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Toxicology in the 21st Century

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28.1 Introduction

Toxicology is the science that started as a branch of pharmacology and deals with the action of toxicants on human health and the environment. Toxicity testing is performed to determine adverse effects, following exposure to a test material, on humans or other target organisms. Toxicological studies on the safety of drugs are required for Phase I of drug development before they are registered by the United States Food and Drug Administration (US FDA). Toxicological studies on the safety of industrial chemicals are also required for submission to the United States Environmental Protection Agency (US EPA) before their registration.

Studies conducted at the end of the 20th century established that human health is a well-woven tapestry of DNA-based threads and environmental principles, and that teasing them apart is no easy task. Investigations during the 20th century have also led to the conclusion that nurture actually influences nature in subtle, but important, ways. Such studies will continue to expand in the 21st century.

28.2 Toxicology in the 20th Century

In the 20th century, fundamental advances were made in toxicology such that it became recognized as a basic science in universities, at regulatory agencies, and among society at large. For the first time, two processes were established to certify scientists as toxicologists, worldwide, by the American Board of Toxicology in 1980 as a Diplomate and the Academy of Toxicological Sciences in 1981, as a Fellow. In the United States, the US EPA was established on 2 December 1973. The EPA has its headquarters in Washington DC and employs 18 000 people countrywide, with 10 regional offices and 27 laboratories through the country. The EPA was charged to regulate chemicals (e.g., industrial, pesticides, and solvents) and to protect human health by safeguarding natural environments of air, water, and land. The National Center for Toxicological Research (NCTR) was established in Jefferson, Arkansas in 1971, while the National Toxicology Program (NTP) was founded in 1978 based at the National Institute of Environmental Health Sciences and is located in the Research Triangle Park (RTP), North Carolina. The chemical Industry Institute of Toxicology (CIIT), established in 1974 by industry, was transitioned in 2007 to the Hamner Institutes for Health Sciences in RTP, North Carolina. Toxicologists in these institutions carry out studies to guarantee the safety of chemical for humans and the environment.

28.2.1 Major Accidents of Human Exposure to Toxic Agents

Radiation Exposure

The 20th century witnessed several major chemical and radiation exposure disasters that had major impacts on human health. The worst nuclear exposure incident in US history occurred near Harrisburg, PA on 28 March 1979 [1] at the Three Mile Island (TMI) nuclear power plant, and this was followed in 1986 by accidents at the Chernobyl nuclear power plant in the Ukraine, USSR [2]. Early in the 21st century, on 11 March 2011, Japan was hit by the Great East Japan Earthquake, followed by the Fukushima Daiichi Nuclear Disaster [3].

During the early days after a nuclear accident, the primary concern should be to prevent the exposure of children to radioactive iodine through inhalation and ingestion, because radioactive iodine preferentially accumulates in the thyroid. In the longer term, another concern is exposure to radionuclides with long half-lives, including cesium-137 and cesium-134, which have physical half-lives of 30 and 2 years, respectively.

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Chemical Exposure

Chemicals have impacted human health in many ways during the 20th century, with exposure to some chemicals having been suspected to cause neurological diseases. Concentrations of some chemicals, such as those present in cigarette smoke or toxic pollutants in the environment, are generally too low to kill a human being outright, but they often cause harm in the long term, such as lung cancer [4]. In contrast, some people who have been exposed to chemicals may appear to be healthy but are so health-impaired that they suffer long-term, chronic and life-long diseases. Concerns related to chemical exposure-induced illnesses that are caused not only by acute, high-level exposure but also to chronic, low-level exposure are described in the following documented accidents that have resulted in adverse health effects.

Organophosphate-Induced Delayed Neurotoxicity (OPIDN) and Good Laboratory Practice (GLP) In 1930, a major disaster took place in Midwestern and Southeastern states in the United States following the consumption of a brand of ginger extract by more than 50 000 individuals that had been adulterated with tri-*ortho*-cresylphosphate (TOCP). As a result, a condition developed that was characterized by ataxia and paralysis [5] and later designated as organophosphate-induced delayed neurotoxicity (OPIDN) [6]. OPIDN is neurologic disorder characterized by central–peripheral axonal degeneration followed demyelination, and accompanied by ataxia that my progress to paralysis [6]. Some 40 years later, 12 workers at Velsicol's Bayport, Texas plant, at which the organophosphorus insecticide, leptophos, was being manufactured, reported serious neurological disorders and were diagnosed by a local physician as having encephalitis or multiple sclerosis [7]. A medical evaluation by NIOSH of the workers concluded that the signs and symptoms of these workers were compatible with OPIDN [7] and was consistent with the findings of experimental studies [6]; the result was a of leptophos. A follow-up investigation indicated that Industrial Bio Test Labs (IBT) had falsely claimed to have performed safety tests on leptophos showing that it did not produce OPIDN. When this and other cases were made public, the FDA proposed regulations on Good Laboratory Practice (GLP) in 1976 [8], followed by the EPA issuing its own redraft of GLP regulations in 1979 [9] and 1980, and publishing the Final Rules in two separate parts (40 CFR 160 and 40 CFR 792) in 1983.

Good laboratory practice refers specifically to a quality system of management controls for research laboratories and organizations, so as to ensure the uniformity, consistency, reliability, reproducibility, quality and integrity of chemical (including pharmaceuticals) nonclinical safety tests, through acute to chronic toxicity tests.

Nerve Agents The 20th century began with the use of mustard gas during World War I against combating armies [10], and ended with the exposure of more than 200 000 US military personnel to low-levels of the nerve agent, sarin [11], which was released from a weapons dump destroyed at Khamassia, Kuwait, at the end of the Persian Gulf War in 1990–1991. As a result of this exposure, these personnel developed debilitating illnesses (known as the Persian Gulf War syndrome) that still haunts hundreds of thousands of veterans. A similar situation occurred in 1994, when terrorists in Japan used sarin gas to attack civilians at midnight in Matsumuto [12], and again at 8:00 am at the Tokyo subway [13, 14]. Those individuals who were exposed to high or low levels of sarin continued to suffer neurological symptoms that were characterized by memory deficits and fibromyalgia, that later was designated organophosphate-induced chronic neurotoxicity (OPICN) [15].

Occupational Exposure

KEPONE INSECTICIDE Another major insecticide poisoning accident took place in a Virginia factory that was manufacturing the chlorinated hydrocarbon insecticide, kepone, and resulted in neuronal injury of the workers involved [16].

N-HEXANE AND METHYL *ISO*-BUTYL KETONE (MIBK) Another chemical-induced nervous system damage occurred in a textile factory at which workers were exposed simultaneously to the hydrocarbon solvents methyl *iso*-butyl ketone and *n*-hexane, causing the development of central–peripheral distal axonopathy [17].

'Natural' Chemical Disasters Bangladesh, a land of 147 570 km^2, has been exposed to various natural disasters such as floods and droughts. Moreover, the population in Bangladesh has been facing yet another environmental hazard, namely the contamination of groundwater with arsenic (As). It is known that more than 60% of the groundwater in Bangladesh contains naturally occurring As, with concentrations often significantly exceeding 10 μg l^{-1}. The sole dependency on groundwater for drinking purposes in rural Bangladesh, which represents almost 75% of total population, has made the situation much worse [18]. Based on estimates of a national population of 164 million, approximately 22 million and 5.6 million people are drinking water with arsenic concentrations >50 μg l^{-1} and

>200 μg l^{-1}, respectively. Although arsenic is a natural element of the Earth's crust, the International Agency for Research on Cancer has classified it as a Group 1 human carcinogen. One of the most disabling effects of chronic exposure to As is an induced neurotoxicity; indeed, As-induced central nervous system (CNS) neurotoxicity has been implicated in causing impaired neurological functions, such as learning and concentration. Moreover, people chronically poisoned by As may suffer from delirium and encephalopathy. In addition to these central effects, peripheral neuropathy is a common complication to chronic As exposure that may lead rapidly to severe ascending weakness, similar to the Guillan–Barré syndrome.

Environmental Chemical Exposure

Love Canal Disaster A major man-made environmental disaster was discovered in the Love Canal area, where new homes were built on disused chemical waste dumps [19]. Another major disaster was the exposure of American military personnel to Agent Orange, a defoliant that contained dioxin and was used widely during the Vietnam War and was implicated in the development of many diseases, including cancer [20].

Industrial Accidents

Bhopal, India Disaster On 3 December 1984, at the Union Carbide India Limited pesticide plant in Bhopal, India, about 32 tons of toxic gases (including methyl isocyanate; MIC) were leaked into the atmosphere, and this led to the world's worst industrial disaster to date [21]. The official death toll was initially recorded at about 5000, although others suggested that 18 000 people had died within two weeks of the disaster. Additional estimations have proposed that about 8000 people have subsequently died from gas-poisoning-related diseases. Many more people became ill and have since been facing chronic health problems such as psychological and neurological disabilities, blindness, and disorders of the skin, vision and breathing. Today, newborn children still suffer from serious birth defects, several generations after the event.

The Gulf War Veterans' Illnesses

Combined Chemical Exposure Of the 750 000 service personnel who participated in the war in the Persian Gulf region between August 1990 and April 1991, approximately 200 000 complained of chronic symptoms that included headache, loss of memory, fatigue, muscle and joint pain, ataxia, skin rash, respiratory difficulties and gastrointestinal disturbances [22]. During the war, service personnel were concurrently exposed to biological, chemical and psychological environments. The reported chemicals exposure included the insect repellent DEET, the prophylactic agent to protect against nerve agent sarin, pyridostigmine bromide (PB), and the insecticide, permethrin. It was hypothesized that the combined exposure to these chemicals had increased their toxicity, because competition for liver and plasma enzymes had led to a reduced breakdown of these chemicals and increased their delivery to the nervous system. The results showed that, whilst exposure to a single compound resulted in minimal toxicity, a combination of two compounds produced a greater neurotoxicity than caused by the individual agents. Moreover, neurotoxicity was greatly enhanced following the concurrent administration of all three agents. Thus, the carbamylation of peripheral esterases by PB reduces the hydrolysis of DEET and permethrin and increases their availability to the nervous system. In effect, BP 'pumps' more DEET and permethrin into the CNS. These results suggest that blood and liver esterases play an important 'buffering' role in protecting against neurotoxicity in the population at large. Consequently, individuals with low plasma esterase activities may be predisposed to neurologic deficit produced by exposure to certain chemical mixtures.

Stress Augments Chemical-Induced Neurotoxicity The exposure of adult rats to a combination of stress and low doses of PB, DEET and permethrin (as a model of Gulf War exposure) produced blood–brain barrier disruption in addition to neuronal cell death in the cingulate cortex, dentate gyrus, thalamus and hypothalamus, as well as severe liver damage [23]. In contrast, exposure to these chemicals or stress alone failed to produce any injury. As the damaged areas of the brain are involved in the maintenance of motor and sensory functions, learning and memory and gait coordination of movements, such injury could lead to many of the complaints made by American service personnel during the Gulf War. Further studies have supported the hypothesis that a combination of chemical exposure with stress causes neuronal cell death via oxidative stress.

Human Diseases

Thalidomide Disaster The mid-20th century witnessed what is now known as the 'thalidomide disaster' in Europe, when pregnant women who had taken a single dose of this hypnotic drug between days 24 and 36 of pregnancy gave

birth to deformed babies [24]. Many thousands of children were born with deformed or missing limbs, mostly in Europe, where this drug was approved by health officials. Noteworthy is the fact that women in the United States had escaped a potential major disaster, because the US FDA never approved the use of this drug.

Parkinson's Disease Many studies have implicated a role of organophosphorus pesticides in the development of human nervous system diseases such as Parkinson's and Alzheimer's [25].

Bowl Diseases Recently, epidemiological reports have indicated an increased prevalence of bowel disease after the approval and use of artificial sweeteners [26, 27].

Autism and Chemical Exposure Since 1990, in the United States, the diagnosis of autism has increased by 172% compared to only 13% and 16% for other nervous system diseases. Autism is a pervasive developmental disorder (PDD) of brain function that appears during the first three years of life. In 1998, the measles-mumps-rubella (MMR) vaccine and/or the antibacterial preservative mercuric compounds, thimerosals (which were added to the vaccine preparation) were implicated in the development of autism in children with bowel disease, and who had developed autism shortly after receiving the MMR vaccine [28]. Follow-up epidemiological studies in the US, UK and Sweden exonerated the MMR vaccine from contributing to the development of autism [29]. Further, the removal of thimerosals from the vaccine did not slow down the increased prevalence of autism.

Male Reproductive Disorders and Decrease in Semen Quality During the past 30–50 years, many disorders of the male reproductive system have been documented. Currently, reproductive disorders of the newborn (e.g., cryptorchidism, or failure of the testes to descend into the scrotum) and hypospadias (urethral abnormalities) and young adult males, as well as low sperm counts and testicular germ cell cancer, are common and/or increasing in incidence [30]. It has been hypothesized that these disorders may comprise a testicular digenesis syndrome (TDS) with a common origin in fetal life, and this proposal has been supported by findings in an animal model of TDS involving fetal exposure to *n*-dibutyl phthalate, as well as by new clinical studies. Changes in semen quality among 14 947 men were reviewed in 61 reports between 1938 and 1991, in which significant decreases were noted in semen count, from 113×10^6 ml^{-1} in 1940 to 66×10^6 ml^{-1} in 1991 (a decrease of 42%), and also in seminal volume, from 3.40 ml to 2.75 ml (a decrease of 19%) [31].

Early Puberty in Girls Early puberty in girls has been reported during recent years. Typically, girls examined in a sample of pediatric practices from across the United States exhibit pubertal characteristics at younger ages, such as developing breasts by the age of 7 or 8 years [32], compared to mean ages of onset of breast development for African-American and white girls of 8.87 years and 9.96 years, respectively. A similar effect was noted for pubic hair development, at 8.78 years and 10.51 years, respectively. The average age of menstruation, of between 12 and 13 years, has not changed. This may be related to increased obesity, because body fat can produce sex hormones or exposure to environmental chemicals that mimic the effects of estrogen. Humans are exposed to estrogens though multiple sources: (i) endogenous sources, such as a low-fiber diet that may cause an increased reabsorption of estrogens from the gastrointestinal tract; (ii) recycled water, which has been shown to be the source of exposure to oral contraceptives such as diethylstilbestrol (DES); (iii) phytoestrogens such as soyabean; and (iv) environmental estrogens such as DDT, tetrachlorodiphenyl dioxin (TCDD) and polychlorinated and polybrominated biphenyls.

28.3 Toxicology in the 21st Century

Humans are not impacted only by their biological programming, as biological programing reacts also to the environment. What is important is not whether a specific gene is present (or not), but whether it has been activated by an external 'trigger.' Today, it is evident now that certain genes do not become active until some form of influence – for example, a stressful experience or trauma – creates the necessary molecular spark that spurs the genes into action. Recent studies shown that stress lowered the threshold level of chemicals to cause neurological deficits. Hence, focusing on a pathological state provides a 'window' into what has gone awry in the intricate and fragile interplay between biology and surroundings, and comparing people with certain disorders to those without, such that the culprit genes can be tracked down more quickly.

28.3.1 Toxicity Testing in the 21st Century

An Overview

Toxicity Testing in the 21st Century: A Vision and Strategy is the title of a report prepared by the US National Research Council in 2007, after being asked by the US EPA to develop, improve, and validate new laboratory tools that could significantly be used to improve society's ability to understand the hazards and risks posed by chemicals [33].

Current Toxicity Testing

Toxicity tests of chemicals have been developed over the past half-century using laboratory animals. These studies have been conducted – and are still being conducted – to evaluate chemicals such as therapeutic drugs, food additives, pesticides and industrial chemicals for their ability to produce cancer, birth defects and other adverse health effects. The test animals are usually treated with higher doses than would be expected for usual human exposures, and assumptions are made and certainty factors used to extrapolate the results to the effects that exposure to lower doses might have on human health. This testing system is lengthy, costly and uses many animals, and has resulted in many chemical not being tested, despite potential human exposure.

Future Toxicity Testing

The new proposed toxicity testing system 'Tox21' relies mostly in understanding 'toxicity pathways' that result from the chemical-induced perturbation of cellular response pathways. Future toxicity testing is expected to benefit from and use recent technology and advanced knowledge. Such knowledge would make it possible to use cells, cellular components and tissues, preferably of human origin rather than living animals. The new tests would investigate changes at the molecular level in order to predict how chemical exposure would lead to certain adverse health effects in the general population, and also in sensitive individuals such as children, pregnant women and the elderly. This would allow the screening of large numbers of chemicals without the use of animals, and at much reduced costs. In short, Tox21 can potentially make testing faster, cheaper, more scientific and more responsive to society's need for an environment that is safe from the risk of chemical injury. A comparison between current and Tox21 toxicity testing assays is provided in Table 28.1.

Administering Tox21

The Toxicology in the proposed 21st century (Tox21) program is a federal collaboration that is coadministered and cofunded by the National Toxicology Program (NTP), based at the National Institute of Environmental Health Sciences (NIEHS) and Center for Computational Toxicology at the EPA's office of Research and Development, both located at the Research Triangle Park, North Carolina. Scientists from both Federal research facilities will utilize data generated from the high-throughput screening (HTS) robotic system at the NIH Chemical Genomics Center (NCGC) to develop cost-effective approaches for prioritizing the estimated 10 000 compounds in the environment and approved drugs that require toxicity testing. The FDA brings expertise and safety information on pharmaceutical drugs and food substances. Both, the FDA and EPA plan to use their knowledge of the products they regulate. The committee has recommended that a new institute be created to oversee the new toxicology testing system, instead of having the work dispersed among different locations and organizations without a core institute. The program time-line is one to two decades at a cost of US$ 1–2 billion. The goal is to quickly and efficiently identify the compounds that have the potential to disrupt processes in the human body that might lead to adverse health effects.

Table 28.1 Current and Tox21 toxicity testing assays.

Current testing	Tox21 testing
In vivo	*In vitro*
High doses	Broad range of doses
Low throughput	High throughput
Expensive	Less expensive
Time-consuming	Less time-consuming
Large number of animals	Virtually no animals
Apical endpoints	Perturbation of toxicity pathways

New Technologies

Among the new technologies that could be used in toxicity testing of chemicals are included:

- High-throughput techniques: These systems, developed by the pharmaceutical industry, use automated methods to test for the biologic activities of thousands of chemicals instead of using animals.
- Systems Biology: This is a very useful approach that utilizes computational models and laboratory data to understand how biologic systems operate.
- Bioinformatics: This field applies computational techniques to large amount of data to understand how cells function.

Using these new techniques will allow toxicologists to better understand how cellular pathways in the human body can carry out normal function. When chemical exposures significantly alter important pathways, they can cause adverse health effects.

New Toxicity Testing Based on Human Biology

The report envisions the development of new toxicity testing based on human biology as follows:

- Tox21 is not designed to predict high-dose animal toxicity.
- It aims to increase efficiency in toxicity testing and decrease animal usage by transitioning into *in-vitro* toxicity pathway assays on human cells or cell lines using robotic HTS with mechanistic quantitative endpoints.
- To use rapid *in-vitro* computational systems biology models to determine the dose–response models of perturbations of pathway function.
- Pharmacokinetic models for each exposure will be used to extrapolate *in-vitro* results to *in-vivo* human blood and tissue concentrations.
- To assess a wide range of doses and interpret the results in relation to structures of biological systems and exposures that are below the threshold level to cause perturbations of these pathways.
- The strategy for this involves:
  i. Identifying toxicity pathways.
  ii. Developing human cell lines and cultures.
  iii. Developing biomarkers for exposure, susceptibility, and human biomonitoring.
  iv. Validating the testing system.
- The current ToxCast *in-vitro* HTS assays of the pharmaceutical industry provide a limited ability to predict *in-vivo* toxic responses.
- The Tox21 approach in case studies is based on toxicity pathways and modes of action, which has the following advantages:
  i. Design essays for purpose, such as use in risk assessment.
  ii. Use test results for regulation.
  iii. Establish the optimal use of *in-vitro* information
  iv. Create a risk/safety-based process that is ready to be used.
  v. Use pathways that are receptor-mediated or related to chemical reactivity.

The New 'Vision' for Toxicity Testing in the 21st Century

Toxicity testing in the 21st century employs a new approach based on the evaluation of perturbations of toxicity pathways identified using a comprehensive system of high-throughput *in-vitro* assays in human cells and cell lines. Dose–response assessments would be carried out using computational models. Risk assessments would focus on maintaining exposures to environmental agents below the level at which significant perturbations of these pathways could occur:

- Chemical characterization.
- Toxicity testing.
- Targeting testing: for the foreseeable future, some animal testing will continue because it is not possible to know how chemicals are transformed in the body using tests in cells alone.

- Dose–response and extrapolation modeling.
- Population-based and human exposure data to collect levels of chemicals in human blood, hair, or other tissues.
- Risk contexts – common decision-making scenarios – for which toxicity testing is being conducted.

Carrying out the Vision of Tox21

To achieve the goals and obtain the benefits of Tox21 proposed testing would require coordinated efforts and resources for several decades by scientists from universities, government, industry, consulting laboratories, and public interest organizations.

- The EPA has established the National Center for Computational Toxicology to develop software and methods for predictive toxicology.
- The NTP that is part of the NIEHS, through its Roadmap for the Future, has initiated a partnership with the Chemical Genomics Center of the National Institutes of Health to develop and begin carrying out high- and medium-throughput screening assays to test more chemicals in less time and at less cost.
- The first phase, established in 2008, used the NIH Chemical Genomics Center (NCGC) robotic HTS system to test 2800 compounds in more than 50 assays [31]. The second phase involves testing 10 000 compounds at the NCGC. The status after five years is that:
  - the vision is holding well, although it is undergoing some refinement;
  - technologies for specific assays are maturing;
  - the testing capacity is growing rapidly in the US; and
  - the development of more quantitative approaches for safety assessment is competing with alternate methodologies.

The report entitled *Toxicity Testing in the 21st Century: A Vision and Strategy* has initiated very useful and meaningful discussions among toxicologists, in academia, government, and industry as well as the private sector. Some of the comments have been published, and listed below is a summary of opinions and comments regarding Tox21.

- The report is more of wishful thinking than is a strategy [34].
- Dose–response analysis is critical
- PBPK modeling: The report did not address mutual interaction from effects on tissues.
- *In-vitro* testing: The whole animal is more than the sum of human cell lines.
- There is a need for someone to see the overall picture.
- The report requires considerable research on unproven methods before replacing the current scheme [35].
- A whole-animal study includes all the cell types and the various interactions through the cell types, tissues, organ and organ systems.
- The biochemical and physiologic processes of whole animal cannot be duplicated by a mixture of cell cultures.
- The ‘Vision’ foresees two components to the paradigm shift away from use of experimental animals and apical endpoints that consist of ‘toxicity-pathway assays’ and ‘targeted testing.’
- The ‘toxicity-pathway assays’ are intended to find cellular and genetic dysfunctions, while ‘targeted testing’ is to design and conduct studies to refine the ‘toxicity-pathway assays.’ This raises questions about determining the dose–response and extrapolation modeling.
- The question of human genetic variation seems to be left ‘dangling’; it is well known that such variation will alter the response to chemical exposure.
- Moving from known, and understood, apical responses to the complicity of integrated cell culture and computer modeling for the purpose of estimating the probability of an adverse effect due to a material under known conditions of use may never possible.

28.4 Future Studies in the 21st Century

Future studies in the 21st century will continue and expand recent investigations aimed at pinning down how genes are turned ‘on’ and ‘off,’ and might shed some light on the ‘nature versus nurture’ debate. So far, this debate has led to the conclusion that nurture actually influences nature in subtle, but important ways. Recent studies have suggested that the gene might be involved either in regulating human health or, conversely, in contributing to the propensity of disease. In the 21st century, genome-wide association of humankind’s 25 000 genes will rapidly become the best way

of identifying DNA hotspots, for example where people suffering from a certain disease differ from those who do not. Such studies have already proved valuable in illuminating the genetic culprits behind everything from Alzheimer's disease to psychoses such as schizophrenia.

Future studies will include the development of biomarkers for the early detection of toxic chemicals, as well as chemical-induced toxicity. These markers should help in providing an early diagnosis, treatment and follow-up of progress, as well as the improvement or recovery of chemically injured individuals.

The development of nanomaterials during the late 20th century and their widespread use in many aspects of life will lead to extensive toxicological studies to determine the risk and hazards related to their exposure. The absorption, distribution, metabolism and excretion of these materials will be studied, while their interaction with tissues at the molecular, cellular and organ levels will be thoroughly investigated.

The use of animals in toxicological studies will be diminished, as new systems are developed for toxicological research. The use of other organisms such as zebrafish, which has been used widely in neurotoxicological studies, will be expanded to other areas of toxicology. *Drosophila*, another organism that is currently used in molecular and cellular biology research, is also an excellent candidate for use in toxicological studies.

28.5 Concluding Remarks

Although agreement exists among research and regulatory toxicologists on the need to improve current toxicity testing systems, various concerns are related to the tests proposed in the Tox21 report. There is a consensus that there will be a need to focus on the effect on low-level exposures, not only for single compounds but also for combinations of chemicals when there is a possibility of concurrent exposure to these compounds. *In-vitro* studies have been used for many years to answer specific questions regarding mechanistic pathways. However, such studies cannot replace *in-vivo* studies of pharmacokinetics and metabolism of test compounds [36] since, once it enters the body, a chemical undergoes a sequence of processes including absorption, distribution, storage, metabolic biotransformation and excretion. A chemical may be metabolized to metabolites that are more toxic or less toxic than the parent compound, and such information cannot be obtained from *in-vitro* studies. There is, however, a need to use fewer animals in toxicity testing, and to complement these with new strategies. A further strategy for toxicity testing is to use biomarkers of the toxic effects of chemicals; these might be found in the blood, urine, saliva, hair, and/or fingernails or toenails. Recently, serum autoantibodies against neuronal and glial proteins have been developed and used as biomarkers for nervous system injury [37, 38].

The 21st century will surely witness an increased use of nanomaterials, which would need to be tested for any adverse effects that they might have on the environment and human health. Indeed, some of these materials are small enough to access the body via inhalation and dermal absorption, and they may also cross the blood–brain barrier.

Some doubt must persist, however, regarding the plans for toxicological research that are being proposed for the 21st century by established toxicologists who have been educated and trained – and have spent much of their careers – during the latter part of the 20th century, and how different these new plans are from current systems. Naturally, toxicologists working over the next few decades will have their own ideas, not to mention new technologies that may not even exist today. But of course, this does not prevent the current generation of toxicologists from having visions, wishful thinking, or even dreams!

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