Joint DG Enterprise/ECVAM Project

Establishment of timetables for the phasing out of animal experiments for cosmetics:

Subgroup 8

UV-induced effects

by
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Executive Summary

Regulative Requirements

According to the current “Notes for Guidance” of the SCCNFP, cosmetic ingredients and mixtures of ingredients absorbing UV light (in particular UV filter chemicals used, e.g. to ensure light stability of cosmetics, or used in sun protection products) need to be tested for acute phototoxicity and photo-genotoxicity potential. Testing for photosensitisation potential (immunological photoallergy) is not specifically required, but it is nevertheless often performed.

Acute Phototoxicity

Due to a thorough multi-stage and multi-centre validation trial (1992-1998) the In Vitro 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) has reached acceptance by the SCCNFP already in 1998. The 3T3NRU PT is recommended by the EMEA/CPMP as basic preclinical test for acute phototoxicity. It was accepted as Annex V Method No. 41 to Dir. 67/548/EEC in the year 2000, and was accepted as new Test Guideline 432 by the OECD in 2002. The 3T3NRU PT is regarded a basic screen to identify acute phototoxic potential.

Two additional tests, formally evaluated in controlled blind trials, the RBC Phototoxicity Test (RBC PT) and the Human 3-D Skin Model In Vitro Phototoxicity Test (H3D PT), are regarded useful and important adjunct tests to overcome some limitations of the 3T3-NRU-PT, namely the fairly low UVB tolerance of the 3T3 fibroblasts and the inability to model the bioavailability of test materials topically applied to the skin. In addition, the Photo-RBC allows evaluation of the phototoxic mechanisms involved. In conclusion, identification of acute phototoxic hazards is regarded sufficiently covered by in vitro tests, so that animal testing for that endpoint can be 100% replaced right now.

Photo-genotoxicity

In the area of photo-genotoxicity, almost the whole battery of in vitro genetic toxicity tests has been (or is currently being) converted into test protocols of photo-genotoxicity tests. Tests exclusively predictive for gene-mutation, e.g. Photo-Ames-Test and Photo-Thymidine Kinase Test (P TKT) have become less important than tests for clastogenic effects (e.g. Photo-Chromosome Aberration Test (P CAT) and Photo-Micronucleus Test (P-MNT). In addition, promising indicator tests like the Photo-Comet-Assay (P COMET) have been developed. Albeit their routine usage, to date none of the new photo-genotoxicity tests has been formally validated. Therefore, the P-MNT and the P-COMET are currently evaluated in a formal interlaboratory validation study. It is expected that these in vitro photo genotoxicity tests methods may be available as accepted methods within the next five years.

Photo-Allergy (-Sensitisation)

In the area of photo-allergy (-sensitisation), like in the area of development of predictive in vitro tests for delayed contact sensitisation (Allergenicity) potential without involvement of light, due to a lacking ability to model the complex mechanisms underlying allergy, currently no promising in vitro methods to predict photo-sensitisation potential are in sight (see chapter on skin sensitisation). One in vitro screening method, which models covalent binding of a light activated chemical to human serum albumin, may become relevant. However, while binding of an excited chemical to proteins is a prerequisite of photoallergy, it is not a sufficient predictor. The only promising Alternatives currently under development are in vivo refinements, like the Photo Local Lymph Node Assay (LLNA). Once a reliable and predictive in vitro test battery and strategy for the assessment of “dark” sensitisation potential will be developed and accepted, adaptation of the test battery into similar photo-sensitisation tests is considered possible.
8.1 ACUTE PHOTOTOXICITY

Chemical phototoxicity is an acute reaction which can be induced by a single treatment with a chemical and ultraviolet or visible radiation. In vivo, the reaction can be evoked in all subjects provided that concentration of chemical and dose of light are appropriate. "Acute" includes both immediate and delayed (e.g. 48 hour) reactions.

Since the year 2000, in Europe in vivo testing in animals for acute phototoxic potential is no longer permitted, since a successfully validated alternative method has been accepted for regulatory purposes. Taking into account the scientific evidence from the validation studies as well as in-house data in laboratories of the industry, consensus has been reached internationally that testing for acute phototoxic potential can be covered by in vitro methods. Due to its high sensitivity and specificity, the validated 3T3 Neutral Red Uptake phototoxicity test (3T3NRU-PT) is the core test, which is usually the only phototoxicity test required. Two additional in vitro tests that have provided promising results in blind trials under the auspices of COLIPA and/or ECVAM, the Red-Blood-Cell Phototoxicity Test (Photo RBC) and the Human 3-D Skin Model Phototoxicity Test (H3D PT), they may be used when sufficient information on the phototoxic potential of a chemical cannot be obtained from results obtained in the 3T3NRU-PT. An important advantage of the Photo RBC is that can be used at high doses of UVB in the irradiation spectrum and provides information on the mechanism of phototoxicity. In the H3D PT metabolic competent primary human skin cells are used in an organotypic structure including a stratum corneum barrier and thus allows to model the bioavailability of the test chemical. Although not formally validated for that purpose yet, the H-3-D PT is currently used in the cosmetic industry to evaluate the relevance of positive results obtained in the 3T3 NRU PT under aspects of bio-availability in human skin (Spielmann et al. 2000).

8.1.1 The In Vitro 3T3 NRU Phototoxicity Test (3T3 NRU-PT)

8.1.1.1 Short description, scientific relevance and purpose

In the in vitro 3T3 NRU PT the cytotoxicity of a chemical when tested in the “dark” IC50 (-UV) is compared to the cytotoxicity measured by the activated chemical exposed to a non-cytotoxic dose of UV or visible light IC-0 (+UV) at wavelengths > 290 nm. In this test photo-cytotoxicity is expressed as the concentration related reduction of uptake of the vital dye Neutral Red (Neutral Red Uptake = NRU) 24 hours after exposure of mouse fibroblasts of the cell line Balb/c 3T3 to the test chemical in the presence of UV irradiation in comparison to 3T3 cells that are exposed to the same chemical but not to UV irradiation.

The Neutral Red Uptake (NRU) cytotoxicity assay with Balb/c 3T3 fibroblasts (Borenfreund and Puerner, 1985) was adapted for phototoxicity testing. In brief, 3T3 cells are incubated with various dilutions of the test-chemicals in 96 well plates for 1 hour. Thereafter they are exposed to UV/vis light (with an effective UVA radiation of 1.67 mW/cm² for 50 minutes, and NRU is determined 24 hours later by measuring the optical density at 540 nm (Spielmann et al. 1994). Concurrently a second set of plates with the same chemicals is kept in the dark and evaluated in parallel. Where possible, the concentration of a test chemical reflecting a 50% inhibition of cell viability (IC 50) is calculated using appropriate non-linear curve fitting models of the concentration-response curve. To discriminate between photo irritant and non-photo irritant chemicals, the photo irritation factor (PIF) was defined as the ratio of the IC50 values, determined in the absence of UVA and the presence of UVA (PIF = IC50 (-UV) : IC50 (+UV)) (Spielmann et al. 1994, 1996). In a more sophisticated data analysis procedure, the mean photo effect (MPE) is determined, which uses a complete comparison of the area under the curve AUC of the concentration response curves obtained with a chemical in the presence and absence of UV light (Holzhütter, 1997, Peters and Holzhütter 2003).
Based on the results of the formal validation study of the 3T3 NRU PT (Spielmann et al. 1998), a prediction model (PM) has been accepted at the international level as described in OECD TG 432 "In Vitro 3T3 NRU phototoxicity test" (OECD 2003): A test substance with a PIF < 2 or an MPE < 0.1 predicts: "no phototoxicity". A PIF >2 and < 5 or an MPE > 0.1 and < 0.15 predicts: "probable phototoxicity" and a PIF > 5 or an MPE > 0.15 predicts: "phototoxicity". For any laboratory initially establishing this assay, the reference materials listed should be tested prior to testing of new substances for phototoxic assessment. A software package for the calculation of the PIF and MPE is available from COLIPA and from the OECD Secretariat (OECD 2003).

8.1.1.2 Developers of the method

The 3T3 NRU PT was developed by members of the COLIPA Task Force on phototoxicity during a prevalidation study (Spielmann et al., 1994). Test development of an in vitro photocytotoxicity test was initiated at the Battelle laboratory in Germany with human skin cells (Maier et al. 1991). At Beiersdorf in Germany, the Neutral Red uptake (NRU) cytotoxicity test using mouse Balb/c 3T3 fibroblasts (Borenfreund and Puerner, 1985) was adapted for phototoxicity and the prediction model (PM) applying the PIF was developed by statisticians at ZEBET (Berlin, Germany) in 1994 from the data obtained with 20 test chemicals during the prevalidation stage of the ECVAM/COLIPA validation study (Spielmann et al.1994) as described in the previous section. The PM applying the MPE was developed by Holzhütter (Humboldt University, Berlin, Germany) from the data of the formal validation study (Holzhütter 1997) and improved software for data handling and analysis was recently developed by Peters & Holzhütter (2003) and funded by ZEBET at the BfR.

8.1.1.3 Known user

The 3T3 NRU PT is at the world-wide level the established standard test for phototoxic assessment of chemicals in laboratories of the cosmetic, chemical and drug industry in Europe, the USA and Japan.

8.1.1.4 Status of validation and/or standardisation

The 3T3 NRU PT has successfully been experimentally validated from 1992-1998 (Spielmann et al., 1994, 1998a, 1998b) and been accepted for regulatory purposes by the EU in the year 2000 (EC 2000), by the OECD in 2002 (OECD 2002), by the EMEA in 2002 (EMEA 2002) and by the US FDA in 2003 (US FDA 2003).

8.1.1.5 Fields and limitations of application

The 3T3 NRU PT is currently accepted for regulatory purposes for chemicals at the world-wide level (EC 2000; OECD 2002) and for cosmetic ingredients in Europe as outlined by the "SCCNFP's Notes of guidance for testing of cosmetic ingredients and their safety evaluation" (SCCNFP 2003). The 3T3 NRU has also been accepted for regulatory purposes by the competent drug authorities in Europe (EMEA 2002), in the USA (US FDA 2003) and in Japan the drug administration of the NIH held a Symposium in December of 2002 on the "Evaluation of in vitro phototoxicity tests" in order to introduce the 3T3 NRU PT for regulatory purposes. The regulatory use of the 3T3 NRU PT in the EU was outlined by ZEBET (BfR, Berlin, Germany) on behalf of ECVAM and the evaluation of the reliability of EU/COLIPA validation studies of this in vitro test was critically assessed during the symposium. The result was favourable for the acceptance of the 3T3 NRU PT as a well validated test that can be used for regulatory purposes in Japan.
An additional evaluation study has shown that due to their higher potential to absorb UV radiation human keratinocytes are less sensitive in photo-cytotoxicity assays than the mouse fibroblast cell line used in the 3T3 NRU PT assay (Clothier et al., 1999).

The 3T3 NRU PT does not provide specific information on the mechanism of phototoxicity and it does not allow to assess the bio-availability of a test compound in the human skin. If this type of information is required, the Photo RBC, applying red blood cells (section 8.1.2), and the H-3-D PT, applying human skin models (section 8.1.3), may be used. The Unilever laboratory in the U.K. has recently reported a step-wise strategy for the use of the 3T3 NRU PT and of human skin models to interpret the results obtained with ingredients of personal care products in in vitro phototoxicity assays (Jones at al., 2003).

8.1.1.6 Recommendations of use in the view of animal replacement

Taking into account EU Directive 86/609/EEC on “The protection of animals used for experimental and other scientific purposes” (EC 1986), safety testing of chemicals and of cosmetic ingredients for phototoxic potential is no longer permitted in EU member countries, since the in vitro 3T3 NRU PT has been accepted as the standard test methods for this endpoint and since two additional in vitro phototoxicity tests complementing the 3T3 NRU PT, the RBC PT and the H3D PT, have undergone pre-validation and are established in laboratories of the cosmetics and chemical industry in the EU as outlined in sections 8.1.2 and 8.1.3.

8.1.1.7 On-going development

Since the validation of the 3T3 NRU PT was successful and regulatory acceptance has been achieved as outlined above, there are currently no on-going validation activities.

8.1.1.8 Efforts needed to complete validation of the method

Since the validation of the 3T3 NRU PT was successful and regulatory acceptance has been achieved as outlined above, there is currently no need to complete any validation activities.

8.1.1.9 Key references

a) international test guidelines and regulations


b) specific references for the 3T3 NRU PT

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Spielmann H; Acute phototoxicity testing.; Environmental Mutagen Research; 23; 45-56; 2001
8.1.2 The Red Blood Cell Phototoxicity Test (RBC PT)

8.1.2.1 Short description, scientific relevance and purpose

Photohaemolysis is one of the oldest and simplest in vitro techniques for screening of putative photosensitizers (Sacharoff and Sachs 1905). Many different protocols applying various light sources and sources for erythrocytes have been reported in the literature. Taking into account this information, Hetherington and Johnson (1984) published a photohaemolysis method for determining the phototoxic potential of drugs and other chemicals.

Additional important endpoints of phototoxicity in red blood cells are free-radical production and the oxidation of haemoglobin. Since methaemoglobin formation is often observed in phototoxicity testing with erythrocytes, both phenomena, photohaemolysis and haemoglobin oxidation, can be tested in a combined RBC (red blood cell) phototoirritation test (RBC PT; Pape et al. 1994, 2001). This approach allows to screen for photosensitizers and to study phototoxic mechanisms at the cellular level in human material. The combined RBC photohaemolysis and photo-haemoglobin oxidation assay is a useful test both for screening and for mechanistic studies. Phototoxic compounds reacting only with DNA will provide negative results in the RBC test, if the generation of active oxygen species (AOS) is not part of the mechanisms of cytotoxicity (photodynamic reactions).

8.1.2.2 Developer of the method

Photohaemolysis is one of the oldest and simplest in vitro techniques for screening of putative photosensitizers (Sacharoff and Sachs 1905). In 1984 Hetherington and Johnson (1984) published a photohaemolysis method for determining the phototoxic potential of drugs and other chemicals (Hetherington and Johnson 1984) and the current protocol of the RBC PT was developed by Pape and co-workers at Beiersdorf in Germany (Pape et al. 1994, 2001). The method was transferred to laboratories of the European cosmetic industry during the COLIPA/ECVAM prevalidation and formal validation studies of in vitro phototoxicity tests conducted from 1992-1998 (Spielmann et al. 1994, 2000).

8.1.2.3 Known user

The RBC PT is established at the laboratory of Beiersdorf AG, Hamburg, Germany (Dr. Wolfgang Pape and Uwe Pfannenbecker) and at the laboratory of Kose Corporation, Tokyo, Japan (Dr. Yuuko Okamoto).

8.1.2.4 Status of validation and/or standardisation

Preliminary data obtained with the RBC PT during the COLIPA/ECVAM prevalidation study were very promising (Spielmann et al., 1994). The positive evaluation is supported by results obtained under blind conditions in three laboratories with the RBC PT test in the validation stage of the ECVAM/COLIPA in vitro phototoirritation validation study (Pape et al. 2001, Spielmann et al., 1998). It was concluded from the results that the RBC PT provides a good overall fit with the in vivo endpoints as well as mechanistic information on two different types of photodynamic reactions (met-Hb formation for type I reactions and photo-haemolytic effects as primary type II reactions).

In summary, when conducted according to the SOP of the prevalidation study, the RBC PT can reliably be performed. Moreover, this test provides relevant mechanistic information on photodynamic reactions, which is useful for the evaluation of the photo-safety of chemicals in a testing strategy that starts with the 3T3 NRU PT test, which does not provide information on photodynamic reactions. An additional advantage of red blood cells is their resistance to
the short-waved UVB-part of sun light, which allows to expose them in the RBC PT to the
entire solar spectrum for prolonged periods of exposure (Spielmann et al. 2000).

8.1.2.5 Fields and limitations of application
The RBC PT is not a stand alone test to assess the phototoxic potential of chemicals. In
contrast to the 3T3 NRU PT, however, the RBC PT can be used at high doses of UVB in the
irradiation spectrum and the RBC PT provides information on several mechanisms of
phototoxicity, which cannot be assessed in the 3T3 NRU PT. Therefore, the RBC PT is a
useful adjunct in vitro test, which should be used complementary to the 3T3 NRU PT, when
mechanistic study have to be conducted. (Spielmann et al. 2000).

8.1.2.6 Recommendations of use in the view of animal replacement
Taking into account EU Directive 86/609/EEC on "The protection of animals used for
experimental and other scientific purposes" (EC 1986), safety testing of chemicals and of
cosmetic ingredients for phototoxic potential is no longer permitted in EU member countries,
since the in vitro 3T3 NRU PT has been accepted as the standard test methods for this
endpoint and since two additional in vitro phototoxicity tests complementing the 3T3 NRU
PT, the RBC PT and the H3D PT, have shown promising results in interlaboratory
assessments under blind conditions, and are established in laboratories of the cosmetics and
chemical industry in the EU as outlined in sections 8.1.1 and 8.1.3.

8.1.2.7 On-going development
We are not aware of on-going research or validation activities to improve the RBC PT.
However, laboratories that are using the RBC PT in-house may provide information on
improvements in the in INVITOX protocol No 81, which was published 10 years ago (Pape et
al 1994).

8.1.2.8 Efforts needed to complete validation of the method
Since the RBC PT is not the standard in vitro test for acute phototoxicity and since the RBC
PT has successfully undergone pre-validation and validation under blind conditions in the
COLIPA/ECVAM validation study of in vitro phototoxicity tests, there is currently no need for
experimental validation.

8.1.2.9 Key references
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Kahn, G. & Fleischaker, B.I.: Red blood cell haemolysis by photosensitizing compounds.
Journal of Investigative Dermatology 56, 85-90 (1971)


8.1.3 Human 3-D Skin Model In Vitro Phototoxicity Test (H3D PT)

8.1.3.1 Short description, scientific relevance and purpose

Reconstituted human skin models (3-D Skin Models) are available commercially, or from a few experienced laboratories, in three different types: dermal models (containing skin fibroblasts), epidermal models (containing skin keratinocytes and a stratum corneum), and full skin models (containing skin fibroblasts, keratinocytes and a stratum corneum). Since the latter two types contain viable, metabolising primary skin cells and a skin barrier, both are frequently referred to as "3-D skin models". Human skin models have been used quite successfully in routine laboratory investigations, since they are relevant to the organ of interest. For in vitro toxicity testing, standardisation and control of 3-D skin models needs to be defined clearly in order to assure that reliable and reproducible data are obtained.

In contrast to normal cell cultures, e.g. mouse fibroblasts that are used in the NRU PT, human skin models allow for topical application of various types of chemicals and preparations and seem to have less limitation concerning solubility problems. Test materials can be applied to 3D skin models undiluted, at extreme pH values or even as 'insoluble' materials.

The first promising data obtained with a H3D PT were reported 1994-1995 with a full skin model (Edwards et al. 1994; Liebsch et al. 1995), and an epidermal model (Roguet et al. 1994). Since the commercial production of the full skin model Skin²™ was stopped in 1996, the test protocol was successfully adapted to the epidermal model EpiDerm™ (Liebsch et al. 1997). The H3D PT applying EpiDerm™ was tested in an ECVAM prevalidation study revealing promising results in three laboratories under blind conditions (Liebsch et al. 1999). The test is currently established in several laboratories of the European cosmetics industry (Jones et al. 1999) and has been successfully adopted to the epidermal model SkinEthic™ (Bernard et al. 1999, Jones et al. 2003). Efforts undertaken to optimise the phototoxicity test protocol and prediction model when transferring it from the full skin model to the epidermal model (Liebsch et al 1997) revealed the basic test protocol and prediction model did not need to be changed.

Several studies (Liebsch et al. 1995; Api 1997) reported that in vivo photoallergens that are in vivo not acute photoirritants at the same time (e.g. coumarin, 6-methyl-coumarin, musk ambrette), are classified correct negative by the skin model phototoxicity tests, while they are positive in the 3T3NRU-PT. Reconstituted human skin models are increasingly investigated for their usability in toxicological hazard identification/safety testing, because their organotypic structure with a functional stratum corneum allows for in vitro tests modeling bioavailability of topically applied substances. Because of the stratum corneum barrier, an important role of the H3D-PT test is the verification / falsification of positive results obtained the 3T3 NRU PT. Simple dermal models, which do not contain a skin barrier, show a sensitivity to phototoxic chemicals, which is similar to photo-cytotoxicity tests, as e.g. the 3T3 NRU-PT (Augustin et al. 1997). They are, therefore, not providing any advantage in a phototoxicity testing strategy.

8.1.3.2 Developer of the method

Two slightly different approaches for the use of skin models in phototoxicity were developed at about the same time (1994 -1995): Roguet et al (1994) developed a protocol for the human epidermis model EPISKIN, and Edwards et al. (1994) and Liebsch et al. (1995) developed a protocol for the full skin model Skin². The basic test design was similar in both methods: investigation of a test substance dose-response on skin cell viability (MTT reduction) in parallel on tissues non-irradiated and irradiated with the highest non-cytotoxic UVA-vis dose. However, the prediction models used in both tests were different. Roguet et al. (1994) compared IC50 values obtained +UVA and -UVA, and Liebsch et al. (1995, 1997, 1999) determined the lowest dose at which a significant phototoxic effect (LOAEL) was observed. Probably due to the fact that the latter method and prediction model had shown
robustness by the successful transfer from the full skin model Skin² to the epidermis model EpiDerm (Liebsch et al. 1997, 1999) without any changes necessary and possible, it is used in several laboratories, also with other human skin / epidermis models (e.g. with SkinEthic: Jones et al. 2003).

8.1.3.3 Known user

The H3D PT is established as an adjunct phototoxicity test in some companies of the Cosmetic Industry, e.g. Beiersdorf AG (Hamburg, D), Unilever (Sharnbrook, UK), as well as in contract testing institutes, e.g. Dr. Schrader Institute (Holzminden, D), Institute for In Vitro Sciences (Gaithersburg, USA), MB Research Laboratories (Spinnerstown, USA).

8.1.3.4 Status of validation and/or standardisation

After development of the method with the model Skin², the H3D PT was evaluated within phase 1 of the EC/COLIPA phototoxicity validation trial by ZEBET on 20 chemicals in a non-blinded study (Liebsch et al. 1994, Spielmann et al 1994, Liebsch et al. 1995). Later, the H3D PT was again evaluated by ZEBET under blind conditions on 30 test chemicals within phase 2 of the EC/COLIPA validation with promising results. This outcome, however, was never published because the model Skin² was not available any more shortly after the end of this study.

Later, in an ECVAM prevalidation study, the H3D PT protocol and prediction model developed with Skin² was transferred to the epidermis model EpiDerm and revealed excellent results in a blind trial in three laboratories. In this study, attempts to optimise the protocol (Liebsch et al. 1997, 1999) revealed that no optimisation was possible. Even the prediction model developed originally for the Skin² H3D PT could not be optimised and predictions achieved with this prediction model were better then those obtained when the PIF or MPE prediction models of the 3T3 NRU PT were applied (Holzhütter, 1998).

As an adjunct test to the 3T3 NRU PT the H3D PT can be regarded valid and sufficiently standardised. However, a formal validation study, specifically designed for the role of this test as described in 8.1.3.5. would help establishing it’s use.

8.1.3.5 Fields and limitations of application

Due to the stratum corneum functional barrier of human skin or epidermis models, the H3D PT is not a stand alone test to assess the inherent phototoxic potential of chemicals. In contrast to the 3T3 NRU PT, the H3D PT may miss the correct detection of phototoxins that cannot enter the skin via topical route but may be sufficiently bioavailable in the skin via systemic pathways, e.g. after oral, or parenteral exposure. The H3D PT may also miss the detection of weakly photoreactive chemicals that induce photoallergic reactions only after repeated exposure. However, because the H3D PT models the bioavailability of chemicals and formulations at topical skin exposure, it is qualified as an adjunct test to further investigate chemicals with (probably false) positive outcomes in the 3T3 NRU PT, as specified in paragraph 54 of new draft OECD Test Guideline 432 (OECD 2002). For assessment of phototoxic hazard this approach is conservative, since the penetration barrier of in vitro skin models is known to be weaker compared with the penetration barrier of skin in vivo.

If the H3D PT is used as an adjunct to the 3T3 NRU PT as described above (only for chemicals intended for topical use) it has hardly any limitation, because it mimics the in vivo situation and can handle solutions, as well as suspensions. The only limitation known is that a test substance may directly reduce MTT and mimic dehydrogenase activity of the cellular mitochondria. This is only a problem if at the time of the MTT test (24 hours after test substance exposure) still sufficient amounts of the test substance are present on (or in) the
tissues. In this case, the (true) metabolic MTT reduction and the (false) direct MTT reduction can be differentiated.

### 8.1.3.6 Recommendations of use in the view of animal replacement

Taking into account EU Directive 86/609/EEC on "The protection of animals used for experimental and other scientific purposes" (EC 1986), safety testing of chemicals and of cosmetic ingredients for phototoxic potential is no longer permitted in EU member countries, since the *in vitro* 3T3 NRU PT has been accepted as standard test method for this endpoint and since two additional *in vitro* phototoxicity tests complementing the 3T3 NRU PT, the RBC PT and the H3D PT, have shown promising results in interlaboratory assessments under blind conditions, and are established in laboratories of the cosmetics and chemical industry in the EU as outlined in sections 8.1.1 and 8.1.2

### 8.1.3.7 On-going development

In an ECVAM funded feasibility study it is currently investigated by ZEBET in co-operation with the Czech Institute for Public Health (SZU) if the H3D PT can be upgraded from a test for phototoxic potential to a test for photo-potency of topically applied substances. In this study, the lowest observed adverse effect levels (LOAEL) determined in the H3D PT will be compared with the LOEAL observed *in vivo* in human photo-patch tests and data obtained with the 3T3 NRU PT.

### 8.1.3.8 Efforts needed to complete validation of the method

If the feasibility study described in 8.1.3.7 shows promising results, the H3D PT should be formally validated to a test for photo-safety / photo-potency of test chemicals (or formulations thereof) with intended topical use on the skin.

### 8.1.3.9 Key references


8.2 PHOTO-CHEMICAL GENOTOXICITY

In vivo testing for photogenotoxicity is rarely performed, since the skin is not utilised in standard approaches. However, transgenic mutagenicity models (such as lacI or lacZ transgenic mice) may be suited for this purpose, because UV radiation induced mutations in skin cells has been demonstrated (Gorelick 1996). Furthermore, the micronucleus test on rat and mouse skin as target organ has been developed and might be applicable for Photo Micronucleus testing as well (Nishikawa 1999 and 2002). Only a few genotoxicity test have been reported for photochemical genotoxicity in vivo. Satoru Itoh et al have demonstrated in vivo photochemical micronucleus induction by a certain quinolone in skin of mice (2002). Furthermore Positive results have been reported for the fluoroquinolone clinafloxacin in the Comet assay (Bulera et al., 1999). However, if in vivo testing seems at all necessary, these assays need further development and validation. In vivo testing of photogenotoxicity potential is not requested by the SCCNFP.

In principle, like with “dark” genetic toxicity tests, photogenotoxicity testing is a domain of in vitro tests. Based on recommendations of Loprieno (1991), for safety testing of cosmetics the SCCNFP proposes a bacterial test for gene mutation and an in vitro test for chromosomal aberrations in mammalian cells be performed in the presence of UV radiation. So far, none of the many in vitro photogenotoxicity tests currently in use has been formally validated. A crucial point, relevance for in vivo, will not be possible to address in these validation studies, because so far only one photogenotoxic chemical (8-MOP) has proven to be photocarcinogenic in vivo in humans.

Many different in vitro photogenotoxicity tests have been adapted for testing the potential of chemicals to damage the DNA after photoactivation. However, it is recommended to focus on those in vitro test systems, which are currently used as standard tests in regulatory testing strategies. Since to date no photogenotoxic chemical is known which exclusively acts through gene mutations, and because the recognised photochemical mechanisms are clastogenic, it is suggested that a test for photochemical clastogenicity (chromosomal aberration or micronucleus test) should have first priority. In addition, the Photo-Comet assay was proposed by an international expert working group as reasonable supplementary test which may give additional information on photogenotoxic properties of a compound (Gocke et al., 2000). The most recent comprehensive review of currently available photogenotoxicity tests has been published by the GUM (German section of the European Environmental Mutagen Society, EEMS) Task Force on photochemical genotoxicity (Brendler-Schwaab et al., 2004).

References to introduction.


8.2.1 Reverse Gene/Point Mutation: Photo Ames Test (P-AMES)

8.2.1.1 Short description, scientific relevance and purpose
Procariontic, bacterial mutation tests are easier and cheaper to perform than any photogenotoxicity tests with eucariontic mammalian cells. Therefore, the P-AMES test was the first in vitro photogenotoxicity test adapted from to the parallel use of light (Jose, 1979) and later proposed for safety testing of cosmetics (Loprieno 1991; COLIPA 1995). In the P-AMES test, a dose dependent, test chemical mediated, increase of reverse mutations from histidine dependent strains to histidine independent wild-type is investigated plus and minus a non-mutagenic UV-vis irradiation.

8.2.1.2 Developer of the method
The first P-AMES was proposed already six years after the publication of Ames test (Jose, 1979). However, the methodology described by Dean et al. (1991) is more frequently used as it was the methodology and recommend by the SCC (Loprieno, 1991).

8.2.1.3 Known user
Several laboratories in Cosmetics and Pharmaceutical Industry, contract laboratories and some academic Institutes are using the P-AMES test.

8.2.1.4 Status of validation and/or standardisation
The P-AMES test has never been formally validated. However, attempts to standardise the many existing protocol modifications have been made (Dean et al., 1991; 1992; Chelat et al., 1993; Henderson et al. 1994; COLIPA, 1995). Albeit this status, the P-AMES test has achieved acceptance by the SCCNFP in the field of cosmetic safety testing.

8.2.1.5 Fields and limitations of application
According to Brendler-Schwaab et al. (2004) the critical point of all P-AMES test protocols is the UV sensitivity of the Salmonella typhimurium or Escherichia coli strains used. Chemicals often have to be pre-irradiated in the absence of the bacteria to achieve doses of light necessary to activate the photogenotoxins. Moreover, the limited predictivity of the regular "dark" AMES test suggests the use of tests employing mammalian cells rather than bacteria, if DNA point mutation tests are considered as part of a relevant test battery.

8.2.1.6 Recommendations of use in the view of animal replacement
While in vivo photogenotoxicity testing is not required for the safety testing of cosmetics, it is hoped that the current in vitro approaches in photogenotoxicity testing will lead to the definition of an in vitro test battery allowing for an assessment of the likelihood of a compound to turn into a photochemical carcinogen upon excitation and activation with UV or visible radiation. However, while pharmaceuticals with a photocarcinogenic potential may be further investigated in vivo because they can be safely used avoiding any light exposure, chemicals with a photocarcinogenic potential will not be acceptable as cosmetic ingredients, so that in vivo testing will not be required in that area.
8.2.1.7 On-going development

Probably because of the decreasing importance of the P-AMES test as a relevant part of sensitive photogenotoxicity testing battery, we are not aware of on-going developments of this test system.

8.2.1.8 Efforts needed to complete validation of the method

The P-AMES test is at the state of an optimised test (i.e. ready for prevalidation and validation). Albeit its acceptance by the SCCNFP a decreasing importance of this test as relevant part of a photogenotoxicity testing battery is obvious, so that it may not be advisable to select this test for entering the expensive procedures of prevalidation and formal validation studies.

8.2.1.9 Key references


Dean S.W., Dunmore R.H., Ruddock S.P., Dean J.C., Martin C.N., Kirkland D.J. (1992) Development of assays for the detection of photomutagenicity of chemicals during exposure to UV light. II. Results of testing three sunscreen ingredients, Mutagenesis 7, 179–182.


8.2.2 Forward Gene/Point Mutation: Photo-Hypoxanthine-Guanine Phosphoribosyl Transferase Assay (P-HPRT) and Photo Mouse Lymphoma assay (P-MLA)

8.2.2.1 Short description, scientific relevance and purpose
In regular ("dark") gentotoxicity testing, *in vitro* mammalian gene mutation tests are integral part of the regulatory required testing battery, and their predictive value is regarded higher than that of bacterial gene mutation tests. The principle is largely similar as with the Ames test: only mutants survive a selective stress (although reverse mutations are detected with the Ames test and forward mutations are detected with the MLA). The following description shows the test principle for one of the mammalian gene mutation tests, the Mouse Lymphoma Assay (MLA):

The L5178YTK+/− mouse lymphoma assay (MLA) detects mutations at the thymidine kinase (TK) locus caused by base pair changes, frameshift and small deletions. Mutant cells, deficient in TK due to the forward mutation in the TK locus (from TK+ to TK−), are resistant to the cytotoxic effect of pyrimidine analogues such as trifluorothymidine (TFT). The mutagenicity of the test agents is indicated by the increase in the number of mutants after treatment.

Other assays of this type are using mutations at enzyme loci of hypoxanthine-guanine phosphoribosyl transferase (HPRT), or a transgene of xanthine-guanine phosphoribosyl transferase (XPRT), and are in general performed with Chinese Hamster cell lines like CHO, CHO-AS52 and more frequently, V79. For review of these methods see Nestmann et al. (1991).

The *in vitro* mammalian cell gene photogenotoxicity tests employ the same protocols, but test chemicals are investigated plus and minus a non-or slightly genotoxic UV-vis radiation.

8.2.2.2 Developer of the method
The developers of the P-HPRT and P-MLA could not exactly been identified.

8.2.2.3 Known user
From the review of Brendler-Schwaab et al. (2004) it is suggested that mammalian cell gene photo mutation tests P-HPRT and P-MLA are established in laboratories Pharmaceutical Industry, Academia, and Public Health Institutes, like the Hadano Research Institute, Food and Drug Safety Centre, Japan (Nakagawa et al., 1997). It is also obvious from the literature that the P-HPRT established in more laboratories than the P-MLA.

8.2.2.4 Status of validation and/or standardisation
The P-HPRT and P-MLA have never been formally validated. Whereas the degree of standardisation for the "dark" versions of the two tests is regarded high (as they have become integral part of many safety testing regulations), the photo modifications of these tests still need standardisation, in particular with regard to the UV-vis radiation used. Moreover, since the number of chemical classes tested in these assays is very limited (predominantly psoralenes and other chemicals used in P-UVA therapy, some fluoroquinolones, and UV filters, Brendler-Schwaab et al., 2004), a thorough data base development phase would be necessary to validate the assays.

8.2.2.5 Fields and limitations of application
The photogenotoxic endpoint covered by the P-HPRT and the P-MLA (gene mutation) is identical with the P-AMES. However, since the mammalian cells are closer to the species of interest (humans), and because the cell lines are more resistant to UV radiation, the likelihood to produce false negative results in the P-HPRT and P-MLA is lower.

8.2.2.6 Recommendations of use in the view of animal replacement

While *in vivo* photogenotoxicity testing is not required for the safety testing of cosmetics, it is hoped that the current *in vitro* approaches in photogenotoxicity testing will lead to the definition of an *in vitro* test battery allowing for an assessment of the likelihood of a compound to turn into a photochemical carcinogen upon excitation and activation with UV or visible radiation. However, while pharmaceuticals with a photocarcinogenic potential may be further investigated *in vivo* because they can be safely used avoiding any light exposure, chemicals with a photocarcinogenic potential will not be acceptable as cosmetic ingredients, so that *in vivo* testing will not be required in that area.

8.2.2.7 On-going development

The group is not aware of current approaches to further develop, prevalidate, or validate the existing protocols of the P-HPRT and P-MLA.

8.2.2.8 Efforts needed to complete validation of the method

The P-HPRT and P-MLA test are in the state of optimised tests (i.e. ready for prevalidation and formal validation studies). However, it is suggested that most regulatory bodies will accept submissions of data from these tests (in place of data from the P-AMES test), provided the protocols are using relevant UV-vis light spectra and doses, and concurrent positive and negative controls. However, if formal validation is anticipated, since the number of chemical classes tested in these assays is very limited (Brendler-Schwaab et al., 2004), a thorough data base development would be necessary to formally validate the assays.

8.2.2.9 Key references


8.2.3 Photo-clastogenicity: Photo Chromosome Aberration Test (P-CAT)

8.2.3.1 Short description, scientific relevance and purpose

Tests for clastogenicity (i.e. inducing mutations at the chromosome level) are essential part of the testing strategy for chemicals, cosmetics and pharmaceuticals. Since so far no photogenotoxic substance is known that is exclusively acting at the gene level, the determination of photo-clastogenicity is very important, as it will have relevance for the assessment of a photo-carcinogenic hazard potential of substances (Müller & Kasper, 1998; Gocke et al., 2000; Brendler-Schwaab et al., 2004).

The purpose of the in vitro P-CAT is to identify agents that cause structural chromosome aberrations in cultured mammalian cells in the presence of a non clastogenic UV-vis radiation. Structural aberrations may be of two types, chromosome or chromatid. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome-type aberrations also occur. The in vitro photo chromosome aberration test may employ cultures of established cell lines (mostly Chinese Hamster lines CHO, V79, CHL), or primary cell cultures (e.g. human lymphocytes).

8.2.3.2 Developer of the method

A developer of the P-CAT could not exactly been identified.

8.2.3.3 Known user

The P-CAT is established in several laboratories pharmaceutical and cosmetics Industry, Academia, and contract testing institutes.

8.2.3.4 Status of validation and/or standardisation

The P-CAT has so far not been formally validated. Albeit this, the P-CAT is recommended and accepted by the SCCNFP for safety testing of cosmetic ingredients. Although several attempts have been made to standardise the protocol of the P-CAT over the past 15 years (Gibson et al., 1986; COLIPA, 1995; Murli et al.; 2002), the P-CAT protocols published show differences, in particular with regard to the UV-vis irradiation spectrum used and number of (UV-) dose levels (Brendler-Schwaab et al., 2004).

8.2.3.5 Fields and limitations of application

The field of the P-CAT is the detection of photo-chemically induced clastogenicity, the most relevant in vitro endpoint for the assessment of photo-carcinogenic potential. So far, limitations are not known – test results seem to independent from the cell lines (or primary cells) used. It may be regarded a “limitation” that cytogenetic chromosome analysis is the most expensive genotoxic endpoint to investigate, which always requires highly trained personnel and the possibilities for apparatus-supported analysis are fairly low.

8.2.3.6 Recommendations of use in the view of animal replacement

While in vivo photogenotoxicity testing is not required for the safety testing of cosmetics, it is hoped that the current in vitro approaches in photogenotoxicity testing will lead to the definition of an in vitro test battery allowing for an assessment of the likelihood of a compound to turn into a photochemical carcinogen upon excitation and activation with UV or visible radiation. However, while pharmaceuticals with a photocarcinogenic potential may be further investigated in vivo because they can be safely used avoiding any light exposure,
chemicals with a photocarcinogenic potential will not be acceptable as cosmetic ingredients, so that in vivo testing will not be required in that area.

8.2.3.7 On-going development

The group is not aware of current approaches to further develop, prevalidate, or validate the existing protocols of the P-CAT.

8.2.3.8 Efforts needed to complete validation of the method

The P-CAT is in the state of an optimised test (i.e. ready for prevalidation and formal validation studies). However, it is recognised that regulatory bodies accept submissions of P-CAT data, provided the protocols are using relevant UV-vis light spectra and doses, and concurrent positive and negative controls. Instead of planning a formal validation study, existing data should be retrospectively analysed. However, if a formal validation is anticipated irradiation for the P-CAT should be defined and standardised before that study.

8.2.3.9 Key references


8.2.4 Photo-clastogenicity: The Photo Micronucleus Test (P-MNT)

8.2.4.1 Short description, scientific relevance and purpose

Tests for clastogenicity (i.e. inducing mutations at the chromosome level) are essential part of the testing strategy for chemicals, cosmetics and pharmaceuticals. Since so far no photogenotoxic substance is known that is exclusively acting at the gene level, the determination of photo-clastogenicity is very important, as it will have relevance for the assessment of a photo-carcinogenic hazard potential of substances (Müller & Kasper, 1998; Gocke et al., 2000; Brendler-Schwaab et al., 2004).

The \textit{in vitro} micronucleus test (MNT) is a mutagenicity test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments i.e. micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from chromosome fragments lacking a centromere or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division. Thus, the micronucleus assay is in principle appropriate for the detection of \textit{clastogenic} and \textit{aneugenic} effects (the latter is not relevant for photogenotoxicity testing).

Of the existing \textit{in vitro} micronucleus test protocols, the method employing Chinese hamster V79 cells (Kalweit et al. 1999), validated in a collaborative study (van der Hude et al. 2000) was successfully adapted to photogenotoxicity testing (Kersten et al. 1999, 2002) by two laboratories. The method is based on concentration-response experiments performed with and without irradiation with a UV-vis sunlight simulation. Currently, the P-MNT is evaluated in a formal interlaboratory validation blind trial, supported by the German Ministry of Research, in five laboratories in Germany and Switzerland.

8.2.4.2 Developer of the method

Two modifications of the P-MNT were published in the same year (Snyder & Cooper, 1999 and Kersten et al. 1999). The P-MNT was currently under formal validation was developed (and to a large extent pre-validated) in a collaborative study between Bayer AG, Wuppertal, and the German Federal Institute for Drugs and medical Devices, BfArM, Bonn (Kersten et al. 1999, 2002).

8.2.4.3 Known user

The test currently established with a common SOP in the five laboratories participating in the formal validation study: Bayer AG (Wuppertal, D), BfArM (Bonn, D), RCC-Cytotest Cell Research (Roßdorf, D), University of Mainz (D), Hoffmann-La-Roche (Basel, CH).

8.2.4.4 Status of validation and/or standardisation

The standardised P-MNT protocol of Kersten et al. (1999) is currently under formal validation since early 2003. The study is co-ordinated by the BfArM in Bonn and independently coached by ZEBET at the BfR (chemical coding and distribution and biostatistics). It will be finalised end of 2005.

8.2.4.5 Fields and limitations of application

The field of the P-MNT is the detection of photo-chemically induced clastogenicity, the most relevant \textit{in vitro} endpoint for the assessment of photo-carcinogenic potential. So far, limitations are not known – test results seem to independent from the cell lines (or primary cells) used. The “limitation” of the alternative photo-clastogenicity assay, the PCAT (high costs of cytogenetic chromosome analysis) is overcome since micronuclei are quicker and...
easier to determine as chromosomal aberrations. The test bears possibilities for apparatus-supported analysis.

8.2.4.6 Recommendations of use in the view of animal replacement

While \textit{in vivo} photogenotoxicity testing is not required for the safety testing of cosmetics, it is hoped that the current \textit{in vitro} approaches in photogenotoxicity testing will lead to the definition of an \textit{in vitro} test battery allowing for an assessment of the likelihood of a compound to turn into a photochemical carcinogen upon excitation and activation with UV or visible radiation. However, while pharmaceuticals with a photocarcinogenic potential may be further investigated \textit{in vivo} because they can be safely used avoiding any light exposure, chemicals with a photocarcinogenic potential will not be acceptable as cosmetic ingredients, so that \textit{in vivo} testing will not be required in that area.

8.2.4.7 On-going development

Apart from the current formal interlaboratory Validation Study, on-going developments of the P-MNT are not known. However, similar activities like the German/Swiss interlaboratory trial may be under way elsewhere.

8.2.4.8 Efforts needed to complete validation of the method

Finalisation of the current formal Validation Study (expected end of 2005).

8.2.4.9 Key references


v.d. Hude W et al. (2000) \textit{In vitro} micronucleus assay with Chinese hamster V79 cells – Results of a collaborative study with in situ exposure to 26 chemical substances, Mutat Res 468, 137-163.

8.2.5 DNA damage Indication: The Photo COMET Test (P-COMET)

8.2.5.1 Short description, scientific relevance and purpose

The ("dark") COMET assay is a method for electrophoretic measurement of DNA strand breaks. The assay can in principle be performed on any eukaryotic cell type and only a small number of cells is necessary. Strand breaks may be introduced directly by genotoxic compounds or through the interaction with reactive oxygen species, or other reactive intermediates. The COMET test is highly sensitive and can detect DNA damage in individual cells. The test is usually performed under alkaline conditions. In the alkaline version of the COMET test, the induction of DNA single strand breaks is measured. The Comet assay was firstly described by Ostling & Johanson (1984), and later further developed by Singh et al. (1988).

The protocol of Singh et al. (1988) has been successfully adapted to photo-genotoxicity testing as is described in several publications (Chetelat et al., 1996; Bulera et al., 1999; Nakagawa et al., 1997; Reavy et al., 1997; Marrot et al., 2001), and later during a research project at Bayer AG (Wuppertal, D) supported by the German Ministry of Research and Technology (Brendler-Schwaab et al., 2003). Based on the positive experience gained in this project, the P-COMET was employed in early 2003 in a formal validation study in which six laboratories are evaluating the P-COMET in a blind trial.

8.2.5.2 Developer of the method

Most probably the group of Chelat et al. (1996)

8.2.5.3 Known user

Apart other possible users the test is currently established with a common SOP in the following six laboratories participating in the formal validation study: Bayer AG (Wuppertal, D), BfArM (Bonn, D), RCC-Cytotest Cell Research (Roßdorf, D), University of Mainz, Wella Cosmital (CH), Novartis Pharma (Basel, CH).

8.2.5.4 Status of validation and/or standardisation

Even though the P-COMET has recently entered a formal validation study which will under blind conditions produce interlaboratory data with a standard P-COMET protocol, the fact that there are several compounds known for which the P-COMET provides negative predictions, while other phototoxicity or photo-genotoxicity tests are providing positive results, has to be seriously taking into account once the relevance of the P-COMET will be independently assessed. Probably, the P-COMET has entered the formal validation study a bit early, because currently different predictions obtained in the indicator test (P-COMET) and other photogenotoxicity tests (like the P-CA, or P-MNT) are not fully understood.

8.2.5.5 Fields and limitations of application

The field of application of the P-COMET is detection of DNA strand breaks induced by a substance and subsequent/concurrent UV-vis irradiation. The P-COMET is on the one hand an extremely sensitive test, and on the other hand, it has produced negative results with some compounds that are providing positive results in other photogenotoxicity tests like the P-MNT. Such chemicals are for example nalidixic acid and 8-MOP of which only the latter result is understood as DNA-DNA crosslinking under UV light (Brendler-Schwaab et al., 2004).
8.2.5.6 Recommendations of use in the view of animal replacement

While in vivo photogenotoxicity testing is not required for the safety testing of cosmetics, it is hoped that the current in vitro approaches in photogenotoxicity testing will lead to the definition of an in vitro test battery allowing for an assessment of the likelihood of a compound to turn into a photochemical carcinogen upon excitation and activation with UV or visible radiation. However, while pharmaceuticals with a photocarcinogenic potential may be further investigated in vivo because they can be safely used avoiding any light exposure, chemicals with a photocarcinogenic potential will not be acceptable as cosmetic ingredients, so that in vivo testing will not be required in that area.

8.2.5.7 On-going development

Apart from the current interlaboratory blind trial, where an agreed P-COMET standard protocol is used, the group is not aware of any other developments.

8.2.5.8 Efforts needed to complete validation of the method

In principle, since the P-COMET is currently evaluated in an inter-laboratory blind trial, the test may, at the expected end of this study (2005) be regarded formally validated. However, before the test can become a recommended part of a photogenotoxicity testing battery, it's relevance and role in a strategy will need further clarification.

8.2.5.9 Key references


Nakagawa, Y. et al. (1997) The photogenotoxicity of titanium dioxide particles, Mutation Res. 394, 125-132.


Selvaag, E., Petersen, A. B., Gniadecki, R., Thorn, T. & Wulf, H-C., (2002) Phototoxicity to diuretics and antidiabetics in the cultured keratinocyte cell line HaCaT: evaluation by
clonogenic assay and single cell gel electrophoresis Comet assay). *Photodermatol, Photoimmunol & Photomed* 18, 90-95.
8.3 PHOTO-ALLERGY (PHOTO-SENSITISATION)

Testing for photosensitisation potential (immunological photoallergy) is not specifically required for Cosmetic ingredients, but it is nevertheless often performed. For in vivo photoallergy testing, no standard testing protocol exists. Protocol designs frequently used are similar to the Guinea Pig Maximisation Test (GPMT), employing additional UV-vis irradiation (e.g. Guillot & Martini, 1985).

In the area of photo-allergy (-sensitisation), like in the area of development of predictive in vitro tests for delayed contact sensitisation (Allergenicity) potential without involvement of light, due to a lacking ability to model the complex mechanisms underlying allergy, currently no promising in vitro methods to predict photo-sensitisation potential are in sight (see chapter on skin sensitisation). One in vitro screening method, which models covalent binding of a light activated chemical to serum albumin, may become relevant. However, while binding of an excited chemical to proteins is a prerequisite of photoallergy, it is not a sufficient stand-alone predictor, but it can help differentiate photoirritants from photoallergens (Barrat & Brown, 1985; Lovell & Jones 2000). The only promising stand-alone Alternatives currently under development are in vivo refinements, like the Photo Local Lymph Node Assay, P-LLNA (Ulrich et al., 1998; Homey et al., 1998), or a combination of the P-LLNA with the Photo-Mouse Ear Swelling test, P-MEST (Vohr et al., 2000). The ECVAM Workshop Report 42 (Spielmann et al., 2000) is still correctly covering the actual status of these methods in detail.

Once a reliable and predictive in vitro test battery and strategy for the assessment of “dark” sensitisation potential will be developed and accepted, adaptation of the test battery into similar photo-sensitisation tests is considered possible.

Because at present, serious predictions on the availability of in vitro alternatives for this endpoint cannot be made, and because, formally for cosmetics testing of photoallergenic potential is not required, this chapter is kept as a summary without sub-chapters.

References


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<td>If at all necessary: Formal validation: 3-5 years</td>
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<td>Test Name (P)</td>
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<td>Optimised</td>
<td>None, but accepted by some authorities</td>
<td>Rarely used in standard battery, additional to bacterial mutations</td>
<td>If at all necessary Formal validation: 4-6 years</td>
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<td>General</td>
<td>Optimised</td>
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*This table estimates the time needed to achieve ESAC endorsement for individual alternative tests assuming optimal conditions. It does not indicate the time needed to achieve full replacement of the animal test, nor does it include the time needed to achieve regulatory acceptance. “Optimal conditions” means that all
necessary resources, for example technical, human, financial and coordination are met at all times in the process and that the studies undertaken have successful outcomes.