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Comments: May 14, 2009
William Stokes, D.V.M. D.A.C.L.A.M.
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Dear Dr. Stokes,

This public comment is provided in response to Federal Register Notice Volume 74, Number 60, pages 14556-14557 requesting comments in the context of the public meeting of an independent scientific peer review panel on alternative ocular safety testing methods that will take place on May 19-21, 2009.

The Procter & Gamble Company fully supports the advancement of alternatives to animal testing. As such, it commends ICCVAM for undertaking this current activity on evaluation of in vitro eye irritation assays validation status and their use in tiered testing strategies for anti-microbial products. It is also noteworthy that the Top/Down-Bottom/Up approach: eye irritation testing strategy to reduce and replace in vivo studies was recently accepted for publication in the peer-reviewed scientific literature (Scott et al. Toxicology In Vitro, accepted for publication).

However, given the extensive industry experience and collective historical data on LVET that exist and which are not fully reflected in the LVET Summary Review Document (SRD), the Procter & Gamble Company would like to raise concerns on this and provide additional technical perspective for consideration by the peer review panel.

This public comment is specifically related to use of historical and published LVET data to support use of this assay as an acceptable in vivo reference standard against which to compare in vitro assays used in a tiered testing strategy for anti-microbial products. It will seek to provide additional information and perspective on specific comments made in the draft ICCVAM SRD: The Low Volume Eye Test (LVET) dated April 1, 2009 that was published on the NICEATM-ICCVAM website.

It is structured to provide a summary of its conclusions followed by specific detailed responses for your consideration to identified focus areas mentioned at different points throughout the LVET SRD on which questions are raised concerning use of LVET as an acceptable reference standard in the context of this ocular methods/approaches review. Each focus area addressed includes line references within the LVET SRD where the questions arise).

Summary of public comment

There exists an extensive historical LVET database that supports use of such existing LVET data as an appropriate in vivo reference standard against which to compare in

vitro assays within the context of the current ICCVAM review “Use of In Vitro Methods in a Tiered Approach for Ocular Hazard Identification of Anti-Microbial Products”. Furthermore, this dataset provides data for several characteristics of the assay that are key to scientific acceptance of historical and available LVET data. This is in the context of domains of applicability for which the data support its use in a WoE approach as a valid and relevant predictor of eye irritation and as such in vivo reference standard against which to compare in vitro assays. These are:

- Anatomical and physiological basis for choice of 10 uL as an appropriate dose volume
- Ability of 10 uL dose volume to effectively discriminate between materials of different eye irritancy potential
- Ability of LVET to detect the range of ocular responses from innocuous to severe
- Ability of LVET to correctly predict known severe human eye irritants
- Over-prediction of the human response by LVET, but to a lesser extent than the Draize test, thereby remaining a conservative evaluation of eye irritation potential
- Correlation of LVET dosing procedure with the human response in clinical studies
- Correlation of LVET data and human experience data from industrial accidents and consumer accidental exposure for the same consumer products

Most recently, use of LVET as an appropriate in vivo reference standard against which the Isolated Chicken Eye (ICE) Test was compared to establish the latter as a suitable in vitro assay to determine eye irritation potential of household cleaning products was accepted for publication in the peer reviewed scientific literature (Schutte et al. Regulatory Toxicology and Pharmacology, accepted for publication).

Comments on identified focus areas within the LVET SRD

1. The nature and range of irritancy of substances tested in LVET. It is reported in the SRD that the majority of LVET data has been generated on surfactant-based mixtures or products which produce only a mild irritant response or no response [lines 280-281].

Furthermore, it is reported that there is no information on the performance of known corrosives in the LVET [lines 285-286].

It is recognised that a significant amount of the historical LVET data available is for surfactant-based materials and surfactant-based products. This reflects the original purpose for development of the LVET as a modified Draize test that better predicts the human response from accidental ocular exposure to detergent and cleaning products. In the development of LVET and its use as the in vivo reference standard in the mechanisms of eye irritation work conducted by Maurer, Jester and others, the range of materials tested in LVET extends well beyond only surfactant-based materials and surfactant-based products.

The work conducted by Griffith et al. (1) in the early stages of its development used a range of chemicals including solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals that were grouped into four irritancy categories (innocuous/non-irritant, moderate, substantial and severe irritant/corrosive) based on human experience derived from literature e.g. Grants Toxicology of the eye (2), occupational incidents within the industrial setting, Poison Control Centre (PCC) data and reports of consumer exposures to detergent and cleaning products. What should not be in question is that known human ocular corrosives/severe irritants were included in this chemical dataset namely acetic acid (10%), NaOH (10%), Ca(OH)2 (100%) and formaldehyde (38%) which have all been identified as ocular corrosives/severe irritants in the human eye. For all of these chemicals, both the LVET and Draize in this study identified them as ocular corrosives/severe irritants.

Similarly, the chemical set used in the mechanisms of eye irritation work by Maurer et al. (3) and Jester (4) included acids, alkalis, alcohols, ketones, peroxides, aldehydes, bleaches, solvents, peroxides as well as surfactants (anionic, non-ionic, cationic). Several of these materials were identified by LVET as being severe eye irritants, some of which again are known human ocular corrosives (e.g. NaOH).

Furthermore, the publication by Cormier et al. (5)

identified 70 parallel LVET and Draize tests conducted on 53 surfactant-based detergent and cleaning and personal care products. Within this historical dataset, LVET identified products that were not classified, irritant and severe irritant. Given the nature of the products, it is logical and expected that most of these products were identified as NC. However, it is important to recognise that LVET was capable of identifying products within this dataset that did merit irritant classifications.

From this it can be concluded that the retrospective historical and available LVET dataset is: 1) based on a range of substances from different chemical classes and consumer products from different product categories; 2) spans the range of irritancy from innocuous to severe and 3) includes known human ocular corrosives.

2. Comparative traditional Draize rabbit data with which to evaluate the accuracy of the LVET are only available for limited types and numbers of substances (i.e. surfactant-containing personal and household cleaning products and comparative human data from clinical studies and accidental exposures proposed to support its accuracy are largely with substances that are mild or non-irritating [lines 300-305].

Parallel datasets that compare the traditional Draize test with LVET for the same substances are available for both surfactant-based and non-surfactant-based substances/products. Such datasets are reported in the publications by Griffith et al. (1) for a range of chemicals that include solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals and by Cormier et al. (5), Freeberg et al. (6, 7, 8) and Gettings et al. (9, 10) for surfactant-based products.

Indeed, it is the original work by Griffith et al. (1) that investigated dose response characteristics with increasing dose volumes (10 uL, 30 uL, 50 uL and 100 uL). These investigators demonstrated statistically that 10 uL was the most effective dose volume for discriminating between substances with different levels of irritancy (defined by National Academy of Sciences (NAS) and Federal Hazardous Substances Act (FHSA) criteria) from innocuous to severe. Furthermore this study identified that: 1) a 10

uL dose volume is capable of detecting the same range of tissues (cornea, iris, conjunctiva) and severity of effects as in the Draize test; 2) correctly classified materials identified as non-hazardous and hazardous (except SLS (40%)) in humans and 3) demonstrated that 30 uL and 100 uL dose volume in rabbits over-classified materials identified as non-hazardous in humans.

The Cormier et al. (5) work used a historical dataset of 70 Draize-LVET studies on surfactant-based products to evaluate, by regression analysis, the linear relationship of LVET to the Draize test. This work established that LVET gives responses that are linearly correlated to the Draize test. Within this historical dataset, LVET identified products that were not classified, irritant and severe irritants.
The studies by Freeberg et al. used parallel Draize-LVET datasets that were compared with clinical data for the same surfactant-based products in one study (7) and with human experience from industrial accidents and follow-up of consumer accidental exposures in two other studies (6, 8). In their correlation of the Draize-LVET dataset to clinical data (7), four different surfactant-based consumer products (undiluted liquid fabric softener, 20% liquid shampoo, 10% liquid hand soap and 4% liquid laundry detergent) were dosed at both 10 uL (LVET dosing) and 100 uL (Draize dosing) in the rabbit and humans. Though formal classifications were not calculated for the products at the time of this study, retrospective classification has identified that rabbit LVET and Draize tests both identified the liquid laundry detergent tested undiluted as a severe eye irritant (R41). This further addresses a comment in the SRD that only non- or only mild irritants have been tested in LVET.

In the Freeberg et al. work that used parallel Draize-LVET datasets compared with human experience from industrial accidents/follow-up of consumer accidental exposures, 29 detergent and cleaning products were included in one study (6) and 14 detergent and cleaning products and personal care products in a second study (8). The formulations that were included in these evaluations were reflective of product formulations in development and/or marketed to consumers and were identified as mild-moderate irritants on the basis of Maximum Average Scores (MAS) and Time-To-Clear for ocular responses.

From this it can be concluded that several historical parallel LVET-Draize datasets are available and published in the scientific literature that cover surfactant-based materials and products as well as different classes of chemicals including solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals. In all of these rabbit LVET-Draize parallel datasets, the Draize test produced more severe responses in terms of ocular tissues involved (cornea, iris, conjunctiva), severity and persistence of ocular effects than LVET. Since this response addresses availability of rabbit LVET-Draize datasets, correlation to the human response is not discussed here but is addressed in point 4 below. The data from these studies also support the conclusion that the range of irritancy of materials addressed in these historical parallel LVET-Draize datasets is from innocuous to severe.

3. A comparison of the substances that have been classified by the Draize rabbit eye test as ocular corrosives or severe irritants that have also been tested in the LVET indicates that the LVET routinely under-predicts the ocular corrosive or severe irritant response in the Draize in many cases by more than one hazard category. This is illustrated by the results of Gettings et al (1996) in their evaluation of 25 surfactant-containing formulations [lines 422-427].
The above statement makes the assumption that the Draize classification is the correct classification for the surfactant-containing formulations tested by Gettings et al. (9, 10) and does not take into consideration that the Draize classification for these surfactant-containing products could be over-predictions. Some examples of the surfactant-containing formulations classified as EPA Category 1 (corrosive) by the Draize test from the Gettings et al. study (9) are a baby shampoo, bath foam, gel cleanser and facial cleansing foam. Such products included in this study were not prototypes nor were they products rejected for marketing due to excessive eye irritation but were formulations that were representative of those product types in the marketplace at that time for which accidental eye exposure would have undoubtedly been expected to occur. Formulation details for these products are publicly available and a review of these formulations

based on their chemical composition would not indicate that these products would be corrosive to the eye. Furthermore, a corrosive classification is not borne out by the human experience that has occurred over many years for these types of surfactant-containing cosmetic products marketed by several companies. It is reasonable to expect that if a baby shampoo was truly corrosive then marketing of such a product over many years by several companies would have resulted in reports of serious eye effects from accidental eye exposure being detected in the human experience. This is simply not the case.

It is also interesting to note that it is often the result of a single in vivo assay that is used in the correlation of in vitro assay data with the in vivo reference standard. This does not take into account the inherent variability of the in vivo test since without the results of multiple tests it is difficult to assess test variability. One of the few studies to take this into account is the CTFA Phase III study which used bootstrap re-sampling to estimate the within group variability for each test material. Since the Draize test has evolved over time from a 6 animal test to the current 3 animal test it is possible, from the CTFA Phase III study, for each test material, to break down the in vivo 6 animal tests into 20 unique combinations of 3 animal groups. It is then possible to determine a classification for each 3 animal group and identify the number of sub-groups in each classification class. To illustrate this point, test material HZE (gel cleanser) has been chosen from the CTFA Phase III study.

This test material is identified in the LVET SRD in Table 4-3 [line 449] as having a classification of EPA Category I based on the Draize test and EPA Category III based on LVET. An analysis of the 20 unique combinations of 3 animal groups from the 6 animal Draize test for this material identifies that 10 of the possible 20 combinations yield a classification of EPA Category I (corrosive) but interestingly the other 10 possible combinations yield a classification of EPA Category IV (non-irritant). This demonstrates that if a single 3 animal Draize test had been conducted there would be an equal chance of identifying the gel cleanser as a non-irritant or corrosive. Conducting this same exercise for test material HZE (gel cleanser) tested in the 6 animal LVET identifies all 20 unique combinations of 3 animal groups as having a classification of EPA Category III.

The example chosen here simply to illustrate the point is one of the more extreme cases but this does demonstrate the importance of understanding variability in the in vivo assays. As such, this does lead to a question on test variability and correct prediction of classification when the in vivo reference standard is subject to such inherent variability.

4. Comparative human data from clinical studies and accidental exposures proposed to support accuracy of LVET are largely with substances that are mild or non-irritating [lines 303-305].

The work of Griffith et al. (1), Cormier et al. (5) and Freeberg et al. (6, 7, 8) discussed above demonstrates that LVET is capable of identifying severe irritants and does so in the experimental setting including for those materials tested that are known to be corrosives/severe irritants in humans.

The purpose of comparing LVET to human data from clinical studies using the same test materials and to human experience data from industrial and consumer accidental exposures was to: 1) understand the predictive capacity of LVET relative to the human response for the consumer product categories involved and 2) determine whether use of LVET provides a conservative evaluation of eye irritation potential that still over-predicts the human response but less so than the Draize test. As such, taking into account both ethical considerations for the conduct of human studies and the nature of the consumer product types involved, it is entirely to be expected that such LVET to human clinical/experience data should have been generated with materials/products that are in the mild-moderate range of irritancy. No other data can be expected here. This does not detract from the wealth of information that can be established from such studies in which mild-moderate irritants have been evaluated in this way. Key conclusions from such studies include the following:

o Draize (100 uL) dosing in the rabbit over-predicted the human response to 100 uL test material.

This was established as early as 1965 and 1969 by Beckley et al. who conducted two in vivo-clinical study

comparisons in which rabbits and humans were exposed to 100 uL of an undiluted dishwashing product in study 1 (11) and a 5% soap solution and undiluted liquid household cleaner in study 2 (12). In both studies, effects in humans were only or primarily conjunctival whereas effects in the rabbit Draize test were more severe (tissue type, severity and persistence of effects).

Freeberg et al. (7) went on the confirm this in an in vivo-clinical study comparison in which four consumer products (100 % liquid fabric softener, 20% liquid shampoo, 10% liquid hand soap and 4% liquid laundry detergent) were tested using LVET (10 uL) and Draize (100 uL) dosing in both rabbits and humans. Effects in humans with Draize (100 uL) dosing were primarily conjunctival and transient whereas effects in rabbits using Draize (100 uL) dosing were more severe (tissue type, severity and persistence of effects).

o LVET (10 uL) dosing in the rabbit over-predicted the human response to 10 uL and 100 uL test material.

This was established in the same in vivo-clinical comparison study conducted by Freeberg et al. (7) as mentioned in the paragraph immediately above. Again effects in humans using LVET (10 uL) or Draize (100 uL) dosing were primarily conjunctival and transient whereas effects in rabbits using LVET (10 uL) dosing were more severe (tissue type, severity and persistence of effects) although less so than with Draize (100 uL) dosing in the rabbit.

Ghassemi et al. (13) went on to confirm this in an in vivo-clinical study comparison in which a liquid household cleaner was tested undiluted in rabbit LVET and in humans using LVET (10 uL) and Draize (100 uL) dosing. Effects in humans were only conjunctival and transient whereas effects in the rabbit LVET were more severe (tissue type, severity and persistence of effects).

o LVET dosing in the rabbit over-predicted the human response using equivalent LVET dosing in humans.

The in vivo-clinical study comparison conducted by Roggeband et al. (14) with two detergent and cleaning products dosed 1 uL of undiluted dishwashing liquid and 3 u

L of undiluted liquid laundry detergent in rabbits and humans. The dosing volume was established based on ethical considerations in a pilot clinical study and then applied to both rabbits and human in the main study. Effects in humans were primarily conjunctival with any corneal effects being minimal and transitory. More severe effects (tissue type, severity and persistence) were observed in the rabbit. For additional perspective, the dishwashing liquid and liquid laundry detergent tested were formulations that were representative of such products in the marketplace at that time. In the EU, both products would be classified as R36 (irritant) based on LVET data.

From all of these studies, irrespective of the classification of the products involved, key conclusions are that: 1) the severity of effects resulting from Draize (100 uL) dosing in the rabbit is greater than that seen with LVET (10 uL) dosing in the rabbit and 2) both LVET (10 uL) and Draize (100 uL) dosing in the rabbit over-predict the human response in terms of ocular tissues involved, severity of effect and persistence of effect, however the degree of over-prediction observed with LVET (10 uL) dosing in the rabbit is less than with Draize (100 uL) dosing in the rabbit.

5. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential since such exposures are likely immediately followed by flushing the eyes with large volumes of water and may not represent the most severe lesion that might be produced by such an exposure [lines 461-464]

Human experience from industrial and consumer accidental exposures is an important source of data that can be integrated in a Weight of Evidence approach to establishment of reference standards. It is recognised that human experience data have strengths and limitations and clearly depend of the quality, robustness and amount of data available.

Three studies that compare LVET with such human experience data are cited in the published scientific literature. The first is a study by Freeberg et al. (6) in which parallel Draize-LVET datasets were compared with human experience from industrial accidents/follow-up of consumer accidental exposures for 29 detergent and cleaning products. This was

followed by a second study for 14 detergent and cleaning products and personal care products (8). The formulations that were included in these evaluations were reflective of product formulations in development/marketed to consumers at that time and were identified as mild-moderate irritants on the basis of MAS scores and Time-To-Clear for ocular responses. Both studies were designed with reporting criteria to maximise quality and consistency of data. Such acceptance criteria included having at least two human exposure data points for each accidental exposure and a known Time-to-Clear for resolution of ocular effects. In the first study (6), for a two year period covering 1979-1980 the authors found 284 exposures to 23 undiluted products that met the defined acceptance criteria. In addition, 231 employee accidental exposure reports involving 24 products were available providing an overall total of 515 reports for 29 products. Using the parameter of Time-to-Clear, analysis of the data identified that in the vast majority of cases, ocular effects resolved within 4 days with no reports of permanent eye damage. Correlation of the rabbit Draize and LVET data for the 29 products involved identified that the LVET data whilst still over-predicting the human response was less so than the Draize test. This was confirmed in the follow-up study by Freeberg et al. (8) in which human experience data were collected over 18 months from mid-1983 to end-1984 for 218 accidental exposures for 14 detergent and cleaning products of 7 different types that met acceptance criteria further refined from the first study. In this second study, the longest time for complete recovery after any human exposure incident was 4 days.

More recently, Cormier et al. (15) reported a similar study comparing LVET to human experience from consumer contacts for a total of 24 products from different categories of detergent and cleaning products over the time period of 1895-1992 for which LVET data were also available. The data from this study confirmed the conclusions of the Freeberg et al. studies (6, 8) by identifying that LVET, while still being over-predictive, better predicts the human response from consumer accidental eye exposure to different categories of consumer products.

These studies are combined with data from other human

experience data sources such as those from: 1) national and regional Poison Control Centres (e.g. Soap and Detergent 1974-75 and 1976 Intermountain Regional Poison Control Centre studies, Pittsburgh Poison Control Centre 1986-1990 study); 2) the National Electronic Injury Surveillance System (NEISS) 1980-1991 study) and 3) individual company and industry association co-ordinated post-marketing surveillance data.

All of this adds up to in excess of 30 years of human experience data that exist for types of consumer products supported by LVET. These human experience data demonstrate that human accidental exposures to such consumer products involve primarily conjunctival effects with any corneal effects being minimal and transitory and with full resolution of ocular effects in the vast majority of cases being within just a few days. This is a very substantial database that should form part of the WoE approach that correlates LVET back to the human response from accidental exposure to consumer products

Indeed there is precedence in the field of herbal medicines in the EU for use of such human experience data in a WoE approach.

To promote consumer safety, the European Commission introduced legislation which requires all unlicensed traditional herbal medicinal products intended for human use to be registered (Directive 2004/24/EC) (16). One of the issues with subjecting herbal medicinal products to the same level of regulatory compliance afforded pharmacologically active medicinal products was the recognition that many traditionally used medicinal substances may have limited formal safety and clinical efficacy data associated with their use and little demonstrated by contemporary clinical and toxicological methodologies and practices. Where this has been demonstrated, such products have received medicinal product marketing authorizations. Retrospective imposition of clinical and toxicological requirements on manufacturers of such products would in all probability remove products from the market that have many years of demonstrable safety associated with established use.

To address this, the European Commission decided to create a legislative framework for a pragmatic assessment of

clinical efficacy and safety based on the principles of well-established use. Under Directive 2004/24/EC (16), if the regulatory authorities determine that sufficient product knowledge exists, applications can be made without the usual dossier information on safety and efficacy associated with medicinal products, and is replaced with a bibliographic review and expert reports to prove that the herbal medicinal product (or an equivalent medicinal product) has been in medicinal use as a traditional medicinal product in the European Union for a period of at least thirty years (or 15 years in the EU plus 15 years outside of the EU).

From this is can be concluded that the extensive human experience database which covers more than three decades is a legitimate data source to support use of LVET as an appropriate in vivo reference standard for the domains of applicability for which such retrospective historical and available data exist.

6. Since its original development, proponents of the LVET have suggested that it is a more appropriate in vivo reference test method for comparisons to in vitro data than is the Draize rabbit eye test. This is primarily based on the assertion that the LVET is more representative of the human response to a potential ocular hazard than the Draize test, given that the site (corneal surface) and volume of exposure used in the LVET more closely resemble that of accidental human exposure than does the Draize [lines 400-405].

Dose volume is one of the most influential factors that contributes to over-prediction of the human response by the Draize test reported in the scientific literature. The volume of test material instilled into the lower conjunctival sac of the rabbit in the Draize test is 100 u L. This amount exceeds the volume capacity of the rabbit eye lower conjunctival sac that can maximally hold ~80 uL without blinking (17). When 100 uL of test material are placed in the lower conjunctival sac of the rabbit eye, the excess would be expected to spill from the eye. This is actually what is observed in the experimental situation by investigators conducting the Draize Test (18).

Since the tear volume in both the rabbit and humans is

very similar at approximately 7 uL (19, 20) and the volume capacity of the human eye is 10 uL after blinking (17, 21), this would indicate, from an anatomical/physiological viewpoint, that 10 uL is an appropriate choice of dose volume for the in vivo rabbit test. Taking these anatomical/physiological data into account, it is clear that the 10 uL volume is more than the volume that can be in direct contact with either the rabbit or the human eye i.e. more than the total tear volume.

In terms of understanding the volume of material that can contact the human eye in an accidental exposure, it is reasonable also to take the blink reflex into account. Spontaneous blinking continues throughout the waking state and ensures that the continuously secreted tears are adequately distributed across the exposed ocular surface at all times. In the human, the spontaneous blink rate is about 12-20 per minute (22, 23) and serves to refresh the tear film at each blink. This is much more frequent than the spontaneous blink rate of about 3 blinks per hour in the rabbit (24). Adversive blinking in response to a foreign material contacting the surface of the eye is a natural, involuntary and extremely rapid, reflex response that is accompanied by a reflex secretion of tears. Since, the blink reflex is poorly developed in rabbits and highly developed in man, this contributes to an increased conservatism in an in vivo test such as the rabbit LVET or Draize test.

Furthermore, the importance of dose volume and location have been recognised by international scientific organisations such as the National Academy of Sciences (NAS). In 1977, a National Academy of Sciences/National Research Council (NAS/NRC) committee on toxicology reviewed toxicological testing methods for household products for the Consumer Product Safety Commission (CPSC) (25). Whilst recognising that in vivo eye irritation methods have historically called for instillation of 100 u L (or solid equivalent) of a test material into the eye of the rabbit, they acknowledged that the comparative data from controlled exposures of humans and rabbits available at that time (e.g. Beckley et al. (11, 12) showed the responses of the rabbit eye to be much more severe and long-lasting injuries. They also acknowledged that the amount of material that actually contacts the ocular tissues in most accidents is probably considerably less

than 100 uL. They concluded that: 1) since the amount contacting the eye may be as important as the product composition in determining the ocular response, there seemed to be no basis for using a single arbitrary dose in an eye test and 2) the high dose of test material in the in vivo rabbit eye test may be an important factor in explaining the differences between the excessive responses observed in the Draize test and real-life responses observed in humans following accidental exposures to certain classes of products. Based on their review, the Committee suggested the possibility to include use of lower dose volumes in the in vivo test as a means to diminish the ocular irritancy response in the rabbit test enabling a better correlation to the estimated human accidental eye irritation response (25). The Committee also commented on the location for placement of the test material indicating that the desired dose should be applied to the eye in a manner that reflects the probable route of exposure. They recommended placement of the test material directly onto the cornea to better reflect conditions of accidental human exposure. Finally, the Committee advocated that advantage should be taken of any accidental human eye splashes with chemicals to establish some basis for comparison with animal data.

As such, it is concluded that choice of 10 uL as the dose volume for LVET is supported by anatomical/physiological considerations between rabbits and humans.

Though the Draize test has been used as the regulatory accepted in vivo eye irritation assay for decades and hence also as the only in vivo reference standard against which to validate in vitro eye irritation methods, there are, as with any assay, generally recognized limitations of the Draize test. Scientific publications describe challenges of the Draize test related to variability, subjectivity of scoring and over-prediction of the human response (26, 27, 28, 29, 30, 31). These challenges, added to concerns about animal welfare and a scientific desire to have available eye irritation assays that are based on better understanding of eye injury at the tissue and cellular level, have led researchers to investigate 3Rs alternative methods both in vivo (refinement) and in vitro (replacement) methods. LVET is a 3Rs refinement method.

As such, the SRD comment detailed above that reads “Since

its original development, proponents of the LVET have suggested that it is a more appropriate in vivo reference test method for comparisons to in vitro data than is the Draize rabbit eye test" would perhaps be better reflected as proponents of LVET suggest that based on retrospective historical and available data that this test method is an appropriate in vivo reference standard for the domains of applicability for which the data support its use in a WoE approach.

In conclusion, there is an extensive dataset of historically available LVET data that supports use of such existing LVET data as an appropriate in vivo reference standard against which to compare in vitro assays within the context of the current ICCVAM review "Use of In Vitro Methods in a Tiered Approach for Ocular Hazard Identification of Anti-Microbial Products". Furthermore, it provides data for several characteristics of the assay that are key to scientific acceptance of available LVET data for domains of applicability for which the data support its use as a reference standard in a WoE approach. These are:

- Anatomical and physiological basis for choice of 10 uL as an appropriate dose volume
- Ability of 10 uL dose volume to effectively discriminate between materials of different eye irritancy potential
- Ability of LVET to detect the range of ocular responses from innocuous to severe
- Ability of LVET to correctly predict known severe human eye irritants
- Over-prediction of the human response by LVET, but to a lesser extent than the Draize test, thereby remaining a conservative evaluation of eye irritation potential
- Correlation of LVET dosing procedure with the human response in clinical studies
- Correlation of LVET data and human experience data from industrial accidents and consumer accidental exposure for the same consumer products

To not use this extensive historical database on LVET to accept this assay as an appropriate in vivo reference standard for domains of applicability for which the available data support its use in a WoE approach against which to compare in vitro assays would indeed be a badly

missed opportunity to support progress to validation of in vitro eye irritation assays.

I thank you for the opportunity to make this public comment and ask that it be made available before the independent scientific peer review panel on alternative ocular safety testing methods that will take place on May 19-21, 2009.

Yours sincerely,

Dr. R.A. Rapaport,
Associate Director,
Product Safety & Regulatory Affairs,
The Procter & Gamble Company

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