemicals, for generating knowledge for toxicity expert systems, and for predicting several physicochemical, ADME, and biological properties of organic compounds. Spectral moments have been extensively used for the modeling of structural factor related with penetration of commercial solvents through living human skin (89), mutagenicity (90), neurotoxicity (91), and carcinogenicity of organic compounds (92-94). TOPS-MODE measures the concentration of structural or physicochemical properties in regions of different sizes in the molecule. The principal advantage of this approach is the possibility of the calculation of quantitative contribution of any fragment to the property (Figure 3) (95), activity or toxicological profile under study.

A model for differentiating strong/moderate skin sensitizers from weak/non-sensitizers using linear discriminant analysis has been published. This model permits to identify those groups, fragments or molecular regions which are responsible for this toxicity, e.g. toxicophores. For instance, it is well known that carbonyl group in aldehydes and ketones represent a structural alert for skin sensitization. In a similar way α,β-unsaturated aldehydes, ketones, esters, etc can undergo Michael addition with proteins which can be traduced into skin sensitization The general flow of knowledge starts when the knowledge is created and then deposited in knowledge archives, such as brain, computer disks or expert systems. TOPS-MODE can be considered as a knowledge generator (Figure 4), and it can be used to help in creating new knowledge that can be then implemented into artificial intelligence tools such as expert systems. As an example we can mention the generation of structural alerts for skin sensitization. Expert systems containing knowledge about skin sensitization, such as DEREK (96), does not contain any structural alert for non-activated double bonds, such as that in styrene. TOPS-MODE has found that this bond contributes positively to skin sensitization in several skin sensitizer compounds (97).

7.2. MARCH-INSIDE approach

The MARCH-INSIDE (MARkov CHain Invariants for Network SImulation and DEsign) was developed by Gonzalez-Diaz and coworkers. This approach is based in the concept of Markov chain theory which constitutes the base of probability states (6, 24, 98), and it consider the external electron layers of any atom core in the molecule (the valence shell) as states of the Markov chains. This model is stochastic per se (probabilistic distribution of electrons in time) but, as mentioned above, actually considers molecular connectivity (the distribution of electrons in space throughout the chemical bonds). The selection of a Markov chain process is not arbitrary (Figure

Table 18. Compilation of the descriptors calculated by DRAGON software 5.3.

ID Block	Block description	Number of descriptors
1	Constitutional descriptors	48
2	Topological descriptors	119
3	Walk and path counts	47
4	Connectivity indices	33
5	Information indices	47
6	2d autocorrelations	96
7	Edge adjacency indices	107
8	Burden eigenvalue descriptors	64
9	Topological charge indices	21
10	Eigenvalue-based indices	44
11	Randić molecular profiles	41
12	Geometrical descriptors	74
13	RDF descriptors	150
14	3D-MoRSE descriptors	160
15	WHIM descriptors	99
16	GETAWAY descriptors	197
17	Functional group counts	154
18	Atom-centered fragments	120
19	Charge descriptors	14
20	Molecular properties	29

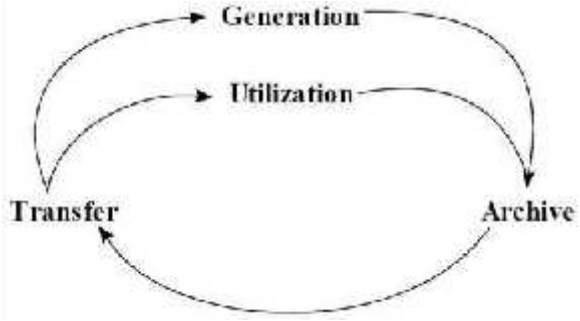

Figure 4. TOPS-MODE as a knowledge generator.

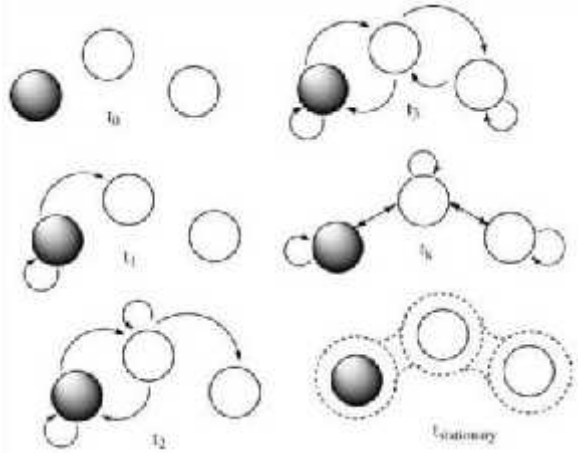

Figure 5. Diagrammatic representation of random electron distribution in a simple Markovian model. The symbol t-stationary represent the stationary time: the time at which electrons reach equilibrium distribution around atoms.

5). Thus, this approach describes the evolution of any molecular system. From quantum physics, it is well known that, if electrons are labeled at an arbitrary initial time, one cannot use these labels to distinguish between them in subsequent moments. Several works have been published using the MARHC-INSIDE approach toward the study of very interesting parameters related with toxicological profiles and subsequently with the risk assessment, including from prediction of side effects of drugs and partition coefficient of organic compounds in different medias to protein related with drug metabolism toxicity (5, 6, 99-104).

7.3. Compilation of different types of molecular descriptors: Dragon software

DRAGON is an application for the calculation of molecular descriptors originally developed by the Milano Chemometrics and QSAR Research Group. These descriptors can be used to evaluate molecular structure-activity or structure-property relationships, as well as for similarity analysis and high-throughput screening of molecule databases. The first release of DRAGON dates back to 1997. Updates and inclusions of new molecular descriptors are regularly made in order to advance research in QSAR. DRAGON has been designed to work both for Windows and Linux systems. There are two versions for Windows, DRAGON professional, which can only work in stand-alone mode and DRAGON plus, which can work both in stand-alone and background mode. For Linux there only is one version, called DRAGON X, which only works in background mode by a command line. DRAGON 5.3 provides more than 1600 molecular descriptors (105) which are divided into 20 logical blocks (Table 18). The user can calculate not only the simplest atom type, functional group and fragment counts, but also several topological and geometrical descriptors. Some molecular properties such as logP, molar refractivity, number of rotatable bonds, H-donors, H-acceptors, and topological surface area (TPSA) are also calculated by using some common models taken from the literature. Moreover, the Lipinski's alert (also known as "the rule of 5") together with some drug-like indices are provided to allow the selection of compounds for biological screening and/or the design of combinatorial libraries. By employing DRAGON descriptor several works have been reported for the study of different toxicological parameters. This includes estimation of blood-brain barrier permeability for diverse organic compounds (106), ADME properties (107), modeling of water quality indices (108), aquatic toxicity of organic chemicals (109) and many other parameters related to risk assessment (110-113).

8. PREDICTION METHODS FOR REGULATORY USES AND DIRECTIONS IN QSAR MODELING IN RUSSIA

The development of reliable computational methods for determination of toxicity of chemical compounds is an intricate process that utilizes knowledge from many different scientific disciplines, including toxicology, chemistry, mathematics and combination of listed sciences like Chemometrics. Prediction of toxicological properties of substances can be performed using different physicochemical parameters. Methods for substantiation, determination and calculation of maximum allowable concentrations and tentative safe levels for different media (air, water, soil) are given in guidelines

called Methodical Instructions. Environmental and occupational exposure levels (standards, norms) for chemicals in Russia are approved by the Chief State Sanitary Doctor and are published as legislative rules in official documents named Hygienic Norms (HN). Maximum allowable concentrations (MAC), tentative safety exposure level (TSEL), tentative safety levels (TSL) and tentative permissible levels (TPL) of chemicals in various environmental media are accepted as a permanent or temporary safe exposure levels. Temporary norms are usually established for certain period of time (for example, 2 years). The determination of temporary norms or safety limits in different media (air, water, soil) is based on calculation methods using regressions equations. Computational methods for sanitary NORMs can be divided into 3 groups:

- On the basis of physico-chemical properties of substances.
- Establishment of safety levels by toxicological parameters from short terms experiments.
- On the basis of safety levels found out in different media. In this case a data transformation obtained in one media is done applying the data to another one.

The first step in risk assessment is estimation of toxicity parameters of acute toxicity such as LD_{50}, LC_{50} (lethal dose and concentration) values, Lim-(threshold of hazardous effect of substances) and others. Safety levels (concentrations or doses) of substances used for regulatory purposes are calculated on the bases of LD_{50}, LC_{50} or Lim. The calculation of LD_{50} or LC_{50} is divided into several sections:

- For volatile organic compounds with boiling point below 200ºC ($t \leq 200$ºC).
- For low-volatile and non-volatile organic compounds with boiling point above 200ºC ($t \geq 200$ºC).
- For inorganic compounds of metals (oxides and salts).

The value of biological endpoint such as toxicity, strongly depends on the aggregation state of substances (solid, liquid, gas) and route of administration (oral, dermal, inhalation). Thus, equations of regressions have been composed with LD_{50} or LC_{50} as a response. As dependent variables the following physical and chemical properties of chemicals have been used:

M - molecular mass

d - density (g/cm^3)

RD - mole refraction

t◦boiling. - boiling point (º C)

t◦melting. - melting point (º C)

C20 - maximum saturated concentration of substances in the air at 20ºC

P - pressure of vapor at 20º - (mm, millimeter of mercury)

S - dissolubility in water (g/l)

K - coefficient of distribution oil/water

M.V. - molecular volume (M/d)

mM – milimole

nD - refraction coefficient

tflash - flashing point (º C)

μ - dipole moment (Debye)

$\Sigma\alpha$ - sum of increments of nuclear quadrupole resonance (NQR)

$\Sigma\sigma$ - sum σ constant of Hammet

In some cases, transformation of data from one species to another one or from animal to human has been done. Safety levels or NORMs can be found on the basis of the acute toxicity values. Numerous regression equations have been composed.

8.1. QSAR methods used in Russia

8.1.1. Hansch and free Wilson methods

Between methods used in Russia we mention Hansch analysis (investigation of the quantitative relationship between the biological activity of a series of compounds and their physicochemical substituent or global parameters representing hydrophobic, electronic, steric and other effects using multiple regression correlation methodology) and Free-Wilson (FW) analysis (a regression technique using the presence or absence of substituents or groups as the only molecular descriptors in correlations with biological activity). For structural series of phenols, a regression equation was reported for acute toxicity in rats in case of oral administration (n = 52, r = 0,887). For MAC in air of work zone regression equations have been reported (n = 15,
r=0,907) using the sum of Hammett electronic substituent constant, reflecting the electron-donating or -accepting properties of a substituent (114). For the same group of substances, 23 phenols were selected and data collected from chronic toxicity experiment in rats in case of oral administration. A correlation was found between MNED (maximum not effective dose) and logarithm of octanol-water partition coefficient P, sum of Hammett substituent constants and *Free-Wilson indices* for the fragments NO_2, CH_3 and Cl. The statistical characteristics in this example were n= 23, r = 0.909, s = 0.577 (115). Later topological descriptors were used for prediction models (116, 117). The molecular topology considers that biological activity is related to the molecular topological characteristics, numerically represented using the distance and connectivity indices.

8.1.2. Pattern recognition technique

Pattern recognition is the identification of patterns in large data sets, using appropriate mathematical methodology. Examples are principal component analysis (PCA), SIMCA, partial least squares (PLS) and artificial neural networks (ANN). A computer-based system was developed for calculation of LD_{50} for drugs on the basis of structural descriptors. Accuracy of prediction shows that in 91% cases, the calculated values don't differ from experimental by more than 3 times.

8.1.3. Hydrogen bond thermodynamics (HYBOT) descriptors

Many program packages have been elaborated by Raevsky and coworkers. They suggested to use hydrogen bond thermodynamics (HYBOT) program for the estimation of hydrogen bonds strength (118) and developed the program package molecular transform analysis (MOLTRA) (by Raevsky, Sapegin, Zefirov), the QSAR discriminant-regression model conformational analysis (CONFAN), dissociation constants (DISCO), and others. HYPOT descriptors have a wide spectrum of application. Predictive models of aquatic toxicity of environmental pollutants with different mechanisms of action were developed on the basis of molecular similarity and HYBOT descriptors. The molecular polarizability and hydrogen bond descriptors for the chemicals of interest and related compounds have been used to calculate any additional contribution in toxicity by means of linear regression relationships. Final comparison of calculated and experimental toxicity values gave good results, with standard deviation close to the experimental error (119). The software program SLIPPER-2001 for prediction of the lipophilicity (logP), solubility (log Sw), and oral absorption of drugs in humans (FA) has been developed. It is based on structural and physicochemical similarity. Reliable results were obtained for simple compounds, for complex chemicals, and for drugs. Thus, the principle of "similar compounds display similar properties" together with estimating incremental changes in properties by using differences in physicochemical parameters results in "structure-property" predictive models, even in the absence of a precise understanding of the mechanisms involved (120).

8.2. QSARs based on ADME and *in vitro* data

Structures and properties of single chemicals usually apply in QSAR models. However, the toxic effects are quite often determined by formation of metabolites in the processes of bioactivation of chemicals by various enzymes. Mechanism-based approach SARs for several toxic effects in various structural series have been developed (121). Due to complexity of processes of biotransformation of chemicals in biological systems, a single reaction of bioactivation cannot account for overall toxicity. Therefore, a logical-combinatorial method of automatic hypothesis generation was developed (122) based on the John Stuart Mills (JSM) logic. The JSM method enables one to predict some property and provide the explanation of this prediction. The prediction is based on the learning using the sets of positive and negative examples. The method does not require big training sets. The standard JSM method does not operate with numerical parameters, but only with chemical structure described by means of special descriptors named as functional code of substructures superposition (FCSS). A new approach has been developed for prediction of the most probable metabolic sites on the basis of statistical analysis of various metabolic transformations. It is related to the prediction of aromatic hydroxylation sites for diverse sets of substrates. Training is performed using the aromatic hydroxylation reactions from the metabolism database (Accelrys). Validation was carried out on heterogeneous sets of aromatic compounds reported in the metabolite database (MDL). The average accuracy of prediction of experimentally observed hydroxylation sites estimated for 1552 substrates from metabolite is 84.5%. The proposed approach is compared with two electronic models for P450 mediated aromatic hydroxylation: the oxenoid model using the atomic oxygen and the model using the methoxy radical as a model for the heme-active oxygen species. For benzene derivatives, the proposed method is inferior to the oxenoid model and as accurate as the methoxy-radical model. It was shown that for hetero- and polycyclic compounds, the oxenoid model was not applicable, and the statistical method was the most accurate (123). An approach based on the oxenoid model of monooxygenase action and semi-empirical quantum chemical calculations was applied to the prediction of aromatic hydroxylation sites of cytochrome P450 substrates. The results were compared with experimental data on the metabolism in mammals and human from metabolite database (124). Knowledge of metabolic pathways of chemical can substantially enhance the accuracy of structure activity analysis.

8.3. Computer program Pass

Computer program PASS (prediction of activity spectra for substances) predicts simultaneously more than three thousand biological activities (main and side pharmacological effects, mechanisms of action, specific toxicities, biotransformations) (125-127). PASS is based on the concept of biological activity spectrum of the compound, which must reflect all kinds of its biological activity resulting from the compound's interaction with biological entities. Since not one compound has been tested experimentally against all known kinds of biological activity, for any real compound known biological activity spectrum contains only part of such information. Biological activity spectrum for the compound under study predicted *in silico* with PASS can identify some additional kinds of biological activity, based on the structural similarity to the sub-sets of compounds, for which the appropriate activities were determined experimentally. Biological activities are described in PASS in qualitative mode ("active" and "inactive"), which provides the possibility of combing the heterogeneous information collected from literature in the PASS training set. Therefore, PASS predictions are based on the results of structure-activity relationships analysis accumulated in the SARBase, which is generated during the training procedure. Currently (PASS 2007 version), PASS training set includes the information about 120000

Table 19. Computer program PASS versions

PASS versions presented by years	Amount of biologically active compounds	Number of biological activity types
1995	~ 10,000	~ 100
1998	~ 30,000	~ 500
2004	~ 57,000	~ 1000
2007	~ 120,000	~ 5000

biologically active compounds with around 5000 kinds of biological activity. These molecules are presented by the completely determined simply connected 2D structural formulae of uncharged molecules. The user can explore the existing SARBase, provided with PASS, or create his own SARBase using in house developed training sets. Since new information about biologically active compounds emerges constantly, continual updating of the existing PASS training set is performed. The first version of PASS (1995) was based on the data for approximately 10000 biologically active compounds with 100 kinds of biological activity; in 1998 these figures came to 30000 and 500, respectively; in 2004 these figures came to approximately 57000 and 1000, respectively; etc. (see Table 19).

8.3.1. Statistical performance of PASS algorithm

In parallel with the extending of PASS training set, PASS algorithm is also modified to provide more accurate results of prediction. The average accuracy of prediction estimated on the basis of leave one out cross-validation (LOO CV) for the whole training set and all predictable kinds of biological activity was around 78% in 1995, 85% in 1998, and 94% in the current version of PASS. PASS 2007 version predicts 3300 kinds of biological activity, while biologically active compounds from the PASS training set are described by 5000 kinds of biological activity. However, some of these biological activities are represented by one or two compounds in the PASS training set, which is not enough to provide an accurate estimation of biological activity (three is the minimum number of compounds currently specified in PASS); also, for some kinds of biological activity accuracy of prediction in LOO CV procedure is less than 70%. Such kinds of biological activity are not included into the default list of PASS predictable activities. Due to the unavoidable incompleteness of any training set, which can be used for biological activity spectra prediction, a robustness of the used algorithm is particularly important. By special computational experiments made with a set of about 20000 principal compounds from the MDDR database it was shown that, despite the random removal of up to 60% of structural or biological information, PASS algorithm still provides a reasonable accuracy of prediction (128).

8.3.2. Descriptors used in PASS

Chemical descriptors used in PASS analysis, called Multilevel Neighborhoods of Atoms (MNA) are described in detail elsewhere (129). They are automatically generated on the basis of MOL-file of a molecule. The list of MNA descriptors currently consists of more than 52,000 different items. The new descriptors added to this list being found in a novel compound refreshing the training set. During the prediction of biological activity spectra the number of new (in relation to the existing SARBase) descriptors is calculated for the compound under study, which provides the possibility of broad definition of PASS applicability domain. If the compound under study contains three or more new descriptors, the results of prediction give a rather crude estimation of potential biological activity spectrum for this compound. MNA descriptors are effectively utilized in SAR, QSAR and similarity analysis for drug-like compounds (129). Recently, new local integrative descriptors (QNA) were proposed, which may provide some advantages in QSAR/QSPR analysis (130). On the basis of QNA descriptors and self-consistent regression computer program GUSAR (general unrestricted structure-activity relationships) is developed (131). For nine data sets, which contain the information about biological activity, toxicity and metabolism for non-congeneric compounds, it was shown that GUSAR provides accuracy of prediction comparable to that of CoMFA, CoMSIA, GRID, HQSAR, EVA and 2D QSAR methods (132).

8.3.3. PASS availability in internet

Since 2000, PASS predictions can be performed via Internet (127, 133). One may obtain the results of PASS INet prediction by submitting the MOL file as an input data or drawing the molecule directly on the display using the MARVIN applet. For about 3000 registered users, this service is provided free of charge, and in 2007 alone, more than 70,000 molecules were submitted for prediction. A dozen papers were published by the independent researchers, in which PASS predictions were later confirmed by the experiments (134).

8.4. Expert system SARET-TERA

8.4.1. SARET

Expert system SARET (structure-activity relationships for environmental toxicology) has been developed for quantitative analysis of structure-property (QSPR), structure-activity (QSAR) and property-property (QPPR) relationships and prediction of toxicity and environmental effects of chemical compounds. It was introduced by Prof. Sergey Novikov, MRC "MEDTOXECO", Department of General Hygiene, Moscow, Russia and by Prof. Vladimir Poroikov, IBMC RAMS, Moscow, Russia, http://www.ibmh.msk.ru (84–85). The expert system SARET consists of:

- SARETbase - data bank that includes toxicological parameters of chemicals.
- SARETmodel - special computer system for modeling and calculations.
- Computer programs for calculation of descriptors (sub-structural, electronic, topological, etc.).
- The integrated risk assessment program for determination of health hazardous of chemicals.

SARETbase includes the information on more than 190 characteristics for 8500 substances: chemical structure, physico-chemical properties (density, boiling and melting points, partition coefficients of octanol/water, etc.),

adverse effect doses and concentrations for acute and chronic exposure, odor thresholds in water and air, character of odor, some of threshold limit values for occupational and environmental exposure (air, water), etc. (135). SARETmodel is designed for statistical analysis of data and calculation of unknown parameters of substances on the basis of (Q)SARs. The application of SARET provides the information necessary to evaluate the hazard of chemicals and to estimate their unknown characteristics. Mathematical models for prediction of toxicological properties of chemicals have been developed. Maximum allowable concentrations for hazard substances in different environmental compartments (air, water, etc.) for different classes of chemical compounds have been calculated. The relationship between physicochemical properties and safe exposure limits has been studied. The new methods for prediction of maximum allowable concentrations for air pollutants have been introduced. The distinguishing characteristics of biological activity of chemicals were taken into account. SARET program was written in DOS. Application of operation system Windows stimulated renovation of prediction programs and development of expert system TERA (tools for environmental risk assessment).

8.4.2. TERA

TERA is aimed at risk assessment of different pollutants. TERAbase is a part of expert system created by prof. S.M. Novikov and coauthors from the A.N. Sysin Research Institute of Human Ecology and Environmental Health of Russian Academy of Medical Sciences. TERA contains information useful for human, environmental and ecological risk assessment and management. TERA includes the information on approximately 200 characteristics for more than 13,000 chemical substances. The information collected in SARET and TERA is verified and specified on the basis of both Russian and foreign literature data including official documents, open publications, and "grey" literature. TERA contains information for 194 mixtures, 182 polymers, 346 dyes, 1080 non-organic compounds, 1407 remedies, 1260 agrochemicals (including pesticides). More than 1000 compounds contained in TERA are not presented in the Registry of Toxic Effects of Chemical Substances (RTECS). TERA contains the following characteristics:

- Chemical structures and their codes (SMILES), the CAS and RTECs numbers.
- Physicochemical properties.
- Human health toxicity values (adverse effect doses and concentrations for acute and chronic exposure).
- Odor thresholds in water and air.
- Skin, eye irritating properties of substances.
- Threshold limit values for occupational and environmental exposure in different media such as maximum allowable concentration used in Russia, safe limits set by American Conference of Governmental Industrial Hygienists (ACGIH), Occupational Safety and Health Administration (OSHA), National Institute for Occupational Safety and Health (NIOSH) and risk assessment values such as Immediately Dangerous to Life and Health (IDLH).
- Target organs and systems.
- Characteristics of specific effects such as carcinogenicity, mutagenicity, teratogenicity, embryotoxicity, etc. Evaluation of carcinogenic potency is given in accordance with Russian classifications as well as those set by the following agencies and bodies: International Agency for Research on Cancer (IARC); National Institute for Occupational Safety and Health (NIOSH); Office of Environmental Health and Hazard Assessment (OEHHA); Occupational Safety & Health Administration (OSHA); American Conference of Governmental Industrial Hygienists (ACGIH); National Toxicology Program (NTP).
- Hazardous classes of chemicals according to international classifications.
- Epidemiological data and human health risk assessment.
- Toxicological properties of substances for different kinds of biosystems, etc.
- Ecological effects such as acute fish, long-term toxicity, acute Daphnid, Alga, terrestrial toxicity.

Besides TERA contains more than 50 special databases, i.e., on cancer slope factors, the regional USA safety levels, reference doses (R_fDs), reference concentrations (R_fCs) from integrated risk information system (IRIS), the EPA superfund health effects assessment summary tables (HEAST), California Environmental Protection Agency (Cal EPA), etc. Exposure standards as defined by World Health Organization (WHO), agencies and regulatory bodies of EU, Canada, Sweden and United States (US) are presented in TERA. This program can be considered as an integrated system which incorporates:

- Calculation of physical and chemical properties.
- Assessment of multi-domain risk.
- Assessment of carcinogenic potency risk.
- Prediction of lead concentrations in blood of fetus, children, adults (system LRISK).
- Health risk connected with lead exposure.
- Prediction of emission of chemical substances and there distribution in different media.
- Parameters used for setting priority of chemical substances in risk assessment.

- Risk assessment using epidemiological data.
- Health risk assessment of air pollutants.
- Health risk assessment of chemicals in case of emergency.
- Evaluation of industrial chemicals emission, etc.

TERA includes additionally bio-kinetic models taken from US Environmental Protection Agency (EPA) and simple risk assessment model CalTOX (CalEPA) that calculates the emissions of a chemical, the concentration of a chemical in soil, and the risk of an adverse health effect due to a chemical. TERA is continuously updating. The new substances structures and properties are inserting into database. The main ongoing activities of TERA are listed below:

- Development of new models for air pollutants emissions.
- Improvement of predictive models on behavior and fate of chemicals in environment.
- Improvement of predictive models on physicochemical toxicological properties of chemicals in relation to human exposure.
- Health care costs calculation in case of exposure to harmful chemicals.

9. SUMMARY AND PERSPECTIVE

The main task of chemical legislations and risk assessment at the international level is harmonization with the GHS. For establishment of common criteria at the world level, it is essential to exchange the experience and knowledge about evaluation of toxicity of chemicals in different countries. The exposition of the Russian and international risk assessment issues helps to a better understanding of the classification criteria for hazardous substances. Also, this fact provides the perception of possible variations and uncertainties in risk value data. Special attention should be paid the regulatory laws for risk assessment in Russia in order to work in all the possible directions that will ensure the correct application of the laws for risk assessment according to the international standard. Understanding risk assessment approaches used in different countries and development of new sciences help to improve chemicals regulations to ensure safety for human health. In this sense, the development of reliable QSARs models in Europe for the regulatory purpose is necessary to the light of the REACH and at the international level in the scope of OECD chemical assessment programs and Globally Harmonized System of Classification and Labeling of Chemicals (GHS). QSAR models for most endpoints will undoubtedly be used to provide us with test expectations for thousands of untested chemicals. Thus, the decision-making procedures for risk assessment and risk management will be easier to develop and the safety will be ensured. In so doing, QSAR will complement the 3Rs (replacement, refinement and reduction of animals in research) with a powerful new tool to minimize animal testing. The integration of QSAR models with *in vitro* methods holds great promise in the prudent use and interpretation of our testing and assessment resources. QSAR methodologies for the study of toxicological properties constitute one of the ways to the Green Chemistry. The development of improved QSAR models will help to choose the optimal decision in future implementation of QSARs for regulatory uses.

10. ACKNOWLEDGEMENTS

The authors are grateful to developers of the CAESAR Project database, which constitutes a valuable tool for the in silico assessment of multiple toxicological profiles of chemicals.

11. REFERENCES

1. N Fjodorova, M Novich, M Vrachko, N Kharchevnikova, Z Zholdakova, O Sinitsyna, E Benfenati: Regulatory assessment of chemicals within OECD member countries, EU and in Russia. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 26, 40-88 (2008)

2. S Erler: Framework for Chemical Risk Management Under Reach. *Smithers Rapra Technology*, Shawbury, Shrewsbury, Shropshire, SY4 4NR (2009)

3. KC Chou: Graphic rule for drug metabolism systems. *Curr Drug Metab* 11, 369-378 (2010)

4. I Garcia, YF Diop, G Gómez: QSAR & complex network study of the HMGR inhibitors structural diversity. *Curr Drug Metab* 11, 307-314 (2010)

5. H Gonzalez-Diaz: Network topological indices, drug metabolism, and distribution. *Curr Drug Metab* 11, 283-284 (2010)

6. H Gonzalez-Diaz, A Duardo-Sanchez, FM Ubeira, F Prado-Prado, LG Pérez-Montoto, R Concu, G Podda, B Shen: Review of MARCH-INSIDE & complex networks prediction of drugs: ADMET, anti-parasite activity, metabolizing enzymes and cardiotoxicity proteome biomarkers. *Curr Drug Metab* 11, 379-406 (2010)

7. MTH Khan: Predictions of the ADMET properties of candidate drug molecules utilizing different QSAR/QSPR modelling approaches. *Curr Drug Metab* 11, 285-295 (2010)

8. M Martinez-Romero, JM Vazquez-Naya, JR Rabunal, S Pita-Fernandez, R Macenlle, J Castro-Alvarino, L Lopez-Roses, JL Ulla, AV Martinez-Calvo, S Vazquez, J Pereira, AB Porto-Pazos, J Dorado, A Pazos, CR Munteanu: Artificial intelligence techniques for colorectal cancer drug metabolism: ontology and complex network. *Curr Drug Metab* 11, 347-368 (2010)

9. Y Mrabet, N Semmar: Mathematical methods to analysis of topology, functional variability and evolution of

metabolic systems based on different decomposition concepts. *Curr Drug Metab* 11, 315-341 (2010)

10. JF Wang, KC Chou: Molecular modeling of cytochrome P450 and drug metabolism. *Curr Drug Metab* 11, 342-346 (2010)

11. WZ Zhong, J Zhan, P Kang, S Yamazaki: Gender specific drug metabolism of PF-02341066 in rats-role of sulfoconjugation. *Curr Drug Metab* 11, 296-306 (2010)

12. J Chen, B Shen: Computational Analysis of Amino Acid Mutation: a Proteome Wide Perspective. *Curr Proteomics* 6, 228-234 (2009)

13. KC Chou: Pseudo amino acid composition and its applications in bioinformatics, proteomics and system biology. *Curr Proteomics*, 6, 262-274 (2009)

14. A Giuliani, L Di Paola, R Setola: Proteins as Networks: A Mesoscopic Approach Using Haemoglobin Molecule as Case Study. Curr Proteomics 6, 235-245 (2009)

15. H González-Díaz, F Prado-Prado, LG Pérez-Montoto, A Duardo-Sánchez, A López-Díaz: QSAR Models for Proteins of Parasitic Organisms, Plants and Human Guests: Theory, Applications, Legal Protection, Taxes, and Regulatory Issues. Curr Proteomics 6, 214-227 (2009)

16. O Ivanciuc: Machine learning Quantitative Structure-Activity Relationships (QSAR) for peptides binding to Human Amphiphysin-1 SH3 domain. Curr Proteomics, 4, 289-302 (2009)

17. LG Pérez-Montoto, F Prado-Prado, FM Ubeira, H González-Díaz: Study of Parasitic Infections, Cancer, and other Diseases with Mass-Spectrometry and Quantitative Proteome-Disease Relationships. Curr Proteomics 6, 246-261 (2009)

18. F Torrens, G Castellano: Topological Charge-Transfer Indices: From Small Molecules to Proteins. Curr Proteomics 6, 204-213 (2009)

19. JM Vázquez, V Aguiar, JA Seoane, A Freire, JA Serantes, J Dorado, A Pazos, CR Munteanu: Star Graphs of Protein Sequences and Proteome Mass Spectra in Cancer Prediction. Curr Proteomics 6, 275-288 (2009)

20. J Caballero, M Fernandez: Artificial neural networks from MATLAB in medicinal chemistry. Bayesian-regularized genetic neural networks (BRGNN): application to the prediction of the antagonistic activity against human platelet thrombin receptor (PAR-1). *Curr Top Med Chem* 8, 1580-1605 (2008)

21. A Duardo-Sanchez, G Patlewicz, A Lopez-Diaz: Current topics on software use in medicinal chemistry: intellectual property, taxes, and regulatory issues. *Curr Top Med Chem* 8, 1666-1675 (2008)

22. MP Gonzalez, C Teran, L Saiz-Urra, M Teijeira: Variable selection methods in QSAR: an overview. *Curr Top Med Chem* 8, 1606-1627 (2008)

23. H Gonzalez-Diaz: Quantitative studies on Structure-Activity and Structure-Property Relationships (QSAR/QSPR) *Curr Top Med Chem*, 8, 1554 (2008)

24. H Gonzalez-Diaz, F Prado-Prado, FM Ubeira: Predicting antimicrobial drugs and targets with the MARCH-INSIDE approach. *Curr Top Med Chem* 8, 1676-1690 (2008)

25. AM Helguera, RD Combes, MP Gonzalez, MN Cordeiro: Applications of 2D descriptors in drug design: a DRAGON tale. *Curr Top Med Chem* 8, 1628-1655 (2008)

26. O Ivanciuc: Weka machine learning for predicting the phospholipidosis inducing potential. *Curr Top Med Chem* 8, 1691-1709 (2008)

27. S Vilar, G Cozza, S Moro: Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. *Curr Top Med Chem* 8, 1555-1572 (2008)

28. JF Wang, DQ Wei, KC Chou: Drug candidates from traditional chinese medicines. *Curr Top Med Chem* 8, 1656-1665 (2008)

29. JF Wang, DQ Wei, KC Chou: Pharmacogenomics and personalized use of drugs. *Curr Top Med Chem* 8, 1573-1579 (2008)

30. B Bhattacharjee, RM Jayadeepa, S Banerjee, J Joshi, SK Middha, JP Mole, J Samuel: Review of Complex Network and Gene Ontology in pharmacology approaches: Mapping natural compounds on potential drug target Colon Cancer network. *Curr Bioinform*, 6, 44-52 (2011)

31. O Chiş, O Dumitru, R Concu, B Shen: Reviewing Yeast Network and report of new Stochastic-Credibility cell cycle models. *Curr Bioinform*, 6, 35-43 (2011)

32. K Dave, A Banerjee: Bioinformatics analysis of functional relations between CNPs regions. *Curr Bioinform*, 6, 122-128 (2011)

33. A Duardo-Sanchez, G Patlewicz, H González-Díaz: A Review of Network Topological Indices from Chem-Bioinformatics to Legal Sciences and back. *Curr Bioinform*, 6, 53-70 (2011)

34. I García, Y Fall, G Gómez: Trends in Bioinformatics and Chemoinformatics of Vitamin D analogues and their protein targets. *Curr Bioinform*, 6, 16-24 (2011)

35. T Ivanciuc, O Ivanciuc, DJ Klein: Quantitative Super - Structure Property Relationships (QSSPR): Reaction poset technique for chromatographic properties of polychlorinated biphenyls (PCBs) *Curr Bioinform*, 6, 25-34 (2011)

36. F Prado-Prado, M Escobar-Cubiella, X García-Mera: Review of Bioinformatics and QSAR studies of β-secretase inhibitors. *Curr Bioinform*, 6, 3-15 (2011)

37. P Riera-Fernández, CR Munteanu, N Pedreira-Souto, A Duardo-Sanchez, H González-Díaz: Definition of Markov-Harary Invariants and Review of Classic Topological Indices and Databases of Biology, Parasitology, Technology, and Social-Legal Networks. *Curr Bioinform*, 6, 94-121 (2011)

38. A Speck-Planche, MNDS Cordeiro: Application of Bioinformatics for the search of novel anti-viral therapies: Rational design of anti-herpes agents. *Curr Bioinform*, 6, 81-93 (2011)

39. SB Wan, LL Hu, S Niu, K Wang, YD Cai, WC Lu, KC Chou: Identification of multiple subcellular locations for proteins in budding yeast. *Curr Bioinform*, 6, 71-80 (2011)

40. R Concu, G Podda, FM Ubeira, H Gonzalez-Diaz: Review of QSAR models for enzyme classes of drug targets: Theoretical background and applications in parasites, hosts, and other organisms. *Curr Pharm Des* 16, 2710-2723 (2010)

41. E Estrada, E Molina, D Nodarse, E Uriarte: Structural contributions of substrates to their binding to P-Glycoprotein. A TOPS-MODE approach. *Curr Pharm Des* 16, 2676-2709 (2010)

42. I Garcia, Y Fall, G Gomez: QSAR, docking, and CoMFA studies of GSK3 inhibitors. *Curr Pharm Des* 16, 2666-2675 (2010)

43. H Gonzalez-Diaz: QSAR and complex networks in pharmaceutical design, microbiology, parasitology, toxicology, cancer, and neurosciences. *Curr Pharm Des* 16, 2598-2600 (2010)

44. H Gonzalez-Diaz, F Romaris, A Duardo-Sanchez, LG Perez-Montoto, F Prado-Prado, G Patlewicz, FM Ubeira: Predicting drugs and proteins in parasite infections with topological indices of complex networks: theoretical backgrounds, applications, and legal issues. *Curr Pharm Des* 16, 2737-2764 (2010)

45. Y Marrero-Ponce, GM Casanola-Martin, MT Khan, F Torrens, A Rescigno, C Abad: Ligand-based computer-aided discovery of tyrosinase inhibitors. Applications of the TOMOCOMD-CARDD method to the elucidation of new compounds. *Curr Pharm Des* 16, 2601-2624 (2010)

46. CR Munteanu, E Fernandez-Blanco, JA Seoane, P Izquierdo-Novo, JA Rodriguez-Fernandez, JM Prieto-Gonzalez, JR Rabunal, A Pazos: Drug discovery and design for complex diseases through QSAR computational methods. *Curr Pharm Des* 16, 2640-2655 (2010)

47. K Roy, G Ghosh: Exploring QSARs with Extended Topochemical Atom (ETA) indices for modeling chemical and drug toxicity. *Curr Pharm Des* 16, 2625-2639 (2010)

48. A Speck-Planche, MT Scotti, V de Paulo-Emerenciano: Current pharmaceutical design of antituberculosis drugs: future perspectives. *Curr Pharm Des* 16, 2656-2665 (2010)

49. JM Vazquez-Naya, M Martinez-Romero, AB Porto-Pazos, F Novoa, M Valladares-Ayerbes, J Pereira, CR Munteanu, J Dorado: Ontologies of drug discovery and design for neurology, cardiology and oncology. *Curr Pharm Des* 16, 2724-2736 (2010)

50. N Fjodorova, M Novich, M Vrachko, V Smirnov, N Kharchevnikova, Z Zholdakova, S Novikov, N Skvortsova, D Filimonov, V Poroikov, E Benfenati: Directions in QSAR modeling for regulatory uses in OECD member countries, EU and in Russia. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 26, 201-236 (2008)

51. P Floyd: Future perspectives in risk assessment of chemicals. In: Chemicals in the environment: Assessing and managing risk. Eds: RE Hester, RM Harrison, *RSC Publishing*, Cambridge CB4 0WF (2006)

52. ESIS-Team: European chemical Substances Information System. Available at: http://esis.jrc.ec.europa.eu/. (2008)

53. RA Merrill: Food safety regulation: reforming the Delaney Clause. *Annu Rev Publ Health* 18, 313-340 (1997)

54. EG Finer: Chemicals risk assessment and management. In: Chemicals in the environment: Assessing and managing risk. Eds: RE Hester, RM Harrison, *RSC Publishing*, Cambridge CB4 0WF (2006)

55. RCEP-Team: Royal Commission on Environmental Pollution, 24th Report, Chemicals in Products, Cm 5827. Available at: http://webarchive.nationalarchives.gov.uk/20060820083451/http://rcep.org.uk/chemicals.htm. (2003)

56. J Patterson, PJ Hakkinen, AE Wullenweber: Human health risk assessment: selected Internet and world wide web resources. *Toxicology* 173, 123-143 (2002)

57. International Programme on Chemical Safety, Environmental Health Criteria 210 1998. Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. Section 6.3.8. Regulatory limits. Available at: http://www.inchem.org/documents/ehc/ehc/ehc210.htm. (1998)

58. The foundations of the state policy for ensuring chemical and biological safety of the Russian Federation for the period up to 2010 and longer terms, approved by President of Russian Federation V.V. Putin on December 4, 2003. *No Pr-2194*. (Russian) (2003)

59. Development of regulation in the field of safety of chemical production. Russian Chemists Union home page. Available at: http://www.ruschemunion.ru/index.php?programsru=16. (2008)

60. Project "HELCOM (Helsinki Commission) data collection strategy". Available at: http://www.ctcspb.ru/en/prvandensprojektai.php?PHPSESSID=5e2dec97ef3720b6f8e4a7d608441f76. (2005)

61. J Ruut, J Simanovska: Analysis of The Existing Chemicals Management System in The Russian Federation. Project BACCON Rus1. Baltic Environmental Forum (BEF). Available at: http://www.norden.org/pub/sk/showpub.asp?pubnr=2006:416. (2005)

62. Russian register of potentially hazardous chemical and biological substances. (Russian). Available at: http://www.rpohv.ru/magazin/b. (2008)

63. AM Beim: Effects of chemical pollutants on aquatic organisms. In: Toxicometry of environmental chemical pollutants. Eds: AA Kasparov, IV Sanotski. *Center for International Projects (GKNT)*, Moscow (1986)

64. YA Rahmanin, VV Semionova, AV Moskuina: Hygienic norms of chemical compounds in the environment. *NPO Professional*, St Petersburg (2006)

65. H Kubinyi: QSAR: Hansch analysis and related approaches. *VCH Publishers*, Weinheim, New York, Basel, Cambridge, Tokyo (1993)

66. C Hansch, A Leo: Exploring QSAR: fundamentals and applications in Chemistry and Biology. *American Chemical Society*, Washington, DC (1995)

67. H van de Waterbeemd: Chemometrics methods in molecular design. *VCH Publishers*, Weinheim, New York, Basel, Cambridge, Tokyo (1995)

68. LG Valerio, Jr.: In silico toxicology for the pharmaceutical sciences. *Toxicol Appl Pharmacol* 241, 356-370 (2009)

69. R Benigni, L Passerini, A Rodomonte: Structure-activity relationships for the mutagenicity and carcinogenicity of simple and alpha-beta unsaturated aldehydes. *Environ Mol Mutagen* 42, 136-143 (2003)

70. MM Mumtaz, PR Durkin: A weight-of-evidence approach for assessing interactions in chemical mixtures. *Toxicol Ind Health* 8, 377-406 (1992)

71. JC Dearden: In silico prediction of drug toxicity. *J Comput Aided Mol Des* 17, 119-127 (2003)

72. AP Worth, CJ Van Leeuwen, T Hartung: The prospects for using (Q)SARs in a changing political environment-high expectations and a key role for the European Commission's joint research centre. *SAR QSAR Environ Res* 15, 331-343 (2004)

73. A Worth, A Bassan, A Gallegos, T Netzeva, G Patlewicz, M Pavan, I Tsakovska, M Vracko: The Characterization of (Quantitative) Structure-Activity Relationships: Preliminary Guidance. EUR 21866 EN European Communities. Available at: http://ecb.jrc.it/DOCUMENTS/QSAR/QSAR_characterisation_EUR_21866_EN.pdf. (2005)

74. JD Walker, J Jaworska, MH Comber, TW Schultz, JC Dearden: Guidelines for developing and using quantitative structure-activity relationships. *Environ Toxicol Chem* 22, 1653-1665 (2003)

75. ED OECD: Report on the Regulatory Uses and Applications in OECD Member Countries of Quantitative Structure-Activity Relationchip (QSAR) Models in the Assessment of New and Existing Chemicals. In: OECD Environment Health and Safety Publications. Series on Testing and Assessment. Paris (2006)

76. E Benfenati: Quantitative Structure-Activity Relationship (QSAR) of Pesticide for Regulatory Purposes. *Elsevier B.V.*, Amsterdam (2007)

77. MT Cronin, JD Walker, JS Jaworska, MH Comber, CD Watts, AP Worth: Use of QSARs in international decision-making frameworks to predict ecologic effects and environmental fate of chemical substances. *Environ Health Perspect* 111, 1376-1390 (2003)

78. MT Cronin, JS Jaworska, JD Walker, MH Comber, CD Watts, AP Worth: Use of QSARs in international decision-making frameworks to predict health effects of chemical substances. *Environ Health Perspect* 111, 1391-1401 (2003)

79. M Cronin: Report on the "Review of the Status of the Development of Alternatives to using Animals in Chemical Safety Testing and Identification of New Areas for Development or Research in the Context of the Proposed REACH Regulation", DEFRA contract number: CPEC 42. Available at: http://www.defra.gov.uk/science/project_data/DocumentLibrary/CB01067/CB01067_2795_FRA.pdf. (2005)

80. L Edler, K Poirier, M Dourson, J Kleiner, B Mileson, H Nordmann, A Renwick, W Slob, K Walton, W G.: Mathematical modeling and quantitative methods. *Food Chem Toxicol* 40, 283-326 (2002)

81. CERTH-Unit: OECD's Database on Chemical Risk Assessment Models. Available at: http://webdomino1.oecd.org/comnet/env/models.nsf. (2006-2012)

82. S Coecke, H Ahr, BJ Blaauboer, S Bremer, S Casati, J Castell, R Combes, R Corvi, CL Crespi, ML Cunningham, G Elaut, B Eletti, A Freidig, A Gennari, JF Ghersi-Egea, A Guillouzo, T Hartung, P Hoet, M Ingelman-Sundberg, S Munn, W Janssens, B Ladstetter, D Leahy, A Long, A Meneguz, M Monshouwer, S Morath, F Nagelkerke, O Pelkonen, J Ponti, P Prieto, L Richert, E Sabbioni, B Schaack, W Steiling, E Testai, JA Vericat, A Worth: Metabolism: a bottleneck in *in vitro* toxicological test development. The report and recommendations of ECVAM workshop 54. *Altern Lab Anim* 34, 49-84 (2006)

83. R Combes, M Barratt, M Balls: An overall strategy for the testing of chemicals for human hazard and risk assessment under the EU REACH system. *Altern Lab Anim* 31, 7-19 (2003)

84. R Gonella-Diaza, V Castiglioni: Computer assisted evaluation of industrial chemical substances according to regulations. Available at: http://www.caesar-project.eu/. (2011)

85. E Estrada: Spectral moments of the edge adjacency matrix in molecular graphs. 3. Molecules containing cycles. *J Chem Inf Comput Sci* 38, 23–27 (1998)

86. E Estrada: Spectral moments of the edge adjacency matrix in molecular graphs. 2. Molecules containing heteroatoms and QSAR applications. *J Chem Inf Comput Sci* 37, 320–328 (1997)

87. E Estrada: Spectral moments of the edge adjacency matrix in molecular graphs. 1. Definition and applications for the prediction of physical properties of alkanes. *J Chem Inf Comput Sci* 36, 844–849 (1996)

88. E Estrada, Y Gutiérrez, MODESLAB 1.5. © 2002-2004. Available at: www.modeslab.com

89. E Estrada, E Uriarte, Y Gutierrez, H Gonzalez: Quantitative structure-toxicity relationships using TOPS-MODE. 3. Structural factors influencing the permeability of commercial solvents through living human skin. *SAR QSAR Environ Res* 14, 145-163 (2003)

90. E Estrada: Structure-mutagenicity relationships in 2-furylethylene derivatives. A molecular orbital study of the role of nitro groups. *Mutat Res* 420, 67-75 (1998)

91. E Estrada, E Molina, E Uriarte: Quantitative structure-toxicity relationships using TOPS-MODE. 2. Neurotoxicity of a non-congeneric series of solvents. *SAR QSAR Environ Res* 12, 445-459 (2001)

92. AM Helguera, G Perez-Machado, MNDS Cordeiro, RD Combes: Quantitative structure-activity relationship modelling of the carcinogenic risk of nitroso compounds using regression analysis and the TOPS-MODE approach. *SAR QSAR Environ Res* 21, 277-304 (2010)

93. A Morales Helguera, M Perez Gonzalez, MNDS Cordeiro, MA Cabrera Perez: Quantitative structure-carcinogenicity relationship for detecting structural alerts in nitroso compounds: species, rat; sex, female; route of administration, gavage. *Chem Res Toxicol* 21, 633-642 (2008)

94. AM Helguera, MP Gonzalez, MNDS Cordeiro, MA Perez: Quantitative structure carcinogenicity relationship for detecting structural alerts in nitroso-compounds. *Toxicol Appl Pharmacol* 221, 189-202 (2007)

95. E Estrada, Y Gutierrez: MODESLAB for WINDOWS. User's Manual. http://www.modeslab.com (2002-2004)