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Act on the Registration and Evaluation of Chemicals (K-REACH) and replacement, reduction or refinement best practices

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Objectives Korea's Act on the Registration and Evaluation of Chemicals (K-REACH) was enacted for the protection of human health and the environment in 2015. Considering that about 2000 new substances are introduced annually across the globe, the extent of animal testing requirement could be overwhelming unless regulators and companies work proactively to institute and enforce global best practices to replace, reduce or refine animal use. In this review, the way to reduce the animal use for K-REACH is discussed.

Methods Background of the enforcement of the K-REACH and its details was reviewed along with the papers and regulatory documents regarding the limitation of animal experiments and its alternatives in order to discuss the regulatory adoption of alternative tests.

Results Depending on the tonnage of the chemical used, the data required ranges from acute and other short-term studies for a single exposure route to testing via multiple exposure routes and costly, longer-term studies such as a full two-generation reproducibility toxicity. The European Registration, Evaluation, Authorization and Restriction of Chemicals regulation provides for mandatory sharing of vertebrate test data to avoid unnecessary duplication of animal use and test costs, and obligation to revise data requirements and test guidelines "as soon as possible" after relevant, validated replacement, reduction or refinement (3R) methods become available. Furthermore, the Organization for Economic Cooperation and Development actively accepts alternative animal tests and 3R to chemical toxicity tests.

Conclusions Alternative tests which are more ethical and efficient than animal experiments should be widely used to assess the toxicity of chemicals for K-REACH registration. The relevant regulatory agencies will have to make efforts to actively adopt and uptake new alternative tests and 3R to K-REACH.

Keywords Korea's Act on the Registration and Evaluation of Chemicals, Replacement, Reduction, Refinement, Alternative to animal test, Safety evaluation

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Introduction

In modern society, chemicals are indispensable for everyday human life. Numerous chemicals are used to maintain and improve the quality of life, including cosmetics, toiletries, detergents, air fresheners, agrochemicals such as pesticides and fertilizers, disinfectants, sterilizers, preservatives and industrial solvents. Chemicals are also employed in the manufacture of various product-comprising ingredients or parts like coatings/paints, photo-resistant treatments, batteries, automobiles, packaging and many more uncountable uses. One recent report estimated that as many as 80000 to 120000 chemicals are currently in use

worldwide, with an additional 2000 chemicals newly introduced each year [1,2]. In Korea, approximately 44000 chemicals are known to be in use and about 300 new chemicals are newly marketed annually [3]. Accordingly, exposure to humans and the environment is unavoidable, which can be accompanied by adverse effects to human health and/or the environment.

The potential danger of chemical exposure and its devastating outcomes has been strikingly exemplified by the recent tragic accident surrounding oligo(2-(2-ethoxy)ethoxyethyl guanidine chloride/polyhexamethyleneguanidine, which were inadvertently used to sterilize humidifiers [4]. These chemicals, which were originally developed as carpet sterilizers, when used in humidifiers, resulted in high doses via inhalational exposure and unanticipated lung fibrosis, which cost 701 innocent lives, mostly newborn babies and nursing mothers [5]. To better protect humans and the environment against exposure to toxic chemicals, the Act on the Registration and Evaluation of Chemicals (K-REACH), was enacted and has been in force since 2015, mandating the registration of a chemical before its release onto the market with relevant information on human health and environmental hazards [6].

Act on the Registration and Evaluation of Chemicals: Data Requirements, Test Guidelines and Skyrocketing Animal Use

K-REACH is the Korean version of the European Registration, Evaluation, Authorization and Restriction of Chemicals (EU-REACH) regulation. K-REACH originally aimed to manage the risk stemming from chemical exposure by providing relevant information on intrinsic hazards and guidance regarding safe use, such as by providing proper classification and labeling [7]. In this respect, it is aligned with the United Nation (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS) [8]. The UN GHS hazard class system categorizes chemicals according to its level of health hazard as an axis and toxic outcomes or endpoints as another. Accordingly, the effects of chemicals on the respective toxic endpoints is evaluated to obtain safety data so the chemical can be properly classified. Depending on the volume or tonnage of the chemical used, the level and range of information required varies, as shown in Table 1. Notably, K-REACH does not incorporate many of the animal welfare provisions contained in its European counterpart regulation, e.g., the Article 1 requirement to pro-

Table 1. Requirement of data for Act on the Registration and Evaluation of Chemicals registration

Field	Tonnage [no. of test][a]			
	≥1-10< [15]	≥10-100< [26]	≥100-1000 < [37]	≥1000 [47]
Physico-chemical property	State of substance Water solubility Melting/freezing point Boiling point Vapor pressure Octanol/water partitioning coefficient Density Particle size analysis	Flammability Explosiveness Oxidation	Viscosity Dissociation constant	
Hazard on human health	Acute toxicity-oral (acute toxicity-inhalation) Ames test (bacterial reverse mutation assay) Skin irritation/corrosion Skin sensitization	Acute toxicity-dermal route or inhalation Eye irritation/corrosion Chromosomal aberration test Genotoxicity test Repeated dose toxicity study (28 d) Screening for reproductive/developmental toxicity	Additional genotoxicity test (reproductive cell and genotoxicity)	Repeated dose toxicity study (90 d) Teratogenicity Two-generation reproductive toxicity Carcinogenicity
Hazard on the environment	Acute toxicity on fish Ready biodegradability Acute daphnia immobilization test	Fresh water algae growth inhibition test Biodegradation as a function of pH	Inherent biodegradability Identification of degradation products Chronic toxicity on fish Chronic toxicity on preferred species daphnia Acute toxicity on terrestrial plants Activated sludge respiration inhibition Adsorption/desorption	Further information on the environmental fate and behavior Chronic toxicity on terrestrial plants Chronic toxicity testing on terrestrial invertebrates Further information on adsorption/desorption depending on the results of the study Long-term toxicity to sediment organisms Bio-concentration

[a]Test items for lower tonnage are also required.

mote alternatives to animal testing, the Article 13 requirement to generate new data wherever possible by means other than vertebrate animal tests, the Article 25 requirement to avoid unnecessary testing, and the Annex XI rules for waiving or otherwise adapting standard data requirements [9]. According to the list of tests required by K-REACH shown in Table 1, for chemical substances manufactured or imported in volumes of 1000 tons or more per year, the data package must include 46 test items; this entails a huge amount of money and resources. Furthermore, to generate health hazard and ecotoxicology packages using conventional test guideline methods, the animal use would be astronomical. In the worst case scenario, the number of animals required per substance would be nearly 6000, just for the assessment of human health effects, at a cost of more than 1.5 billion Korean won. The number of animals required and the costs for each test are listed in Table 2.

Given that about 300 chemicals are newly introduced into Korean market every year, the use of animals would be enormous without any drastic changes. Statistics published recently by the Korean Ministry of Agriculture, Food and Rural Affairs (MAFRA) reflects a 36.7% increase in experimental animal use between 2012 and 2015 (2012, 1834000; 2013, 1967000; 2014, 2412000; 2015, 2507000) [10]. This problem is not unique to Korea. Following the enactment of REACH in the EU, the European Chemicals Agency has estimated that 30000 substances will be registered, requiring upwards of 3.9 million animals to be used in tests costing €1.6 billion (US$ 2.3 billion) [11,12]. However, others have suggested that the European Chemicals Agency's estimates may represent a substantial underestimation of both chemical registrations and animal use, projecting on the order of 68000 substance registrations and use of 54 million animals, respectively [13]. Thus, the European public has demanded swift action by authorities and industry to ensure effective sharing of existing data, uptake of validated alternatives to animal tests, and other scientifically supported replacement, reduction or refinement (3R) best practices.

Limitation and Problems of Conventional Animal Experiments

Conventional animal experiments have been developed and used on the premise that the responses of animals in the laboratory to chemicals can provide information to predict those of humans in the real world. However, this basis is somewhat incorrect with respect to species differences in genetic expression, absorption, distribution, metabolism and excretion, organ susceptibility, immune responses and resistance or tolerance to xenobiotics. This point has been explicitly criticized by Leist and Hartung [14], who stated that "the human is not a 70 kg rat." Indeed, rats, one of the most widely exploited experimental animal species, have different metabolic capacity and immune responses to humans, which seriously undermines the predictability of the experimental data [15]. Another important point which diminishes the utility of animal experiments is that extreme conditions are generally employed as the "worst case scenario" in view of chemical exposure levels (extremely high doses ranging from 10-fold to 1000-fold higher than human exposure levels) [16], exposure route (forced eyelid eversion for ocular irritant administration and closure for persistent exposure, or injection into sutured oral pouches to simulate oral mucosal exposure), stress (unrealistically stressful conditions like cold or hot conditions), or disease states (no animal naturally exhibits an asthma-

Table 2. Number of animals required for the assessment of human health effects

	Cost[a] per substance	Primary species	Second species	Primary species (second cohorts)
Acute toxicity for oral	2948571[b]	24		
Acute toxicity for dermal or inhalation	3338333[b]	40		
Skin irritation and corrosion	3186000[b]	3		
Skin sensitization	13195000[b]	45		
Eye irritation and corrosion	3186000[b]	3		
Repeated dose toxicity (28 d)	78637500[b]	60	60	
Repeated dose toxicity (90 d)	90000000[c]	120	100	
In vivo mutagenicity/genotoxicity	9580517[b]	25-80		
Carcinogenicity	1000000000[c]	500	500	
Two generation	270000000[c]	200	240	
Reproductive toxicity				
Reproductive toxicity screening	62000000 -70000000[c]	675		
Total	1540071921			5975

GLP, good laboratory practice; CRO, contract research organization.
[a]Unit Korean won.
[b]Ministry of Environment 2016 (average price of 15 GLP-CROs).
[c]From a single GLP-CRO.

like syndrome that is similar to the disease in humans) [17].

Although these experimental approaches may save time (using a high dose to curtail the time factor) and cost, they frequently produce unrealistic and inaccurate dose-response data for the prediction of human responses. Exemplifying this, endocrine disrupting chemicals generally exhibit unconventional non-linear, bell-shaped or U-shaped dose-response patterns that cannot be properly evaluated in high-dose animal experiments [18]. The dose-limiting toxicity of drug candidates observed in animal tests mostly do not appear in human clinical trials and the poor predictability of animal experiments has been observed across diverse target tissues [19]. Due to the failure of clinical trials by the appearance of unscreened toxicity during preclinical trials, enormous amounts of money and time are wasted [20]. In this context, the development of more human-relevant and advanced methods is necessary to replace or at least to supplement traditional animal tests.

In addition to the major scientific drawbacks described above, animal tests have been criticized for their inherent cruelty, for being excessively time-intensive and resource-intensive, restrictive in the number of substances or mixtures that can be tested, and of little value in understanding the mechanistic underpinnings of toxicity in the species of ultimate interest.

New Methods as Replacements for Conventional Animal Tests

The last century has witnessed unprecedented scientific and technological advances in biology, represented by human whole genome sequencing [21], the birth of functional genomics [22], computational biology, and high-speed robot automation of cell-based (*in vitro*) screening systems [23]. These innovations are being incorporated into a wide range of health and molecular/cellular biological research sectors, providing renewed vigor and inspiration in these areas. Numerous novel and revolutionary biochemical and molecular tools have been developed through this innovation, including high throughput assays, quantitative real time-polymerase chain reaction, flow cytometry, high content assays, gene transfection, reporter gene assays and tissue engineering. in fact, these new tools have helped to understand how toxicants disrupt the normal physiology of the human body at the cellular and molecular levels, which has contributed to the birth of molecular and mechanistic toxicology [24]. The resulting predictions regarding human safety and the risk of a chemical are potentially more relevant to people than animal tests. In line with this, the Toxicology for the 21st Century or "Tox21" strategy has been embarked on with a grand vision "to innovate virtually all routine toxicity testing to be conducted in human cells or cell lines" [25]. These non-animal based new testing methods enable safety evaluations of a much larger number of substances, in a more rapid, efficient and cost-effective way. Most importantly, these methods are likely to be more relevant to toxicity in humans, as well as capable of identifying the cellular mechanisms of toxicity using fewer or no animals [26,27].

Development of Non-animal Based Alternatives and Their Adoption by Regulatory Science

Safety and regulatory science is one of most conservative and slowest-moving sectors in taking up new and novel methods, since many countries with diverse cultural and socioeconomic backgrounds must comply with new regulation through so-called "international and inter-regional harmonization". Moreover, there are various stakeholders with conflicting interests even within a single country. This is understandable, since important policies like drinking water standards, sewage control, emission limits, inclusion on positive or negative lists, and authorizing the use of chemicals are based upon safety evaluations and risk assessments, which often cost enormous amounts of money to achieve compliance, with serious risks to public health or the environment in the absence of compliance. Prior to implementation, a newly developed method must be compared regarding its relevance and reliability to the original gold standard method or targeted toxicity endpoint in humans in a validation trial, which can take as long as 10 years from the initial research and development steps [28]. The results of validation studies are then subject to regulatory adoption processes, involving appraisal by experts with multi-disciplinary backgrounds, often with the participation of more than one regulatory body from multiple countries.

International regulatory bodies or collaborative organizations that can represent a large number of countries, like the Organization for Economic Cooperation and Development (OECD), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and the International Organization for Standardization have participated in these review and appraisal processes to accommodate the opinions of member countries. Of these, the OECD is the most active in publishing and updating new test guidelines (TGs), along with integrated approaches to testing and assessment and other guidance materials, which 35 member countries can follow as the standardized methods. Many *in vitro* or *in vivo* tests with animal replacement, reduction or refinement potential have been included as TGs recently, as shown in Table 3 [29], indicating 3R concepts applied to TGs, which are classified according to toxic endpoints. While non-animal tests have been

Table 3. OECD TGs and number of animal reduction by an alternative test methods based on 3R

Toxic endpoint	OECD TG No.	Title	*In vivo*/ *in vitro*	3R	Year	No. of animals used
Acute toxicity	420	Acute oral toxicity: fixed dose procedure	*In vivo*	Refinement	2002	20
	423	Acute oral toxicity: acute toxic class method	*In vivo*	Reduction	2002	12
	425	Acute oral toxicity: up-and-down procedure	*In vivo*	Refinement/ reduction	2008	9
Skin irritation and corrosion	404	Acute dermal irritation/corrosion	*In vivo*	Refinement	2015	3-4
	430	*In vitro* skin corrosion: transcutaneous electrical resistance test method	*In vitro*	Replacement	2015	0
	431	*In vitro* skin corrosion: reconstructed human epidermis test method	*In vitro*	Replacement	2015	0
	435	*In vitro* membrane barrier test method for skin corrosion	*In vitro*	Replacement	2015	0
	439	*In vitro* skin irritation: reconstructed human epidermis test method	*In vitro*	Replacement	2015	0
Skin sensitization	429	Skin sensitization: local lymph node assay	*In vivo*	Reduction	2010	24
	442A	Skin sensitization: local lymph node assay: DA	*In vivo*	Refinement/ reduction	2010	24
	442B	Skin sensitization: local lymph node assay: BrdU-ELISA	*In vivo*	Refinement/ reduction	2010	24
	442C	In chemico skin sensitization: direct peptide reacitivity assay	*In vitro*	Replacement	2015	0
	442D	*In vitro* skin sensitization: ARE-Nrf2 luciferase test method	*In vitro*	Replacement	2015	0
	442E	*In vitro* skin sensitisation: human cell line activation test	*In vitro*	Replacement	2016	0
Eye irritation and corrosion	405	Acute eye irritation/corrosion	*In vivo*	Refinement	2012	1-3
	437	Bovine corneal opacity and permeability test method	*In vitro*	Replacement	2013	0
	438	Isolated chicken eye test method	*In vitro*	Replacement	2013	0
	460	Fluorescein leakage test method for identifying ocular corrosives and severe irritants	*In vitro*	Replacement	2012	0
	491	Short time exposure *In vitro* test method for identifying; i) chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage	*In vitro*	Replacement	2015	0
	492	Reconstructed human cornea-like epithelium test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage	*In vitro*	Replacement	2015	0
Dermal/ percutaneous absorption	428	Skin absorption: *in vitro* method	*In vitro*	Replacement	2004	0
Mutagenicity/ genotoxicity	471	Bacterial reverse mutation test	*In vitro*	Replacement	1997	0
	473	*In vitro* mammalian chromosomal aberration test	*In vitro*	Replacement	2014	0
	474	Mammalian erythrocyte micronucleus test	*In vivo*	Refinement	2014	23-35
	475	Mammalian bone marrow chromosomal aberration test	*In vivo*	Refinement	2014	45
	476	*In vitro* mammalian cell gene mutation tests using the hprt and xprt genes	*In vitro*	Replacement	2015	0
	478	Rodent dominant lethal test	*In vivo*	Refinement	2015	96-120
	483	Mammalian spermatogonial chromosomal aberration test	*In vivo*	Refinement/ reduction	2015	45
	487	*In vitro* mammalian cell micronucleus test	*In vitro*	Replacement	2014	0
	488	Transgenic rodent somatic and germ cell gene mutation assays	*In vivo*	Refinement	2013	25
	489	*In vivo* mammalian alkaline comet assay	*In vivo*	Refinement/ reduction	2014	25~35
	490	*In vitro* mammalian cell gene mutation tests using the thymidine kinase gene	*In vitro*	Replacement	2015	0
Reproductive toxicity	421	Reproduction/developmental toxicity screening test	*In vivo*	Refinement/ reduction	2015	90
	422	Combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test	*In vivo*	Refinement/ reduction	2015	90
	443	Extended one- generation reproductive toxicity study	*In vivo*	Refinement/ reduction	2012	140
Photo-induced toxicity	432	*In vitro* 3T3 NRU phototoxicity test	*In vitro*	Replacement	2004	0
Carcinogenicity	453	Combined chronic toxicity/carcinogenicity studies	*In vivo*	Reduction	2009	560

OECD, Organization for Economic Cooperation and Development; TG, test guidelines; 3R, replacement, reduction or refinement; BrdU-ELISA, bromodeoxyuridine-enzyme-linked immunosorbent assay; ARE-Nrf2, antioxidant responsive element binded NF-E2-related factor 2; DA, developed by Daicel Chemical Industries, Ltd; NRU, neutral red uptake.

actively developed and widely used in skin or eye irritation tests, the alternative tests are not yet established for acute toxicity or reprovductive toxicity.

OECD TGs on toxicity tests were first developed in early 1980s,

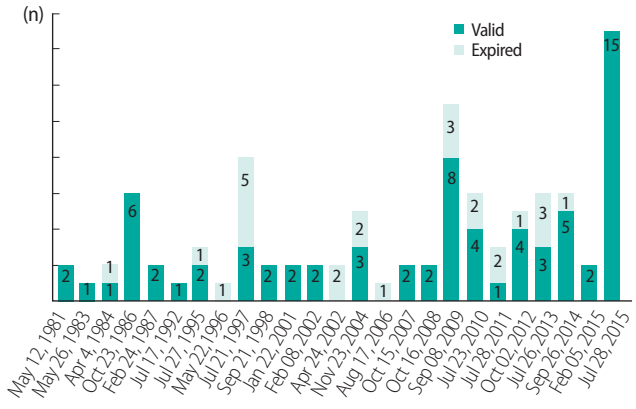

Figure 1. Number of revision or updates of Organization for Economic Cooperation and Development test guideline by Dec 2015.

and have been revised continuously by 3R concepts and with the progression of science. From the 1980s to the early 2000s, mainly *in vivo* methods using animals were adopted as TGs, and the pace of development or the appearance of new methods was slow. However, since the late 2000s, the TGs have been annually revised with 3R concepts, and many new alternative animal tests have been adopted (Table 3), and many old or obsolete TGs can now be abandoned. The number of TG revisions by year is shown in Figure 1 [27].

Human biology-based *in vitro* methods are typically more relevant and predictive, less time-consuming, can accommodate larger number of chemicals or mixtures than traditional animal experiments, and can address the mechanism of toxicity using

Table 4. Category and tools employed for OECD TGs based on non-animal based methodology

Category	OECD TG No.	Toxi endpoints	Title	System and tools employed
Ex vivo	437	Eye irritation/corrosion	Bovine corneal opacity and permeability test method	Isolated corneas from the eyes of cattle slaughtered (UV absorbance/Opacimeter)
	438	Eye irritation/corrosion	Isolated chicken eye test method	Eyes of slaughtered chicken (slit-lamp microscopes)
	460	Eye irritation/corrosion	Fluorescein Leakage test method for identifying ocular corrosives and severe irritants	Confluent monolayer of Madin-Darby canine kidney (UV absorbance)
In vitro	430	Skin corrosion	*In vitro* skin corrosion: transcutaneous electrical resistance test method	Rat skin disk (voltohmmeter)
	431	Skin corrosion	*In vitro* skin corrosion: reconstructed human epidermis test method	Reconstructed human epidermis (3D tissue engineering)
	435	Skin corrosion	*In vitro* membrane barrier test method for skin corrosion	Artificial membrane
	439	Skin irritation	*In vitro* skin irritation: reconstructed human epidermis test method	Reconstructed human epidermis (3D tissue engineering)
	442D	Skin sensitization	*In vitro* skin sensitization: ARE-Nrf2 luciferase test method	Cell line which contains the luciferase gene under the transcriptional control of a constitutive promoter fused with an are element (reporter gene assay)
	442E	Skin sensitization	*In vitro* skin sensitisation: human cell line activation test	Human monocytic leukaemia cell line THP-1
	491	Eye irritation/corrosion	Short time exposure *in vitro* test method for identifying i) chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage	Statens seruminstitut rabbit cornea cells
	492	Eye irritation/corrosion	Reconstructed human cornea-like epithelium test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage	Reconstructed human cornea-like epithelium (3D tissue engineering)
	428	Dermal/percutaneous absorption	Skin absorption: *in vitro* method	Viable or non-viable human (animal) skin
	471	Mutagenicity/genotoxicity	Bacterial reverse mutation test	Salmonella typhimurium, escherichia coli
	473	Mutagenicity/genotoxicity	*In vitro* mammalian chromosomal aberration test	Mammalian somatic cells
	476	Mutagenicity/genotoxicity	*In vitro* mammalian cell gene mutation tests using the HPRT and XPRT genes	Mammalian cells (ex. cho, chl)
	487	Mutagenicity/genotoxicity	*In vitro* mammalian cell micronucleus test	Mammalian cells (ex. blood lymphocytes)
	490	Mutagenicity/genotoxicity	*In vitro* mammalian cell gene mutation tests using the thymidine kinase gene	L5178Y tk+/-3.7.2C cells for the mouse lymphoma assay, TK6 tk+/- cells for the TK6 assay
	432	Photo-cytotoxicity	*In vitro* 3T3 NRU phototoxicity test	Balb/c 3T3 cells
In chemico	442C	Skin sensitization	*In chemico* skin sensitization: direct peptide reactivity assay	Synthetic peptides (LC/UV)

OECD, Organization for Economic Cooperation and Development; TG, test guideline; UV, ultraviolet; 3D, three-dimensional; HPRT, hypoxanthine-guanine phosphoribosyl transferase; XPRT, xanthine phosphoribosyl transferase; NRU, neutral red uptake; LC, liquid chromatography.

the framework of an adverse outcome pathway (AOP). By systemically categorizing the biological events leading to adverse effects into key events between two points, i.e., a molecular initiating event and an adverse outcome, the AOP describes an adverse outcome following exposure to a chemical by a series of key events and key event relationships that describe the causal relationships between the key events. AOPs are instrumental for establishing the toxic mechanism of an adverse outcome and the utilization of safety test data at the *in vitro*, *in vivo* and human levels for risk assessments and regulatory applications. In the best case scenario, the number of animals saved by employing multiple *in vitro* tests along with limited *in vivo* tests will be in the thousands, which will significantly contribute to animal ethics without compromising predictive capacity or risking human health or environmental protection.

Pros and Cons of *In Vitro* Alternatives to Animal Tests

Ultimately and ideally, all animal experiments, including those with refined or reduced use of animals, are to be changed into non-animal based methods. These methods can be largely categorized into *in chemico, ex vivo* and *in vitro* methods. Approved OECD TGs fall into these categories, as shown in Table 4.

Since these methods largely employ test-tube or multi-well plate formats, the throughput is much higher than traditional animal experiments. For example, the 3D reconstructed human cornea-like epithelium (RhCE) method can evaluate the ocular irritancy of 10 test substances in one run, which takes three days from the delivery of the model [28]. This test is equivalent to 10 tests using the *in vivo* Draize rabbit eye irritation test, costing the lives 30 of rabbits and taking at least 10 days from the delivery of the rabbits [30]. This test can be harnessed to assess multiple combinations of test conditions like different mixture ratios, diverse exposure scenarios and the addition of metabolic capacity using a feasible amount of time and money [27]. Moreover, through targeting a single molecular event on the AOP framework, the conclusions of alternative methods may be more direct and straightforward, which is critical to address the mechanism of toxicity and extrapolation into human responses. For example, in the direct peptide reactivity assay, an *in chemico* skin sensitization test that addresses the haptenization response of a substance, positive results indicate that the chemical is reactive and can form protein adducts [31].

Of course, there are limitations and shortcomings of non-animal methods as well. First, most of the alternatives have not advanced into the level of risk assessment, since they only give a qualitative "yes" or "no" answer, namely hazard identification. Even though some methods give quantitative data, their utility for potency classification or risk assessment has not been fully validated [32]. In addition, since the alternatives address mostly a single key event in the series of events constituting a larger AOP, they cannot provide the full mechanistic information regarding the final outcome of the initial exposure [33]. In this case, a combination of multiple *in vitro* assays can be used as an integrated approach to testing and assessment (IATA) [34]. Indeed, considerable efforts have been directed toward developing a standardized IATA scheme.

Barriers to Acceptance of Non-animal Based Alternatives in Act on the Registration and Evaluation of Chemicals and Recommendations

Although many validated and internationally recognized non-animal methods and strategies are now available and ready for immediate use in regulatory frameworks such as K-REACH, their uptake by Korean authorities continues to be less than optimal. Whereas EU-REACH data requirements and guidance have been or are being amended to incorporate all available OECD 3R TGs and related best practices, such is not yet the case with K-REACH [35,36]. This is in part due to the lack of a "mandatory 3R" requirement under K-REACH and other legislation for the uptake of new methods. In addition, there is a lack of inter-ministerial cooperation regarding the uptake of new methods. For example, the Korean Centre for the Validation of Alternative Methods has been established under the Ministry of Food and Drug Safety (MFDS), but does not currently include participation from other regulatory or research agencies in the development, validation or regulatory review of non-animal based methods [37], indicating the inter-ministerial inefficiency and passive attitudes towards new and more advanced methods. Delayed implementation of 3R-based alternatives, due to these inefficiencies, would lead to the unnecessary sacrifice of laboratory animals, even though they are not better than new alternatives for the protection of human health and the environment.

There are also duplicate experiments for substances that have already been tested and assigned GHS classifications, including chemicals that are known corrosives, fatal if inhaled or probable carcinogens. The scientific basis for duplicating well-established test results and regulatory classifications is incomprehensible, particularly where vertebrate animal testing would be involved, and most notably where such testing (e.g., of corrosives) would result in the most extreme pain and suffering for the animals involved.

To ensure that the Korean regulatory framework for chemicals, and the general approach to the validation and adoption of

3R best practices, do not fall farther behind those of other developed nations, K-REACH data requirements and TGs must be immediately and frequently amended on regular basis to incorporate all relevant OECD 3R TGs and related best practices, including mandatory data sharing, chemical grouping and read-across to ensure maximum possible reduction of vertebrate animal use and no duplication of existing test results. One overarching regulatory body shall be formed to include all Korean ministries with a stake in alternative methods and safety assessment, including the MFDS, the Ministry of Environment (MOE), and the MAFRA to facilitate inter-ministerial cooperation toward the development of a comprehensive government strategy and funding framework for the reduction and replacement of animals used for toxicity testing and life sciences research [38].

Conclusion

The implementation of K-REACH will contribute to the safe use of chemicals by identifying human health effects and ecotoxicity before their introduction to human society and the environment. However, a huge number of laboratory animals are required to comply with the current Korean guidelines, which is sometimes unnecessary and can be replaced with alternative methods. Therefore, in order for K-REACH to be more practically implemented, 3R concepts and alternative to animal tests (AATs) should be actively accepted, which has been advanced in the developed countries. Using AATs for safety assessment is scientific, highly reliable and predictable and may be more human-relevant without inflicting pain and death in laboratory animals. Moreover, the application of AATs may be more economical and effective than animal tests. The implementation of AATs is also a concern in other chemical sectors such as the food, cosmetics, drug and agrochemical industries. In this regard, the collaboration of the MOE, the MFDS, and MAFRA in Korea is critical. Korean regulatory authorities should actively communicate and collaborate to follow global trends in safety assessments and rapidly developing alternative tests.

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Conflict of Interest

The authors have no conflicts of interest associated with material presented in this paper.

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