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Progress Toward Replacing Animals in Toxicity Testing for Cosmetics

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This paper is submitted to satisfy the course requirement for Food and Drug Law.

Abstract: In the 1980's, animal rights activists successfully motivated the cosmetic industry to begin researching alternatives to animal tests. The European Union has taken action to stimulate development and validation of alternatives to animal testing through the Sixth and Seventh Amendments to the Cosmetics Directive. In this paper, I will briefly describe the history of the search for alternatives to animal testing for cosmetics. I will then discuss the progress that has been made toward developing and validating replacement alternatives in the eleven toxicological safety testing areas; alternatives are needed in all eleven areas in order for animal tests to be totally replaced.

Introduction

For many years, European and American consumers have expressed moral concerns over animal testing of cosmetics.¹ The political momentum against animal testing gained publicity and visibility in May 1980 when 300 people, some dressed in bunny costumes, demonstrated outside Revlon's New York offices.² In response to continued protests and political pressure, Revlon gave a \$250,000 per year grant to Rockefeller University to research alternatives to animal testing.³ The protestors then moved onto Avon and those protests resulted in the creation of the Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University.⁴ Many other cosmetic companies also responded to consumer concerns and protests by investing

¹An Overview of Animal Testing Issues, The Humane Society of the United States, at 8, available at http://www.hsus.org/web-files/PDF/ARI/ARIS_An_Overview_Of_Animal_Testing_Issues.pdf. (“The use of animals to test drugs and other therapeutic agents has the support of a majority of the American public, but there is much less support for the animal testing of products that are deemed less essential, such as cosmetic or household cleaning products. For example, 60 percent of a sample of 1,000 American adults opposed the use of animals in cosmetics testing, compared to 43 percent and 20 percent opposing animal testing of over-the-counter medicines and prescription drugs respectively (Ward, 1990). About 90 percent of the sample said they would purchase cosmetics that had not been tested on animals.”); EBRA Bulletin, European Biomedical Research Association, Jan. 1996, available at, <http://www.ebra.org/bulletin/jan04.96.html#1> (Animal rights and animal welfare groups in the UK have advocated for a ban on the use of animals in the testing of cosmetics and cosmetic ingredients since 1987 and the movement spread throughout Europe in 1990.).

²Henry Spira, Fighting to Win, in In Defense of Animals 194 (Peter Singer ed., Basil Blackwell 1985), available at, <http://www.animal-rights-library.com/texts-m/spira01.htm>.

³Id.

⁴Id.

significant funds into researching alternatives to animal testing.⁵

In 1993, the European Parliament took legislative action to aide and encourage the search for alternatives to animal testing for cosmetics by adopting the Sixth Amendment to the Cosmetics Directive (the Cosmetics Directive was adopted in 1976 to regulate the manufacture and sale of cosmetic products in the European Union).⁶ The Sixth Amendment banned the marketing of cosmetics containing ingredients that had been tested on animals starting January 1, 1998.⁷ To facilitate the development and validation of alternatives, the European Union created the European Centre for the Validation of Alternative Methods (ECVAM).⁸ ECVAM accepted a tremendous task under the Sixth Amendment because the 1998 marketing ban would be postponed if satisfactory alternatives to animal tests had not been validated by the beginning of 1997.⁹ The United States followed Europe in 1997 by creating the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and its supporting center the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to research and validate alternatives to animal testing.¹⁰

Despite these efforts, the European Parliament was not satisfied that alternatives were being developed fast enough.¹¹ Therefore, in 2003, the European Parliament adopted the Seventh Amendment to the European

⁵Id.; Carmen Fleetwood, In Vitro Testing is Coming to Aid, If Not Yet Succeed, the Guinea Pig, Wall Street Journal, Sept. 19, 2004, at B.2F (For example, Proctor and Gamble has invested more than \$170 million on alternatives to animal testing over the past two decades and continues to spend \$10-13 million per year on alternatives.).

⁶Azalea P. Rosholt, The Seventh Amendment Directive – An unnecessary measure to a necessary end – Possible legal challenges to directive 2003/15/EC, 60 Food Drug L.J. 421, 424-25 (2005); EBRA Bulletin, supra note 1.

⁷Id.

⁸Alison Abbott, More than a Cosmetic Change, Nature, Nov, 10, 2005, at 144.

⁹EBRA Bulletin, supra note 1 (quoting the specific wording of the Sixth Amendment to the Cosmetics Directive: “If there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular in those cases where alternative methods of testing, despite all reasonable endeavors, have not been scientifically validated as offering an equivalent level of protection, for the consumer, taking into account [Organisation for Economic Co-operation and Development] OECD toxicity guidelines, the Commission shall, by 1st January 1997, submit draft measures to postpone the date of implementation of this provision, for a sufficient period, and in any case for no less than two years. . .”).

¹⁰Overview, ICCVAM, available at <http://iccvam.niehs.nih.gov/about/overview.htm>; Animal Testing, U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, June 9, 2005, available at, <http://www.cfsan.fda.gov/~dms/cos-205.html>.

¹¹Rosholt, supra note 6, at 429 (stating “the Seventh Amendment attempts to legislate an accelerated development of

Union Cosmetics Directive.¹² The Seventh Amendment phases in a ban on the sale of cosmetic products tested on animals and a ban on the sale of cosmetic products containing ingredients tested on animals.¹³ Although the Seventh Amendment has been criticized for creating unreasonable deadlines¹⁴ and for being unnecessary,¹⁵ it has provided a catalyst for the development and validation of alternatives to animal testing for cosmetics.¹⁶ In this paper, I will discuss the progress that has been made toward developing and validating alternatives to animal testing.

Legal Rules for Cosmetics Safety Assessment

Consumers in the United States and Europe have come to expect the cosmetic products they use on a daily basis to be safe.¹⁷ Despite consumer beliefs, American laws do not inspire much confidence regarding cosmetic product safety because the Food Drug and Cosmetic Act of 1938 does not require that cosmetic manufacturers or marketers test their products for safety.¹⁸ However, the FDA does strongly urge cosmetic

alternative testing methods for the cosmetic industry”).

¹²Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003: Amending Council Directive 76/768/EEC on the 66 Official Journal of the European Union 26 (2003), available at <http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/L066/L06620030311en00260035.pdf>; See also The European Cosmetic Directive 76/768/EEC – 7th Amendment, available at <http://www.a-t-c.org.uk/pages/index.cfm?FuseAction=ShowPage&sec=6&page=191>.

¹³New methods replace animal testing for cosmetics, European Innovation, March, 2005, available at <http://aoi.cordis.lu/article.cfm?article=1502>. (Specifically, the Directive provides for a ban on animal testing of finished products (applicable from September 11, 2004), and a complete ban on animal testing of cosmetic ingredients as soon as alternative methods are validated and adopted by EU legislation. The final deadline of March 11, 2009 applies even if alternative tests are not available yet. A marketing ban on animal-tested products also applies, from September 11, 2004 or as soon as alternative methods are validated and adopted and again by March 11, 2009 at the latest, with the exception of three study areas where development of alternatives will unavoidably take longer – alternative tests for repeated dose toxicity, reproductive toxicity and toxicokinetics need not be introduced until March 11, 2013 (and perhaps later if technical problems arise).)

¹⁴Rosholt, supra note 6, at 429; Geoff Meade, Animal Tests Ban Deadline ‘Unrealistic’, The Press Association, Sept. 21, 2004 (“experts say the timetable is unrealistic and that it is not feasible to replace animal testing with other techniques within 10 years”).

¹⁵Rosholt, supra note 6, at 446 (“The Experimental Animals Protection Directive includes a recommendation to encourage the development of validated reduction and refinement alternatives across the board for all industries in addition to full replacement alternatives. . .”, thus making the Seventh Amendment redundant legislation).

¹⁶See Abbott, supra note 8, at 144-46.

¹⁷Rosholt, supra note 6, at 421 (“No one expects to be blinded or disfigured by a cosmetic product, even if the product is misused”).

¹⁸U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, Cosmetic Handbook

manufacturers to conduct whatever tests are appropriate to substantiate the safety of their cosmetics and if the safety of a cosmetic is not adequately substantiated, then the product may be considered misbranded unless the label states: “Warning – The safety of this product has not been determined.”¹⁹ Although American cosmetics safety laws are weak, this labeling requirement “is likely to persuade manufacturers to conduct [safety] testing to avoid such labelling [sic]” and the industry has adopted a self-regulation program, which reviews the safety of cosmetic ingredients.²⁰

Europeans, on the other hand, have legal authority to expect cosmetic products to be safe because Article 2 of the European Union Cosmetics Directive declares: “A cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use...”²¹ Moreover, Article 7a. 1(d) states that when assessing the safety of the cosmetic product, the manufacturer shall consider the general toxicological profile of the ingredients, their chemical structure and their level of exposure.²²

Europe has identified eleven toxicological testing areas that a cosmetic must pass to be considered safe.²³ Thus, to comply with European safety laws and the Seventh Amendment, alternatives to animal testing must be validated in the following areas: 1) Acute toxicity; 2) Skin Irritation and corrosion; 3) Eye irritation; 4) skin sensitization; 5) Skin absorption and penetration; 6) Subacute and subchronic toxicity; 7) Genotoxicity and mutagenicity; 8) UV-induced toxic effects; 9) Toxicokinetics and metabolism; 10) Carcinogenicity; and

(1992), available at, <http://www.cfsan.fda.gov/~dms/cos-hdb1.html>.; But cf. Simon Pitman, FDA launches electronic registration system for cosmetic formulations, Decision News Media, Dec. 2, 2005, available at, <http://www.cosmeticsdesign.com/news/ng.asp?id=64303-fda-vcrp-regulation> (discussing the FDA’s recently launched Voluntary Cosmetic Registration Program which aims to enhance the safety labeling of cosmetic products).

¹⁹21 C.F.R. 740.10 (1975).

²⁰Ian Indans, The use and interpretation of in vitro data in regulatory toxicology: cosmetics, toiletries and household products, 127 Toxicology Letters 177 (Feb. 28, 2002); Judith E. Foulke, Cosmetic Ingredients: Understanding the Puffery, FDA Consumer, May 1992, available at, <http://www.fda.gov/fdac/reprints/puffery.html> (Cosmetic Ingredient Review (CIR) is sponsored by the Cosmetic Toiletry and Fragrance Association).

²¹European Commission, The Rules Governing Cosmetic Products in the European Union (1999), available at, <http://www.leffingwell.com/cosmetics/vol1.en.pdf>; Marleen Pauwels & Vera Rogiers, Safety Evaluation of Cosmetics in the EU: Reality and challenges for the toxicologist, 151 Toxicology Letters 7 (June 15, 2004).

²²Id.

²³Pauwels & Rogiers, supra note 21.

11) Reproductive and developmental toxicity.²⁴

The European cosmetic safety requirements coupled with the approaching Seventh Amendment deadline has stimulated a frenzy of European efforts toward validating non-animal toxicological safety tests.²⁵ The work researching and validating alternatives that has been done in Europe under the requirements of the European Union “has had a spillover effect in the United States, where the issue [of finding alternatives to animal testing] had otherwise lost some momentum in the 1990’s.”²⁶ In fact, once an alternative method is validated by ECVAM, it is typically put under an expedited review process by ICCVAM for validation in the United States.²⁷

The Process of Developing and Validating Alternative Toxicological Tests

Toxicological tests evaluate the effects of chemical exposure on the health and morality of living creatures.²⁸ Toxicity tests have traditionally been performed on animals despite admission that “Most animal tests over or underestimate toxicity, or simply don’t mirror toxicity in humans very well.”²⁹ Moreover, non-animal toxicity tests must go through a rigorous validation process to prove that the test accurately predicts a specific effect in humans, even though the animal tests traditionally relied upon never underwent any scientific validation process.³⁰ The validation process

²⁴New Methods Replace Animal Testing for Cosmetics, *supra* note 13.

²⁵See Abbott, *supra* note 8, at 145-46.

²⁶An Overview of Animal Testing Issues, *supra* note 1, at 18.

²⁷Megan Erin Gallagher, Toxicity Testing Requirements, Methods and Proposed Alternatives, 26 *Environ. L. & Pol’y J.* 253, 265 (2003).

²⁸*Id.* at 253; Stacy E. Gillespie, Animal Testing A Cover-Girl Face Does Not Have to Begin with Animal Cruelty: Chapter 476 Gives Legal Force to Methods, 32 *McGeorge L. Rev.* 461, 463 (2001).

²⁹Abbott, *supra* note 8, at 144-45; Accord Gallagher, *supra* note 27, at 254 (stating “Many animal tests have led to results that are inaccurate in humans, and some have led to death and deformity caused by products that initially appeared to be nontoxic to nonhumans.”); Gillespie, *supra* note 28, at 464-65.

³⁰Abbott, *supra* note 8, at 145; An Overview of Animal Testing Issues, *supra* note 1, at 8.

typically requires testing a new protocol in three or four different external laboratories. The test chemicals are analysed by personnel who do not know the compounds' identities. If a test proves reproducible, it is sent for peer review by ECVAM's Scientific Advisory Committee, whose members include scientists, representatives from all member states of the European Union and relevant industrial and animal-welfare groups. Once approved by the committee, the test is adopted by the European Chemical Bureau, also located in Ispra, and then sent to the [Organization of Economic Co-operation and Development] OECD for the all-important global validation.³¹

Researchers developing alternative test methods follow the Three Rs: reduction, refinement, and replacement, advocated by the British researchers Russell and Burch in their 1959 book The Principles of Humane Experimental Technique. Reduction occurs when new methods allow the use of fewer animals. Refinement occurs when a method is changed to minimize the animals' pain and distress. Replacement occurs when a new method or new methods are able to take the place of an animal test entirely.³³ Replacement is the ultimate goal of animal rights advocates;³⁴ and under the Seventh Amendment to the Cosmetics Directive, replacement tests are the only acceptable alternatives.³⁵ Therefore, I will focus my attention on the progress being made in validating replacement alternatives to animal testing for cosmetics.

The Cosmetic Industry Has Already Done Some of the Leg-Work

Animal rights conscious cosmetic companies³⁶ have been developing and implementing alternative safety assessment methods for years (in addition to giving grants to outside researchers).³⁷ These companies have been conducting "in house validation" studies by comparing the results obtained through alternative methods

³²History of Alternatives, Altweb, available at, <http://altweb.jhsph.edu/history.htm>.

³³See Id.

³⁴Karen Lee Stevens, Animal Testing Alternatives, All For Animals (2000), available at, <http://www.allforanimals.com/alternatives1.htm>.

³⁵Rosholt, supra note 6, at 446.

³⁶Cosmetic companies are also economically motivated to develop and implement non-animal tests because the cost of animal upkeep in laboratories can be expensive. See Fleetwood, supra note 5.

³⁷Tony Vinas, P&G Seeks Alternatives to Animal Tests, Industry Week, July 2004; Jacques LeClaire & Odile de Silva, Industry Experience with Alternative Methods, 102-103 Toxicology Letters 575 (Dec. 28, 1998) (L'OREAL has been using alternative methods for almost 30 years).

to historical data from *in vivo* animal tests.³⁸

When ECVAM began conducting formal validation studies of alternative methods under the instruction of the European Union, the cosmetic companies eagerly submitted their testing techniques for consideration: “The cosmetics industry is committed to develop and help validate alternative safety tests of particular relevance to cosmetics.”³⁹ For example, when ECVAM scheduled a prevalidation study of alternative methods to test skin irritation, L’Oreal proposed a prediction model based on the assessment of cutaneous irritancy of chemicals on EPISKIN, a reconstructed epidermis model.⁴⁰ As a result of this coordinated effort, ECVAM validated EPISKIN as an alternative method for testing skin corrosion.⁴¹ The cosmetic industry also has wide experience in the field of percutaneous absorption which it shared and discussed with regulatory bodies.⁴²

Validated Alternatives That Replace Animal Tests for Cosmetics

As of November 2005 a total of seventeen alternative tests had been validated by ECVAM.⁴³ However, only eleven use *in vitro*⁴⁴ methods and thus replace *in vivo* tests; the remaining six validated alternatives refine *in vivo* tests to reduce the number of animals used, but do not replace animals.⁴⁵ An additional forty alternative tests are currently going through the peer review stage of validation with even more alternative

³⁸LeClaire & de Silva, supra note 37.

³⁹Id.; Accord New Methods Replace Animal Testing for Cosmetics, supra note 13 (quoting Dr. Thomas Hartung of ECVAM as saying “Many [cosmetic companies] are interested in participating [in the validation of alternative methods] at an early stage because they will be able to use these innovative methodologies for predictive testing, agent discovery and agent profiling even before the tests become mandatory. They promise higher throughput, lower costs and better public acceptance – and thus offer real market advantages.”).

⁴⁰Id.

⁴¹Michael K. Robinson, John P. McFadden & David A. Basketter, Validity and Ethics of the Human 4-h Patch Test to Assess Acute Skin Irritation, 45 Contact Dermatitis 1 (July 2001), citing J.H. Fentem et al., The ECVAM International Validation Study on In Vitro Tests for Skin Corrosivity, 12 Toxicology In Vitro 483 (1998).

⁴²LeClaire & de Silva, supra note 37.

⁴³Abbott, supra note 8, at 145 (it is not clear that all seventeen validated tests can be used as alternatives for the cosmetic industry rather than alternatives for non-cosmetic products).

⁴⁴“*In vitro* testing is defined as ‘a biological process made to occur in a laboratory vessel or other controlled experimental setting rather than within a living organism. . . .’” Gillespie, supra note 28, at 467, quoting Random House Unabridged Dictionary 1004 (2d. ed, 1993).

⁴⁵Gillespie, supra note 28, at 467.

testing methods to come.⁴⁶ As these statistics reveal, *in vitro* methods have been the most successful source of replacement tests validated to date. In this section, I will discuss the current validation status and future prospects in each of the eleven toxicological testing areas outlined above. I will describe the alternative tests in the areas of skin corrosion and irritancy, eye irritation, skin absorption and penetration, and UV-induced toxicity in greater detail because these are the areas where alternatives are more fully developed and the most progress has been made toward total animal replacement.

Acute Toxicity

The traditional method of testing acute toxicity is the Lethal Dose 50 test (LD50).⁴⁷ The LD50 is used to find the lethal dose for 50% of the sample of animals tested.⁴⁸ During the test period the animal is forced to inhale, ingest or is exposed in some other way (such as intravenous or dermal exposure) to the test substance.⁴⁹ Despite long-term research efforts, no replacement alternatives have been validated by ECVAM or ICCVAM.⁵⁰ The LD50 has been replaced by less traumatic, but still fatal alternatives.⁵¹

The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) has been working on *in vitro* alternatives to acute toxicity tests since 1989.⁵² MEIC has found that *in vitro* human cell lines can predict acute toxicity in humans for most chemicals tested.⁵³ MEIC narrowed their battery of tests down to the most predictive four

⁴⁶Id. (it is not clear how many of these alternatives are replacement alternatives and it is unclear how many of them can be used as alternatives for the cosmetic industry).

⁴⁷John McArdle, The Search for Alternatives in Cosmetic Testing, American Anti-Vivisection Society, available at, <http://www.sniksnak.com/aavs4.html>; Joanne Zurlo, Deborah Rudacille & Alan M. Goldberg, Animal and Alternatives in Testing: History, Science, Ethics, Johns Hopkins University Center for Alternatives to Animal Testing (2002), available at, http://caat.jhsph.edu/pubs/animal_alts/animal_alts.htm.

⁴⁸Zurlo, Rudacille & Goldberg, *supra* note 47; Spira, *supra* note 2.

⁴⁹Gallagher, *supra* note 27, at 258; An Overview of Animal Testing Issues, *supra* note 1, at 15.

⁵⁰In Vitro Methods for Assessing Acute Systemic Toxicity, ICCVAM, available at, <http://iccvam.niehs.nih.gov/methods/invitro.htm>.

⁵¹McArdle, *supra* note 47.

⁵²Id. (MEIC created 61 *in vitro* assays for assessing acute toxicity); See also Laura Gribaldo et al., Acute Toxicity, Report for Establishing the Timetable for Phasing Out Animal Testing for the Purpose of the Cosmetics Directive, available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/1%20Acute%20Tox.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/1%20Acute%20Tox.pdf) (describing the numerous *in vitro* methods for testing acute toxicity); The MEIC Program, available at, <http://www.cctoxconsulting.a.se/meic.htm>.

⁵³McArdle, *supra* note 47.

in vitro tests and they recommend using the four tests in combination to test products for acute toxicity.⁵⁴

Many of MEIC's methods are considered prevalidated even though it is uncertain how much longer it will take for ECVAM and ICCVAM to formally validate these methods.⁵⁵

In addition to MEIC's research, project A-cute-tox is currently working to develop a simple and robust *in vitro* testing strategy for predicting acute oral toxicity in humans.⁵⁶ If this project is successful, it could totally replace animal acute toxicity tests:

the time estimated to achieve complete animal replacement for acute toxicity is strongly dependent on the outcome of this project [A-cute-tox], which represents the first attempt to create an integrated strategy to be validated with the purpose to predict human systemic toxicity, and it cannot be less than 10 years.⁵⁷

Currently, ECVAM and ICCVAM's supporting center NICEATM are jointly working on the *In Vitro* Cytotoxicity Validation Study seeking to validate two *in vitro* basal cytotoxicity assays proposed as alternatives to *in vivo* acute toxicity alternatives.⁵⁸ The two methods under evaluation are the neutral red uptake assay using mouse cell lines and the neutral red uptake assay using human cells.⁵⁹ However, these two alternatives will only be used to predict starting doses for *in vivo* acute toxicity tests; thus, they are not total replacement alternatives.⁶⁰

Skin Irritation and Corrosion

The traditional method of testing skin irritation and corrosion is the Draize rabbit skin irritation test.⁶¹ The

⁵⁴Id.

⁵⁵Gribaldo et al., supra note 52.

⁵⁶Id.; Sandra Coecke et al., The value of alternative testing for neurotoxicity in the context of regulatory needs, *Environmental Toxicology & Pharmacology* (2005), available at, <http://caat.jhsph.edu/dnt/background/Coecke%20et%20al.pdf>; Simon Pitman, EU pushes for alternatives to animal testing, *DECISION MEDIA NEWS*, Aug. 24, 2005, available at, <http://www.cosmeticsdesign-europe.com/news/ng.asp?n=62071-ecvam-animal-testing>.

⁵⁸*In Vitro* Methods for Assessing Acute Systemic Toxicity, supra note 50.

⁵⁹Id.

⁶⁰Id.

⁶¹Robinson, McFadden & Basketter, supra note 41.

Draize skin irritancy test is performed by shaving the rabbit and then abrading the skin by pressing adhesive tape against the skin and quickly stripping it off until several layers of skin are removed.⁶² Although the Draize test has been criticized for not being able to reliably predict human skin reactions, it has been used for over fifty years.⁶³

The Transcutaneous Electrical Resistance (TER) Assay, an *in vitro* test for assessing the corrosivity of chemicals, was validated by ECVAM in March 1998 (and by ICCVAM in May 2004 after expedited review).⁶⁴ The TER Assay evaluates corrosivity based on the observation that corrosive substances reduce the electrical resistance of the skin.⁶⁵ The TER Assay only reveals whether the substance is corrosive or not; it does not discriminate between levels of corrosiveness.⁶⁶ Although the TER Assay is considered a replacement alternative, it uses rat skin cells and does involve killing animals to obtain fresh skin cells.⁶⁷

EpiDerm and EPISKIN⁶⁸ are three dimensional *in vitro* tissue cultures of human skin used to test chemical corrosivity.⁶⁹ They were validated by ECVAM in March 1998 (and by ICCVAM in May 2004 after expedited review).⁷⁰ To administer the test, a chemical is applied to the tissue cultures and cell death is recorded

⁶²Animals in Product Testing: Animal Tests, National Anti-Vivisection Society, available at, <http://www.navs.org/site/PageServer?pagename=ain.pt.animal.tests>; Gallagher, supra note 27, at 258.

⁶³Animals in Product Testing: Animal Tests, supra note 62; Robinson, McFadden & Basketter, supra note 41.

⁶⁴An Overview of Animal Testing Issues, supra note 1, at 20; Dermal Corrosivity Assays: EPISKIN, EpiDerm and the Rat Skin Transcutaneous ICCVAM, available at <http://iccvam.niehs.nih.gov/methods/epiderm.htm>.

⁶⁵An Overview of Animal Testing Issues, supra note 1, at 20.

⁶⁶Indans, supra note 20.

⁶⁷An Overview of Animal Testing Issues, supra note 1, at 20 (noting that although rats must be killed for the TER Assay it reduces the number of animals needed per chemical from 3 to 1 and it is a refines traditional corrosiveness tests because the animals are not exposed to any pain or distress from the corrosivity test).

⁶⁸Corrositex is another artificial skin *in vitro* test validated by ICCVAM and ECVAM to test skin corrosivity of acids, bases and acid derivatives, but since Corrositex is not as sensitive as EPISKIN or EpiDerm, it does not completely replace animal skin corrosivity tests. Instead, Corrositex is used to weed out highly corrosive substances, but if a substance passes Corrositex, it is still tested on an animal. Gallagher, supra note 27, at 269-70; Indans, supra note 20.

⁶⁹Gallagher, supra note 27, at 269.

⁷⁰An Overview of Animal Testing Issues, supra note 1, at 21; Dermal Corrosivity Assays: EPISKIN, EpiDerm and the Rat Skin Transcutaneous supra note 42.

throughout a defined exposure period.⁷¹ The validation studies revealed that EPISKIN is useful for testing cosmetics, sunscreens and other topical products.⁷² EpiDerm and EPISKIN are superior to the TER Assay because they can discriminate between level of corrosiveness and because no animal must be killed to perform the test.⁷³

Currently, no alternative tests are validated for testing skin irritation.⁷⁴ However, EpiDerm is undergoing validation studies and is expected be approved as an alternative test for skin irritation in the near future.⁷⁵ Others believe that skin irritancy testing is safe and ethical to conduct on humans once a product has been determined non-corrosive using *in vitro* methods.⁷⁶ For example, the 4-h human patch test has been shown to be low in risk; subjects experience mild to moderate reactions at the site of exposure and recover quickly.⁷⁷ To administer the test, a small amount of the test substance is applied to a patch placed against to upper outer arm of the human subject.⁷⁸ After the exposure period, the patch is removed and the irritation response is measured.⁷⁹

Eye Irritation

The traditional eye irritation test, called the Draize eye irritation test was developed by the FDA after a series of reports that women were suffering permanent eye injuries from cosmetic products.⁸⁰ One woman even went blind after eyelash dye destroyed her eyeballs.⁸¹ The Draize eye irritation test is performed by

⁷¹Gallagher, supra note 27, at 269.

⁷²An Overview of Animal Testing Issues, supra note 1, at 21.

⁷³Indans, supra note 20 (Human skin equivalence models discriminate between R34 (causes burns) and R35 (causes severe burns))

⁷⁴Id. (noting that although no skin irritation tests have been validated, if a product or ingredient fails the *in vitro* corrosivity tests it is not testing on animals); Thomas Welss, David A. Basketter & Klaus R. Schroder, In vitro skin irritation: facts and future. State of the art review of mechanisms and models, 18 *Toxicology In Vitro* 231 (2004).

⁷⁵Fleetwood, supra note 5; See also New Methods Replace Animal Testing for Cosmetics, supra note 13.

⁷⁶Robinson, McFadden & Basketter, supra note 41; McArdle, supra note 47.

⁷⁷Robinson, McFadden & Basketter, supra note 41.

⁷⁸Id.

⁷⁹Id.

⁸⁰Abbott, supra note 8, at 144; Gillespie, supra note 28, at 464.

⁸¹Abbott, supra note 8, at 144, Rosholt, supra note 6, at 421.

placing the test substance in one eye of a rabbit, without local anesthetic, while the other eye is used as a control.⁸² Clips are placed on the eyelids to hold the eyes open and to keep the animals from blinking the product away and the animals are placed in restraining stocks to prevent them from moving during the test period.⁸³ Eye irritation levels are then observed over several days with maximum irritation being total destruction of the eye.⁸⁴

The Draize eye irritation test was the test that prompted the animal rights activists to protest Revlon and other cosmetic companies dressed as bunnies.⁸⁵ Thus, when CAAT was created, their primary mission was to discover an alternative to this test.⁸⁶ However, thus far neither ECVAM nor ICCVAM have validated a non-animal eye irritancy test.⁸⁷ Reductions and refinements of *in vivo* tests have been implemented, but these changes have not eliminated the need to use live animals.⁸⁸ Despite the slow progress, many eye irritancy replacement alternatives are near final validation by ECVAM and ICCVAM and alternatives are already being used in the industry.⁸⁹ It is unlikely that a single *in vitro* test will be able to replace the Draize eye test, but a battery of *in vitro* alternatives used together can probably replace *in vivo* eye irritancy tests in the near future.⁹⁰

Several *in vitro* techniques use isolated eyes, corneas and lenses freshly obtained from slaughterhouses or

⁸²An Overview of Animal Testing Issues, supra note 1, at 11.

⁸³Animals in Product Testing: Animal Tests, supra note 62.

⁸⁴An Overview of Animal Testing Issues, supra note 1, at 11.

⁸⁵Id.; Spira, supra note 2.

⁸⁶An Overview of Animal Testing Issues, supra note 1, at 12.

⁸⁷McArdle, supra note 47; Indans, supra note 20.

⁸⁸Eskes, et al., Eye Irritation, 33 Alternatives to Laboratory Animals 47 (2005) (for example, the low volume eye test (LVET) uses less test substance in the Draize eye test to reduce the pain endured by the rabbit, but this refinement method has not yet been validated).

⁸⁹In Vitro Test Methods for Detecting Ocular Corrosives and Severe Irritants, ICCVAM, November 2005, available at, <http://iccvam.niehs.nih.gov/methods/eyeirrit.htm>; New Methods Replace Animal Testing for Cosmetics, supra note 13 (stating that there are 14 *in vitro* alternatives being used in the industry or being developed); Fleetwood, supra note 5.

⁹⁰Eskes, et al., supra note 88, at 68.

from animals used in other toxicological studies.⁹¹ The test substance is administered to the eye and the eye is observed for toxic reaction at any level.⁹² For example, the Bovine Corneal Opacity and Permeability (BCOP) Assay is used by several cosmetic companies and has been shown well suited for identifying substances which are moderately and severely irritating to the eye.⁹³ But, the BCOP assay has not proven as sensitive in distinguishing among mild irritants; and therefore, it is suggested that the BCOP assay be used in combination with other tests.⁹⁴ The Isolated Rabbit Eye (IRE) test and the Chicken Enucleated Eye Test (CEET) have both only proven useful for identifying severely irritating substances.⁹⁵ Although the isolated eye methods are considered replacement alternatives, animal rights activists have two problems with them: (1) live animals are still needed to test for mild irritation once a substance passes these tests and (2) animals must be killed to perform these tests.⁹⁶

Another method for testing eye irritation is the Hen's Egg Test on the Chorio-allantoic Membrane (HET-CAM) Assay which exposes the chorio-allantoic membrane of fertilized chicken eggs to test chemicals.⁹⁷ The test is performed before the fertilized egg has developed nerve tissue and therefore is believed to cause no pain.⁹⁸ To perform the test, the test substance is applied to the membrane and then the membrane is observed over a 5-minute period for hemorrhage, lysis and coagulation.⁹⁹ This test has been found useful for identifying mild and non-irritating substances and because of its limited range it is recommended for use in conjunction with other eye irritancy tests.¹⁰⁰ The Chorio-allantoic Membrane Vascular Assay (CAMVA) and the Chorio-allantoic Membrane: Trypan Blue Staining (CAM-TB) Test are related methods which can

⁹¹Id. at 48 (bovine, porcine, chicken or rabbit organs are all used).

⁹²Id. at 49.

⁹³Id. at 49-50; LeClaire & de Silva, supra note 37.

⁹⁴Id. at 50.

⁹⁵Id. at 51-52.

⁹⁶An Overview of Animal Testing Issues, supra note 1, at 13-14.

⁹⁷Id. at 13; Eskes, et al., supra note 88, at 53; McArdle, supra note 47.

⁹⁸Eskes, et al., supra note 88, at 53.

⁹⁹Id.

¹⁰⁰Id. at 54.

also be used in combination with other eye irritancy tests.¹⁰¹

Reconstructed human tissue models are *in vitro* methods that offer completely animal free replacements for testing eye irritancy.¹⁰² For example, EpiOcular is a multi-layered culture of human cells that mimics the structure of the human cornea.¹⁰³ EpiOcular is most often used to identify mild or moderate irritants, but can also identify potentially severe irritants (EpiOcular cannot differentiate between degrees of severe irritancy).¹⁰⁴ EpiOcular is near final validation¹⁰⁵ and is already being used by the cosmetics, personal care, household and industrial chemical industries.¹⁰⁶ Other human tissue models in the process of being developed and tested are the SkinEthic *In Vitro* Reconstituted Human Corneal Epithelium (HCE) Model and the Gillette HCE-T Tissue Construct Model.¹⁰⁷

Cell based cytotoxicity alternatives measure cell viability to predict eye irritancy.¹⁰⁸ For example, the Neutral Red Uptake (NRU) Assay measures the ability of a substance to inhibit the uptake of a dye marker of cell viability.¹⁰⁹ After exposing the cells to the test substance, the dye is applied and researchers observe how much dye the cells uptake; uptake is decreased if the cells have been altered by the test substance.¹¹⁰ The NRU is useful for identifying moderate to severely irritating substances and can be used in combination with *in vitro* methods able to test mild irritancy such as the HET-CAM.¹¹¹ The related Neutral Red Release (NRR) Assay is able to differentiate degrees of mildness in the mild to very mild range following brief, high concentration contact with a test substance.¹¹²

¹⁰¹Id. at 54-55.

¹⁰²McArdle, supra note 47.

¹⁰³Id.

¹⁰⁴Eskes, et al., supra note 88, at 56.

¹⁰⁵Fleetwood, supra note 5.

¹⁰⁶Eskes, et al., supra note 88, at 56.

¹⁰⁷Id. at 57.

¹⁰⁸Id. at 58; An Overview of Animal Testing Issues, supra note 1, at 13.

¹⁰⁹Eskes, et al., supra note 88, at 58.

¹¹⁰Id.

¹¹¹Id. at 58-59.

¹¹²Id. at 59- 60 (also discussing the Red Blood Cell (RBC) Haemolysis Test).

Cell function assays measure the effects of a test substance by observing changes in cell function.¹¹³ The Fluorescein Leakage (FL) Test assesses the effects of a test substance on an *in vitro* cell model of the corneal epithelium.¹¹⁴ The cells are exposed to the test substance and then tested for changes in permeability.¹¹⁵ The FL test has shown promise when used in combination with other non-animal eye irritation tests.¹¹⁶ The Silicon Microphysiometer (SM) Assay measures changes induced by chemical substances on metabolic activity of cells.¹¹⁷ The cells are grown in a pH sensitive chamber which causes the cells to release acidic metabolic products establishing a baseline metabolic rate.¹¹⁸ The cells are then exposed to the test substance and changes in the metabolic rate are measured.¹¹⁹ The SM assay has been found reliable for classifying certain types of substances as innocuous-mild, mild-moderate or moderate-severe.¹²⁰

The IRRITECTION assay is the updated protocol of the EYTEX system.¹²¹ This biochemical assay is based on the premise that eye irritation and corneal opacity from chemicals is the result of perturbation or denaturation of corneal proteins.¹²² To perform the test, changes to a protein solution are observed following exposure to the test substance.¹²³ However, IRRITECTION needs to undergo further research before it could be considered for validation.¹²⁴

Finally, the Pollen Tube Growth (PTG) Assay exposes pollen from tobacco plants to a test substance for 18 hours.¹²⁵ The mass of pollen tubes produced during this period is photometrically determined to ascertain the concentration of test substance that reduces pollen tube growth to 50% of the control.¹²⁶ This test was

¹¹³Id. at 61.

¹¹⁴Id.

¹¹⁵Id.

¹¹⁶Id.

¹¹⁷Id. at 62; An Overview of Animal Testing Issues, supra note 1, at 14.

¹¹⁸Eskes, et al., supra note 88, at 62.

¹¹⁹Id.

¹²⁰Id.

¹²¹Id. at 63; An Overview of Animal Testing Issues, supra note 1, at 14.

¹²²Eskes, et al., supra note 88, at 63.

¹²³Id.

¹²⁴Id. at 64.

¹²⁵Id.

¹²⁶Id.

shown to correlate well with the Draize eye irritation test for substances with very low to moderate irritation potentials.¹²⁷ However, more validation studies need to be performed on this method.¹²⁸

Skin Sensitization

Skin sensitization tests are used to identify potential allergic reactions to products; a serious safety consideration for cosmetic manufacturers.¹²⁹ The traditional method of testing skin sensitization involved exposing guinea pigs' skin to the substance, intentionally increasing the animals' sensitivity and observing the animals' reaction.¹³⁰ Currently, the only validated alternative is the Local Lymph Node Assay (LLNA), which also uses animals and requires those animals to be killed.¹³¹

Despite the fact that the LLNA is the only validated alternative for testing skin sensitization, *in vitro* reconstructed human skin models are being studied as potential replacement alternatives.¹³² Research teams in Europe have made progress with a human reconstructed epidermis model containing Langerhans cells (immunological cells in the skin).¹³³ Further research and refinement of the *in vitro* skin sensitization tests are needed and it is likely that more than one alternative sensitization test will be needed to replace animal tests considering the complexity of *in vivo* skin sensitization reactions.¹³⁴

In addition to *in vitro* research, *in silico* systems are being developed to test skin sensitization and validation is expected within five to six years.¹³⁵ *In silico* systems use computer models or computer simulations to

¹²⁷Id.

¹²⁸Id. at 65.

¹²⁹McArdle, supra note 47.

¹³⁰Id.

¹³¹Id. (the LLNA is both a reduction and a refinement alternative test, but not a replacement); Murine Local Lymph Node Assay (LLNA), ICCVAM, available at, <http://iccvam.niehs.nih.gov/methods/llna.htm>; An Overview of Animal Testing Issues, supra note 1, at 20.

¹³²McArdle, supra note 47.

¹³³R. Schmidt, C. Duval & M. Regnier, Reconstructed Human Skin: Alternative Models for the Safety and Efficacy Assessment of Dermato-Cosmetic Products, 27 Alternatives to Laboratory Animals 81 (1999) (abstract), available at, <http://www.invitroderm.com/abstracts/schmidt.html>; LeClaire & de Silva, supra note 37.

¹³⁴New Methods Replace Animal Testing for Cosmetics, supra note 13.

¹³⁵Id.

replace physical experiments.¹³⁶ *In silico* systems may never fully replace physical experiments, but new substances can be pre-screened using computer models and if the computer determines the substance is likely to be dangerous, then the substance can be rejected without further testing.¹³⁷

Skin Absorption and Penetration

The traditional animal test for assessing skin absorption and penetration is performed by applying the test substance to the surface of a rat's skin for a specified period of time.¹³⁸ The animal is then terminated and the amount of dose associated with the skin, carcass and in excreta is calculated.¹³⁹

In vitro alternatives using reconstructed skin models (similar to EpiDerm and EPISKIN) can also be used to test skin absorption and penetration.¹⁴⁰ Although these alternatives have not undergone validation by ECVAM or ICCVAM, OECD Guidelines already exist for using alternative *in vitro* skin absorption and penetration tests; based on OECD acceptance these methods can be assumed to be internationally accepted.¹⁴¹ To perform the *in vitro* skin absorption and penetration test, the test substance is applied to the reconstructed skin for a specified period of time.¹⁴² The receptor fluid, the fluid under the surface of the reconstructed skin, is then sampled to determine the mass (and possibly the rate) of absorption.¹⁴³ These *in vitro* alternatives are limited by the fact the reconstructed skin models cannot fully simulate the effects of peripheral blood flow, but "skin absorption is primarily a passive process and studies undertaken using appropriate *in vitro* experimental conditions have produced data for a wide range of chemicals that

¹³⁶Alternative Testing Methods: European research to replace and reduce animal tests, June 23, 2004, [available at, http://europa.eu.int/comm/research/press/2004/pr2306en.cfm](http://europa.eu.int/comm/research/press/2004/pr2306en.cfm).

¹³⁷Grant Buckler, A Different Kind of Laboratory Mouse, Nov. 20, 2004, [available at, http://www.digitaljournal.com/news/?articleID=4045](http://www.digitaljournal.com/news/?articleID=4045).

¹³⁸Guidance Document for the Conduct of Skin Absorption Studies: OECD Series on Testing and Assessment, March 2004, at 13, [available at, http://appli1.oecd.org/olis/2004doc.nsf/43bb6130e5e86e5fc12569fa005d004c/4ba210c177892390c1256e4e00379b6a/\\$FILE/JT00](http://appli1.oecd.org/olis/2004doc.nsf/43bb6130e5e86e5fc12569fa005d004c/4ba210c177892390c1256e4e00379b6a/$FILE/JT00)

¹³⁹Id.

¹⁴⁰Id.

¹⁴¹Timetables for the phasing-out of animal testing in the framework of the 7th Amendment to the Cosmetics Directive (Council Directive 76/7/ Commission of the European Communities (2004), at FN 6, [available at, http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/sec_2004_1210.en.p](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/sec_2004_1210.en.p)

¹⁴²Id.

¹⁴³Id.

demonstrate the usefulness of this method.”¹⁴⁴

Subacute and Subchronic Toxicity

For cosmetic products oral, dermal and inhalation subacute (28 days) and subchronic (90 days) repeated dose studies are important safety tests.¹⁴⁵ The currently accepted testing methods use rodents and there are no accepted or validated alternative methods for replacing *in vivo* tests.¹⁴⁶ Finding replacement alternatives represents an enormous scientific and technical challenge because of the complexity of the information collected from *in vivo* tests: “the eventual replacement of *in vivo* repeat-dose toxicity tests must involve some integration of *in vitro* data on target organ toxicity with *in vitro/in silico* data. . .”¹⁴⁷

Preliminary research has been done to develop *in vitro* models to test subacute and subchronic toxicity in the liver, kidneys, central nervous system, lungs and haematopoietic system.¹⁴⁸ And, the Predictomics project is currently working to develop *in vitro* systems capable of predicting long-term renal and hepatic toxicities in humans.¹⁴⁹ However, these alternatives are at the early stages of research and development and no alternative methods are yet available for actual implementation.¹⁵⁰ Moreover, “none of the *in vitro* models we currently have is ideal for any of the target organs and therefore some efforts should be put to optimise the models available and to look for good *in vitro* models in those cases w[h]ere fewer models are available (e.g. *in vitro* models to study lung toxicity).”¹⁵¹

Genotoxicity and Mutagenicity

¹⁴⁴Id. at 12.

¹⁴⁵Pilar Prieto et al., 3.6 Subacute and subchronic toxicity, in Report for Establishing the Timetable for Phasing Out Animal Testing for the Pur (2004), available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/6%20Subac-subchr%20tox.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/6%20Subac-subchr%20tox.pdf).

¹⁴⁶Id. at 2.

¹⁴⁷Id. at 2-3.

¹⁴⁸Id. at 4.

¹⁴⁹Coecke et al., supra note 56.

¹⁵⁰Prieto et al., supra note 145 at 11.

¹⁵¹Id. at 15.

Mutagenicity refers to a substance's ability to induce permanent mutations in the structure of genetic material.¹⁵² Genotoxicity refers to a substance's ability to interact with DNA.¹⁵³ Researchers are looking for three different things when they test a substance for genotoxicity and mutagenicity: gene mutations, clastogenicity and aneuploidy.¹⁵⁴ Currently, there is no single validated *in vitro* test that can provide information on all three endpoints; therefore, a battery of tests is needed to fully assess the genotoxic and mutagenic profile of a test substance.¹⁵⁵ Many of the *in vitro* methods for assessing mutagenicity and genotoxicity are accepted by regulatory authorities worldwide and commonly used by the cosmetic industry (in a standard battery of *in vitro* tests), but *in vivo* tests are still used as a back-up when needed.¹⁵⁶

The *in vitro* tests for mutagenicity and genotoxicity accepted by regulatory authorities and recommended for use as part of an *in vitro* battery of tests include: the Mammalian chromosome aberration test, the Bacterial reverse mutation test, the Mammalian cell gene mutation test, and the unscheduled DNA synthesis (UDS) in mammalian cells.¹⁵⁷ The *In vitro* mammalian micronucleus assay is also recommended for use as part of an *in vitro* battery of tests, but it has not yet been formally accepted by regulatory authorities.¹⁵⁸ The *In vitro* Comet assay is used by the cosmetic industry to test for DNA damage, but it has not been validated and is not currently undergoing any validation studies.¹⁵⁹

The *Saccharomyces Cerevisiae* gene mutation assay and the *Saccharomyces Cerevisiae* mitotic recombination assay are accepted by regulatory authorities, but are no longer recommended for use in the standard battery

¹⁵²Maurier et al., Genotoxicity/mutagenicity, in Establishment of Timetables for the Phasing out of Animal Experiments for Cosmetics, (2004), at 1, available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/7%20genotox-mutagen.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/7%20genotox-mutagen.pdf).

¹⁵³Id.

¹⁵⁴Id. at 2.

¹⁵⁵Id.

¹⁵⁶Id. at 3-4.

¹⁵⁷Id. at 6-11.

¹⁵⁸Id. at 13.

¹⁵⁹Id. at 14.

of tests.¹⁶⁰ The Sister chromatid exchange (SCE) assay in mammalian cells is another *in vitro* mutagenicity test that is rarely used today, but has been accepted by regulatory authorities.¹⁶¹

Three dimensional reconstructed skin models by SkinEthic and EpiDerm are used by the cosmetic industry to test for photomutagenicity, but they have not yet been validated for this purpose.¹⁶² Much more research is needed before any *in vitro* skin models will be validated to test for photomutagenicity.¹⁶³

Despite the numerous *in vitro* methods for testing mutagenicity and genotoxicity, mentioned above, *in vitro* tests are limited by the fact that they cannot show toxicokinetic or metabolic interactions.¹⁶⁴ Considering the slow progress in developing alternatives in toxicokinetics and metabolism (see below), it is unlikely that *in vivo* tests for genotoxicity and mutagenicity can be fully replaced in the near future.¹⁶⁵ Nevertheless, the current battery of *in vitro* tests is being used to dramatically reduce the number of *in vivo* tests needed.¹⁶⁶

UV-Induced Toxicity

The 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) was the first replacement test validated in Europe.¹⁶⁷ To perform the test, the test substance is added to an *in vitro* culture of skin cells which is then irradiated with ultraviolet (UVA) light (which simulates sunlight).¹⁶⁸ The rate at which cells die before

¹⁶⁰Id. at 8-9.

¹⁶¹Id. at 12.

¹⁶²Id. at 16.

¹⁶³See Id. at 16-17.

¹⁶⁴See Id. at 22.

¹⁶⁵Id. (estimating that full replacement will not be feasible within the next 12 years).

¹⁶⁶Id.

¹⁶⁷Abbott, *supra* note 8, at 146 (in overleaf “The Validation Game”); An Overview of Animal Testing Issues, *supra* note 1, at 21; LeClaire & de Silva, *supra* note 37, at 190.

¹⁶⁸Abbott, *supra* note 8, at 146 (in overleaf “The Validation Game”); McArdle, *supra* note 47; See also Manfred Liebsch et al., UV-induced effect, in Establishment of Timetables for the Phasing Out of Animal Experiments for Cosmetics (2004), available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/8%20UV%20induced%20effects.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/8%20UV%20induced%20effects.pdf) (for a more technical description of the method).

and after irradiation is monitored to assess phototoxicity.¹⁶⁹ ICCVAM has also determined that the 3T3 phototoxicity test is predictive of phototoxicity in humans and animals.¹⁷⁰ This method has been officially accepted for all types of products, not just cosmetics.¹⁷¹

The Red Blood Cell Phototoxicity Test (RBC PT) is an *in vitro* test that can identify photosensitizers and study the phototoxic mechanisms at the cellular level.¹⁷² This test provides information about photodynamic reactions which is useful in evaluating overall safety.¹⁷³ A testing strategy can start with the 3T3 NRU PT test and then use the RBC PT test to provide the photodynamic information not provided by the 3T3 NRU PT test.¹⁷⁴

EpiDerm, the reconstructed human skin model, is also being studied for its potential ability to test phototoxicity.¹⁷⁵ EpiDerm may be suitable for testing substances that cannot be tested using the 3T3 NRU PT Test.¹⁷⁶ It can also be used to further investigate substances which resulted in potentially false positives under the 3T3 NRU PT Test.¹⁷⁷ *In vitro* reconstructed skin models are already used by the industry, in combination with the other tests mentioned, to evaluate sunscreen efficacy and safety.¹⁷⁸

Toxicokinetics and Metabolism

Toxicokinetics and metabolism studies show researchers how a test substance is absorbed into an organism, distributed throughout the organism, metabolized and excreted.¹⁷⁹ Currently, research is being done

¹⁶⁹Id.

¹⁷⁰In Vitro 3T3 NRU Phototoxicity, ICCVAM, available at, http://iccvam.niehs.nih.gov/methods/3t3_nru.htm.

¹⁷¹McArdle, supra note 47.

¹⁷²Liebsch et al., supra note 168.

¹⁷³Id.

¹⁷⁴Id.

¹⁷⁵Id.; McArdle, supra note 47; Fleetwood, supra note 5.

¹⁷⁶McArdle, supra note 47.

¹⁷⁷Liebsch et al., supra note 168.

¹⁷⁸LeClaire & de Silva, supra note 37.

¹⁷⁹New Methods Replace Animal Testing for Cosmetics, supra note 13.

to develop both *in vitro* and *in silico* methods for assessing toxicokinetics and metabolism.¹⁸⁰ Although significant progress has been made, it is expected that complete replacement of *in vivo* tests will take over ten years.¹⁸¹

Researchers are using a three tiered strategy to ensure that alternatives are developed to provide all necessary information formerly obtained through animal tests.¹⁸² Tier 1 tests will be used to determine the likelihood that an organism will have systemic exposure to a test substance via the dermal, oral or inhalation routes.¹⁸³ Tier 2 tests will be used to determine the distribution of the compound after systemic exposure by developing ways to test plasma level, excretion, tissue distribution and metabolism.¹⁸⁴ Lastly, Tier 3 tests will be used to determine the overall potency of a compound.¹⁸⁵ Finding non-animal alternatives for testing excretion is proving the most difficult task, but it has been suggested that excretion testing is less important for cosmetics than for chemicals and pharmaceuticals.¹⁸⁶

Carcinogenicity

Carcinogenicity tests are rarely performed in the cosmetic industry, but non-animal alternatives to testing carcinogenicity are nevertheless being researched for use in testing other substances.¹⁸⁷ However, to date, no alternative tests have been validated and companies testing for carcinogenesis continue to use animals (typically rodents).¹⁸⁸ Moreover, because carcinogenesis is a complex, multi-step process, it is very difficult to build all the phases into a single *in vitro* model.¹⁸⁹ Several *in silico* systems and models for predicting

¹⁸⁰Id.; Sandra Coecke et al., Subgroup of Experts on Toxicokinetics and Metabolism in the context of the follow-up of the 7th Amendment on the Cosmetics Directive, in Report for Establishing the Timetable for Phasing out Animal Testing for the Purpose of the Cosmetics Directive (2004), available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/9%20Toxicokinetics%20and%20Metabolism.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/9%20Toxicokinetics%20and%20Metabolism.pdf).

¹⁸¹Id.

¹⁸²Id.

¹⁸³Id.

¹⁸⁴Id.

¹⁸⁵Id.

¹⁸⁶Id.

¹⁸⁷Daniela Maurier et al., Carcinogenicity, in Establishment of Timetables for the Phasing out of Animal Experiments for Cosmetics Directive (2004), available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/10%20Carcinogenicity.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/10%20Carcinogenicity.pdf).

¹⁸⁸Id.

¹⁸⁹Id.

carcinogenicity have been developed, but prediction success using these systems has been poor.¹⁹⁰

Thus far, the two *in vitro* tests that have been proposed are the Cell transformation assay and the Gap junction intercellular communication (GJIC).¹⁹¹ Although these tests provide some information about carcinogenic substances, they are insufficient replacements for animal tests.¹⁹² Given the present state of developing alternative carcinogenicity tests, “the experts were unable to suggest a deadline for full replacement.”¹⁹³

Reproductive and Developmental Toxicity

Reproductive toxicity refers to the adverse effects of a substance on any aspect of the reproductive cycle.¹⁹⁴ Due to the complexity of human reproduction cycle, there are numerous toxicity endpoints that must be tested, making it impossible for a single *in vitro* system to replace animal tests.¹⁹⁵ However, the cycle can be broken down into biological components that can be studied individually or in combination.¹⁹⁶ Promising *in vitro* models are already available for certain reproductive toxicity testing endpoints.¹⁹⁷ Although *in vivo* tests cannot be fully replaced until non-animal tests are developed for all aspects of reproductive toxicity testing, the individual tests can be used to reduce the number of animal tests needed.¹⁹⁸

¹⁹⁰Id.

¹⁹¹Id.

¹⁹²See Id.

¹⁹³Id.

¹⁹⁴Coecke et al., supra note 56; Susanne Bremer et al., Document of the subgroup “Reproductive and developmental toxicity”, in Establishment of Timetables for the Phasing out of Animal Experiments for Cosmetics (2004), available at, [http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/\(5\)%20Chapter%203/11%20Repro%20Developmental%20Tox.pdf](http://pharmacos.eudra.org/F3/cosmetic/doc/AnTest/(5)%20Chapter%203/11%20Repro%20Developmental%20Tox.pdf).

¹⁹⁵Id.

¹⁹⁶Bremer et al., supra note 194.

¹⁹⁷Id.

¹⁹⁸See Id.

Research toward finding more non-animal alternatives for testing reproductive toxicity is being studied by ReProTect, a European project aimed at developing non-animal alternative strategies for detecting reproductive toxicants.¹⁹⁹ ReProTect is exploring both *in vitro* and *in silico* methods.²⁰⁰ They have broken down the entire reproductive cycle and are in the process of developing suitable tests.²⁰¹

To date, ECVAM has validated three embryotoxicity tests: the whole embryo culture (WEC) test, the micromass (MM) test, and the embryonic stem cell test (EST); and ICCVAM has validated the frog embryo teratogenesis assay.²⁰² However, these tests are limited in that they only cover single aspect of developmental toxicity and many of them still rely on intact rodents as their source.²⁰³

Conclusion

In conclusion, progress toward developing and validating replacement alternatives to animal testing for cosmetics is slow, but far from stagnant. The deadlines imposed by the Seventh Amendment to the Cosmetics Directive in Europe have stimulated significant advances in *in vitro* and *in silico* replacement alternatives. Some alternatives have already been validated or internationally accepted and many more are in the process of being validated. Moreover, new research projects are being funded to develop non-animal alternatives for even the most complicated toxicity tests. Although the validation process through ECVAM and ICCVAM is slow and conservative, the cosmetic industry is putting non-animal alternatives into practice as they develop, sparing animals' lives while not compromising the safety of cosmetic products.

¹⁹⁹Coecke et al., supra note 56.

²⁰⁰Id.

²⁰¹Bremer et al., supra note 194 (numerous tests have been identified, but much more research is needed).

²⁰²Id.

²⁰³Id.