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"Review On: Alternative To In vivo Experimental Study In Pharmacology "

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ABSTRACT

Animal testing means non-human animal in experiments. 50 to 100 million vertebrates including non rodent and rodent are used for experimentation in education and research. The non judicious use of this animal (rabbit,rat, pig,mice) is affecting the ecosystem so most are searching for alternative. Because of this growing concern of animal many countries like Europe and North America has passed the legislation to prevention of animal. Use of model like computer program, tissue and body substance of normal animal and human, use of microorganism, primary cell culture and cell lines are included during this variety of experiments. Advancement of research and development in medical technology had increased the number of animals used for research. Every year many animals are used for research. The distress,pain, suffering is experienced by the animal during these experiments. It's debating a issue for a long time besides the major concern of ethics.Many further disadvantages of beast trial are there like force, time consuming protocol and high cost are a number of the disadvantages. Hence, various alternative to animal testing were proposed to overcome the drawbacks and to avoid unethical procedure 3R's (reduction, refinement, replacement) is applied for laboratory use of animal. There has been small shift far from animal that's whole animal testing to an vitro and non animal technique in recent years. Many new technologies are being made a number of them don't seem to be validated. There are number of recent development in toxicity testing which is playing a important role. Toxicity is locate in Alternative manner.The process of acceptance of an alternate test method is in steps like development, validation, formal test method, regulatory acceptance detection of animal test. Alternative means a drug and chemical testing to the some level. A brief account of other alternative, advantages related to this are discussed during this review with example.

KEY WORDS - Alternative; 3R's; Toxicity

1.INTRODUCTION

Animal testing called in vivo testing where the utilization of non-human animals. There are 100 million vertebrates which are used worldwide for the purpose of the research. This research are conducted in University,companies, Behavioural studies, xeno -transplantation, cosmetic testing, drug testing, biomedical research. Animals like mice, rabbit, guinea pig, fish, hamesters are mostly used during experimentation because of which animals are harmed the ecosystem is being affected so, the scientists are attempting to seek out the selection method for animal testing. The majorly used alternative are the In-vivo, In-vitro studies of animal and cell culture & silicon computer stimulation. This alternative need to be developed more. Drug testing may well be a due to find a new replacement treatment for infectious and Non-infectious disease. Animals are important in this, they are used for vaccine and antibiotics (Giacomollo and segalat, 2010; Hendriksen, 2009, 2007). In year 2011 3.71 million animals were used for research (www.ispca.org.uk). The year 2009 in USA total number of animals used were 1,131,076; in Germany it reached to 2.13 million in year 2001 (Rusche, 2003). This huge no of animals comes from breeding centres all thes are known as a class-A delears. For the experiments full animals body is used some cases in which animal dies as to get a result of experiment (example LD50 analysis) Animal suffer from pain, death, distress during this experiments. Animals have their right against the pain and hence using them is unethical (Rollin, 2003).There are so many laws & acts are passed to chop back the use of animal example organization for animal right was made by Royal society for the preventation of animals, Prevention of cruelty to animal act was Formed by UK in 1876 (Balls, 1994). The varied organization like CPCSEA, ICH, OECD, NIH provided guidlines against animal care. (Rollin 2003).

2.THREE RS: REDUCTION, REFINEMENT AND REPLACEMENT

The principle of Human experimental technique (Russell and Burch 1959) introduce the term replacement reduction and refinement which also know as"alternative "or "alternative method" that is reduction" in the total number of animals used for experiment" refined in such way pain should be minimized and "if possible should be replaced with alternative". (Ranganatha and Kuppast 2012; zurloetal 1996). Animal substitute described as "any clinical technique using non-sentient fabric which might also additionally update use of aware residing vertebrates in animal experimentation". Types of substitute have been outstanding as "relative" & "absolute" .Relative substitute is like animals are used however they may be now no longer uncovered to any distress. No use of animals at any experimetal degree is idefined as absolule strategy (Balls, 1994).

2.1.Reduction

Reduction refers to method which means to minimise, the number of animals used in experiment for example- in vitro cell culture, it is one of the best way to reduce animals by working on in-vitro cell culture, Pilot studies are also good way silicon computer stimulation can be used. Live animals and embroye used for embroye development and its study Hepatocyte culture provides information of drugs metabolism and elimination. Inclusions is also method which helps to minimize the use of animals (kimber et.al., 2001)

2.2.Refinment

This is method to minimise the pain, suffering experienced by research animals, which improve the welfare of animal. Scientists should refine the animals.Under the stress there may be change in hormone of animals which may lead to Fluctuations in the result. so, refinment should be used improve the life of animals by improving the quality of research (Hendriksen, 2009). Example mice which respond like human tissues this model minimised stress of animals (De Silva et al., 1996).

2.3.Replacement

Replacement is the substitution for higher animals. Allernative used such computer model, new imaging anlysis, cell culture, in vitro model (Balls, 2002).computer model where the working of heart is Studied (Gipson and sugrue, 1994). In vitro model provide great opportunities to study the cell response in a closed system. In vitro cell culture been replaced in many countries with skin irritancy test and eye irritancy test. Another example is, insuline extraction from pancrease of pig and cow needed to be checked for the efficacy & purity now it's been replaced by the chromatography technique. (Foreman et al., 1996).

3.ALTERNATIVE METHOD.

Several methods been suggested to avoid the use of animals in experimentation alternative like drug & chemical testing upto some extend. There are some non-animals methods also.

3.1.Cell and tissue culture

In vitro cell and tissue culture is one which involves the growth of cell outside the body in laboralary terrain and this can be used as a volition to beast trials. The tissue and cells can be removed from an creatures and can kept in specific terrain for several months, days and many times. The culturing illustration eurkoryotic cell is used cover for beast exploration in laboratories worldwide, culture cell been developed to monoclonal antibodies.The testing of cosmetics directly on animals is replaced or mimimized by in vitro cell growth. for example eye irritancy test previously Draize test was used which required rabbit .Ke Ping Xu and coworkers suggested the Alternative of bovine corneal organ culture. The bovine was cultured upto three weeks in laboratory with various anlytical method used to evalute the toxicological effect on chemical iritancy (xu et al., 2000).

The localised toxic effect of skin corrosion and Skin irritation, human skin equivalent test was replaced with animal based irritative and corresive study. EpiDerm from Mattek and Episkin and skinEthic RHE model from human skin cell which have been produce to culture a model of human skin. one of the technology 30 cell culture known as a organoids or miniorgan have replaced with animal model for some research organoids was used in to model disease and test new drug. organoids were derived from 3 kinds of human cell ESCS, ASES, IPSCS. organoids were grown in vitro and mimic the function of various organ (Russell and Burch)

3.2.Computer models.

computer models have been given model to human metabolism.It helps to design new drug softwares computer stimulation includes model on Asthma and Computer Aided drug design (CADD) is used to find the (receptors side of binding activity which avoids the unwanted testing and biological activity with the help of this we can fined new drug specific site of binding and then animal test as a final test (vedani ,1991) Hence, Number of experiments are lowered and objective (3 R's are acheived) Structure Activity Relationship (SAR) is another programe which predict the biological activity of drug candidates (Knight et al., 2006).Computer Assisted learning (CAL) is a interactive computer assited learning tool without the associatio of real experimental tool (Dewhurst et al., 1994). The SAR is another popular tool. All biological activity of drug with chemical moiety are attached. Quantitative Structure Activity Relationship (QSAR) gives a relationship between the biological activity and Physiochemical properties of the drug molecule.

3.2.1.SAR

Structure Activity Relationship (SARs) confer with any definable relationship between a molecular point of a chemical and its exertion. Simple' rule- grounded' bracket schemes, cut-off criteria or general rules-of-thumb are the only exemplifications. Lipinski's Rule of Fives, which is intended to screen out medicine campaigners with potentially poor oral immersion, is may be the foremost well- known of those.22 Lipinski's Rule states that chemicals with a molecular weight above 500 Da, a logarithm of the octanolwater partition measure (log P) above 5, over 5 hydrogen bond benefactors or over 10 chemical bond acceptors, are associated with low oral immersion. Simple rules have also been developed for other parcels of interest — for illustration, if the volume of nitrogen plus oxygen tittles in a veritably patch is a lower quantum than or up to five, it's the implicit to access the blood – brainbarrier.While there are easily numerous exceptions to similar general rules, numerous have been obsessed extensively for primary webbing purposes, specially in early medicinedevelopment.There are multitudinous samples of software (freely available and marketable) which will induce simple physicochemical parcels for chemicals, apply rules-of-thumb or cut-off criteria, and astronomically classify chemicals into categorical classes. Freely available web- grounded operation SwissADME, from the Swiss Institute of Bioinformatics (http//www.swissadme.ch/index.php), is one similar illustration. Also, Molinspiration (freely available at http//www.molinspiration.com/) readily identifies chemicals with potentialbLipinski Rule of Fives violations.

Structural Alerts (SAs) is deduced from simple relationship between molecular features and it's known as exertion (toxin), and that they're frequently used to screen chemicals for implicit hazard. During this approach, the molecular structures of chemicals known to be related to a named toxin are delved, so as to spot whichsub-structural features or fractions are related to the exertion. A mechanistic explanation can also be posited, wherepossible.These fractions are frequently habituated define SAs, i.e. specific molecular features that, if present in a veritably chemical of interest, are reflective of the eventuality to evoke a poisonous effect. In1988, Ashby and Tennant published a'poly-carcinogen' patch, i.e. a academic molecular structure that incorporated the SAs for carcinogenicity. Work has been ongoing during this area for numerous times, to identify fresh SAs related to DNA list, that could be associated with mutagenic/ carcinogenic exertion.

Numerous SAs have also been defined to help the identification of chemicals with the eventuality for protein list which will be related to skin and/ or respiratorysensitisation.The presence of those functional groups in other chemicals (for which test data do not feel to be available) indicates the eventuality of that chemical to evoke toxin via DNA or protein list. When relating SAs, it's important to be suitable to rationalise the observed exertion/ toxin in terms of the mechanistic chemistry behind the chemical-natural commerce, so as to justify the vaticination.

The sweet amines give an illustration of how such an print is rationalised. Sweet amines can suffer metabolism (via N-hydroxylation and O-acetyl transferase) to make a reactive nitrenium ion. The electrophilic nitrenium ion interacts with nucleophilic groups on DNA to produce a DNA adduct, a process related tomutagenicity.SAs for skin and respiratory sensitisation (associated with antipathetic dermatitis and airway acuity, independently) are extensively used within assiduity — for illustration, to prognosticate implicit toxin of private care and ménage products.

In medicine development, cautions related to the conformation of reactive metabolites, hepatotoxicity,etc. are of major concern, as these are frequently related to late stage or maybepost-marketing pullout of drug. Still, it's important to notice that the presence of a structural alert does not inescapably equate with a natural response. Modulating factors may meliorate or potentiatebthe response in vivo — as an illustration, a emulsion with an alert might not be bioavailable or, from a

chemical structure perspective, significant steric interference may intrude with access to a full of life point. Also, it's possible that metabolic deactivation/ activation may render a potentially poisonous chemicalnon-toxic, and contrarily. SAs are frequently grouped together to form' profilers', chemically also being screened against these profilers to spot groups of chemicals that partake common point (s). this idea is bandied further below, in regard to the functionality of the QSAR Toolbox.

SAs have been decoded within colorful prophetic toxin software and web- grounded operations. Toxtree uses SAs, decision trees and QSARs are used to prognosticate poisonous hazards, reactivity and implicit metabolism. It encodes the Cramer rules and thus the revised Cramer decision tree, which relate to oral systemictoxicity.Through the application of structural information, chemicals are allocated to a toxin class (where Class I relates to low toxin, Class II intermediate and complication III high toxin). Within Toxtree, the Kroes Threshold of Toxicological Concern (TTC) decision tree will be used to establish whether a substance is assessed for oral systemic toxin by using the TTC approach. TTC could be a conception that establishes the quantum of exposure for all chemicals (with or without toxin data) below which there would be no perceptible threat to mortal health. The strategy incorporates Cramer bracket rules and rules for vaticination of genotoxic carcinogens; it also requires information pertaining to the estimated diurnal input. The Verhaar scheme for prognosticating medium of action of fish acute toxin is also decoded within Toxtree. Chemicals are placed into classes I to V, Class I representingnon-polar anesthetics, Class II polar anesthetics, Class III reactive chemicals and complication and IV specifically acting chemicals; Class V is employed for chemicals that can not be placed to classes I –IV.Other functionalities within Toxtree use SAs and physico-chemical information to prognosticate implicit for carcinogenicity (genotoxic and non genotoxic), mutagenicity, skin sensitisation, protein and DNA list, also as skin and eye vexation/ erosion, biodegradability and cytochrome P450- intermediated drug metabolism.

OCHEM is another freely available web tool, which includes (in addition to the contrary capabilities described herein) compendiums of SAs related to different venom (e.g. skin sensitisation or environmental endpoints) against which a target chemical are frequentlyscreened.However, for farther information on the alert is handed, like the literature source from where the alert information was deduced, If an alert is linked within the target chemical. The advantages of using SAs are that they are transparent and might be readily interpretable, if developed from a mechanistic base. Work is ongoing to spot farther cautions related to poisonous goods and to grasp the mechanisms behind the relations. One disadvantage of the approach relates to how the absence of any SAs for toxin within a chemical's structure are frequently interpreted, as this can not be considered as substantiation of safety. The use of webbing to descry implicit toxin within the early stages of development (including particular care products, manufacturing interceders or medicines) means that those phrasings probably to be related to significant toxin are frequently linked before within the development process. Only campaigners that are more likely to be successful are taken forward, while those presenting toxin issues are discontinued. This eventually reduces the number of chemicals tested on creatures.

3.2.2.QSAR

QSARs Since the pioneering work of Hansch etal. in quantitative structure – exertion connections (QSARs) are used to demonstrate the quantitative relationship between parcels of interest for a chemical and descriptors that are deduced from its chemical structure. Similar models are applied to the vaticination of (eco) toxin and medicine energy, and have also been used to prognosticate physico-chemical parcels (where they are named as quantitative structure – property relationship (QSPR) models). The gospel of QSAR or QSPR modelling may be explained in reference to its three indigenous conditions, outlined belowThe first demand is for quantitative measures of theproperty of the chemical that is to be modelled (i.e. endpointvalues), for a series of affiliated chemicals. Endpoints includebiological exertion or toxin — for case, half minimal effective attention for a medicine (EC50), or cure for 50 of test organisms (LD50); ADME parameters, similar as the chance of mortal intestinal immersion (HIA); or physico-chemical

parcels, like induratingpoint.The alternate demand is to get, or gain, descriptors deduced from knowledge of the chemicals' structures for the series of chemicals oriented induce the model. It's now possible to come up with thousands of descriptors, and care must be taken to confirm that spurious connections do not feel to be developed by the objectification of too numerous, or inapplicable, descriptors into the model. Generally, readily interpretable descriptors are favoured in generating QSARs, particularly where these are habituated inform safety assessment or nonsupervisory cessions. Still, there are numerous samples of QSARs supported statistical correlations. Although these can be delicate to interpret, they will be useful defenses in early product development. Constantly used descriptors include those regarding partitioning — as an illustration, the logarithmbof the octanolwater partition measure (log P), waterless solubility; and tissueblood partition portions. These indicate relative lipophilicity/ hydrophilicity of chemicals, and have been shown to relate with the inflexibility of chemicals to cut natural membranes and hence reach a point of action. Size and shape descriptors — as an illustration, molar volume, relative molecular mass, topological indicators and face area are frequently incorporated, as they will reflect the capability of the chemical to succeed in a point of action or interact with a target within the body. Electronic goods, like hydrogen cling capability, Energy of the stylish Enthralled Molecular Orbital (EHOMO), Energy of gemstone bottom Unoccupied Molecular Orbital (ELUMO) and moment, are frequently usedto indicate implicit reactivity or binding at a lively point. For illustration, Schwo ¨ bel etal. 42 reviewed the application of descriptors, supported electrophilic reactivity, for prognosticating toxin associated with the commerce of natural nucleophiles with electrophilic xenobiotics (e.g. DNA list and skin sensitisation). The final demand of a QSAR could be a statistical fashion that is used to demonstrate the correlation between the exertion (toxin, or other property of interest) with thedescriptor values. Numerous statistical styles are used, ranging from simple simple retrogression, where one descriptor is related to an exertion, or multiple retrogression toward the mean, where several descriptors are used.

3.2.3.Predictive Software

There are numerous exemplifications where being QSAR models have been incorporated within prophetic software. For illustration, EPISUITE (freely available from the US EPA) enables a variety of endpoints to be prognosticated, supported the chemical's structure and operation of a set of integral QSAR models. The endpoints include physico-chemical parcels, like log P, waterless solubility, Henry's law constant, dermal uptake and toxin to environmental species (i.e. via the ECOSAR operation that predicts acute and habitual toxin of chemicals towards algae, submarine pets and fish). VEGA HUB also provides access to a spread of freely available QSAR models that were moreover developed as part one in every of the multitudinous EU systems (similar as CAESAR), or attained from TEST (the Toxicity Estimation Software Tool from the US EPA). There are four orders of prophetic models, videlicet

.1) toxin (e.g. mutagenicity, carcinogenicity, experimental and reproductive toxin, oestrogen receptor list, skin sensitisation and hepatotoxicity models);

.2) ecotoxicity (e.g. fish, Daphnia and freak acute toxin models);

.3) environmental fate (e.g. bioconcentration, half- life, biodegradability and continuity models); and physico-chemical property models (e.g. log P vaticination).

Detailed and accurate reporting of QSAR models and prognostications deduced therefrom are important, if the prognostications are to be used confidently, especially for nonsupervisory cessions. VEGA,is the software, provides detailed reports supported a standardised QSAR and QMRF. These are representative exemplifications to point the capabilities of similar software, the list is by no means total. As an affair of the ANTARES design (AlternativeNon-Testing styles Assessed for REACH Substances), a comprehensive list of software available for prognosticating physicochemical, (eco) toxicological, environmental fate and ADME parcels was preliminarily reported (http//www.antares- life.eu/index.php? sec ¼ modellist). A further moderen review by Kar and Leszczynski51 describes a variety of freely available tools for ADMET

vaticination. The Computational Chemistry List (http//www.ccl.net/chemistry/links/software/index.shtml) also provides an outsized compendium of accessible coffers for QSAR modelling. Also noteworthy, is that the open source KNIME platform, through which a community of druggies has developed and participated multitudinous' bumps'for a large range of chemoinformatic operations. Further information (anddownload access) is offered at https//www.knime.com/.

3.3.Alternative organism / tissue

Due to ethical issue the choice has been come.Vertebrates like guinea pig, rats,dogs etc. have been confined. Thus, use of other organisms has been .Different model organisms are used to replace experimental creatures(Table 1).

Table1.Selected samples of organisms as druthers for laboratory use of creatures

Alternative Organisms	**Remarks**
Escherichia coli	Model for molecular and Inheritable study
Bacillus subtilis	Model for cellular isolation
Dictyostelium discoideum	Model for inheritable studies
Neurospora crassa Model	Model for inheritable study, circardian meter and metabolic regulation studies
Schizosaccharomyces pombe	molecular and inheritable studie model
Amphimedon queenslandica	Studies on elaboration, experimental biology and relative genomics
Drosophila melanogaster	neurology exploration,
Aplysia sp./ocean slug	Neurobiology
Hydra	To know the system of morphogenesis and about rejuvenescence

3.3.1.Lower vertebrates

An isolated chicken ileum (V.R undale 2012)found that chicken ileum is giving an honest response to Ringer Lock. As cock ileum contains histaminic receptors, tachykinins suitable for experiments like PA2 value, bioassay lleum. Cock lleum would be good alternative within the tissue experimentation where animals is saved.

An isolated chicken intestine (Neetu Prince, vinay Oommen and Anand Bhaskar 2018) observed the response of the chicken intestine with various drugs & chemical and which was the same as the response of mammalian tissue.

The Guinea pig as a model for spordiac Alzheimer's Disease (AD): The impact of cholestrol Intake an expression of AD-related gene (2013) Guinea pig Showes closer sequence and Similarities.

Danio rerio, common name zebra fish. It's embroye and larvae was developed and used for cell culture testing in plates & petri plates. Genome sequence of zebra fish is option for a molecular and genetic research. It's wide application in cancer investigation, heart diseas. Human disease modeling in zebra fish are often wont to ameliorate disease phenotype and malfunctions on development of organ (Peferson el al., 2008)

3.3.2.Invertebrates

Eathworm cooling properties of the gizzard . Rhymatic movement of the warm preparation neurogenic and peristalsis in nature was found. Abolished by nicotine but not by atropine.

Muscle within crop and gizzard is contract by the Acetylcholine. And this effect is abolished by the atropine. Excitability isn't lost after cooling.

Peristalsis is achieved by the continual liberation of Acetylcholine in warm preparation and this is often absent in cold preparation. Disappearance of rhythmic activity in these is because of loss of acetylcholine synthesis.

Warm crop and gizzard produces pottasium contraction which is enhanced by eserine, and this is not abolished by the nicotine or by atropine.Higher dose of pottasium results to inhibition. After cooling the mortor response is lost .

Drosophila Melanogaster (fruit fly) widely studied (Gilbert, 2008) It is important tool for Invistagation of neurodegenerative disease like Parkinson's, Alzheimers disease. (Bonin and Fortini, 2003; Iijima and Iijima Ando,2008). Drugs working on CNS is similiar to the mammals. The brain of fly is extraordinary more then 100,000 neurons mediates various complex behaviors like feeding, sleep, learning & memory) (Pandey and Nichols, 2011; wolf and Rockman. 2008, Rothenfluh and Heberlein,2002) . 75% of gene in fruit fly are homolog the human disease (Reiter et al., 2001; wilson-sanders, 2011). The function of various organs like heart, lungs,kidney, reproductive tract and gut are same like mammals. (Rothenfluh and Heberlein, 2002)

Caenorhabditis elegans (eukaryolic nematode). It characteristics is that its complele: life cycle is about 2-3 weeks, Embroyogenesis acheives in 12 hrs and adult is made within 2.5 days. It's simple cellular and transparent, laureate Brenner slected it as a model onganism (Barr, 2003, strange, 2007).

3.3.3.Micro-organism

Saccharomyces cerevisiae (Brewing yeast) . yeast can grown is liquid solid culture from a single cell on the solid media. It is very easy to grow and analyze (Mell and Burgess, 2002). Brewing yeast used for understanding cell death programming and useful in cancer research. (Madeo et al.,2002).

4.IMPLEMENTATION OF ALTERNATIVE METHOD.

Relief of an creatures in laboratory is lengthy and laborious process. Fig 1. shows the way that bear to be taken before the beast test, but the figure process is generally repeated. for case needs validated system and accepted for replacing creatures to check chemical and so so as of repetation for relief of beast employed in medicine testing. This is frequently because of the fear of the choice may not work due to chemical differences.

The stage where the choice is formed, optimizied and tested intially that stage is named as Development. Academic plays important part duringthis.The Alternative charities, Indispensable centres like UK National centre for 3 RS play a pivotal part in backing. Numerous medicinal companies ornamental companies devlope indispensable, and that they produce their own spin-off companies or buying being one illustrationL'oreal bought the rights to Episkin in 1997 For useing their own mortal skin vexation model (Auplat, 2012). Academic- driven development may struggle to understand the regultory chain. Artificial driven development also inward looking. Companies get satisfied if the strategy is suitable for the end purpose of webbing substance.

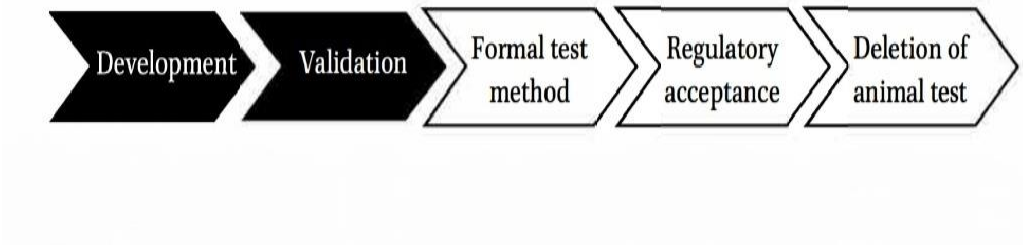

Figure 1. The process of acceptance of an indispensable test system.

Way in black are primarily wisdom driven, way in white are primarily nonsupervisorydriven.Validation is system where the singly assessed to insure it's accurate and dependable. System is vital if it's progress to acceptance. System should be validated by internationally agreed principles, but they aren't always we'll understood. It's crucial demand is that it should show same result with same procedure whenever it's carried. It requires labour and it's precious. Confirmation stage need to be repeted if effects go wrong. Beast test is noway validated it causes problem during confirmation, assessors realize that creatures data is inaccurate that they can not be trusted. (Balls, 2006)

Formal test system, once it's proven that indispensable styles is validated also coming stage is to write up how the system should be performed as a formal test.

Regulatory acceptance, this doesn't happens automatically and not extensively appreciate. Regional controllers agencies assess whether the system is used for their sector.

Omission of beast test, legislation to replace conditions for beast test with the volition needs several times and process isn't started until the very end of the process. Political pressure is demanded for the omission of beast test. For illustration, dealay of 7 times in which there was formal system of volition to rabbit skin vexation test.

The development and confirmation stage can be done with applicable backing and collaboration. Where as formal test system, nonsupervisory acceptance, omission of beast test are primarily depend on political will and nonsupervisory enforcement.

5.RECENT DEVELOPMENTS IN ALTERNATIVE TO TOXICITY TESTING

In once times there was a dramatic increase the development of volition (Liebschet.al., 2011).Replacement are more observed in the filled of the toxicity and received the most attention. Toxicity testing is in small proportion to the animal testing (Daneshian et. al.,2015 according to them 80% in Europe). Table 2. provides the status of alternative testing used for chemical saftey. Two things show up this table, first topical endpoint of the replacement and that is its effects on the part of the body. Second, many test gained approval from organization even if they are in combination with other test .Approval is gained from the organisation for Economic co-operation and Development (OECD).

Table 2.Alternatives for standard toxin tests for chemical safety

Endpoint	Animal test	Alternative tests	Regulatory acceptance
Acute toxicity	Rats are exposed to a high dose, no of them are excepted to die.(OECD TG 402,403,420,425,436)	Cell based death , extent of cell death in presence of substance	Used in combination with other information only
Eye irritation/ corrosion	rbbits eye substance is placed it is monitored for 3 weeks (OECD TG 405)	Human corneal epithelial model based on excited human cornea or skin measures the extend of cell death in presence of substance and can detect non - irritants. Excised eyes of hen and cattel can detect non-irritant and severe irritant.	OECD TG 437 and 438(ex vivo, 2009) Testing strategies gives yet to be formally accepted.
Carcinogenicity	Rat and mice are fed with substance for few years that is 2 years to see if theyare affected with cancer. (OECD TG 451,452)	Cell transformation assay(CTA) based on cellular changes to rodent cells have been in use for 50 years and can detect	CTA aasay have failed to gain international regultory acceptance now used on screening purpose. (OECD 2015,2016)

		90% of cancer activity.	
Skin absorption	Substance is rubbed on the back of rats, on next day they are killed (OECD TG 427)	Ex -vivo skin based model measures the substance passed through excised skin.	OECD TG 428(2004)
Skin irritation /corrosion	On back of rabbit substance is rubbed, they are killed 2 weeks later. (OECD TG 404)	In vitro human skin model is reconstituted and it measures the extent of cell death in presence of substance.	OECD TG 431(2004) and 439(2010)
Skin sensitization	On shaved skin of guinea pig the substance is rubbed whi are subjectly assed for allergies (guinea pig maximization test)	Adverse outcome pathway(AOP) for skin allergy covers the several test.The binding of the substance to proteins (in chemico) measured by direct peptide reactivity assay(DPRA), in vitro keratinocyte assay and human cell line activation test (h-CLAT).	OECD TG 442c(DPRA, 2015) ;442d(keratinicyte assay, 2015)
Mutagenicity/ Genotoxicity	In rat or mice the substance is forced or injected for 14 days, they are killed to look at the effect on their cell (OECD TG 474,475,483,486,488 ,489)	In vitro tests, including bacteria, cell micronucleus and gene mutation test are available.	OECD TG 471 (1997) ;473(1997). 476(1997);487(2010);490(2015).Positive result but still follow up in vivo
Repeated dose	Substance is rubbed on the shave skin of rats every day, and they are forced to inhale substance for	Chips model are developed in vitro lab test is did.. Silicon techniques	Silicon techniques is accepted only not others.

	28 to 90 days, before being killed (OECD TGS 407-413)	like read across is used.	
Reproductive toxicity	Pregnant female rabbit and rats are killed with their unborn babies.	Silicon techniques are tested. The in vitro Embryonic stem cell(EST)is did.	Read across test of silicon computer is accepted (OECD TG 455,2012;457, 2012;456, 2011) . EST is failed.

Public outrage for animals testing for cosmetic started in 1970s and it gained momentum in the 1980s.The formal encouragement to use alternative in the European Commission (Eu) set stone by Eu Directive on animal testing in 1996.

6.FUTURE ALTERNATIVE

Some of the future alternatives like -

6.1.organs on a chip

The wyss institule for Biologically Inspired Engineering (US) develop in-vitro organs for screening of drug which eliminated the use of animals for testing.

6.2.Human toxome

Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council (2007) charted a long range plan for loxicity testing. The major components of that includes the use of cell-based assay, conduction of targeted testing.

7.CONCLUSION

Animals saftey is an issue like a human welfare. Action should be taken for 3 Rs during laboratory use of animals. There are various alternative, we need to implement them with effective manner. Various computer model, bioinformatics, in vitro cell culture,tisuee replacement is necessary . Using alternative gives a dependable outcomes. This integrated approach result in minimum use of animals in scientific procedures.

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