Title

‘Safety’ Testing of Products for Human Use: Irrefutable necessity or morally indefensible false sense of security?

Author

Buyukmihci, NC

Publication Date

2023-03-23

Peer reviewed

‘Safety’ Testing of Products for Human Use: Irrefutable necessity or morally indefensible false sense of security?[1]

Nedim C Buyukmihci, V.M.D.[2]

Summary

This paper discusses the harmful and fatal use of non-human animals in so-called safety testing of products that are destined for human use (the same principles would be applicable to those destined for other animals). It argues and demonstrates that such use is not scientifically valid if the results are applied to humans. Moreover, not only does this testing result in extreme suffering and death for non-humans, dependence on this has also caused substantial harm to humans. Alternatives to such use are discussed in the context of being more defensible morally and scientifically.

Keywords: alternative, animal welfare, compassion, ethics, kindness, morality, non-human animal, scientific method, species differences, toxicity testing

Virtually anything which is ingested by, injected into or applied onto humans, or which may accidentally come into contact with them, is ‘safety’ tested by using and killing millions of non-human animals (animals[3]) annually. This includes therapeutic agents such as antibiotics, personal hygiene preparations, cosmetics, household cleaners and industrial solvents, to name a few. The same holds true for products which are used in the environment such as pesticides, fertilisers and machine lubricants.

There are several parameters used in testing these substances: chronic toxicity, carcinogenicity, teratogenicity and acute toxicity. In this paper, I will address only two of the methods used in the last category. The principles with respect to unreliability and the concerns for animal welfare and morally defensible behaviour by humans are the same in all cases.

Acute toxicity testing almost always is associated with extreme pain and suffering because of the very nature of the tests used. Anaesthetics or analgesics are *not* used. Two of the ways in which a substance is evaluated for its acute effects are the lethal dose 50% (LD_{50}) and topical irritancy tests[4]. Surviving animals generally are killed at the termination of the tests.

The LD_{50} typically involves the administration, by injection or forced ingestion, of various doses of the material to groups of animals[5]. Two or more species of animals and as many as several hundred each may be used for every substance or concentration of substance tested. The dose at which 50% of the animals die is called the LD_{50} and is generally expressed as the amount of the material per unit of body weight. Sometimes the material is not very toxic and the animals die from the volume of the material forced into their stomachs. The animals who die, often after agonising illness, may be considered the lucky ones. Those who become sick and do not die suffer longer. Depending on the material being tested, the animals may experience severe abdominal pain, muscle cramps, convulsions, vomiting, diarrhoea, gastrointestinal ulcers with bleeding, loss of kidney function, or other painful or distressing conditions.

---

1 The intent of this brief review is to demonstrate that reliance on animals is unnecessary, as well as immoral, to ensure a product is safe for human use. Although this document is not being regularly updated and some of the references may be considered 'dated', the arguments made are still valid for this issue. Additional strong moral arguments against subjecting non-consenting beings to harm and death is the subject of another manuscript (Buyukmihci 2022-12-01).

2 Emeritus Professor of Veterinary Medicine, University of California and Emeritus Diplomate, American College of Veterinary Ophthalmologists; contact: ncbuyukmihci@ucdavis.edu;

3 Purely for the sake of convenience, I may refer to animals other than humans as "animals", recognising that all are animals of one kind or another; there is no intention to imply that any, even a human, is morally superior or intrinsically more valuable than another.

4 Bosshard 1985; Draize et al 1944

5 Sperling & McLaughlin 1976; Zbinden & Flury-Roversi 1981

The problem with this test, besides one of abject inhumanity, is that the numbers generated essentially are meaningless. The LD_{50} is a statistical value which is valid only for the exact conditions under which it was derived and only for the animals in whom it was determined[6]. Changes in ambient temperature, degree of stress, or amount of food or water, for example, can alter the LD_{50} by ten times or more. Furthermore, the LD_{50} changes drastically from one species to another or even from one strain to another of the same species. The LD_{50} of a substance in rabbits or rats in no way is an indicator of the acute toxicity of the substance in a human. Drug interactions, a common problem in the clinical setting, are not addressed by this test. Moreover, a number indicating 50% *mortality* has minimal clinical relevance. A minimum lethal dose or a maximum tolerated dose, in *humans*, would have much more meaning to the practising physician. Data of this type can easily be obtained from various poison control centres[7]. There are other alternatives for this and other types of toxicity tests, as well[8].

In the Draize eye irritancy test, *any* compound which might intentionally or by accident gain access to the eye is tested by being placed onto the eyes of conscious, restrained rabbits. The animals are observed over a period of several days to see if there is an adverse reaction to the substance. There may be no reaction or there may be irritation ranging from minor to severe. In the worst situation, the cornea may ulcerate and perforate. Because the cornea is one of the most sensitive tissues in the body – rich in nerve endings – irritation or ulceration produces considerable pain. The rabbits usually are restrained in stocks which hold the animals by the neck and prevent them from rubbing their eyes. They cannot, therefore, in any way mitigate the discomfort or pain produced by the material placed in their eyes.

As an ophthalmologist and scientist, it is my professional opinion that the Draize eye irritancy test has little, if any, relevance to human safety. It is fraught with technical and biological problems which make extrapolation of results to the human situation not only tenuous, but also dangerous. It is the subject of a brief review by me elsewhere[9]. The same problems compromise the 'usefulness' of the skin irritancy tests done on animals. In these, the animals are shaved and a substance is held in contact with the skin using a bandage. The area is examined later to determine if there was irritation. Depending on the substance tested, there can be substantial corrosion of the skin.

From a practical standpoint, therefore, the tremendous suffering which some of the animals must endure in toxicity tests is unnecessary. Their misery in no way guarantees the safety of humans.

There are numerous alternative methods to obtain data to predict whether a particular material will be safe for human use[10]; see also the cited information in the paper on the Draize test[11]. So-called organs-on-a-chip are particularly of interest[12] as is the VPROMPT project at Vanderbilt University[13]. These various alternative methods are more reliable and more humane than tests

6 Kaufmann [sic] & Cohen 1987; Morrison et al 1968; Sperling & McLaughlin 1976; Zbinden & Flury-Roversi 1981
7 Werner 1983
8 Bassi et al 1993; Ciapetti et al 1992; Dierickx & Ekwall 1992; Fiskesjö & Levan 1993; Grundt & Nyland 1992; Hazard 1993; Kerszman 1993; Mäkelä & Isomaa 1992; Peloux et al 1992; Sapora et al 1993; Sbarbati-Del Guerra et al 1993; Schambye et al 1992; Valentino et al 1993
9 Buyukmihci 2023
10 Andrews 2014; Anon 1989-01-01,2016-12-01,2022-08-28; Arenholt-Bindslev et al 1992; Babich & Borenfreund 1989; Bigelow 2014; Boue-Grabot et al 1992; Carrara et al 1992; Cook et al 1992; Douglas 1982; Evans et al 2009; Henderson 2010; Kruszewski et al 1992; Mackar & Spencer 2011; Neves et al 2013; Pitman 2014; Renzi et al 1993
11 Buyukmihci 2023
12 Anon 2007-12-17,2019-08-14; Benam et al 2016; Clark 2015; Kremen 2010; Ma et al 2015
13 Anon 2016-11-19

using animals. In some cases the methods only represent a refinement in the test or a reduction in the numbers of animals used and are unacceptable morally. In other cases, however, there is evidence that a total replacement, using a number of *in vitro* tests, is possible.

It often is stated that a proposed alternative to an animal-based test must first be ‘validated'. This means that the proposed alternative must be reasonably close in predicting what would be the result using the animal test. There are at least two systematic errors with this approach. One, as mentioned, is that available data indicate many of the standard tests such as the Draize test are not reliable indicators of human reactivity. Another is that, to my knowledge, there has been no validation of the standard tests themselves. They have been accepted as the standard with no rigorous attempt at verifying their reliability. Therefore, although it is true that new methods of determining toxicity should be ‘validated’, the standard should be against known reactions to various categories of substances by *humans*, not other animals.

From a scientific and human safety perspective, results from toxicity tests on animals largely are irrelevant, unpredictable and potentially dangerous because people would tend to react differently to many substances[14]. One example is the case of paraquat[15]. This chemical was introduced in 1960 as a herbicide. It was believed to have low toxicity because the LD_{50} in the rat was 120 mg/kg body weight. By 1972, however, more than 400 people had died from exposure to this chemical. From these tragic deaths, it was estimated that the lethal dose in humans was as little as 4 mg/kg body weight.

In addition, this type of testing cannot predict individual or familial tendencies for adverse reactions. For example, the antibiotic chloramphenicol is relatively safe in animals, but causes illness and death from aplastic anaemia in susceptible people. The amount necessary to do this in some individuals is so small that even the tiny amount applied through eye ointments can be fatal[16].

Ironically, products are still manufactured and distributed for human use even though they are demonstrated to be toxic to animals. For example, during ‘safety’ testing of the artificial sweetener saccharin, it was found that rodents developed cancer. Despite this, the test results were, in essence, ignored, and the product was marketed, albeit with a warning label. Perhaps thousands of animals suffered and were killed for a trivial, non-essential product, and the data generated were pre-empted by economic interests.

In another example, a nail polish remover containing acetonitrile, which was tested on animals, was released for use and resulted in the death of a human child[17]. The acute toxicity data with respect to this chemical's effects on rodents were reported in the article, but were of no use to the child who succumbed to the chemical.

In yet another example, a tuberculosis vaccine that failed in non-human primates was still given to human babies[18].

It would be far more pragmatic and reliable to gain data by learning from the numerous

14 Anon 1991-09-17,2022-08-28; Baldrick 2011; Boomgaarden 2014; Brinkworth et al 2012; Bruner et al 1993; Carter & Griffith 1965; Chapin et al 1993; Coleman 2011; Cookson 2007; Davis 1979; D'Mello 1993; Dorman et al 1993; Eastwood et al 2010; Gartner 2005; Ledford 2013; Makino et al 2022; Mathews 2007; Neergaard 1993; Oksenberg et al 1992; Perel et al 2007; Pouliot et al 2022; Pritchard 2008; Roche Pharmaceuticals 2009; Spearow et al 1999; Vince 2006
15 Van Heijst 1991
16 Fraunfelder et al 1993
17 Caravati & Litovitz 1988
18 Newell & Malnick 2017

unplanned human exposures to various substances[19]. Physicians deal daily with accidental poisonings or exposure of the body surfaces to various chemicals. The data generated by these observations are critical to our ability to predict what another person might expect and to develop treatment measures. We have a practical as well as moral obligation to record and centralise this information so that it can be universally shared.

What is the solution to the problem of safety testing? As with most complicated situations, there are no easy nor universally accepted answers to the question. Federal agencies, such as the Food and Drug Administration and Environmental Protection Agency, for example, have stated that they do not require nor encourage the LD_{50}. There are no regulations which require the use of the Draize test[20]. The FDA Modernization Act will allow companies the option to use alternative, humane and human-relevant methods to test experimental drugs before human clinical trials[21]. Many companies are modifying the LD_{50} and other tests so that the number of animals used is less[22]. There are numerous 'cruelty-free' products readily available. These are safe and reasonable alternatives to those tested on animals. Although some of the companies claiming to have discontinued testing their products on animals buy their raw ingredients from suppliers who still test them on animals, most such companies do not.

Much, if not most, 'safety' testing is done on products which are designed to be an 'improvement' over an existing one. Whereas this normally would be appropriate in a free enterprise system, the fact that someone – animals – must suffer and die as a result makes it unconscionable. Companies also appear to be doing this type of testing to limit their liability for complications following the use of their products. This may be, however, to little avail. The data derived from animal testing may not be admissible in court when a human brings action against a company due to injury from a particular product[23].

What can you do to reduce the pain and suffering involved in 'safety' testing? Alert others to the truth about the products they are using. When the facts are known, compassionate people will substitute cruelty-free alternatives. Alert your local market to the facts and ask them to at least carry some of the alternative products. Whereas food co-ops traditionally have been open to this, many of the large grocery chains now routinely carry such products. Make the pledge to use cruelty-free products whenever possible. Follow up by letting the producers of other products know that you have switched over and why. With increased economic pressure, there will be increased efforts by the large companies to develop cruelty-free products.

From a moral perspective, toxicity testing using animals cannot be condoned. The animals used are living, feeling creatures who are capable of suffering in ways similar to us. They have lives and interests independent of ours. There are no morally relevant differences between them and us which make it acceptable to use them for purposes to which we would not consider subjecting ourselves.

Cited information[24]:

1. Andrews, James 2014-02-10 "New shellfish toxicity test could save thousands of mice"

19 Werner 1983
20 Anon 1982-04-01
21 FDA Modernization Act 2022
22 Weiss 1988
23 Gleeson 1987
24 In this paper, I have cited only a few references to document various points because the literature on this subject is substantial.

Food Safety News https://www.foodsafetynews.com/2014/02/new-shellfish-safety-test-readies-to-save-thousands-of-mice/#.WDAaOVxMhMt Accessed 2019-10-27

2. Anonymous 1982-04-01 "CPSC: Exclusive use of Draize test not required" Lab Animal 11:19
3. Anonymous 1989-01-01 "Testskin: An analysis" The Alternatives Report 1:1-6
   "Testskin combines Living Dermal Equivalent (LDE) and Living Skin Equivalent (LSE); LSE behaves like normal skin after grafting onto animals. Has good potential for estimating human dermal irritancy."
4. Anonymous 1991-09-17 "Heart medicine to be withdrawn: Irregular-heartbeat drug tied to high death rate, study found" Sacramento Bee
5. Anonymous 2007-12-17 "Biochip mimics the body to reveal toxicity of industrial compounds" PhysOrg.com https://www.physorg.com/news117133544.html Accessed 2019-10-27
6. Anonymous 2016-11-19 "VPROMPT: Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology" Vanderbilt University https://www.vanderbilt.edu/vprompt/ Accessed 2019-09-02
7. Anonymous 2016-12-01 "New device creates 3D livers in a droplet" National Institute of Biomedical Imaging and Bioengineering https://www.nibib.nih.gov/news-events/newsroom/new-device-creates-3d-livers-droplet Accessed 2019-07-27
8. Anonymous 2019-08-14 "Tissue Chip for Drug Screening" National Center for Advancing Translational Sciences https://ncats.nih.gov/tissuechip Accessed 2019-09-02
9. Anonymous 2022-08-28 "The OOC alternative" Laboratory News https://www.labnews.co.uk/article/2091979/the-ooc-alternative Accessed 2022-08-30
   "...animal experimentation is slow, expensive and requires cross-species translation to extrapolate predictions to humans. Furthermore, it carries important ethical considerations. Translating findings to these critical in vivo settings remains a challenge due to inherent differences between species and an insufficient understanding of the underlying pathophysiology of human diseases."
10. Arenholt-Bindslev, Dorthe; Bleeg, Henry S. and Richards, Alan 1992-01-01 "Toxicity of sodium dodecyl sulphate and other detergents in cultures of human oral mucosa epithelium" Alternatives to Laboratory Animals 20(1):28-38 http://www.atla.org.uk/toxicity-of-sodium-dodecyl-sulphate-and-other-detergents-in-cultures-of-human-oral-mucosa-epithelium/
11. Babich, Harvey and Borenfreund, Ellen 1989-06-01 "Development of an alternative to the Draize rabbit skin test" International Foundation for Ethical Research Newsletter 3(2):1-2
    "Showed the neutral red test, using human cells, to be "...reproducible, sensitive, rapid, and economical and can be readily incorporated into the testing protocols of industrial and pharmaceutical laboratories.""
12. Baldrick, Paul 2011-03-01 "Safety evaluation of biological drugs: What are toxicology studies in primates telling us?" Regulatory Toxicology and Pharmacology 59(2):227-236 https://dx.doi.org/10.1016/j.yrtph.2010.10.005
13. Bassi, Anna Maria; Bosco, Ornella; Brenci, Sabrina; Adamo, Daniela; Penco, Susanna; Piana, Sandra; Ferro, Margherita and Nanni, Giorgio 1993-01-01 "Evaluation of the Cytotoxicity of the First 20 MEIC Chemicals in Two Hepatoma Cell Lines with Different Xenobiotic Metabolism Capacities" Alternatives to Laboratory Animals 21(1):65-72 https://doi.org/10.1177%2F026119299302100111
    "These findings suggest that hepatoma cell lines possessing various specific enzyme activities could be usefully employed in a battery of tests designed to reproduce in vitro the wide range of biochemical properties expressed by the cells in the whole organism."
14. Benam, Kambez H.; Villenave, Remi; Lucchesi, Carolina; Varone, Antonio; Hubeau, Cedric; Lee, Hyun-Hee; Alves,Stephen E.; Salmon, Michael; Ferrante, Thomas C.; Weaver, James C.; Bahinski, Anthony; Hamilton, Geraldine A. and Ingber, Donald E. 2016-02-01 "Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro"

Nature Methods 13(2):151-157 https://dx.doi.org/10.1038/nmeth.3697

15. Bigelow, Bruce V. 2014-08-13 "Organovo to offer preclinical drug tests based on 3-D liver tissue" Xconomy.com. https://www.xconomy.com/san-diego/2014/08/13/organovo-to-offer-preclinical-drug-tests-based-on-3-d-liver-tissue Accessed 2019-10-27
16. Boomgaarden, Wolfgang 2014-09-11 "Animal testing can mislead drug discovery and development" PharmaInformatic http://www.pharmainformatic.com/Animal_testing_can_mislead_drug_discovery_and_development.pdf Accessed 2023-03-23

    "...PharmaInformatic, a German biotech company, compared study results on drug-uptake ("Oral Bioavailability") in animals and humans for a large number of approved and established drugs.

    Results showed that oral bioavailability in animals is inconsistent with the values reported for humans and large differences can exist."
17. Bosshard, E. 1985-02-01 "Review on skin and mucous-membrane irritation tests and their application" Food and Chemical Toxicology 23(2):149-154 https://dx.doi.org/10.1016/0278-6915(85)90007-9

    "Moreover discrepancies in the response of the rabbit and human eye impede the extrapolation from animal data to man."
    "Anatomical, physiological and biochemical differences between animals and humans prevent direct prediction of the expected irritancy in man from data on experimental animals."
    "...the information that is necessary to prevent a possible hazard to man is the identification of an irritant or a corrosive substance. If this straightforward goal is kept in mind, in vitro techniques will soon provide reliable data for the estimation of the irritancy potential in man."
18. Boue-Grabot, M.; Halaviat, B. and Pinon, J. Francois 1992-04-01 "A simple method for cytotoxicity studies of non-hydrosoluble substances. Possible application as an alternative to the Draize test for cosmetics and toiletries" Alternatives to Laboratory Animals 20(2):307-312 http://www.atla.org.uk/a-simple-method-for-cytotoxicity-studies-of-non-hydrosoluble-substances-possible-application-as-an-alternative-to-the-draize-test-for-cosmetics-and-toiletries/

    "From the results of this preliminary study, it appears that the method could provide an alternative to animal studies on the ocular tolerance of cosmetics and toiletries."
19. Brinkworth, J.F.; Pechenkina, E.A.; Silver, J. and Goyert, S.M. 2012-12-01 "Innate immune responses to TLR2 and TLR4 agonists differ between baboons, chimpanzees and humans" Journal of Medical Primatology 41(6):388-393 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3603697/
20. Bruner, R.H.; Kinkead, E.R.; O'Neill, T.P.; Flemming, C.D.; Mattie, D.R.; Russell, C.A. and Wall, H.G. 1993-01-01 "The toxicologic and oncogenic potential of JP-4 jet fuel vapors in rats and mice: 12-month intermittent inhalation exposures" Fundamental and Applied Toxicology 20(1):97-110 https://doi.org/10.1006/faat.1993.1012

    "The study did not demonstrate target organ toxicity or carcinogenesis which could be extrapolated to other species."
21. Buyukmihci, Nedim C 2022-12-01 "Serious Moral Concern Is Not Species-limited" https://escholarship.org/uc/item/6604b7qj
22. Buyukmihci, Nedim C 2023-03-22 "The Draize Eye Irritancy Test" https://escholarship.org/uc/item/5ds7v9tj Accessed 2023-01-31
23. Caravati, E. Martin and Litovitz, Toby L. 1988-12-16 "Pediatric cyanide intoxication and death from an acetonitrile-containing cosmetic" JAMA 260(23):3470-3473 https://dx.doi.org/10.1001/jama.1988.03410230088034
24. Carrara, Maria; Cima, Lorenzo; Cerini, Roberto and Carbonare, Maurizio Dalle 1992-01-01 "A new in vitro model for predicting the toxicity of cosmetic products" Alternatives to

Laboratory Animals 20(1):138-143
“A method has been developed whereby cosmetic products which are not soluble in water or in alcohol can be brought into contact with cell cultures by being placed in a cell culture insert, which is then placed in the cell culture well.”

25. Carter, R. Owen and Griffith, John F. 1965-01-01 "Experimental bases for the realistic assessment of safety of topical agents" Toxicology and Applied Pharmacology 7(suppl 2):60-73 https://dx.doi.org/10.1016/0041-008X(65)90113-4
With respect to animal data, "Their use, however, for predicting human responses in an absolute sense is tenuous. Only through employment of human subjects in competent procedures can the uncertainty involved in the extrapolation of animal test results to man be avoided."
26. Chapin, Robert E.; Morrissey, Richard E.; Gulati, Dushyant K.; Hope, Esther; Barnes, Leta H.; Russell, Susan A. and Kennedy, Sarah R. 1993-07-01 "Are mouse strains differentially susceptible to the reproductive toxicity of ethylene glycol monomethyl ether? A study of three strains" Fundamental and Applied Toxicology 21(1):8-14 https://dx.doi.org/10.1006/faat.1993.1065
“Most rodent reproductive toxicology studies utilize strains of high fecundity. ... The data show that the most fecund strain (Swiss) was affected the least by exposure to EGME, while the least fecund strain (C3H) suffered the greatest declines in fertility. These differences might alter interspecies extrapolation factors, or the permissible exposure levels for humans.”
27. Ciapetti, Gabriela; Cenni, Elisabetta; Cavedagna, Daniela; Pratelli, Loredana and Pizzoferrato, Arturo 1992-01-01 "Cell culture methods to evaluate the biocompatibility of implant materials" Alternatives to Laboratory Animals 20(1):52-60
"Despite some inherent limitations of the cell culture techniques, they are an accurate and reliable method of predicting the biological compatibility of materials to be implanted in vivo."
28. Clark, Nick 2015-06-22 "Medical technology that could eliminate need for animal testing wins design award" The Independent https://www.independent.co.uk/news/science/medical-technology-that-could-eliminate-need-for-animal-testing-wins-design-award-10337887.html Accessed 2022-11-23
29. Coleman, Robert A. 2011-02-01 "Efficacy and safety of new medicines: a human focus" Cell and Tissue Banking 12(1):3-5 https://dx.doi.org/10.1007/s10561-010-9200-x
“The introduction of safe and effective new medicines is proving ever more difficult, a problem arguably due at least in part to over-reliance on experimental animal-based test systems. In light of the increasing awareness of the lack of predictiveness of such non-human approaches, the necessity to focus on human-based test methods is clear.”
30. Cook, Jeffery R.; Gabriels, Joseph; Patrone, Laura M.; Rhoads, Laura, S. and Van Buskirk, Robert G. 1992-04-01 "A human epidermal model that can be used in an automated multiple endpoint assay" Alternatives to Laboratory Animals 20(2):313-323 http://www.atla.org.uk/a-human-epidermal-model-that-can-be-used-in-an-automated-multiple-endpoint-assay/
“Our data suggest the feasibility of using multilayered human tissues for automated multiple endpoint analysis.”
31. Cookson, Clive 2007-09-14 "Flaws in use of animal tests for new drugs" Financial Times https://www.ft.com/content/e5e81b22-62d6-11dc-b3ad-0000779fd2ac Accessed 2019-10-27
“The use of animal tests in drug development is fundamentally flawed, according to new evidence presented at the BA (British Association for the Advancement of Science) Festival of Science in York on Friday.”
32. Davis, Lloyd E. 1979-11-01 "Species differences as a consideration in drug therapy" Journal of the American Veterinary Medical Association 175(9):1014-1015 https://www.ncbi.nlm.nih.gov/pubmed/521363

"It is unwise to extrapolate information concerning drugs from one species to another."

33. Dierickx, Paul J. and Ekwall, Björn 1992-04-01 "Long-term cytotoxicity testing of the first twenty MEIC chemicals by the determination of the protein content in human embryonic lung cells" Alternatives to Laboratory Animals 20(2):285-289 http://www.atla.org.uk/long-term-cytotoxicity-testing-of-the-first-twenty-meic-chemicals-by-the-determination-of-the-protein-content-in-human-embryonic-lung-cells/
    "Together with other preliminary MEIC validation results, this study indicates that a good alternative cytotoxicity test may be found for the prediction of human long-term toxicity."
34. D'Mello, G.D. 1993-01-01 "Behavioural toxicity of anticholinesterases in humans and animals - A review" Human & Experimental Toxicology 12(1):3-7 https://doi.org/10.1177/096032719301200101
    "Understanding of the behavioural mechanisms mediating antiChE-induced changes observed in animals is not sufficient to enable extrapolation to humans."
35. Dorman, David C.; Dye, Janice A.; Nasisse, Mark P.; Ekuta, Jethro; Bolon, Brad and Medinsky, Michele A. 1993-04-01 "Acute methanol toxicity in minipigs" Fundamental and Applied Toxicology 20(3):341-347 https://doi.org/10.1006/faat.1993.1044
    "Based on results following a single dose, female minipigs do not appear to be overtly sensitive to methanol and thus may not be a suitable model for acute methanol-induced neuro-ocular toxicosis."
36. Douglas, William H.J. 1982-05-01 "New methodology" Alternative Methods in Toxicology 1(1)
    "The ideal in vitro test must utilize human corneal tissue in order to obviate the need to extrapolate laboratory animal eye irritancy to human situation.
    Unquestionably, the Draize alternative is sensitive to toxicities which result from a broad range of doses of test substances. The observed ED50 [effective dose 50%] of the most toxic material tested differs from the ED50 of the least toxic material by a factor of more than one million...Within this broad range of doses, the toxicities of many substances have been determined with sufficient precision that a statistically sound conclusion can be drawn...With the Draize test, this degree of precision has never been achieved.
    The preliminary studies support the contention that a simple, well designed, cell culture alternative can duplicate the findings of in vivo Draize experiments and extend these by overcoming the quantitative, practical, and humanitarian problems associated with Draize testing."
37. Draize, John H.; Woodard, Geoffrey and Calvery, Herbert O. 1944-11-01 "Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes" The Journal of Pharmacology and Experimental Therapeutics 82(3):377-390 http://jpet.aspetjournals.org/content/82/3/377
38. Eastwood, D.; Findlay, L.; Poole, S.; Bird, C.; Wadhwa, M.; Moore, M.; Burns, C.; Thorpe, R. and Stebbings, R. 2010-10-01 "Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells" British Journal of Pharmacology 161(3):512-526 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2990151/
    "In 2006, a life-threatening 'cytokine storm', not predicted by pre-clinical safety testing, rapidly occurred in all six healthy volunteers during the phase I clinical trial of the CD28 superagonist monoclonal antibody (mAb) TGN1412. To date, no unequivocal explanation for the failure of TGN1412 to stimulate profound cytokine release in vitro or in vivo in species used for pre-clinical safety testing has been established. Here, we have identified a species difference almost certainly responsible for this disparate immunopharmacology. ...we identify a species difference in CD28 expression on the CD4+ effector memory T-cell subset as being most likely responsible for the failure of pre-clinical safety testing of TGN1412 in cynomolgus macaques."
39. Evans, E.R.; Skipper, P.J.A. and Shone, C.C. 2009-10-01 "An assay for botulinum toxin

types A, B and F that requires both functional binding and catalytic activities within the neurotoxin" Journal of Applied Microbiology 107(4):1384-1391 https://doi.org/10.1111/j.1365-2672.2009.04325.x

40. FDA Modernization Act 2022 https://www.congress.gov/bill/117th-congress/senate-bill/5002/text Accessed 2023-03-23
41. Fiskesjö, Geirid and Levan, Albert 1993-04-01 "Evaluation of the first ten MEIC chemicals in the Allium test" Alternatives to Laboratory Animals 21(2):139-149 http://www.atla.org.uk/evaluation-of-the-first-ten-meic-chemicals-in-the-allium-test/
    "...we found that this test correlates well with several other tests (e.g. MIT-24 cell test, and tests with mice, rats or humans in vivo). ... The present results indicate that the eukaryotic higher plants may serve as highly useful test systems for biological risk evaluation."
42. Fraunfelder, F.T.; Morgan, R.L. and Yunis, A.A. 1993-06-15 "Blood dyscrasias and topical ophthalmic chloramphenicol" American Journal of Ophthalmology 115(6):812-813 https://dx.doi.org/10.1016/S0002-9394(14)73653-0
43. Gartner, John 2005-07-22 "Vioxx suit faults animal tests" Wired https://archive.wired.com/medtech/health/news/2005/07/68260?currentPage=all Accessed 2019-10-27
44. Gleeson, John Gerald 1987-10-01 "Exclusion of animal data as evidence of chemically-induced disease" For the Defense 25-29
45. Grundt, Inger K. and Nyland, Harald 1992-04-01 "The use of microglia activation in the evaluation of neurotoxicity" Alternatives to Laboratory Animals 20(2):271-274
46. Hazard, Holly E. 1993-09-01 "Transportation moves on animal alternative" The Animals' Agenda 13(4):14-15
47. Henderson, Mark 2010-05-04 "New stem cells will reduce the need for animal testing" The Times Online https://www.thetimes.co.uk/tto/science/medicine/article2499416.ece Accessed 2019-10-27
48. Kaufmann [sic], Stephen R. and Cohen, Murry J. 1987-02-01 "The clinical relevance of the LD50" Veterinary and Human Toxicology 29(1):39-41 https://www.ncbi.nlm.nih.gov/pubmed/3824874
49. Kerszman, Gustaw 1993-04-01 "Of bacteria and men: Toxicity of 30 MEIC chemicals to bacteria and humans" Alternatives to Laboratory Animals 21(2):233-238
    "A highly significant correlation could be established for the first 30 MEIC chemicals between [minimal inhibitory concentrations] in bacteria and acute lethal blood concentration in humans. A significant correlation was also established for sets of MEIC chemicals between MIC in bacteria and the relevant endpoint concentrations in various tests employing human cells."
50. Kremen, Rachel 2010-01-20 "Cosmetics testing without animals" Technology Review https://www.technologyreview.com/biomedicine/24384/
51. Kruszewski, Francis H.; Hearn, Laura H.; Smith, Kyle T.; Teal, Janice J.; Gordon, Virgnia C. and Dickens, Michael S. 1992-01-01 "Application of the EYTEX™ system to the evaluation of cosmetic products and their ingredients" Alternatives to Laboratory Animals 20(1):146-163 http://www.atla.org.uk/application-of-the-eytex-system-to-the-evaluation-of-cosmetic-products-and-their-ingredients/
    "100% sensitivity and 85% predictability were described by the data, indicating the efficiency of EYTEXtm in identifying known irritants. ... The EYTEXtm system protocols, when used appropriately, can provide a conservative means of assessing the irritant potential of most cosmetic formulations and their ingredients."
52. Ledford, Heidi 2013-07-16 "Animal studies produce many false positives" Nature http://dx.doi.org/10.1038/nature.2013.13385
53. Ma, Zhen; Wang, Jason; Loskill, Peter; Huebsch, Nathaniel; Koo, Sangmo; Svedlund, Felicia L.; Marks, Natalie C.; Hua, Ethan W.; Grigoropoulos, Costas P.; Conklin, Bruce R. and Healy, Kevin E. 2015-07-14 "Self-organizing human cardiac microchambers mediated

by geometric confinement" Nature Communications 6:7413 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4503387/

54. Mackar, Robin and Spencer, Geoff 2011-03-10 "New robot system to test 10,000 chemicals for toxicity" NIH News https://www.nih.gov/news/health/mar2011/niehs-10.htm Accessed 2019-10-27
55. Mäkelä, Jan-Henrik and Isomaa, Boris 1992-04-01 "The possible use of isolated human platelets in in vitro cell toxicology" Alternatives to Laboratory Animals 20(2):258-261
56. Makino, Chie; Watanabe, Akiko; Kato, Manabu; Shiozawa, Hideyuki; Takakusa, Hideo; Nakai, Daisuke; Honda, Tomoyo and Watanabe, Nobuaki 2022-03-19 "Species differences between rats and primates (humans and monkeys) in complex cleavage pathways of DS-8500a characterized by 14C-ADME studies in humans and monkeys after administration of two radiolabeled compounds and in vitro studies" Drug Metabolism and Pharmacokinetics 45():100459 https://doi.org/10.1016/j.dmpk.2022.100459
57. Mathews, Anna Wilde 2007-03-30 "Recent cases point to the limitations of animal drug tests" The Wall Street Journal https://www.wsj.com/articles/SB117519602221153510 Accessed 2019-10-27
58. Morrison, J.K.; Quinton, R.M. and Reinert, H. 1968-01-01 "The purpose and value of LD50 determinations" In Modern Trends in Toxicology, Volume 1, Boyland, E. and Goulding, Roy (eds), 1-17 pp London: Butterworths
    “Thus comparison of LD50 results obtained in different laboratories or even within the same laboratory at different times may be misleading.”
59. Neergaard, Lauran 1993-09-01 "Drug trial yields its deadly secret" Pittsburgh Post-Gazette https://www.newspapers.com/newspage/89588474/ Accessed 2019-10-27
    Although fialuridine passed toxicity tests in animals, was killing people when used in them.
60. Neves, Bruno Miguel; Rosa, Susana Carvalho; Martins, João Demétrio; Silva, Ana; Gonçalo, Margarida; Lopes, Maria Celeste and Cruz, Maria Teresa 2013-03-18 "Development of an in vitro dendritic cell-based test for skin sensitizer identification" Chemical Research in Toxicology 26(3):368-378 https://dx.doi.org/10.1021/tx300472d
61. Newell, Claire and Malnick, Edward 2017-09-03 "Oxford University scientists gave babies trial TB vaccine 'that did not work on monkeys'" The Telegraph http://www.telegraph.co.uk/news/2017/09/03/oxford-university-scientists-gave-babies-trial-tb-vaccine-did/ Accessed 2020-03-13
62. Oksenberg, Donna; Marsters, Scot A.; O'Dowd, Brian F.; Jin, Hui; Havlik, Sona; Peroutka, Stephen J. and Ashkenazi, Avi 1992-11-12 "A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT1B receptors" Nature 360(6400):161-163 https://dx.doi.org/10.1038/360161a0
63. Peloux, Anne-Francoise; Fédérici, Christian; Bichet, Nicole; Gouy, Daniel and Cano, Jean-Paul 1992-01-01 "Hepatocytes in primary culture: An alternative to LD50 testing? Validation of a predictive model by multivariate analysis" Alternatives to Laboratory Animals 20(1):8-26
64. Perel, Pablo; Roberts, Ian; Sena, Emily; Wheble, Philipa; Briscoe, Catherine; Sandercock, Peter; Macleod, Malcolm; Mignini, Luciano E.; Jayaram, Pradeep and Khan, Khalid S. 2007-01-27 "Comparison of treatment effects between animal experiments and clinical trials: systematic review" British Medical Journal 334(7586):197 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781970/
65. Pitman, Simon 2014-10-09 "Baxter Laboratories develops new in-vitro skin testing method" Cosmetics design-asia.com https://www.cosmeticsdesign-asia.com/Regulation-Safety/Baxter-Laboratories-develops-new-in-vitro-skin-testing-method Accessed 2019-10-27
66. Pouliot, Mylène; Bussiere, Jeanine; Coppi, Aldo; Holbrook, Kristen; Shelton, Amy; Sparapani, Samantha; Maher, Jonathan; Zabka, Tanja S.; Boulay, Emmanuel and Authier, Simon 2022-03-01 "Polysorbate 80-Induced Anaphylactoid Reaction and the Effects on Cardiovascular Function: Dose Threshold and Species Comparison" International Journal

of Toxicology 41(2):99-107 https://doi.org/10.1177/10915818211072780
Long-tailed macaques reacted differently in toxicity tests of polysorbate 80 to the other species tested (dogs and pigs), so no way to know which species could be predictive of reaction in people.

67. Pritchard, J. Fred 2008-12-10 "Risk in CNS drug discovery: focus on treatment of Alzheimer's disease" BMC Neuroscience 9(suppl 3):S1 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2604886/
68. Renzi, Daniela; Valtolina, Marinella and Forster, Roy 1993-01-01 "The evaluation of a multi-endpoint cytotoxicity assay system" Alternatives to Laboratory Animals 21(1):89-96
    "The aim of this study was to evaluate a multi-endpoint cytotoxicity screening method using V79 cells based on four different endpoints of cytotoxicity: trypan blue exclusion, reduction of XTT, neutral red uptake and total protein content. In addition, cell morphology was routinely observed after each treatment. Seven compounds were studied, which can be divided into five classes: protein synthesis inhibitors (cycloheximide, actinomycin D); inhibitors of cell division (bleomycin, vincristine); membrane-active compounds (Triton X-100); lysosomotropic agents (ammonium chloride); and general toxicants (sodium chloride). We obtained a variety of different toxicity profiles, which may be useful in defining the mechanisms of toxic action of these compounds. The multi-endpoint screening system proved to be readily applicable, robust and rapid, and gave reliable toxicity results over a wide range of chemical concentrations."
69. Roche Pharmaceuticals 2009-01-01 "XENICAL - orlistat capsule" Roche Pharmaceuticals https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020766s026lbl.pdf Accessed 2020-04-27
    "Because animal reproductive studies are not always predictive of human response, XENICAL is not recommended for use during pregnancy."
70. Sapora, Orazio; Maggi, Antonella; Maione, Barbara; Pazzaglia, Simonetta and Tabocchini, Maria-Antonella 1993-01-01 "K562. A human cellular system capable of undergoing in vitro differentiation: Measurement of genotoxic parameters useful for cytotoxicity evaluation" Alternatives to Laboratory Animals 21(1):50-56
71. Sbarbati-Del Guerra, Rosella; Cascone, M. Grazia; Tricoli, Mario and Cerrai, Piero 1993-01-01 "In vitro validation of poly(ester-ether-ester) block copolymers as biomaterials" Alternatives to Laboratory Animals 21(1):97-101
    "The cell culture method proved to be very effective for testing both the cytotoxicity and the cytocompatibility of new synthetic materials..."
72. Schambye, Hans T.; Pedersen, Fritz B. and Wang, Palle 1992-04-01 "Cytotoxicity of continuous ambulatory peritoneal dialysis (CAPD) solutions. A biocompatibility study involving human polymorphonuclear granulocytes rather than laboratory animals" Alternatives to Laboratory Animals 20(2):275-279
73. Spearow, Jimmy L.; Doemeny, Paul; Sera, Robyn; Leffler, Rachael and Barkley, Marylynn 1999-08-20 "Genetic variation in susceptibility to endocrine disruption by estrogen in mice" Science 285(5431):1259-1261 https://dx.doi.org/10.1126/science.285.5431.1259
    "Large (more than 16-fold) differences in susceptibility to disruption of juvenile male reproductive development by 17-estradiol (E2) were detected between strains of mice...Product safety bioassays conducted with animals selected for fecundity may greatly underestimate disruption of male reproductive development by estradiol and environmental estrogenic compounds."
74. Sperling, Frederick and McLaughlin, Joseph L. 1976-07-01 "Biological parameters and the acute LD50 test" Journal - Association of Official Analytical Chemists 59(4):734-736 https://www.ncbi.nlm.nih.gov/pubmed/939738
75. Valentino, Matteo; Monaco, Francesca; Pizzichini, Maria Antonietta and Governa, Mario 1993-01-01 "The use of the vitality and chemotaxis of human polymorphonuclear leukocytes for the in vitro estimation of the acute toxicity of the first ten chemicals from the

MEIC list" Alternatives to Laboratory Animals 21(1):73-80
“Spearman rank correlation analysis revealed significant correlations between our results and those from in vitro experiments conducted in other laboratories, as well as with data concerning mouse, rat and human lethal doses.”

76. Van Heijst, A.N.P. 1991-02-01 "The advancement of the science of clinical toxicology" Veterinary and Human Toxicology 33(1):43-44 https://www.ncbi.nlm.nih.gov/pubmed/2017865
77. Vince, Gaia 2006-04-05 "Drug trial horror – the official interim report" New Scientist https://www.newscientist.com/article/dn8956-drug-trial-horror-the-official-interim-report/
78. Weiss, Rick 1988-01-16 "Test tube toxicology" Science News 133(3):42-45 https://web.a.ebscohost.com/ehost/detail/detail?vid=2&sid=1e314573-cca8-47c4-b51a-d966c3af7545%40sessionmgr4007&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3d%3d#AN=8845000&db=a9h
79. Werner, B. 1983-01-01 "The use of acute toxicity data in poison information centres" Acta Pharmacologica et Toxicologica 52(s2):263-268 https://dx.doi.org/10.1111/j.1600-0773.1983.tb02694.x
80. Zbinden, G. and Flury-Roversi, M. 1981-04-01 "Significance of the LD50-test for the toxicological evaluation of chemical substances" Archives of Toxicology 47(2):77-99 https://doi.org/10.1007/bf00332351