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METHODOLOGY

REACH, animal testing, and the precautionary principle

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Abstract: Relatively little is known about the toxicity of the many chemicals in existence today. This has prompted European Union regulatory authorities to launch a major chemicals testing program, known as Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Although the driving force behind REACH is ostensibly based on the precautionary principle, in practice, the evidence suggests that it is oriented more toward risk assessment than precaution. In addition, the test methods used to assess chemical risk also raise questions about the efficacy of REACH in achieving its stated aims of protecting human health and the environment. These tests rely in large part on animal models. However, based on empirical evidence and on well-established principles of evolutionary biology and complex systems, the animal model fails as a predictive modality for humans. In turn, these concerns raise significant ethical and legal issues that must be addressed urgently. Immediate measures should include a major biomonitoring program to reliably assess the chemical burden in European Union citizens as a means of prioritizing the most dangerous substances present in the environment. Blood and urine biomarkers are useful tools with which to implement biomonitoring and to help guide public policy. An ecological paradigm, based on pollution prevention, rather than pollution control and risk assessment of individual chemicals, represents a superior strategy, to prevent global chemical pollution and toxicity risks to human health.

Keywords: precautionary principle, risk, chemicals, animal tests, biomonitoring

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Introduction

> People, not chemicals, have the right to be presumed innocent until proven guilty. People also have the right not to be experimented on without informed consent; no one has ever been given the opportunity to grant or deny their consent before being exposed to the [toxic] burden that now contaminates us all.[1]

REACH is the acronym for "Registration, Evaluation, Authorisation and Restriction of Chemicals" in the European Union (EU), which entered into force on June 1, 2007.[2] The program addresses the potential impacts of chemical substances on human health and the environment, and has been described by various stakeholders as the most complex legislation in the Union's history and the most important in the last 20 years. The administrative implementation of REACH is largely overseen by the European Chemicals Agency (ECHA),[3] which helps companies to comply with the legislation, while the required test methods are described in Organisation for Economic Cooperation and Development (OECD) guidelines.[4] These guidelines represent internationally agreed test methods to determine the safety of chemicals. In addition

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to human health and environmental effects, these guidelines include tests to cover physical–chemical properties of chemicals and their degradation and accumulation in the environment. Although within the EU and internationally REACH is widely regarded as being modeled on the precautionary principle,[2,5] in practice, it is based more on risk management, as this article will show; the precautionary principle and the management of risk are two fundamentally different concepts. In addition, we will demonstrate that much of the methodology that this risk assessment is based on (animal models) is invalid for predicting human response. In turn, these significant shortcomings raise fundamental questions about the role of REACH.

There are more than 7 million recognized chemicals in existence, of which at least 80,000 are in common use worldwide[6] and for which potential toxicity remains largely unknown.[7,8] Since the early 1970s, there has been a growing debate among EU member states about how best to regulate the use of industrial chemicals, particularly with respect to risk and hazard. "Hazard" is associated with a chemical's intrinsic ability to cause adverse effects while "risk" refers to the probability that such effects will occur in the various applications in which the chemical will be used and discharged (exposure scenarios).[9] Simply put: risk = hazard × exposure.

Despite the clear distinction between these two terms, their use may sometimes be clouded by cultural or political motives. For example, the UK is more likely to consider risk and benefit trade-offs in regulation than a country like Sweden, which tends to be more precautionary.[10] Nordic countries, such as Denmark and Sweden, have actively promoted integrated chemicals policies and successfully used a variety of voluntary and mandatory policy tools to reduce reliance on harmful substances and to develop safer substitutes.[11]

However, the overarching consideration in any regulatory framework dealing with human hazard identification is the methodology on which it is based. In the case of the EU chemicals testing program REACH, the methodology is clearly set out in the OECD test guidelines,[12] which sets forth principles of good laboratory practice and mutual acceptance of data for use by government and industry. In addition to the OECD test guidelines, guidance on information requirements and chemical safety assessment are supplemented by the ECHA.

This paper will examine the EU chemicals regulation program with respect to scientific, legal, and ethical considerations.

REACH and the precautionary principle

"When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically" (from the January 1998 Wingspread Statement on the Precautionary Principle[13]).

The "precautionary principle," as broadly defined, states that if an action or policy has a suspected risk of causing harm to the public or to the environment, in the absence of clear scientific consensus, then the burden of proof that it is not harmful falls on those taking the action.[14] The precautionary principle is mentioned in paragraph 9 in the introduction to REACH and is also detailed in paragraph 2 of Article 191 of the Treaty on the Functioning of the European Union, which states:

> Union policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Union. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay.[15]

According to Kriebel et al:

> If there is certainty about cause and effect, as in the case of lead and children's health, then acting is no longer precautionary, although it might be preventive. In essence, the precautionary principle provides a rationale for taking action against a practice or substance in the absence of scientific certainty rather than continuing the suspect practice while it is under study, or without study. Instead of asking what level of harm is acceptable, a precautionary approach asks: How much contamination can be avoided? What are the alternatives to this product or activity, and are they safer? Is this activity even necessary?[16]

REACH is clearly at odds with the precautionary principle in stating that

> Under the REACH regulation, even if a substance presents a risk to human health or the environment, authorisation may be granted if the socio-economic benefits are proven to outweigh risks arising from its use and if there are no suitable alternatives.[17]

Risk assessment is based on setting an acceptable level of harm while perpetuating a "business as usual" approach,

in contrast with the precautionary principle, which calls for dynamic change toward sustainability.[18] REACH clearly reveals its risk assessment character on the issue of substances of very high concern (SVHC). These are substances considered to be particularly hazardous for human health and the environment. To date, the candidate list for registration of SVHC numbers just 73 chemicals[19] of a total of 143,000 chemicals registered by ECHA.[20] Considering that REACH entered into force on June 1, 2007 and that it has taken nearly 5 years simply to register – not remove or replace – these 73 SVHC, there is cause for concern that the administrative process for eliminating such chemicals will be too slow and too cumbersome to prevent avoidable harm to human health and damage to the environment. This concern has been echoed by Environment Commissioner Janez Potočnik, who admitted in March 2010 that there were still no SVHC on the substitution list.[17]

The task of evaluating thousands of individual chemicals for their acute and chronic effects on various human organ systems as well as on the environment is daunting but not impossible in an age of platform-based high-throughput robotized analytical systems.[21] However, the very real need to evaluate combinations of chemicals and chemical mixtures presents a very different challenge. According to Vyvyan Howard, a toxicopathologist, to test the most common 1000 chemicals in unique combinations of three would require 166 million experiments.[22] One alternative to risk management is an ecological paradigm centered on the precautionary principle and that favors "prudent pessimism" over "hazardous optimism" in the presence of scientific uncertainty.[23] Although the precautionary principle may be considered by some to be too vague to function as a regulatory standard, an ecological paradigm sets out clear guidelines.[1] These include clean production and zero discharge. Clean production places the emphasis on pollution prevention rather than pollution control by requiring industry to make use of the most benign available methods in addition to avoiding the release of hazardous materials by preventing their production in the first place.[24] The policy of zero discharge would prohibit the release of dangerous substances into the environment.

Animal test requirements under REACH

The stated aim of the EU chemicals testing program is to "improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances as well as the free circulation of substances on the internal market while enhancing competitiveness and innovation."[25] A significant aspect of this evaluation of chemicals involves animal tests, with between 9 million and 54 million animals currently estimated as necessary to meet requirements.[26] The original estimates for the implementation and scope of REACH were based on chemical production data corresponding to the time when an EU chemicals program was under discussion during the 1980s and early 1990s. According to some European Commission (EC) scientists, the probable cost of the chemicals testing program for that era would be in the region of €1.6 billion and would require approximately 2.6 million animals.[26]

What was not predicted at the time of these calculations were factors such as the dramatic increase in the production of new chemicals, the inclusion of additional testing requirements (for example, reaction intermediates), and a significant increase in the number of EU member states, all of which would lead to vastly different estimates. For example, the first phase of REACH envisaged the registration of 30,000 chemical substances produced or imported into the EU in quantities of more than 1 tonne per year. The total number of substances submitted by the 2008 deadline for registration was 143,000, although EC authorities anticipate that this figure will ultimately decrease.[20] Indeed, ECHA's Dissemination Database contains just 4326 unique substances at the present time (March 2012).[27] Overall, however, it has become clear that the cost of REACH today is much higher than original estimates, in terms of budget and animal numbers.

Whereas the REACH regulation sets out the general conditions for the registration, evaluation, authorization, and restriction of chemicals, the actual test methods for the determination of toxicity and other health effects (Table 1) are described by OECD Council Regulation No 440/2008 of May 30, 2008.[28]

The use of animals for the safety testing and human risk assessment of chemical substances raises ethical and scientific issues. Acute and repeated dose toxicity testing in vertebrates are acknowledged as causing suffering and usually the death of the animal. Society in general is uncomfortable with the use of animals in research and testing. An EU wide survey involving 42,655 participants conducted by the EC in 2006[29] found that a majority of EU citizens considered the use of animals to be unacceptable under any circumstances to "develop and test chemicals for industrial, household and agricultural use for their safety for human, animal and the environment" (question 22). In addition to the ethical and

Table 1 Methods for the determination of toxicity and other health effects.[135]

Test type	Test name	Animal species/organism involved	Mode of action
Acute toxicity	Acute oral toxicity	In vivo: "preferred rodent species is the rat although other rodent species may be used"	Available from: http://www.oecd.org/dataoecd/17/51/1948378.pdf. Accessed May 28, 2012
	Acute toxicity (inhalation)	In vivo: rat (as above). Ten animals to be used for each concentration. Animals are exposed to one limit concentration or a series of concentrations over multiple time durations. Usually two animals per concentration each time interval are used. Animals are observed for at least 14 days. In the traditional LC50 (median lethal concentration), animals are exposed to 1 limit concentration or to 3 concentrations, at least, for a predetermined duration, generally of 4 hours	Available from: www.oecd-ilibrary.org/environment/test-no-403-acute-inhalation-toxicity_9789264070608-en. Accessed May 28, 2012
	Acute toxicity (dermal)	In vivo: rodents (rat, rabbit, or guinea pig may be used). For each dose at least five animals (of the same sex) are used. The substance is applied to the skin (not less than 10% of the body surface area) in graduated doses to several groups of animals, one dose being per group. At least three dose levels are recommended to be used to produce a dose–response curve. A limit test of at least 2000 mg/kg is proposed. The observation period is at least 14 days	Available from: www.oecd-ilibrary.org/environment/test-no-402-acute-dermal-toxicity_9789264070585-en. Accessed May 28, 2012
	Acute toxicity: dermal irritation/corrosion	In vivo: albino rabbit is the preferred animal. The substance to be tested is applied in a single dose to an area of skin of approximately 6 cm^2 of the rabbit. The exposure time is 4 hours. Residual test substance is then removed. The dose is 0.5 mL (liquid) or 0.5 g (solid) applied to the test site. The method consists of two tests: the initial test and the confirmatory test (used if a corrosive effect is not observed in the initial test). Animals are examined for signs of erythema and edema over 14 days. The dermal irritation scores are evaluated with the nature and severity of lesions, and their reversibility or lack thereof. When responses persist to the end of the 14-day observation period, the test substance is considered an irritant	Available from: www.oecd-ilibrary.org/environment/test-no-404-acute-dermal-irritation-corrosion_9789264070622-en. Accessed May 28, 2012
	Acute toxicity: eye irritation/corrosion	In vivo: albino rabbit preferred. Substance is applied in a single dose in the conjunctival sac of one eye of each animal. The other untreated eye is used as control. The initial test uses one animal; the dose level depends on the substance. A confirmatory test is advised if a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. The duration depends on how long is needed to evaluate fully the magnitude and reversibility of the effects observed. The eyes should be examined at 1 hour and 24, 48, and 72 hours after test substance application. The ocular irritation scores are evaluated in conjunction with the nature and severity of lesions and their reversibility or lack thereof	Available from: www.oecd-ilibrary.org/environment/test-no-405-acute-eye-irritation-corrosion_9789264070646-en. Accessed May 28, 2012
	Skin sensitization	In vivo: guinea pig is preferred, but also mouse. For the guinea pig maximization test (GPMT) at least ten animals in the treatment group and five in the control group are used. For the Buehler test, a minimum of 20 animals is used in the test group and at least ten animals in the control. The animals are initially exposed to the substance, then to a rest period, and an induction period (10–14 days), for an immune response to develop. Following this, the animals are exposed to a challenge dose. The GPMT is made over 23–25 days, the Buehler test over 30–32 days. The concentration of substance used for each induction exposure should be well tolerated systemically and the highest to cause mild-to-moderate skin irritation. For the challenge exposure, the highest nonirritant dose should be used	Available from: www.oecd-ilibrary.org/environment/test-no-406-skin-sensitisation_9789264070660-en. Accessed May 28, 2012

Repeated dose toxicity	Repeated dose (28 days) toxicity (oral)	In vivo: rodents (rat preferred). Test made during one limited period (one dose level daily during 28 days). At least ten animals (five female and five male) are used for each dose level and at least three tests groups. The substance is given by gavage or via the diet or drinking water. A limit test may be performed if no effects would be expected at a dose of 1000 mg/kg body weight per day (bw/day). Clinical and functional observations, body weight and food/water consumption measurements, hematology and clinical biochemistry tests are made; as well as gross necropsy and histopathology, as animals will be killed at the end of the test	Available from: www.oecd-ilibrary.org/environment/test-no-407-repeated-dose-28-day-oral-toxicity-study-in-rodents_9789264070684-en. Accessed May 28, 2012
	Repeated dose (28 days) toxicity (inhalation) (subacute inhalation toxicity: 28-day study)	In vivo: adult rodent, preferred species rat (as above). Groups of at least five male and five female exposed 6 hours per day for 28 days to: a) the test animal at three or more concentration levels, b) filtered air (negative control), and/or c) the vehicle (vehicle control). Animals are generally exposed 5 days per week but exposure for 7 days per week is also allowed.	Available from: http://www.oecd.org/document/29/0,3746,en_21571361_43392827_45356509_1_1_1_1,00.html and www.oecd-ilibrary.org/environment/test-no-412-subacute-inhalation-toxicity-28-day-study_9789264070783-en. Both accessed May 28, 2012
	Repeated dose (28 days) toxicity (dermal)	In vivo: adult rat, rabbit, or guinea pig. Two tests: main test and limit test. At least ten animals (five female and five male) with healthy skin used at each dose level (at least three). The highest dose level to result in toxic effect not producing fatalities. The limit test is one dose level of at least 1000 mg/kg body weight. The method consists of repeated application of the substance during a limited period (several hours daily during 21/28 days). At the end, animals are killed	Available from: www.oecd-ilibrary.org/environment/test-no-410-repeated-dose-dermal-toxicity-21-28-day-study_9789264070745-en. Accessed May 28, 2012
Genetic toxicology: mutagenicity and aberration	Mutagenicity – in vitro mammalian chromosome aberration test	In vitro: mammalian somatic cell lines	Available from: www.oecd-ilibrary.org/content/book/9789264071261-en. Accessed May 28, 2012
	Mutagenicity – in vivo mammalian bone marrow chromosome aberration test	In vivo: usually rodents (rats, mice, and Chinese hamsters [*Cricetulus griseus*])	Available from: www.oecd-ilibrary.org/content/book/9789264071308-en. Accessed May 28, 2012
	Mutagenicity – in vivo mammalian erythrocyte micronucleus test	In vivo: erythrocytes from bone marrow and/or peripheral blood cells of animals, usually rodents (mice or rats)	Available from: www.oecd-ilibrary.org/content/book/9789264071285-en. Accessed May 28, 2012
	Mutagenicity: reverse mutation test using bacteria	In vitro: bacteria	Available from: www.oecd-ilibrary.org/content/book/9789264071247-en. Accessed May 28, 2012
	Mutagenicity testing and screening for carcinogenicity gene mutation – saccharomyces cerevisiae or: saccharomyces cerevisiae, gene mutation assay	In vitro: yeast	Available from: www.oecd-ilibrary.org/environment/test-no-480-genetic-toxicology-saccharomyces-cerevisiae-gene-mutation-assay_9789264071407-en. Accessed May 28, 2012

(*Continued*)

Table 1 (*Continued*)

Test type	Test name	Animal species/organism involved	Mode of action
Genetic toxicology: mutagenicity and those involving genetic mutations	Mitotic recombination assay in *Saccharomyces cerevisiae*	In vitro: yeast	Available from: www.oecd-ilibrary.org/content/book/9789264071421-en. Accessed May 28, 2012
	Mutagenicity – in vitro mammalian cell gene mutation test	In vitro: cultured mammalian cells	Available from: www.oecd-ilibrary.org/content/book/9789264071322-en. Accessed May 28, 2012
	DNA damage and repair – unscheduled DNA synthesis in mammalian cells in vitro	In vitro: cells	Available from: www.oecd-ilibrary.org/environment/test-no-482-genetic-toxicology-dna-damage-and-repair-unscheduled-dna-synthesis-in-mammalian-cells-in-vitro_9789264071445-en. Accessed May 28, 2012
	Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo	In vivo: Rats are commonly used, at least three animals per group. Test based on incorporation of tritium-labeled thymidine, 3H-TdR, (during 3–8 hours) into the DNA of liver cells of animals. Substances are administered as a single treatment by gavage using a stomach tube or a suitable intubation cannula. At least two dose levels are used. A limit test is advised if no effects would be expected at a dose of 2000 mg/kg bw/day	Available from: www.oecd-ilibrary.org/environment/test-no-486-unscheduled-dna-synthesis-uds-test-with-mammalian-liver-cells-in-vivo_9789264071520-en. Accessed May 28, 2012
	Sister chromatid exchange assay in vitro	In vitro: mammalian cells	Available from: www.oecd-ilibrary.org/content/book/9789264071384-en. Accessed May 28, 2012
	Sex-linked recessive lethal test in *Drosophila melanogaster*	In vivo: *Drosophila melanogaster* insects, offsprings, germline mutations	Available from: www.oecd-ilibrary.org/content/book/9789264071346-en. Accessed May 28, 2012
	Mutagenicity – in vitro mammalian cell gene mutation test	In vitro: cells	
	DNA damage and repair – unscheduled DNA synthesis – mammalian cells in vitro	In vitro: cells	
	Sister chromatid exchange assay in vitro	In vitro: cells	
	Sex-linked recessive lethal test in *Drosophila melanogaster*	In vitro: cells	
	In vitro mammalian cell transformation tests	In vitro: cells	

Genetic toxicology: lethal, genetic, and aberration tests	Rodent dominant lethal test	In vivo: rats or mice, males and females germinal tissues and organs	Available from: www.oecd-ilibrary.org/content/book/9789264071360-en. Accessed May 28, 2012
	Mammalian spermatogonial chromosome aberration test	In vivo: male Chinese hamsters and mice	Available from: www.oecd-ilibrary.org/content/book/9789264071469-en. Accessed May 28, 2012
	Mouse spot test	In vivo: mouse, embryos to 4 weeks	Available from: www.oecd-ilibrary.org/content/book/9789264071483-en. Accessed May 28, 2012
	Mouse heritable translocation assay	In vivo: mouse, germ lines	Available from: www.oecd-ilibrary.org/content/book/9789264071506-en. Accessed May 28, 2012
	Sub-chronic oral toxicity test: repeated dose 90-day oral toxicity study in rodents	In vivo: mouse, rat, at least ten males and ten females	Available from: www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en. Accessed May 28, 2012
	Sub-chronic oral toxicity test repeated dose 90-day oral toxicity study in nonrodents	In vivo: the dog (the beagle is frequently used). At least eight animals (four female and four male) should be used for each test group	Available from: www.oecd-ilibrary.org/environment/test-no-409-repeated-dose-90-day-oral-toxicity-study-in-non-rodents_9789264070721-en. Accessed May 28, 2012
	Sub-chronic dermal toxicity study 90-day repeated dermal dose study using rodent species	In vivo: usually rats, at least 20 animals per sex	Available from: www.oecd-ilibrary.org/content/book/9789264071209-en. Accessed May 28, 2012
	Sub-chronic inhalation toxicity: 90-day, repeated inhalation dose study	In vivo: rodents (usually rats). Groups of ten male and ten female rodents are exposed for 6 hours per day over 90 days (13 weeks)	Available from: www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en. Accessed May 28, 2012
Chronic toxicity	Prenatal developmental toxicity study	In vivo: pregnant test animal and on the developing organism, rodent (rat suggested) and nonrodent (rabbit suggested, but can also be dog)	Available from: www.oecd-ilibrary.org/content/book/9789264070820-en. Accessed May 28, 2012
	Chronic toxicity studies	In vivo: usually rats, at least 20 animals per sex, while for nonrodents (eg, dogs) a minimum of four per sex per group is recommended. Daily exposure, may vary if oral, dermal or inhalation. The duration of the exposure period should be 12 months, after which animals are killed	Available from: www.oecd-ilibrary.org/environment/test-no-452-chronic-toxicity-studies_9789264071209-en. Accessed May 28, 2012

(*Continued*)

Table 1 (*Continued*)

Test type	Test name	Animal species/organism involved	Mode of action
Carcinogenicity	Combined chronic toxicity/ carcinogenicity test	In vivo: rat typically used. Each dose group and control group for the carcinogenicity test uses at least 50 animals of each sex, for the chronic toxicity at least ten animals of each sex	Available from: www.oecd-ilibrary.org/content/book/9789264071223-en. Accessed May 28, 2012
Reproduction toxicity	One-generation reproduction toxicity test	In vivo: usually rat or mouse. Each test and control group contains a number of animals to obtain about 20 pregnant females at or near term, three test groups, at least, are recommended	Available from: www.oecd-ilibrary.org/environment/test-no-415-one-generation-reproduction-toxicity-study_9789264070844-en. Accessed May 28, 2012
	Two-generation reproduction toxicity study	In vivo: usually rat or mouse. Each test and control group contains a number of animals to obtain about 20 pregnant females at or near term; three test groups, at least, are recommended. Parent generation (5–9 weeks old), offspring, and administration of the substance is continued to first generation offspring during their growth into adulthood, mating and production of a second generation (until weaning)	Available from: www.oecd-ilibrary.org/environment/test-no-416-two-generation-reproduction-toxicity_9789264070868-en. Accessed May 28, 2012
Toxicokinetics	Toxicokinetics delayed neurotoxicity of organophosphorus substances following acute exposure	In vivo: adult domestic laying hen (*Gallus gallus domesticus*), aged 8–12 months, recommended group is twelve hens at least, the positive control group at least six hens. The dose level of the main study is recommended to be high as possible, with a maximum dose level of 2000 mg/kg bw/day	Available from: www.oecd-ilibrary.org/environment/test-no-418-delayed-neurotoxicity-of-organophosphorus-substances-following-acute-exposure_9789264070905-en. Accessed May 28, 2012
	Delayed neurotoxicity of organophosphorus substances 28 day repeated dose study	In vivo: domestic laying hens (aged 8–12 months) for 28 days, observed at least daily until 14 days after the last dose. At least twelve hens and three groups recommended to be used. The highest dose level should be chosen with the aim of inducing toxic effects, thereafter descending	Available from: www.oecd-ilibrary.org/environment/test-no-419-delayed-neurotoxicity-of-organophosphorus-substances-28-day-repeated-dose-study_9789264070929-en. Accessed May 28, 2012
Skin corrosion	In vitro skin corrosion: transcutaneous electrical resistance test	In vitro: skin taken from killed rats aged 28–30 days old	Available from: www.oecd-ilibrary.org/content/book/9789264071124-en. Accessed May 28, 2012
	In vitro skin corrosion: human skin model test	In vitro: test material (solid or liquid) is applied to a three-dimensional human skin model, comprising at least a reconstructed epidermis with a functional stratum corneum. Does not require the use of live animals or animal tissue for the assessment of skin corrosivity	Available from: www.oecd-ilibrary.org/content/book/9789264071148-en. Accessed May 28, 2012
Phototoxicity	In vitro 3T3 NRU phototoxicity test	In vitro: Balb/c 3T3 cells	Available from: www.oecd-ilibrary.org/content/book/9789264071162-en. Accessed May 28, 2012
Skin sensitization	Skin sensitization: local lymph node assay	In vivo: generally in mouse. A minimum of four animals is used per dose group, with a minimum of three concentrations of the substance and a negative control group and a positive control. The experimental schedule of the assay is during 6 days, then the animals are killed and a cell suspension of lymph node cells is prepared	Available from: www.oecd-ilibrary.org/environment/test-no-429-skin-sensitisation_9789264071100-en. Accessed May 28, 2012

Neurotoxicity	Neurotoxicity study in rodents	In vivo: usually rat. Daily oral administration, by gavage (in the diet, in drinking water or by capsules) of the substance. As a separate study, at least 20 animals (ten females and ten males) are used in each dose. At least three dose groups and one control group. The recommended dosing regimen is 28 days, sub-chronic (90 days), or chronic (1 year or longer)	Available from: www.oecd-ilibrary.org/environment/test-no-424-neurotoxicity-study-in-rodents_9789264071025-en. Accessed May 28, 2012
Skin absorption	Skin absorption: in vivo method	In vivo: rat is commonly used; at least four animals of one sex are recommended. The substance is radiolabeled and applied, for a fixed period of time, to the clipped skin of animals at one or more dose levels. Samples are taken during the study and animals are killed at the end of the test and blood collected for analysis	Available from: www.oecd-ilibrary.org/environment/test-no-427-skin-absorption-in-vivo-method_9789264071063-en. Accessed May 28, 2012
	Skin absorption: in vitro method	In vitro: skin from human or animal sources can be used. Viable skin is preferred, but nonviable skin can also be used	Available from: www.oecd-ilibrary.org/environment/test-no-428-skin-absorption-in-vitro-method_9789264071087-en. Accessed May 28, 2012

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Abbreviation: bw/day, body weight per day; UDS, unscheduled DNA synthesis; NRU, Neutral Red Uptake.

animal welfare concerns, there are also methodological issues that should be addressed, particularly Council Regulation No 440/2008 on test methods for the determination of toxicity of chemicals.

The following sections of this article will examine why animal models are invalid for predicting human outcome.

Empirical evidence comparing human with animal toxicity

Whether an animal model can be used to predict human response can be tested, using indicators such as sensitivity, specificity, and positive and negative predictive values (Table 2). The few data available on human accidental and deliberate poisoning and overdose of industrial chemicals provide only limited information on human toxicity.[30] Also, industrial chemicals are not subjected to human clinical trials, for ethical reasons.

However, there is a considerable body of evidence available from the pharmaceutical industry to provide a good indication of whether animal models can predict human outcome. The observation is based on data from pharmaceutical drug development, where adverse drug effects seen in humans during clinical trials and post-marketing surveillance are compared retrospectively with toxic effects seen in animals during preclinical testing.[31–35] This can best be demonstrated by a specific example. In the case of teratogens, out of 1500 chemicals that resulted in drug-induced birth deformities in laboratory animals, only 40 had human correlates, yielding a positive predictive value (PPV) of 3%.[36] Another element to be considered when using animals to detect possible teratogenicity due to chemicals is Karnofsky's law, which states that any compound can be teratogenic if given to the right animal species at the right dosage and at the right time during gestation.[37] Similarly, all known adequately studied human carcinogens have been shown to be carcinogenic in at least one animal species,[38] which poses the same scientific dilemma as Karnofsky's law of teratology.

The lifetime rodent bioassay (LRB) is the regulatory standard in predicting human cancer risk, even though it has never been subjected to formal validation as an assay for human carcinogens.[39] Originally developed in the 1940s and 1950s,[40–42] its underlying principles have remained largely unchanged since that time. A major drawback of the LRB is the high false-positive rate with respect to human carcinogenicity potential.[43–47]

There is mounting skepticism within the scientific community about the relevance of the rodent bioassay to the risk of human cancer. In a study cited by Ennever and Lave in 2003, only three out of six known human carcinogens caused

cancer in both rats and mice, while one of the known human carcinogens did not cause cancer in either rats or mice.[48] According to both the US National Toxicology Program (NTP) and the World Health Organization's International Agency for Research on Cancer (IARC), a substance is designated as a "known human carcinogen" (IARC class 1) based on strong data from human epidemiologic studies.[49] The NTP has currently designated 54 substances as known human carcinogens, while the IARC has designated 66 (IARC 2006, NTP 2005).[50]

The importance of accurate identification of cancer-causing chemicals is central to the objectives of REACH. In a report prepared for the EC on the expected role of REACH in reducing cancer deaths resulting solely from occupational exposure to chemicals, the authors conservatively concluded that the economic benefits over 30 years would amount to between €18 billion and €54 billion.[51] Regulatory authorities rely to a large degree on animal carcinogenicity data in formulating human hazard assessments. However, the animal data has yielded conflicting results, as evidenced, for example, by the classification systems of the US Environmental Protection Agency (EPA) and the IARC.[52,53] Knight et al found that for 111 chemicals considered by the EPA to lack human data but to possess animal data, EPA and IARC classifications were significantly different.[54]

Systematic reviews of animal models

The systematic review is currently a favored method of evaluating the efficacy of medical treatments. The definition of a "systematic review" is "the conscientious, explicit, judicious use of current best evidence in making decisions about the care of individual patients."[55] Today, the systematic review has wider application, which includes animal studies. The poor predictability of animal models in translational human studies has prompted calls for more systematic reviews to improve results.[56,57] Much of the criticism of animal models is based on poor research methodology, including lack of standardization, species selection, sample size, blinding, and randomization.[58] Several collaborations have attempted to address the situation, in the form of standardized checklists.[59,60] However, despite significant improvements in methodology, the translation rates of some important therapies continue to elude translational application to the clinic.

Notable examples of these include the search for a vaccine against human immunodeficiency virus (HIV) and the search for neuroprotective drugs. In the case of the former, approximately 100 vaccines have been shown effective against an HIV-like virus in animal models but none have prevented HIV in humans.[61–63] In the case of neuroprotection, more than 1000 drugs have been shown effective in animal models but none have been effective in humans.[64,65] Systematic reviews are only as good as the data they review. If the scientific hypothesis that underpins the studies under review is invalid, the methodology becomes irrelevant. We will discuss this in more detail in the following sections.

Evolutionary biology and complexity

The species is the principal unit of evolution and can be defined in terms of its reproductive isolation.[66] Reproductive isolation is intrinsically caused by incompatibilities between genes from different species.[67] Identifying the genes and determining their functions brings us closer to understanding the relationship between isolating mechanisms and the process of speciation.[68,69] It is precisely this species-specific gene function that makes interspecies extrapolation impossible to predict in any complex system. Living systems, especially mammals, are examples of complex systems. Complex systems have very specific characteristics that influence the ability of one complex system to predict the response of another.[70–72]

One essential feature of complex systems is their nonlinear response to perturbations (such as chemical insult).[72] Another is that they are dependent on initial conditions (for example, gene expression levels).[73] Different strains of mice may respond very differently to gene deletion.[74,75] Similarly, humans may differ in their response to drugs and chemicals due to gender[76,77] or ethnicity.[78,79] Even monozygotic twins may respond differently to perturbations.[80] Another essential property of complex biological systems is their robustness.[81,82] Robust systems are resistant to changes in the environment because they can adapt and have redundant components, which can act as a backup if individual components fail. A further characteristic of complex systems is their modularity. By virtue of subsystems that are physically and functionally insulated, it is less likely that failure in one module will spread to other parts with potentially deleterious consequences. At the same time, this modularity does not prevent different compartments from communicating with each other.[83]

Finally, complex systems are more than the sum of their parts, illustrated by the fact that they exhibit "emergence," meaning that new properties of a complex system arise from the interactions of the parts. "Emergent properties resist any

attempt at being predicted or deduced by explicit calculation or any other means."[84] These new properties cannot be determined even in light of full knowledge of the component parts. Greek and Shanks explain that evolution, complexity theory, and genetics demonstrate why animal testing cannot be an effective means of predicting what a drug or a chemical will do in humans.[38] The fact that rodents and humans represent differently complex systems with unique evolutionary trajectories invalidates the use of one complex system (the rodent) to predict the response of another complex system (the human). Complex biological systems, especially mammals, are not amenable to reductionism. In his criticism of reductionist thinking, van Regenmortel states:

> The reductionist method of dissecting biological systems into their constituent parts has been effective in explaining the chemical basis of numerous living processes. However, many biologists now realize that this approach has reached its limit. Biological systems are extremely complex and have emergent properties that cannot be explained, or even predicted, by studying their individual parts. The reductionist approach – although successful in the early days of molecular biology – underestimates this complexity and therefore has an increasingly detrimental influence on many areas of biomedical research, including drug discovery and vaccine development.[84]

Uncertainty of data extrapolation

Using indicators such as sensitivity, specificity, and positive and negative predictive values, it is possible to statistically assess the value of an animal model with respect to predicting human response (Table 2). This requires humans and animals to be exposed to the same chemical insult or mixture of chemicals, under controlled conditions. In the case of industrial chemicals within the context of REACH, this would very seldom occur. Examples of such rare events would include the accidental release of a cloud of 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) in Seveso in 1976[85] and the Bhopal tragedy in 1984 involving the release of methyl isocyanate.[86] In contrast, there are considerably more examples of controlled exposure to a substance in pharmaceutical drug development, where preclinical toxicity in animals is compared with adverse drug reactions seen clinically in humans. As shown earlier, the PPV in such cases is considerably less than one would expect from a coin toss. It should be noted that the poor performance of animal models in regulatory toxicology persists despite the use of adjustment factors.[87,88]

Table 2 Calculating values for a binary classification test

		Gold standard	
		GS+	GS–
Test	T+	TP	FP
	T–	FN	TN
Sensitivity = TP/TP + FN			
Specificity = TN/FP + TN			
Positive predictive value = TP/TP + FP			
Negative predictive value = TN/FN + TN			

Abbreviations: T–, test negative; T+, test positive; FP, false positive; TP, true positive; FN, false negative; TN, true negative; GS–, gold standard negative; GS+, gold standard positive.

For interspecies extrapolation, industry and regulatory authorities have resorted to the use of various algorithms and assessment factors "in the range 10–10,000."[89]

The limitation of using adjustment factors is perhaps best known to regulators from rodent studies. The goal of the NTP for carcinogenicity testing using the LRB is to predict human carcinogenicity using a correction factor that is required to translate high doses in rodents to typically low doses in humans. However, according to Pritchard et al, there is no definitive link that has been made to connect the responses of animals in cancer assays to dose–response effects seen in humans.[49] This view is echoed by Gad: "Extrapolation of rodent carcinogenicity data to humans remains one of the greatest challenges of modern toxicology."[90]

The example of bisphenol A (BPA) as a chemical in REACH

The first phase of REACH requires the registration of high-volume substances (those manufactured in excess of 1000 tonnes per year) and SVHC, including potential carcinogens, mutagens, or reproductive toxins,[91] whose deadline for registration was December 1, 2010. The chemical BPA falls within the high-volume category as it is produced in amounts of 3 billion kg per year.[92] In addition, there is evidence to suggest that BPA could be classed as a carcinogen, mutagen, or reproductive toxin. Evidence of developmental effects of endocrine-disrupting chemicals on wildlife and humans began to appear in the early 1990s[93–95] and one of the compounds to come under scientific scrutiny was BPA.[96] A considerable amount of research has subsequently been devoted to the effects of this chemical in utero and the effect of low-level exposure of endocrine disruptor chemicals.[97–102]

The traditional view in toxicology has been that "the dose makes the poison" (Paracelsus).[103] The comparatively

new field of developmental toxicology has brought with it important new insights into the effects of perturbations, including those of exogenous chemicals on the unborn. Also significant is the effect of low versus high doses of endocrine disruptor chemicals on susceptible molecular receptors. Le and colleagues showed that very small amounts (<1 ppt) of BPA, are capable of affecting developing neurons in vitro.[104] Of particular significance with respect to species differences between humans and animals is that gestation is divided into two major periods:

> In humans the embryonic phase constitutes 20% of the whole gestation period and the fetal phase 80% whereas in mice and rats the exact opposite is seen.[105]

The example of BPA is instructive with respect to REACH because it illustrates some of the confusion caused among regulatory authorities arising from current reliance on animal models, as well as underpowered or limited human studies.[106–110] In 2009, Beronius and colleagues published the findings of a literature study in which conclusions regarding health risks of BPA varied between assessments ranging from "there is no risk to any part of the population" to "there is risk to the entire population."[111] The survey found that differences in conclusions by regulatory bodies were mainly influenced by the evaluation of low-dose effects and the uncertainties surrounding the significance of these data for health risk assessment. Indeed, published studies exist in support of either position (all or nothing risk) in the scientific literature. As an example, Ryan and colleagues demonstrated that pharmacologically relevant doses of the human oral contraceptive ethinyl estradiol were damaging to the reproductive morphology and function of the female rat while BPA was not.[112] At the other end of the spectrum, vom Saal and Hughes reported adverse effects in mice dosed below the predicted "safe" or reference dose of 50 µg/kg/day BPA.[113] The official "no observed adverse effect level" for BPA in the USA and Europe is currently 5 mg/kg body weight per day (bw/day).[114,115] This figure, which is intended to serve as a benchmark for international health authorities, is based on studies in rats and mice.[116,117] Negishi et al observed considerable differences in distribution, metabolism, and excretion of BPA between rodents and nonhuman primates as well as differences between monkeys and chimpanzees.[118] There are also differences in response to BPA based on the strain of rats used. For example, while BPA stimulates prolactin secretion in Fischer 344 rats, it does not in Sprague Dawley rats.[119]

The *Toxicological and Health Aspects of Bisphenol A: Report of Joint FAO/WHO Expert Meeting* published in November 2010, states:

> Although a large number of studies on the toxicity and hormonal activity of BPA in laboratory animals have been published, there have been considerable discrepancies in outcome among these studies with respect to both the nature of the effects observed as well as the levels at which they occur. This has led to controversy within the scientific community about the safety of BPA, as well as considerable media attention.[120]

The expert meeting recommended the need for additional human data:

> The major remaining research need is additional human pharmacokinetic studies performed to high standards of analytical sensitivity and method validation that provide accurate and precise time-dependent measurements of aglycone and conjugated forms of BPA in conjunction with complete analysis of urinary excretion. These data are essential for filling some identified data gaps and thereby minimizing uncertainty through mass balance evaluation as well as classical pharmacokinetic and PBPK [physiologically based pharmacokinetic] modelling approaches to human metabolism and disposition of BPA.[120]

In the example of BPA, the Canadian health authorities have set the gold standard by invoking the precautionary principle and being the first country in the world to declare BPA to be a "toxic substance."[121]

Human exposure models

While animal models are not predictive for humans, several emerging technologies hold promise for providing data that is directly relevant to human health. Possibly the most important first step – biomonitoring of human populations – is currently underway in the EU, albeit on a small scale.[122] Manno et al define "biomonitoring" as

> the repeated, controlled measurement of chemical or biological markers in fluids, tissues or other accessible samples from subjects exposed or exposed in the past or to be exposed to chemical, physical or biological risk factors in the workplace and/or the general environment.[123]

Biomonitoring lends itself to the identification of biomarkers in human populations, either as indicators of exposure, effect, or susceptibility (for a fuller discussion of this subject,

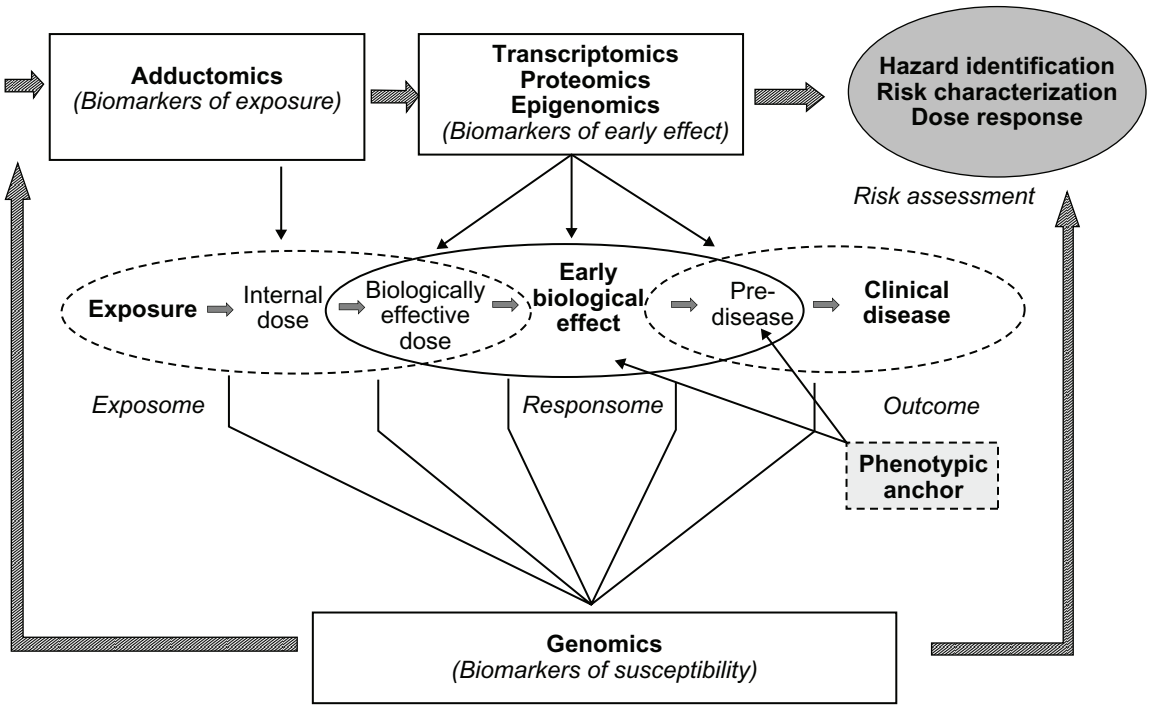

Figure 1 Application of human toxicogenomic studies to risk assessment.
Reprinted from McHale CM, Luoping Z, Hubbard AE, Smith MT. Toxicogenomics profiling of chemically exposed humans in risk assessment. Mutat Res. 2010;705(3): 172–183. With permission from Elsevier.[126]

see Silins and Högberg[124]). The study of biomarkers[125] in conjunction with other human-oriented technologies, such as genomics,[126,127] epigenomics,[128] induced pluripotent human stem cells,[129,130] epidemiology, and human physiologically based pharmacokinetic/physiologically based toxicokinetic modeling,[131] will all contribute to better-informed public health policies with respect to chemical exposure and preventive measures. Figure 1 is illustrative of some of these concepts.

The importance of biomonitoring cannot be underestimated.[132] A survey published in 2005 revealed the presence of 287 industrial chemicals in human umbilical cord blood, including 209 never before detected in the newborn.[133,134] As already discussed, it is well known that toxicity can be modified by simultaneous or sequential exposure to multiple agents in the environment, including synergistic effects.[124] In view of these circumstances, pollution prevention is clearly preferable to pollution control.

Conclusion

The only way to empirically compare animal and human response to toxic insult is when both animals and humans are exposed to the same chemical, or mixture of chemicals, under identical and usually tragic circumstances, as occurred in Seveso and Bhopal. The only realistic situation comparable to such a chemical exposure scenario is that seen in preclinical toxicity tests in animals and adverse drug reactions seen in humans during pharmaceutical drug development. The empirical evidence in this context yields a PPV less than that of a coin toss. There appears to be a fundamental disconnect between the evidential lack of prediction of animal data and the "tick box" mentality prevalent within the regulatory arena.

REACH is at odds with the precautionary principle, in large part because of the inherent flaws in its risk assessment paradigm, which ignores empirical evidence as well as fundamental principles of evolutionary biology and complex systems that invalidate the animal model. Although REACH does put the burden of proof on manufacturers to demonstrate the safety of their products, it then "scores an own goal" by obliging manufacturers to conform to invalid test methods to predict human health outcomes.

Human biomonitoring, in conjunction with chemical policies that reduce reliance on harmful substances and develop safer substitutes, should be an integral part of a precautionary and preventive strategy. The current chemical burden present in the human population is proof that urgent measures must be taken by national and international governments to avoid further global chemical pollution and, additionally, to

ensure that human-specific tests to assess the effects of these substances are developed and implemented.

Disclosure

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