Appendix A

Publicly Available Protocols for the IRE Test Method

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Appendix A1

INVITTOX Protocol 85. The Rabbit Enucleated Eye Test Method of Dr. Lesley Earl

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THE RABBIT ENUCLEATED EYE TEST

The isolated eye of a rabbit is exposed to the test compound and assessed for corneal swelling, corneal opacity and fluorescein retention in order to evaluate the eye irritation potential of the compound.

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NOTE
*The protocol presents the standard operation procedure used in the Home Office UK/EEC Validation Study for Alternatives to the Draize Test. It should be noted that this protocol might need to be modified in light of experience gained in the study. Additional information added in the course of producing this **INVITTOX** protocol, e.g. this note, is presented in italics.*

Critical Assessment

The use of isolated rabbit eyes for the assessment of eye irritation potential is a recognized alternative to the Draize eye irritation test, because it closely models the *in vivo* test system, but does not require the exposure to take place in the eyes of living animals. The procedure permits evaluation of undiluted test materials, i.e. as they could enter the *in vivo* eye test, and in this respect provides a major advantage over many other *in vitro* procedures. This test system could be used as part of an *in vitro* test battery for the assessment of eye irritancy, to provide a higher level of testing after initial screening in simple cell culture-based systems. It will not, however, provide information about potential effects of the test substance on the conjunctivae, nor about the rate of recovery from the insult.

An interlaboratory trial (Whittle *et al.*, 1992), using test substances with various degrees of eye irritation potential, showed good agreement between three laboratories, with 22 out of 27 substances being rated within the same one out of the four irritancy ratings used. This consistency occurred in spite of the fact that the three laboratories adopted different *in vitro* grading systems. Two exposure periods were used, 10 seconds and 60 seconds. When results were compared with *in vivo* data, a better correlation was obtained with results from the 10-second exposure. Better predictions of *in vivo* effects were obtained with liquid test substances than with solid test substances.

Basic Procedure

Objectives
The purpose of the test is to assess the irritation potential of substances applied to the isolated rabbit eye. However, the method does not provide information on the effects of materials on the conjunctiva of the eye, or on any recovery of the cornea from damage, which might occur in the eye *in vivo*.

Summary of test method
The eyes of rabbits are enucleated immediately after death of the animal and are mounted in a temperature-controlled chamber which provides optimum conditions for the continuation of *in vivo* physiology. The eyes are left in the maintenance chamber long enough to stabilise. Test materials are then applied in a single dose to the cornea for 10 seconds. The effects of this treatment are assessed at predetermined intervals by four methods:

1) Assessment of corneal opacity.
2) Measurement of corneal thickness to determine corneal swelling.
3) Assessment of the rate at which fluorescein penetrates into the cornea.
4) Histological examination of the cornea to assess any damage to the corneal epithelium.

The degree of damage to the cornea is recorded over a 4 hour period. The stability of the test system during the study is confirmed by the concurrent observation of an untreated eye. At the end of the experiment, the preparation and examination of histological sections of the cornea can be used to confirm the level of corneal damage.

Procedure Details

Preparation of the *in vitro* model

Selection of animals
Eyes from New Zealand White rabbits are used in this study. Suitable eyes show no opacity of the cornea and no imperfections on the corneal surface based on detailed macroscopic and slit-lamp examination. Sufficient animals are required to provide at least three eyes for each test material to be tested, plus one other eye to serve as an untreated control.

Equipment
The equipment used has been described previously (Burton, York and Lawrence, ***Food and Cosmetics Toxicology***, **19**: 471-480, 1981).

Equipment required is as follows:

Fine surgical scissors
Surgical enucleating scissors
Forceps

Perspex clamps for holding eyes: the clamp has an upper arm that can be moved up and down to accommodate the eyeball, and stainless steel pins embedded in the upper arm and base to hold the eye in place. The pins protrude only to about 1 mm, so as to avoid puncturing the globe. The upper arm is cut away, if required, to permit saline to drip onto the upper surface of the cornea.

Superfusion apparatus: this is a perspex maintenance chamber with six cells, each holding one eye. The walls of each cell are made of black perspex for optimal slit-lamp observation. A stainless steel tube leading from each cell is connected to a peristaltic pump and is used to supply isotonic saline at a constant flow to the cell. The perspex clamp with the eye is positioned within the compartment so that saline from the steel tube drips onto the cornea. Saline is pumped out of the cell via two stainless steel drainage tubes in the rear bottom corners. A sliding door at the front of each cell allows for access to the eye. A water jacket surrounds the maintenance chamber and receives water pumped in from a temperature-controlled water bath. The stainless steel tubes used for saline delivery to the cells pass through the water jacket so that the saline can be warmed to the correct temperature.
Slit lamp microscope, e.g. Haag-Streit AG, Liebefeld-Bern, Switzerland
Depth Measuring Attachment for slit lamp, e.g. Attachment No. 1, Haag-Streit

Prior to preparation of the eyes, the water heater/circulator and the remote thermometer are switched on. After a heating period, the heater control is adjusted to give a stable air temperature within a closed cell of the maintenance chamber of 32°C (± 2°C). The peristaltic pump provides a flow rate of saline to each cell of less than 1 ml/min.

Dissection

N.B. Some training is required in order to carry out this dissection. Care is required to avoid loss of intraocular pressure. A trained dissector can expect to lose one out of every three-four eyes dissected, on account of damage. Spare eyes should be prepared to make up for any loss.

The following dissection procedure is carried out on each selected rabbit:

a) The animal is killed by the injection of pentobarbitone solution into the ear vein.

b) Immediately after death, a few drops of physiological saline are applied to the eyes to prevent them drying during dissection.

c) Each eye is dissected by deflecting the nictitating membrane and cutting away the conjunctivae using angled forceps and curved scissors. The eyeball is proptosed by applying pressure above and below the eyeballs. The remaining conjunctival tissue, the orbital muscles and the optic nerve are cut and the eyeball is lifted from the orbit.

d) Adherent tissue is dissected from the globe of the eyeball, and the eyeball is rinsed with physiological saline.

Supply of tissue

If it is impractical or undesirable to use eyes from rabbits killed at the testing facility, then eyes may be obtained from a local industrial laboratory and transported with minimum delay under maintained conditions to the testing facility.

Eyes are enucleated from dead rabbits in the previously described way at the supplying laboratory. Those animals that had previously been used for experimental purposes by the laboratory, were either used in skin irritation tests, to supply tissues other than eyes or as control (untreated) animals. After removal, the eyes are placed in a large insulated flask. The temperature is maintained by sealing 1 litre of water (37°C) in a plastic bag within the flask. To prevent drying of the enucleated eyes, each eye is thoroughly wetted with saline and the humidity maintained by a quantity of freestanding water (37°C) in the bottom of the flask. The eyes are transported to the testing facility (not more than 1 hour) for the continuation of the procedure. The eyes are then placed in the superfusion chambers.

Pretreatment incubation
After dissection, the eyeball is then mounted in a vertical position in a clamp which holds the eye firmly, but without excessive pressure. The clamp is positioned in a cell of the maintenance chamber. The saline drip tube of the cell is positioned so that the drops of saline fall onto the upper margin of the cornea and irrigate the whole surface of the cornea. All the eyes necessary for the test are dissected out and mounted in the chamber in this way.

Immediately after the eye is positioned in the chamber, it is stained with fluorescein solution (1% fluorescein sodium BP, Smith and Nephew Pharmaceuticals, Romford, Essex, UK - or equivalent) for a few seconds, after which it is rinsed with saline to establish if there has been any damage during dissection, i.e. if there is any evidence of penetration of fluorescein into the eye. If the cornea has been damaged during dissection, that eye is rejected as unsuitable for use and a further eye is prepared as a replacement. The corneal thickness of undamaged eyes is then measured (Slit reading -1).

The eyes are maintained in the chamber to equilibrate for 45-60 minutes, after which the corneal thickness is measured again (Slit reading 0). If Slit reading 0 exceeds Slit reading -1 by more than 5%, then that eye is rejected from the experiment.

Treatment
Three eyes are treated with each test material and one eye remains untreated as a control.

Prior to application of the test materials, the eye, held in its clamp, is removed from the chamber and positioned with the cornea uppermost.

Liquid test materials
0.1 ml of the test material is applied to the central part of the cornea. After 10 seconds, the test liquid is removed from the cornea by rinsing the surface with a 20-ml syringe of saline. The eye is then replaced in the chamber. The saline drip is repositioned as before.

Solid test materials

Test materials which are not in a powder or fine granular form should be ground prior to treatment. 25 mg of the test material is sprinkled evenly over the whole surface of the cornea. After 10 seconds, all the test material is removed from the corneal surface by rinsing with 20 ml of saline at room temperature. If particles of test material adhere to the corneal surface, then the cornea is rinsed further. If the particles cannot be removed, even after excess rinsing, this should be noted. The clamped eye is then returned to the maintenance chamber, and the saline drip is repositioned as before.

Assessments
The cornea of each treated eye and the control eye is assessed by the methods detailed below:

Corneal opacity
A slit-lamp biomicroscope is used to examine the cornea for the degree of opacity (the most dense area is taken for reading) using the following scoring system:

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through opacity	4

Assessments are carried out immediately after treatment, at 30 minutes, and at 1, 2, 3 and 4 hours after treatment.

Corneal thickness
The thickness of the cornea is measured using a slit-lamp biomicroscope fitted with a depth-measuring attachment, or an ultrasonic pachymeter. Refer to slit-lamp manual for instructions on corneal thickness measurements. The definitive values obtained for each eye are recorded and the degree of corneal swelling caused by treatment is calculated as a percentage of the corneal thickness of the eye immediately before treatment (Slit reading 0). Assessments are carried out at 30 minutes, and at 1, 2, 3, and 4 hours after treatment.

Slit-lamp examination of the cornea
Using the slit-lamp set with a narrow slit, the treated corneas are examined for evidence of damage based on reflection of light from different parts of the slit image. The effects are assessed on the following scale:

Slit image identical to control eye	0
Light reflection from one or more regions of the slit image	1

Increased reflection of light suggests some form of corneal damage has occurred.

Assessments are carried out 30 minutes after treatment, and at 1, 2, 3 and 4 hours after treatment.

Penetration of fluorescein into the cornea
One drop of fluorescein solution is applied onto the cornea of each eye for 10 seconds and then rinsed off with saline. The cornea is then examined using a slit-lamp biomicroscope and the staining and diffusion characteristics are assessed according to the following criteria:

No staining	0
Bright green staining of anterior edge of cornea but no penetration	1
Bright green anterior edge to cornea, gradual diffusion of stain through cornea	2

Assessments are carried out at 30 minutes and 4 hours after treatment.

NB In circumstances where grade 3 or grade 4 corneal opacities are present, evaluation of fluorescein penetration is unnecessary.

Supplementary observations
The parameters detailed above provide the minimum requirements for evaluation of effects on the isolated rabbit eye. Solutions of solid substances may also be tested. Further parameters, such as the histological examination of the epithelium, may be recorded. Photography of the eye may also be useful for comparison of responses.

Recording data
The attached score sheet is used to record the individual raw data for each chemical. Further descriptive information can be recorded on a separate sheet, which should be marked with the study number, the compound being evaluated, the date of the experiment and the signature of the operator.

Amendments

Experimental variation
Where the investigator determines that an individual eye has elicited a different response from the other two, similarly treated, eyes, the experiment must be repeated. The data from all six eyes will then be used to calculate the mean values to be used in the overall assessment of damage.

Swelling of control eyes
Control (untreated) eyes are used as an indication of the stability of the test system equipment. They should remain stable without significant change in corneal thickness during the 4 hour experimental observation period. If the corneal thickness of a control eye changes by more than 7% during the 4 hour observation period, then the experiment must be rejected and repeated.

Results

Interpretation
The opacity scores that are obtained with this method are defined in the same way as the Draize corneal opacity scores. The other scores, with the exception of corneal swelling, are numerical representations of qualitative descriptions. No one score is sufficient in itself to assess the effect of a test substance. Damage is assessed by means of different parameters, depending on the nature of the effects observed. For example, when severe opacity is the primary effect in the test, opacity and its time of onset are the important factors to be evaluated. On the other hand, when no opacity is observed, other factors, such as corneal swelling are used in the assessment.

References

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The *in vitro* assessment of severe eye irritants.
Fd. Cosmet. Toxicol. **19**: 471-480.

York M., Lawrence R.S., and Gibson G.B. (1982)
An *in vitro* test for the assessment of eye irritancy in consumer products - preliminary findings.
Int. J. Cosmet. Sci. **4**: 223-234.

Whittle E., Basketter D., York M., Kelly L., Hall T., McCall J., Botham P., Esdaile D., and Gardner J. (1992)
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Appendix A2

Table of IRE Protocols from the Reviewed Literature

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Table of IRE Protocols from the Reviewed Literature

Reference	Unilever Safety & Environmental Assurance Centre (SEAC)	SafePharm Laboratories, Contract Research Organization in the United Kingdom (SOT 2003/2004 posters and Appendix to Unilever protocol)	INVITTOX Protocol #85 (EC/HO Validation Study)	Chamberlain et al. (1997) -- IRAG Evaluation (1 data set)	Cooper et al. (2001)
TEST METHOD COMPONENT					
Eye selection and preparation performed at testing laboratory	Not noted	Not noted	Not noted	Note: Procedure based on Burton et al. (1981). Submitted data based on Lewis et al. (1994)	Not noted
Rabbit strain	New Zealand White	New Zealand White of either sex	New Zealand White	Not noted	Not noted
Eyes inspected on live animal and method of inspection	Suitable eyes show no opacity of the cornea and no imperfections on the corneal surface based on macroscopic and slit-lamp examination	Biomicroscopic examination of cornea using slit-lamp; assessment of corneal uptake of sodium fluorescein; measurement of corneal thickness using ultrasonic pachymeter	Cornea examined for opacity and surface imperfections with slit lamp	Not noted	Not noted
Method of killing animal	Pentobarbitone solution injected into ear vein	Pentobarbitone solution injected into ear vein	Pentobarbitone solution injected into ear vein	Not specified; "humanely sacrificed"	Not noted
Eye dissection	Some training is required in order to carry out this dissection. Care is required to avoid loss of intraocular pressure. Immediately after animal death, saline is applied to eye to prevent drying during dissection. Nictitating membrane and conjunctiva are cut away, and the eyeball is proptosed by applying pressure above and below the eyeball. Orbital muscles and the optic nerve are cut and the eyeball is lifted from the orbit. Excess tissue is dissected from the eyeball. Eyeball is rinsed with physiological saline.	Similar to INVITTOX protocol	Saline applied to eye to prevent drying during dissection. Training recommended. Nictitating membrane and conjunctiva are cut away, and the eyeball is proptosed by applying pressure above and below the eyeball. Orbital muscles and the optic nerve are cut and the eyeball is lifted from the orbit. Excess tissue is dissected from the eyeball.	Not noted	Performed on the premises of the rabbit supplier
Eyes purchased from supplier					
Supplier	Eyes are enucleated in the supplier's facility from rabbits used for other testing purposes (i.e., skin irritation tests, untreated control animals, or tissue supply for studies not involving the eye)	Not noted	Rabbits used for other testing purposes in the supplier's laboratory (i.e., skin irritation tests, untreated control animals, or tissue supply for studies not involving the eye)	Not noted	Eyes were enucleated from animals that had been used for other purposes at a nearby laboratory, then transported to the testing facility with minimum delay
Maintenance of eyes during shipment	After removal, eyes are placed in a large insulated flask. The temperature is maintained by sealing 1 L of water (37°C) in a plastic bag within the flask. Each eye is thoroughly wetted with saline and humidity maintained by free-standing water (37°C) in the bottom of the flask. Eyes are transported to the testing facility within 2 hours.	Not noted	After removal, eyes are placed in an insulated flask, that is maintained at 37°C. Saline is applied to eyes, and added to the bottom of the flask to maintain humidity. Eyes are transported to testing facility within 1 hour.	Not noted	Not noted
Pretreatment equilibration in superfusion apparatus	Eye is mounted in a vertical position in metal clamp that holds the eye firmly, but without excessive pressures. The clamp has metal rings on which the eye sits; it is positioned in a cell of the maintenance chamber. The saline drip tube of the cell is positioned so that drops of saline fall onto the upper margin of the cornea and irrigate the whole surface of the cornea. Peristaltic pump provides a flow rate of saline to each cell of 0.1 - 0.2 mL/min.	Enucleated eyes mounted in perspex clamps and placed in superfusion chamber for equilibration. Peristaltic pump supplies 0.9% saline solution at approximately 32°C	Eye is mounted in a vertical position in a clamp with stainless steel pins embedded in the upper arm and base to hold the eye in place. The pins protrude to about 1 mm, so as to avoid puncturing the globe. Each holder is placed in a cell of a maintenance chamber; saline is dripped onto the cornea at a rate of less than 1 mL/minute.	Eyes are maintained in a superfusion system which maintains them bathed with saline at a constant temperature	On arrival at the testing facility, eyes were placed clamps and mounted in a maintenance chamber; the anterior corneal surface was bathed with a saline drip
Duration	45 - 60 minutes	30 or more minutes	45 - 60 minutes	45 - 60 minutes	Short period to stabilize; otherwise not specified
Temperature	31°C (± 1°C)	32°C (± 1.5°C)	32°C (± 2°C)	About 32°C	31°C

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TEST METHOD COMPONENT					
Method of detecting damaged enucleated eyes prior to use in test	Immediately after the eye is positioned in the chamber, it is stained with 1% fluorescein sodium BP for a few seconds, after which it is rinsed with saline; if any fluorescein penetrates into the eye, the eye is rejected for use and a suitable replacement prepared	Eyes are re-examined after 30 minutes to ensure damage was not caused during dissection. Eyes are rejected if corneal thickness has increased greater than 10% relative to the *in vivo* measurement or if the cornea has stained with fluorescein sodium drops.	1% fluorescein sodium BP applied for a few seconds and rinsed with saline; if any fluorescein penetrates into the eye, the eye is rejected	Enucleated eyes are examined with a slit lamp before use in a test and any with abnormalities are rejected	Eyes were observed during the stabilization period, and any damaged eyes were discarded
First corneal thickness measurement (when performed)	Corneal thickness measured after fluorescein test with slit/pachymeter reading set at -1	*In vivo* then after equilibration.	Corneal thickness measured after fluorescein test with slit reading set at -1	Corneal thickness measured after slit lamp examination with the depth measuring attachment for the slit lamp.	Pretreatment corneal thickness measurement performed, but no details provided
Additional corneal thickness measurements prior to treatment	After equilibration, corneal thickness is measured again (slit/pachymeter reading set at 0). If slit reading 0 exceeds slit reading -1 by more than 4%, the eye is rejected from the experiment.	After equilibration, just before treatment.	After equilibration, corneal thickness is measured again (slit reading set at 0). If slit reading 0 exceeds slit reading -1 by more than 5%, the eye is rejected.	Repeated measurements (to the nearest 0.01 units) are made at the corneal apex while the eye is in the superfusion apparatus. After equilibration, corneal thickness is measured again, and any eyes that have swollen more than 4% relative to the first reading are rejected.	Not noted
Treatment of eyes					
No. of eyes used/test substance	3	3	3	2	3
No. of untreated controls	1	2	1	Not noted	1
Liquid substances	Viscous liquids should be layered onto the cornea to ensure even coverage.	-	-	-	Shampoo formulations
Amount applied	1) 20 µL of test material is applied to the upper margin of the cornea every 10 seconds up to 60 seconds (120 µL total amount applied). Usually, application of liquids to the eye is *in situ* with the eye clamped in the maintenance chamber. The saline drip tube is deflected from the eye during treatment. ***OR*** 2) 20 µL of test material is applied for 10 seconds.	0.1 mL applied evenly to the cornea	The eye in its clamp is removed from the superfusion chamber for treatment; eye is treated with cornea facing upward. 0.1 mL applied to central part of cornea (prior to application of test material, the eye, held in its clamp, is removed from the chamber and positioned with the cornea uppermost	0.1 mL applied to cornea	20 µL of test material applied to the cornea every 10 seconds up to 60 seconds (120 µL total amount applied)
Concentration tested	100%	100%	100%	100%	Formulations were tested at 100% and as 10% (w/v) solutions in distilled water
Exposure duration	10 seconds or 60 seconds	10 seconds	10 seconds	10 seconds	60 seconds
Rinsing procedure	Test material is removed from the cornea with at least 20 mL of physiological saline from a syringe. The saline drip is repositioned to irrigate the eye as before.	Test material is washed off cornea using 20 mL of saline solution warmed to approximately 32°C	Cornea rinsed with 20 mL of saline	Cornea rinsed with 20 mL or more of warmed saline	Not noted

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TEST METHOD COMPONENT					
Solid substances	The eye to be treated is removed from the maintenance chamber fixed in its clamp and positioned horizontally in a petri dish.	-	Solutions of solids may be tested in addition to finely ground or powder forms	-	None tested
Form of solid	Not noted	Not noted	Test materials are applied as a powder or fine granular form	Not noted	Not noted
Amount applied	50 mg	0.1 mL or a maximum of 100 mg sprinkled evenly over the cornea	25 mg	25 mg applied to cornea	Not noted
Concentration tested	Not noted	Not noted	Not noted	Not noted	Not noted
Exposure duration	"Specified exposure period"	10 seconds	10 seconds	10 seconds	Not noted
Method of application	Sprinkled evenly over entire surface of cornea	Sprinkled evenly over entire surface of cornea	Sprinkled evenly over entire surface of cornea	Not noted	Not noted
Rinsing prodedure	All particles are removed from the corneal surface by rinsing with at least 20 mL of physiological saline from a syringe. The clamped eye is returned to the maintenance chamber and saline drip repositioned to irrigate the eye.	Test material is washed off cornea using 20 mL of saline solution warmed to approximately 32°C	Cornea rinsed with 20 mL of saline at room temperature; the cornea is rinsed further if particles stick to surface; if particles cannot be removed completely, this is noted	Cornea rinsed with 20 mL or more of warmed saline	Not noted
Endpoints assessed					
Corneal opacity					
Timepoints after treatment	0.5, 1, 2, 3 and 4 hours after treatment	1, 2, and 4 hours after treatment	0.5, 1, 2, 3 and 4 hours after treatment	Not noted	At regular intervals (not specified) up to 4 hours
Scoring system used	Most dense area taken for reading; macroscopic and microscopic examinations conducted. 0 = No opacity or Normal; 1 = Scattered or diffuse area, details of iris clearly visible or Very slight; 2 = Easily discernible translucent area, details of iris slightly obscured or Slight; 3 = Nacreous (gray/white) area, no details of iris visible, size of pupil barely discernible or Moderate; 4 = Opaque cornea, iris not discernible through opacity or Severe	McDonald-Shadduck system used, which measures the severity of corneal cloudiness and the area of the cornea involved. CORNEAL CLOUDINESS: 0 = Normal cornea; 1 = Some loss of transparency; 2 = Moderate loss of transparency; 3 = Involvement of the entire thickness of the stroma (endothelial surface still visible); 4 = Involvement of the entire thickness of the stroma (endothelial surface not visible). AREA: 0 = normal cornea with no area of cloudiness; 1 = 1 - 25% of stromal cloudiness; 2 = 26 - 50% area of stromal cloudiness; 3 = 51 - 75% area of stromal cloudiness; 4 = 76 - 100% area of stromal cloudiness	Draize system for scoring corneal opacity; 0 = no opacity, 1 = scattered or diffuse, 2 = discernible transluscent area, 3 = nacreous area, and 4 = opaque cornea	Not noted	Draize system for scoring corneal opacity; 0 = no opacity, 1 = scattered or diffuse, 2 = discernible transluscent area, 3 = nacreous area, and 4 = opaque cornea
Instrumentation	Slit lamp biomicroscope is used to examine cornea for degree of opacity	Slit lamp biomicroscope is used to examine cornea for degree of opacity	Slit lamp biomicroscope is used to examine cornea for degree of opacity	Not noted	Not noted
Corneal thickness					
Timepoints after treatment	0.5, 1, 2, 3 and 4 hours after treatment	1, 2, and 4 hours after treatment	0.5, 1, 2, 3 and 4 hours after treatment	Not specifed in report; intervals up to 5 hours after application of test substance	At regular intervals (not specified) up to 4 hours
Instrumentation	Slit lamp biomicroscope fitted with a depth-measuring device, or an ultrasonic pachymeter	Ultrasonic pachymeter (DGH Technology Incorporated, Solana Beach, California)	Slit lamp biomicroscope fitted with a depth-measuring device, or an ultrasonic pachymeter	Slit lamp biomicroscope fitted with a depth-measuring device	Ultrasonic pachometer (Teknar Ophthsonic pachometer, Mentor O&O Inc., MA, USA)

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TEST METHOD COMPONENT					
Method of evaluating degree of swelling as a result of treatment	Value obtained for each eye is recorded; degree of corneal swelling caused by treatment is calculated as a percentage of the corneal thickness of the eye just prior to treatment (slit reading 0)	Not described	Value obtained for each eye is recorded; degree of corneal swelling caused by treatment is calculated as a percentage of the corneal thickness of the eye just prior to treatment (slit reading 0)	Corneal thickness is measured and expressed as percentage of corneal swelling relative to pretreatment corneal thickness value (a continuous variable)	Corneal thickness is measured and expressed as percentage corneal swelling throughout the 4 hour observation time using the pretreatment thickness value
Fluorescein penetration/staining					
Timepoints after treatment	60 minutes	4 hours after treatment (assessment of corneal uptake of sodium fluorescein)	0.5 and 4 hours after treatment (Not conducted when grade 3 or 4 corneal opacities are present)	Not noted	Performed, but few details provided
Method of application	1 drop of fluorescein solution is applied to the cornea for 10 seconds, then is rinsed off with saline	Not described	1 drop of fluorescein solution is applied to the cornea for 10 seconds, then is rinsed off with saline	Not noted	Not noted; the extent to which fluorescein penetrated the cornea was assessed visually by using a Zeiss slit lamp
Scoring system used	N = negligible (occasional punctate staining with no diffusion of stain into the stroma); M = marginal (punctuate staining across cornea with some evidence of slight diffusion into cornea); D = distinct (pale continuous staining of the epithelium with slow diffusion into the stroma); L = bright area of stain to extreme outer edge of cornea, with no penetration into cornea; S = intense staining of the epithelium and anterior stroma with very rapid diffusion into the remainder of the stroma; E = intense staining of very badly damaged cornea, which appears yellow/orange as opposed to bright green of previous grades; O = other effect	0 = Absence of fluorescein staining. 1 = Slight fluorescein staining confined to a small focus. 2 = Moderate fluorescein staining confined to a small focus. 3 = Marked fluorescein staining that may involve a larger portion of the cornea. 4 = Extreme fluorescein staining. (More detail provided in Appendix to Unilever protocol)	Staining and diffusion characteristics are assessed as follows: 0 = no staining, 1 = bright green staining of anterior cornea edge but no penetration, 2 = bright green anterior edge to cornea and gradual diffusion of stain through cornea	Not noted	Fluorescein penetration is expressed using a graded scoring system (not specified)
Macroscopic examination of cornea	Not noted	Not noted	Not noted	Any changes in the normal appearance of the cornea are carefully noted	Not noted
Timepoints after treatment	Not noted	Not noted	0.5, 1, 2, 3 and 4 hours after treatment	Not noted	Not noted
Instrumentation	Not noted	Not noted	Slit lamp	Not noted	Not noted
Histology performed?	After the final assessments and measurements have been taken (240 minutes), each eye is removed from its chamber cell, and the cornea is dissected, fixed, processed, and embedded in paraffin wax for sectioning. Sections are cut and stained. Corneal evaluation is divided into 2 distinct areas: epithelial and stromal response.	Not noted	Histological examination of corneal epithelium is noted as a supplementary observation that may be performed	Not noted	After 4 hour observation period, the corneas were excised and fixed for histological assessment of epithelial and stromal responses; the number of epithelial cell layers that had eroded and evidence of other histopathological changes were recorded

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Reference	Unilever Safety & Environmental Assurance Centre (SEAC)	SafePharm Laboratories, Contract Research Organization in the United Kingdom (SOT 2003/2004 posters and Appendix to Unilever protocol)	INVITTOX Protocol #85 (EC/HO Validation Study)	Chamberlain et al. (1997) -- IRAG Evaluation (1 data set)	Cooper et al. (2001)
TEST METHOD COMPONENT					
Other observations	Slit-lamp examination of the cornea at 0.5, 1, 2, 3, 4 hours after treatment. Using the slit-lamp set with a narrow slit, the treated corneas are examined for evidence of damage based on reflection of light from different parts of the slit image. The effects are scored as follows: N = normal; BG = more reflection than control eye, most intense at anterior margin decreasing gradually towards the posterior margin; BD = distinct bright line on anterior margin and little reflection from remainder of cornea; BT = intense reflection throughout cornea reflecting presence of significant primary opacity. Increased reflection of light suggests some form of corneal damage has occurred.	Corneal epithelium observations	Slit-lamp examination of the cornea at 0.5, 1, 2, 3, 4 hours after treatment. Using the slit-lamp set with a narrow slit, the treated corneas are examined for evidence of damage based on reflection of light from different parts of the slit image. The effects are scored as follows: 0 = slit image identical to control eye; 1 = light reflection from one or more regions of the slit image. Increased reflection of light suggests some form of corneal damage has occurred. Photography of the eye may be useful for comparing responses	-	-
Criteria for an acceptable test	There are no criteria set for the control eyes post treatment; the eyes are checked pretreatment and this has been found to be sufficient to weed out any damaged eyes. If, however, there is an unusual degree of change in the control whether by swelling, macro, or even micro observation, the test would be repeated, with consideration made on a case-by-case basis.	Not described	Control eyes should remain stable without > 7% change in corneal thickness during the 4 hour observation period	Not noted	Not noted
Irritancy classification	Normal = no effects; Very slight = No significant effects on any category (<11% swelling and/or 1-2 cell layers lost); Slight = Any unusual effect, slight opacity (>11% swelling and/or 3-4 cell layers lost); Moderate = Slight/moderate opacity and/or >25% swelling and/or 5-6 cell layers lost; Severe = Moderate/severe opacity and/or >35% swelling and/or 7-8 cell layers lost.	Any parameter that meets or exceeds the following cut-off values indicates a severe eye irritant. *Cut-off Values to Detect Severe Eye Irritants*: Maximum corneal opacity (corneal cloudiness x area) ≥ 4; Maximum fluorescein uptake (intensity x area) ≥ 4; Mean corneal swelling (60, 120, 240 minutes) ≥ 25%; Corneal epithelium observations = any with pitting, mottling or sloughing	Damage is assessed by means of different parameters, depending on the effects observed.	Any chemical causing >15% corneal swelling at any time after treatment is considered to have the potential to cause severe ocular irritation *in vivo*	The classification is generally based on the weight of evidence from the opacity score, the % corneal swelling, and the number of epithelial cell layers eroded, with any one endpoint triggering the higher classification. Very slight irritant (opacity = 0, or corneal swelling < 11%, or 0-2 epithelial cell layers lost); Slight (opacity = 1-2, or corneal swelling = 12-25%, or 3-4 epithelial cell layers lost); Moderate (opacity = 2-3, or corneal swelling = 26-35% or 5-6 epithelial cell layers lost); Severe (opacity = 3-4, or corneal swelling = >35% or 7-8 epithelial cell layers lost)
Conducted in compliance with GLPs	Not noted	Not noted	Not noted	Not noted	Not noted
Other Notes	-	-	-	-	-

Table of IRE Protocols from the Reviewed Literature

Reference	Gettings et al. (1996)
TEST METHOD COMPONENT	
Eye selection and preparation performed at testing laboratory	Not noted
Rabbit strain	New Zealand White
Eyes inspected on live animal and method of inspection	Not noted
Method of killing animal	Not noted
Eye dissection	Performed on the premises of the rabbit supplier
Eyes purchased from supplier	
Supplier	A supplier was used, but specific supplier not noted
Maintenance of eyes during shipment	Eyes were transported to the laboratory under humid conditions at 31°C
Pretreatment equilibration in superfusion apparatus	On receipt at testing facility, each eye was mounted in a vertical position in a perspec clamp. The clamp was positioned in a cell of a maintenance chamber at 31°C and the corneal surface bathed with a saline drip.
Duration	Approximately 30 minutes
Temperature	31°C

Table of IRE Protocols from the Reviewed Literature

Reference	Gettings et al. (1996)
TEST METHOD COMPONENT	
Method of detecting damaged enucleated eyes prior to use in test	Eyes were stained with 2% fluorescein, examined using a slit lamp, and those retaining fluorescein were discarded
First corneal thickness measurement (when performed)	Corneal thickness measured after slit lamp examination with the depth measuring attachment for the slit lamp (slit reading -1)
Additional corneal thickness measurements prior to treatment	After equilibration, corneal thickness is measured again (slit reading set at 0). If slit reading 0 exceeds slit reading -1 by more than 4%, the eye was rejected.
Treatment of eyes	
No. of eyes used/test substance	3
No. of untreated controls	1
Liquid substances	Surfactant-based formulations
Amount applied	20 µL of test material was applied to the upper margin of the cornea every 10 seconds up to 60 seconds (120 µL total amount applied)
Concentration tested	100%
Exposure duration	60 seconds
Rinsing procedure	Test material was removed by rinsing with 20 mL saline

Table of IRE Protocols from the Reviewed Literature

Reference	Gettings et al. (1996)
TEST METHOD COMPONENT	
Solid substances	-
Form of solid	Not noted
Amount applied	Not noted
Concentration tested	Not noted
Exposure duration	Not noted
Method of application	Not noted
Rinsing prodedure	Not noted
Endpoints assessed	
Corneal opacity	
Timepoints after treatment	Immediately after treatment and at 0.5, 1, 2, 3, and 4 hours after treatment
Scoring system used	Macroscopic examination; Scoring system not described
Instrumentation	Not noted
Corneal thickness	
Timepoints after treatment	At 0.5, 1, 2, 3, and 4 hours after treatment
Instrumentation	Corneal thickness measured with the depth measuring attachment for the slit lamp

Table of IRE Protocols from the Reviewed Literature

Reference	Gettings et al. (1996)
TEST METHOD COMPONENT	
Method of evaluating degree of swelling as a result of treatment	Post-treatment corneal thickness values were compared with the pretreatment value and expressed as the percentage increase in thickness
Fluorescein penetration/staining	
Timepoints after treatment	1 hour after treatment
Method of application	Fluorescein solution is applied and initial staining of cornea and diffusion into corneal stroma assessed by slit lamp
Scoring system used	Not noted
Macroscopic examination of cornea	Slit lamp examination using both open and narrowed slit settings to assess any damage to the corneal epithelium
Timepoints after treatment	Immediately after treatment and 0.5, 1, 2, 3, 4 hours after treatment
Instrumentation	Slit lamp
Histology performed?	Performed but not described

Table of IRE Protocols from the Reviewed Literature

Reference	Gettings et al. (1996)
TEST METHOD COMPONENT	
Other observations	-
Criteria for an acceptable test	Not noted
Irritancy classification	Report states that "test materials were classified into four groups ranging from no significant effects to maximal response." However, no other information was provided.
Conducted in compliance with GLPs	Not noted
Other Notes	-

Table of IRE Protocols from the Reviewed Literature

Reference	Jones et al. (2001)	Koeter and Prinsen (1985)	Lewis et al. (1994a)	Price and Andrews (1985)	Whittle et al. (1992) - method A
TEST METHOD COMPONENT					
Eye selection and preparation performed at testing laboratory	Not noted	Rabbits that had been used in primary skin irritation or eye irritation studies were used as eye donors	Not noted	Not noted	Interlaboratory study of 3 laboratories, but not all labs used same methods
Rabbit strain	Not noted	New Zealand White	New Zealand White albino	Not noted	New Zealand White
Eyes inspected on live animal and method of inspection	Not noted	Only animals that were in good health and free of any eye defects were used	Eyes were examined *in vivo* for suitability before testing	Rabbits with microscopically normal eyes were selected and corneal thickness was measured using a Zeiss photoslit-lamp microscope, specially modified to take photographs through the pachometer	Corneal thickness of eyes was measured *in vivo*
Method of killing animal	Not noted	Not noted	Animals were humanely killed; no other information provided	An iv overdose of sodium pentobarbitone	Lethal dose of pentobarbitone sodium was administered via the marginal ear vein
Eye dissection	Performed on the premises of the rabbit supplier	Not noted	Immediately after death, a few drops of saline (0.85%) were applied to the eyes to prevent them from drying during dissection. The eyes were dissected carefully, the eyeball was proptosed, the adjacent conjuntival tissue, orbital muscles and the optic nerve were cut, and the eyeball was lifted from the socket.	Dissected as described in Burton et al. (1981)	Immediately after death, each eye was dissected carefully but rapidly, avoiding contact with or drying of the corneal surface
Eyes purchased from supplier					
Supplier	Eyes were enucleated from animals that had been used for other purposes at a nearby laboratory, then transported to the testing facility with minimum delay	Not noted	Not noted	Not noted	Not noted
Maintenance of eyes during shipment	Not noted	Not noted	Not noted	Not noted	Not noted
Pretreatment equilibration in superfusion apparatus	On arrival at the testing facility, eyes were placed in clamps and mounted in a maintenance chamber; the anterior corneal surface was bathed with a saline drip	Not noted	Each eyeball was mounted in a vertical position in a perspex clamp held within a chamber that was fitted with a pump that delivered saline (about 32°C) at regular intervals to the surface of the cornea	The apparatus used to maintain eyes was similar to that described in Burton et al. (1981). Enucleated eyes were lightly supported by clamps within temperature-regulated chambers and warm saline was dripped continuously over their surfaces.	The eye was mounted in a perspex clamp within a temperature-controlled superfusion chamber, such that the cornea was in a vertical position facing the observer. Each compartment of the chamber was equipped such that isotonic saline solution dripped onto the cornea and flowed down over the cornea surface
Duration	Short period to stabilize; otherwise not specified	Not noted	45 - 60 minutes	Approximately 30 minutes	30 - 45 minutes
Temperature	31°C	Not noted	About 32°C	Not noted	32 ± 1.5°C

Table of IRE Protocols from the Reviewed Literature

Reference	Jones et al. (2001)	Koeter and Prinsen (1985)	Lewis et al. (1994a)	Price and Andrews (1985)	Whittle et al. (1992) - method A
TEST METHOD COMPONENT					
Method of detecting damaged enucleated eyes prior to use in test	Eyes were observed during the stabilization period, and any damaged eyes were discarded	All eyes were examined with a slit-lamp microscope just before treatment	A pretreatment measurement of corneal thickness was taken using a slit lamp and pachymeter (Carl Zeiss, 30 SL)	Eyes were examined and only those within an *in vitro* corneal thickness measurement within 2 machine units of the *in vivo* reading were used.	After equilibratioin, two drops of 1% (w/v) fluorescein solution were applied to the eye and washed off with saline after a few seconds. Corneal thickness was measured. Eyes were rejected if they either retained fluorescein stain or had a corneal thicknesss 4% or greater than *in vivo* reading.
First corneal thickness measurement (when performed)	Pretreatment corneal thickness measurement performed, but no details provided	Pretreatment corneal thickness measurement performed, but no details provided	Just before equilibration period	*In vivo*. First performed on enucleated eye just after equilibration period.	*In vivo*. First performed on enucleated eye just after equilibration period.
Additional corneal thickness measurements prior to treatment	Not noted	Not noted	Just after equilibration period. The percentage corneal swelling was calculated and any eyes that had swollen more than 4% relative to the first reading were rejected.	Not noted	Not noted
Treatment of eyes					
No. of eyes used/test substance	3	4	2	6 or more	3 eyes
No. of untreated controls	1	2	Not noted	Used, but a specific number not noted	1 eye
Liquid substances	Shampoo and conditioner formulations	-	-	-	-
Amount applied	20 µL of test material applied to the cornea every 10 seconds up to 60 seconds (120 µL total amount applied)	100 µL	100 µL applied directly to the cornea	100 µL of test substance was dripped onto the surface of the eye	100 µL applied to the eye using a 1 mL syringe
Concentration tested	All formulations were tested at 100% and the shampoos were also tested as 10% (w/v) solutions in distilled water	Not noted	100%	100%	100%
Exposure duration	60 seconds	5 - 10 seconds	10 seconds	Approximately 10 seconds	10 seconds
Rinsing procedure	Not noted	The corneal surface was rinsed thoroughly with approximately 20 mL of isotonic saline	Test chemical was removed by rinsing the surface of the cornea with at least 20 mL warmed saline	Excess test substance was washed off using warm saline (usually 5 drops from an eye dropper, but sometimes a greater volume and/or force was used, if necessary)	Test substance was washed off using saline at about 32°C

Table of IRE Protocols from the Reviewed Literature

Reference	Jones et al. (2001)	Koeter and Prinsen (1985)	Lewis et al. (1994a)	Price and Andrews (1985)	Whittle et al. (1992) - method A
TEST METHOD COMPONENT					
Solid substances	None tested	-	-	None tested	-
Form of solid	Not noted	Not noted	Not noted	Not noted	Not noted
Amount applied	Not noted	100 mg	25 mg applied directly to the cornea	Not noted	25 mg applied directly to the cornea
Concentration tested	Not noted	Not noted	100%	Not noted	100%
Exposure duration	Not noted	5 - 10 seconds	10 seconds	Not noted	10 seconds
Method of application	Not noted	Solids were dusted onto the eyes	Not noted	Not noted	For solids, the eye was removed from the superfusion chamber, and placed so that the cornea faced upwards
Rinsing prodedure	Not noted	The corneal surface was rinsed thoroughly with approximately 20 mL of isotonic saline	Test chemical was removed by rinsing the surface of the cornea with at least 20 mL warmed saline	Not noted	While the eye was still outside the superfusion apparatus, the solid test substance was washed off with saline; then the eye was returned to its chamber
Endpoints assessed					
Corneal opacity					
Timepoints after treatment	At regular intervals (not specified) up to 4 hours	30, 75, 120, 180, 240 minutes	Before dosing and at 0.5, 1, 2, 3, 4, 5 hours after dosing	Not evaluated	Immediately after treatment and at 30, 60, 120, 180, 240 and 300 minutes
Scoring system used	Draize system for scoring corneal opacity; 0 = no opacity, 1 = scattered or diffuse, 2 = discernible transluscent area, 3 = nacreous area, and 4 = opaque cornea	0 = no effect or negligible effect, 1 = slight degree of corneal opacity, 2 = moderate degree of corneal opacity, 3 = marked degree of corneal opacity (the final score = the sum of scores for each of the 4 eyes and was interpreted as follows: 1-5 = slight effects, 6-9 = moderate effect, 10-12 = severe effect)	The cornea of each eye was assessed by macroscopic examination for evidence of opacification of the cornea; no additional information was provided	Not noted	Area most dense used for scoring. No opacity = 0; scattered or diffuse areas, details of iris visible = 1; easily discernible translucent area, iris slightly obscured = 2; severe corneal opacity, iris not visible, pupil barely discernible = 3; complete corneal opacity, iris invisible = 4.
Instrumentation	Not noted	Not noted	Not noted	Not noted	Not noted
Corneal thickness					
Timepoints after treatment	At regular intervals (not specified) up to 4 hours	30, 75, 120, 180, 240 minutes	Before dosing and at 0.5, 1, 2, 3, 4, 5 hours after dosing	1, 2, 3, 4, 5 hours	Immediately after treatment and at 30, 60, 120, 180, 240 and 300 minutes
Instrumentation	Ultrasonic pachometer (Teknar Ophthsonic pachometer, Mentor O&O Inc., MA, USA)	Depth-measuring device mounted on a slit-lamp microscope	Not noted	Zeiss photoslit-lamp microscope, equipped with a pachometer, specially modified to take photographs through the pachometer	Not noted

Table of IRE Protocols from the Reviewed Literature

Reference	Jones et al. (2001)	Koeter and Prinsen (1985)	Lewis et al. (1994a)	Price and Andrews (1985)	Whittle et al. (1992) - method A
TEST METHOD COMPONENT					
Method of evaluating degree of swelling as a result of treatment	Corneal thickness is measured and expressed as percentage corneal swelling throughout the 4 hour observation time using the pretreatment thickness value	Corneal thickness is measured and expressed as percentage corneal swelling throughout the 4 hour observation time using the pretreatment thickness value; the interpretation of the observed swelling was based on the mean maximum swelling for all 4 eyes and also on the time of occurrence	The mean percentage corneal swelling relative to the pretreated (control) value was calculated for each treated pair of eyes	Not noted	Not noted
Fluorescein penetration/staining					
Timepoints after treatment	Performed, but few details provided	Before treatment and 30 minutes after treatment	4 hours	If used, fluorescein was applied 4 hours after dosing	240 minutes posttreatment
Method of application	Not noted; the extent to which fluorescein penetrated the cornea was assessed visually by using a Zeiss slit lamp	2% fluorescein sodium solution was applied to the surface of the cornea for a few seconds followed by rinsing with isotonic saline	Not noted	Not noted	Not noted
Scoring system used	Fluorescein penetration is expressed using a graded scoring system (not specified)	0 = none or a few cells permeable, 1 = small number of cells permeable, 2 = individual cells and areas of the cornea permeable, 3 = entire cornea permeable (the final score = the sum of scores for each of the 4 eyes and was interpreted as follows: 1-5 = slight effects, 6-9 = moderate effect, 10-12 = severe effect)	Not noted	The rate and degree of penetration of the stroma were assessed	No fluorescein retention = 0; small number of cells retaining fluorescein = 1; individual cells and areas of the cornea retaining fluorescein = 2; large areas of the cornea retaining fluorescein =3
Macroscopic examination of cornea	Not noted	Pitting of corneal epithelial cells, loosening of epithelium, roughening of the corneal surface, and sticking of the test substance to the cornea; the final score for these effects was subjective and represented the mean value of all 4 eyes	Not noted	Any qualitative changes in the appearance of the cornea were noted and/or photographed	During exposure, eyes were examined for any macroscopic signs of damage
Timepoints after treatment	Not noted	Not noted	Not noted	Not noted	Not noted
Instrumentation	Not noted	Not noted	Not noted	Not noted	Not noted
Histology performed?	After 4 hour observation period, the corneas were excised and fixed for histological assessment of epithelial and stromal responses; the number of epithelial cell layers that had eroded and evidence of other histopathological changes were recorded	Not noted	Not noted	Not noted	After 300 minutes posttreatment, lab A and lab B removed the corneas from the eyes, fixed the corneas in Bouins fixative, mounted them in wax blocks, and sectioned using standard histological techniques. The number of cell layers eroded from the corneal epithelium was noted.

Table of IRE Protocols from the Reviewed Literature

Reference	Jones et al. (2001)	Koeter and Prinsen (1985)	Lewis et al. (1994a)	Price and Andrews (1985)	Whittle et al. (1992) - method A
TEST METHOD COMPONENT					
Other observations	-	-	-	-	-
Criteria for an acceptable test	Not noted	Not noted	Not noted	Not noted	Not noted
Irritancy classification	The classification is generally based on the weight of evidence from the opacity score, the % corneal swelling, and the number of epithelial cell layers eroded, with any one endpoint triggering the higher classification. Very slight irritant (opacity = 0, or corneal swelling < 11%, or 0-2 epithelial cell layers lost); Slight (opacity = 1-2, or corneal swelling = 12-25%, or 3-4 epithelial cell layers lost); Moderate (opacity = 2-3, or corneal swelling = 26-35% or 5-6 epithelial cell layers lost); Severe (opacity = 3-4, or corneal swelling = >35% or 7-8 epithelial cell layers lost)	The final *in vitro* irritancy grade was assessed by averaging the final scores of permeability, corneal opacity, corneal swelling, and the macroscopic effects	Any chemical causing more than 15% corneal swelling at any time after treatment was considered to have the potential to cause severe ocular irritancy *in vivo*	Grade I = <20% increase in corneal thickness in 5 hours, Grade II = ≥20% increase in corneal thickness in 5 hours, Grade III = ≥20% increase in corneal thickness in 2 hours, Grade IV = ≥20% increase in corneal thickness in 1 hour. The grade is increased by 1 if eyes stain with fluorescein. The grade for a test substance is the overall mean for 6 eyes.	LAB A: No significant effects (<11% swelling, 0-2 epithelial cell layers eroded) = 1; effects but no opacity (>11% corneal swelling and/or 3-4 epithelial cell layers eroded) = 2; slight-moderate opacity and/or >25% corneal swelling and/or 5-6 epithelial cell layers eroded = 3; immediate opacity or moderate-severe opacity that develops over time and/or >35% swelling and/or 7-8 epithelial cell layers = 4. LAB B: Grading was based on a subjective judgement of the measured parameters, each of which influenced the grading to a greater or lesser extent, such that the significance of the % corneal swelling > epithelial cell erosion ≥ corneal opacity > fluorescein retention. LAB C: <20% corneal swelling within 5 hours = 1; ≥20% corneal swelling by 5 hours = 2; ≥20% corneal swelling within 2 hours = 3; ≥20% coreal swelling within 1 hour or if corneal opnacity was visible to the naked eye = 4
Conducted in compliance with GLPs	Not noted	Not noted	Not noted	Not noted	Not noted
Other Notes	-	-	-	-	Each laboratory adopted an approach to the assessment of results based on previous experience with the technique in their laboratory.

Table of IRE Protocols from the Reviewed Literature

Reference	Whittle et al. (1992) - method B	York et al. (1994)	CEC (2001)
TEST METHOD COMPONENT			
Eye selection and preparation performed at testing laboratory	Interlaboratory study of 3 laboratories, but not all labs used same methods	Not noted	Interlaboratory study of 3 laboratories, but not all labs used same methods
Rabbit strain	New Zealand White	Not noted	New Zealand White
Eyes inspected on live animal and method of inspection	Corneal thickness of eyes was measured *in vivo*	Not noted	Corneal thickness measured in vivo in all laboratories
Method of killing animal	Lethal dose of pentobarbitone sodium was administered via the marginal ear vein	Not noted	Lethal dose of Euthesate or sodium pentobarbitol via the marginal ear vein
Eye dissection	Immediately after death, each eye was dissected carefully but rapidly, avoiding contact with or drying of the corneal surface	Not noted	Immediately after death, each eye was dissected in approximately two minutes with extreme care to avoid touching the corneal surface. Left sufficient length of optic nerve to prevent rupture and loss of intra-ocular pressure
Eyes purchased from supplier			
Supplier	Not noted	Eyes were purchased from another establishment where rabbits had been used for other purposes that would not adversely affect the eyes.	For I.H.S. Proefstations voor Veeteelt (Merelbeke, Belgium)
Maintenance of eyes during shipment	Not noted	Eyes were dissected immediately after animal's death, and transported quickly to testing facility under warm, moist conditions.	Not noted
Pretreatment equilibration in superfusion apparatus	The eye was mounted in a perspex clamp within a temperature-controlled superfusion chamber, such that the cornea was in a vertical position facing the observer. Each compartment of the chamber was equipped such that isotonic saline solution dripped onto the cornea and flowed down over the cornea surface	After each eye had been mounted in the perfusion chambers, the procedures were consistent with Burton et al. (1981)	45-60 Minutes at 32 C
Duration	30 - 45 minutes	Not noted	45-60 minutes
Temperature	32 ± 1.5°C	Not noted	32 ± 1.5°C

Table of IRE Protocols from the Reviewed Literature

Reference	Whittle et al. (1992) - method B	York et al. (1994)	CEC (2001)
TEST METHOD COMPONENT			
Method of detecting damaged enucleated eyes prior to use in test	After equilibratioin, two drops of 1% (w/v) fluorescein solution were applied to the eye and washed off with saline after a few seconds. Corneal thickness was measured. Eyes were rejected if they either retained fluorescein stain or had a corneal thicknesss 4% or greater than *in vivo* reading.	Not noted	Fluorescein sodium 2% (w/v) applied to corneal surface for a few seconds and then rinsed off with 5-10 mL of isotonic saline at 32 ° C
First corneal thickness measurement (when performed)	*In vivo*. First performed on enucleated eye just after equilibration period.	Not noted	Not noted
Additional corneal thickness measurements prior to treatment	Not noted	Not noted	After fluorescein staining for damage assessment, then post-equilibration, then at 30, 75, 120, 180a nd 240 minutes after test substance application (Shell used 60 instead of 30 and 75 minutes)
Treatment of eyes			
No. of eyes used/test substance	3 eyes	1 Eye for 10 sec. treatment + 1 eye for 60 sec. Treatment	3 Eyes for each test substance
No. of untreated controls	1 eye	1 Eye	1 Eye
Liquid substances	-	Not tested	-
Amount applied	20 µL of test material applied to the cornea every 10 seconds up to 60 seconds (120 µL total amount applied over 6 applications)	Not noted	100 µL was applied to the cornea for 10 seconds; then rinsed with 20 mL of isotonic saline
Concentration tested	100%	Not noted	100% unless otherwise specified
Exposure duration	60 seconds	Not noted	10 seconds
Rinsing procedure	Not noted	Not noted	20 mL isotonic saline

Table of IRE Protocols from the Reviewed Literature

Reference	Whittle et al. (1992) - method B	York et al. (1994)	CEC (2001)
TEST METHOD COMPONENT			
Solid substances	-	Eyes were removed from the temperature-controlled chambers and arranged so that the cornea faced upwards.	-
Form of solid	Not noted	Not noted	Not noted
Amount applied	25 mg applied directly to the cornea	50 mg	100 mg
Concentration tested	100%		100% unless otherwise specified
Exposure duration	60 seconds	10 seconds and 60 seconds	10 seconds
Method of application	For solids, the eye was removed from the superfusion chamber, and placed so that the cornea faced upwards	Sprinkled over the cornea.	Sprinkled to cover the entire cornea
Rinsing prodedure	While the eye was still outside the superfusion apparatus, the solid test substance was washed off with saline; then the eye was returned to its chamber	The test material was rinsed from each eye using an excess (usually 20 mL) of warm isotonic saline then returned to its chamber, and the saline perfusion restarted	The test material was rinsed from each eye using 20 mL of warm isotonic saline then returned to its chamber, and the saline perfusion restarted
Endpoints assessed			
Corneal opacity			
Timepoints after treatment	Immediately after treatment and at 30, 60, 120, 180, 240 and 300 minutes	Immediately after treatment and at 4 hours	Immediately after treatment and at 60 , 120, 180, and 240 minutes; except Shell used 60, 120, 240 and 300 minutes and I.H.E used 60, 120, 180 and 240 minutes
Scoring system used	Area most dense used for scoring. No opacity = 0; scattered or diffuse areas, details of iris visible = 1; easily discernible translucent area, iris slightly obscured = 2; severe corneal opacity, iris not visible, pupil barely discernible = 3; complete corneal opacity, iris invisible = 4.	Opacification scored immediately after treatment and maximum corneal opacity. Based on Draize et al. (1944) for corneal assessment of corneal opacity *in vivo*	Area most dense used for scoring. No opacity = 0; scattered or diffuse areas, details of iris visible = 1; easily discernible translucent area, iris slightly obscured = 2; severe corneal opacity, iris not visible, pupil barely discernible = 3; complete corneal opacity, iris invisible = 4.
Instrumentation	Not noted	Not noted	Not noted
Corneal thickness		Maximum corneal swelling	Maximum corneal swelling
Timepoints after treatment	Immediately after treatment and at 30, 60, 120, 180, 240 and 300 minutes	4 hours after treatment	30 ,75, 120, 180 and 240 minutes after treatment of eyes; except Shell used 60, 120, 180, 240 minutes
Instrumentation	Not noted	Slit lamp with a pachometer attachment	Slit lamp by TNO-CIVO and Shell; ultrasonic pachometer at I.H.S.

Table of IRE Protocols from the Reviewed Literature

Reference	Whittle et al. (1992) - method B	York et al. (1994)	CEC (2001)
TEST METHOD COMPONENT			
Method of evaluating degree of swelling as a result of treatment	Not noted	Not noted	Percent increase in thickness at each time point relative to Tzero was calculated
Fluorescein penetration/staining			
Timepoints after treatment	240 minutes posttreatment	Performed, but few details provided	30, 240 minutes
Method of application	Not noted	Not noted	Drops of 2% (w/v) fluorescein sodium applied to cornea for a few seconds, then rinsed off with 5-10 mL of isotonic saline at 32ºC
Scoring system used	No fluorescein retention = 0; small number of cells retaining fluorescein = 1; individual cells and areas of the cornea retaining fluorescein = 2; large areas of the cornea retaining fluorescein =3	Assessment was qualitative	No fluorescein retention = 0; small number of cells retaining fluorescein = 1; individual cells and areas of the cornea retaining fluorescein = 2; large areas of the cornea retaining fluorescein =3
Macroscopic examination of cornea	During exposure, eyes were examined for any macroscopic signs of damage	Not noted	During exposure, eyes were examined for any macroscopic signs of damage
Timepoints after treatment	Not noted	Not noted	Not noted
Instrumentation	Not noted	Not noted	Not noted
Histology performed?	After 300 minutes posttreatment, lab A and lab B removed the corneas from the eyes, fixed the corneas in Bouins fixative, mounted them in wax blocks, and sectioned using standard histological techniques. The number of cell layers eroded from the corneal epithelium was noted.	Histological evaluation of loss of corneal epithelial cells was performed.	No

Table of IRE Protocols from the Reviewed Literature

Reference	Whittle et al. (1992) - method B	York et al. (1994)	CEC (2001)
TEST METHOD COMPONENT			
Other observations	-	-	-
Criteria for an acceptable test	Not noted	Not noted	Not noted
Irritancy classification	LAB A: No significant effects (<11% swelling, 0-2 epithelial cell layers eroded) = 1; effects but no opacity (>11% corneal swelling and/or 3-4 epithelial cell layers eroded) = 2; slight-moderate opacity and/or >25% corneal swelling and/or 5-6 epithelial cell layers eroded = 3; immediate opacity or moderate-severe opacity that develops over time and/or >35% swelling and/or 7-8 epithelial cell layers = 4. LAB B: Grading was based on a subjective judgement of the measured parameters, each of which influenced the grading to a greater or lesser extent, such that the significance of the % corneal swelling > epithelial cell erosion ≥ corneal opacity > fluorescein retention. LAB C: <20% corneal swelling within 5 hours = 1; ≥20% corneal swelling by 5 hours = 2; ≥20% corneal swelling within 2 hours = 3; ≥20% coreal swelling within 1 hour or if corneal opnacity was visible to the naked eye = 4	Emphasis was placed on the development of corneal opacity that was visible immediately after the test material was rinsed from the treated eye.	Although independent standard methods were used in each laboratory to perform the calculations, an overall *in vitro* irritancy grade was assigned as follows: A = Not irritant; B = Slightly irritating; C = Moderately irritating; D = Severely irritating.
Conducted in compliance with GLPs	Not noted	Not noted	Not noted
Other Notes	Each laboratory adopted an approach to the assessment of results based on previous experience with the technique in their laboratory.	-	Each laboratory adopted an approach to the assessment of results based on previous experience with the technique in their laboratory.

Appendix B

Chemical and Product Class Information for the Substances Tested in the IRE Test Method

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Appendix B1

Chemical and Product Class Information for the Substances Tested in the IRE Test Method

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Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
Acetaldehyde	75-07-0	ORGANIC	Flammable liquid used in manufacture of acetic acid, perfumes, and flavors
Acetic acid	64-19-7	CARBOXYLIC ACID	Food preservative and acidifier, Solvent, Manuf of acetates, acetyl compounds, cellulose acetate, rayon, plastics and rubber in tanning
Acetone	67-64-1	KETONE	Solvent; Antiseptic; Chemical intermediate; Raw material
Acetone (F33)	67-64-1	KETONE	Solvent; Antiseptic; Chemical intermediate; Raw material
2-(Acetyloxy)-1-phenylethanone (F27)	-	KETONE, ESTER	Raw material
Allyl alcohol (F34)	107-18-6	ALCOHOL	Resins, Plasticizers, War gas, Allyl compounds
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methylethyl)-gamma-phenyl-, iodide (F14)	-	ONIUM, AMIDE	Active pharmaceutical ingredient
1-(5-Amino-2-methoxyphenyl) piperazine hydrochloride (F20)	-	HETEROCYCLE, AMINE	Chemical intermediate
tetra-Aminopyrimidine sulfate	5392-28-9	AMINE, HETEROCYCLE, SALT, INORGANIC	Developer
Ammonium nitrate	6484-52-2	SALT, INORGANIC, ONIUM	Fertilizer; Chemical intermediate; Industrial explosive
Ammonium nitrate (F36)	6484-52-2	SALT, INORGANIC, ONIUM	Fertilizer; Chemical intermediate; Industrial explosive
L-Aspartic acid	70-47-3	AMINO ACID	Organic intermediate; Fungicides; Germicides
Benzalkonium chloride	8001-54-5	INORGANIC SALT, ONIUM	Surfactant (cationic), Bactericide, Fungicide, Preservative
Benzalkonium chloride (1 %)	8001-54-5	ONIUM	Surfactant (cationic), Bactericide, Fungicide, Preservative
Benzalkonium chloride (10%)	8001-54-5	ONIUM	Surfactant (cationic), Bactericide, Fungicide, Preservative
Benzalkonium chloride (5%)	8001-54-5	ONIUM	Surfactant (cationic), Bactericide, Fungicide, Preservative
3-((Benzylthio)methyl)-6-chloro-,1,1-dioxide (F29)	-	ETHER, SULFUR COMPOUND, ORGANIC	Active pharmaceutical ingredient
Brij 35	9002-92-0	ALCOHOL	Detergent, Solubilizer, Emulsifier, Lubricant
Butanol	71-36-3	ALCOHOL	Solvent; Chemical intermediate; Flavor ingredient
iso-Butanol	78-83-1	ALCOHOL	Solvent; Chemical intermediate; Flavor ingredient
n-Butanol (F35)	71-36-3	ALCOHOL	Solvent; Chemical intermediate; Flavor ingredient

Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
2-Butoxyethyl acetate	112-07-2	ESTER	Solvent, Textile dyeing and printing, Leather treatment, Production of placticizers, Stabilizer
N-Butyl acetate	123-86-4	ESTER	Solvent; Synthetic flavor ingredient
n-Butyl acetate (F39)	123-86-4	ESTER	Solvent; Synthetic flavor ingredient
g-Butyrolactone	96-48-0	HETEROCYCLE, LACTONE	Synthetic intermediate; Solvent
Captan 90 concentrate	133-06-2	IMIDE, SULFUR COMPOUND, ORGANIC	Pesticide
4-Carboxybenzaldehyde	619-66-9	CARBOXYLIC ACID, ALDEHYDE	Manufacturing impurity (polyester); Developer intermediate
Cetylpyridium bromide (F37)	140-72-7	ONIUM, HETEROCYCLE	Surfactant (cationic), Germicide, Laboratory reagent
Cetylpyridinium bromide (0.1%)	140-72-7	ONIUM, HETEROCYCLE	Surfactant (cationic), Germicide, Laboratory reagent
Cetylpyridinium bromide (10%)	140-72-7	ONIUM, HETEROCYCLE	Surfactant (cationic), Germicide, Laboratory reagent
Cetylpyridinium bromide (6%)	140-72-7	ONIUM, HETEROCYCLE	Surfactant (cationic), Germicide, Laboratory reagent
5-Chloro-2,4-disulfamoyl chloroacetanilide (F22)	-	AMIDE	Raw material
Chloroform	67-66-3	HYDROCARBON, HALOGENATED COMPOUND, ORGANIC	Solvent
5-Chloro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (F18)	-	SULFUR COMPOUND, ORGANIC	Raw material
5-Chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[B]thiophene-2-sulfonamide monohydrochloride (F4)	-	HETEROCYCLE, ETHER, AMIDE	Active pharmaceutical ingredient
Chlorhexidine	55-56-1	AMIDINE	Disinfectant; Mouthwash; Anti-infective agent
Cyclohexanol	108-93-0	ALCOHOL	Solvent; Chemical intermediate
Dibenzoyl-L-tartaric acid	2743-38-6	CARBOXYLIC ACID	Optical resolution agent
Dibenzyl phosphate	1623-08-1	ESTER, ORGANOPHOSPHOROUS COMPOUND	Not classified
Dibutyltin chloride	683-18-1	ORGANOTIN COMPOUND	Molluscicide, Slime control in paper mills, Wood preservative, disinfectant, Biocide in cooling systems, Leather and textile processing
3,4-Dichloroaniline hydrochloride (F11)	-	AMINE, ORGANIC SALT, CYCLIC HYDROCARBON	Chemical intermediate
2,6-Dichlorobenzenesulfonyl chloride (F1)	6579-54-0	ORGANIC SALT, CYCLIC HYDROCARBON, SULFUR COMPOUND	Raw material
2,6-Dichlorobenzoyl chloride	4659-45-4	ACYL HALIDE	Anti-infective; Anti-fungal; Preservative
2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (F16)	96568-04-6	ESTER, KETONE, HETEROCYCLE	Chemical intermediate
1-(3,4-Dichlorophenyl)-5-isopropylbiguanide HCl (F6)	537-21-3	AMIDINE	Active pharmaceutical ingredient
3,4-Dimethoxybenzaldehyde (F19)	120-14-9	ALDEHYDE	Raw material
2,2-Dimethylbutanoic acid	595-37-9	CARBOXYLIC ACID	Pharmaceutical metabolite

Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
Dimethyl carbonate (F30)	616-38-6	CARBOXYLIC ACID	Raw material
2,5-Dimethylhexanediol	110-03-2	ALCOHOL	Intermediate for pharmaceticals, pesticides, perfumes
Dimethyl sulfoxide	67-68-5	SULFUR COMPOUND	Solvent, Antifreeze, Paint and varnish remover,
Ethanol	64-17-5	ALCOHOL	Solvent; Beverages; Antifreeze agent
Ethyl acetate	141-78-6	ESTER	Solvent; Synthetic flavoring
2-Ethyl-1-hexanol	104-76-7	ALCOHOL	Solvent; Plasticizer
Ethyl-2-methylacetoacetate	609-14-3	KETONE, ESTER	Not classified
Ethyl trimethyl acetate	3938-95-2	ESTER	Solvent
Fluorescein, sodium	518-47-8	POLYCYCLIC COMPOUND, CYCLIC HYDROCARBON	Phthalic indicator dye; used to detect corneal lesions in ophthalmology
p-Fluoraniline	371-40-4	AMINE	Intermediate for herbicides; Dyes
Fomesafen, acid form (solid)	72128-02-0	IMIDE, ETHER, NITRO COMPOUND	Pesticide
Glycerol	56-81-5	ALCOHOL	Solvent; Plasticizer; Lubricant; Emollient; Drug vehicle
Glycerol	56-81-5	ALCOHOL	Solvent; Plasticizer; Lubricant; Emollient; Drug vehicle
Glycerol (F41)	56-81-5	ALCOHOL	Solvent; Plasticizer; Lubricant; Emollient; Drug vehicle
n-Hexane	110-54-3	ACYCLIC HYDROCARBON	Solvent; Adhesive; Gasoline additive
n-Hexanol	111-27-3	ALCOHOL	Solvent; Chemical intermediate; Synthetic flavor ingredient
3-Hydroxy-2-phenyl-4-quinolinecarboxylic acid (F24)	485-89-2	HETEROCYCLE, CARBOXYLIC ACID, ALCOHOL	Chemical intermediate
HZA-1	-	FORMULATION	Shampoos, Hair care
HZB-1	-	FORMULATION	Soaps and surfactants
HZC-1	-	FORMULATION	Shampoos, Hair care
HZD-1	-	FORMULATION	Shampoos, Hair care
HZE-1	-	FORMULATION	Soaps and surfactants
HZF-1	-	FORMULATION	Shampoos, Hair care
HZG-1	-	FORMULATION	Shampoos, Hair care
HZH-1	-	FORMULATION	Soaps and surfactants, cosmetics
HZI-1	-	FORMULATION	Soaps and surfactants
HZJ-1	-	FORMULATION	Shampoos, Hair care
HZK-1	-	FORMULATION	Soaps and surfactants
HZL-1	-	FORMULATION	Soaps and surfactants
HZM-1	-	FORMULATION	Shampoos, Hair care
HZN-1	-	FORMULATION	Shampoos, Hair care
HZP-1	-	FORMULATION	Shampoos, Hair care
HZQ-1	-	FORMULATION	Soaps and surfactants
HZR-1	-	FORMULATION	Soaps and surfactants
HZS-1	-	FORMULATION	Soaps and surfactants
HZT-1	-	FORMULATION	Soaps and surfactants
HZU-1	-	FORMULATION	Soaps and surfactants
HZV-1	-	FORMULATION	Shampoos, Hair care
HZW-1	-	FORMULATION	Soaps and surfactants
HZX-1	-	FORMULATION	Shampoos, Hair care
HZY-1	-	FORMULATION	Shampoos, Hair care
HZZ-1	-	FORMULATION	Soaps and surfactants
Imidazole	288-32-4	HETEROCYCLE	Anti-fungal; Enzyme inhibitor
1H-Indole-2,3-dione (F28)	91-56-5	HETEROCYCLE	Raw material
Iodine chloride with pyridine (1:1) (F10)	6443-90-9	HETEROCYCLE	Raw material

Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
di-Isopropyl aminoethyldiphenyl acetamide (F15)	-	AMINE, AMIDE	Raw material
Isopropyl dicyanamide (F9)	35695-36-4	AMINE, NITRILE	Chemical intermediate
Maneb	12427-38-2	AMINE, SALT, ORGANIC, UREA	Pesticide
Mebrophen hydramine HCl (F5)	13977-28-1	ETHER, AMINE, SALT	Active pharmaceutical ingredient
Mercuric chloride	7546-30-7	INORGANIC	Topical antiseptic/disinfectant; corrosive agent
2-Methoxyethanol	109-86-4	ALCOHOL	Solvent
1-(2-Methoxyphenyl)piperazine hydrogen sulfate (F2)	-	HETEROCYCLE, SULFUR COMPOUND, ORGANIC SALT	Raw material
Methyl acetate	79-20-9	ESTER	Solvent; Chemical intermediate; Synthetic flavor ingredient
6-(Methylamino)-2-pyridine ethanol formate (1:1) (salt) (F17)	-	HETEROCYCLE, AMINE, CARBOXYLIC ACID, SALT	Chemical intermediate
Methyl cyanoacetate	105-34-0	ESTER, NITRILE	Adhesive; Pharmaceutical intermediate
Methylcyclopentane	96-37-7	HYDROCARBONS, CYCLIC	Solvent
4,4'-Methylenebis-(2,6-di-tert-butylphenol) (F43)	118-82-3	SULFUR COMPOUND, ORGANIC	Raw material
Methyl ethyl ketone	78-93-3	KETONE	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals
Methyl ethyl ketone (F38)	78-93-3	KETONE	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals
Methyl isobutyl ketone	108-10-1	KETONE	Solvent; Synthetic flavor; Drycleaning
1-Naphthalene acetic acid	86-87-3	CARBOXYLIC ACID, POLYCYCLIC COMPOUND	Pesticide
1-Naphthalene acetic acid, Na salt	61-31-4	SALT, ORGANIC, POLYCYCLIC COMPOUND, CARBOXYLIC ACID, SALT	Pesticide
2-Nitro-4-propoxyaniline (F23)	-	NITRO COMPOUND, AMINE, ETHER	Chemical intermediate
2-Nitro-4-thiocyanoaniline (F7)	54029-45-7	NITRO COMPOUND,AMINE,SULFUR COMPOUND, ORGANIC	Chemical intermediate
2-Nitro-4-thio-N-propylaniline (F21)	54393-89-4	AMINE,NITRO COMPOUND,SULFUR COMPOUND, ORGANIC	Chemical intermediate
n-Octanol	111-87-5	ALCOHOL	Solvent; Fragrance
tetra-N-Octylammonium bromide (F8)	14866-33-2	ONIUM	Raw material
2-(4-Oxopentyl)-1H-isoindole-1,3 (2H)-dione (F26)	3197-25-9	HETEROCYCLE	Chemical intermediate
(S)-1-Phenyl-N-propylamine (F13)	3789-59-1	AMINE, ORGANIC	Raw material
Polyethylene glycol 400	25322-68-3	ALCOHOL, ETHER	Surfactant (nonionic), Lubricant, Plasticizer, Solvent
Polyethylene glycol 400 (F44)	25322-68-3	ALCOHOL, ETHER	Surfactant (nonionic), Lubricant, Plasticizer, Solvent
Potassium cyanate	590-28-3	SALT, INORGANIC	Herbicide; Pharmaceutical intermdiate
Promethazine HCl	58-33-3	AMINE, HETEROCYCLE, SULFUR COMPOUND, ORGANIC	Antihistamine; Anti-nausea drug
iso-Propanol	67-63-0	ALCOHOL	Solvent; Aerosol formulations (ingredient)

Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
Propylene glycol (F42)	57-55-6	ALCOHOL	Antifreeze, Solvent, Emulsifier, Resins, Inhibitor of fermentation and mold growth
Pyridine	110-86-1	HETEROCYCLE	Solvent; Intermediate for pharmaceuticals, dyes, pesticides
4,4'-Pyridylpiperidine (F12)	-	HETEROCYCLE	Raw material
Quinacrine	69-05-6	AMINE, HETEROCYCLE, POLYCYCLIC COMPOUND	Anti-infective (anti-helmentic)
Silver nitrate	7761-88-8	INORGANIC	Germicide
Sodium dicyanamide (F3)	-	AMIDE	Raw material
Sodium dodecyl sulfate	151-21-3	ORGANIC SALT, CARBOXYLIC ACID, SALT	Anionic detergent, Emulsifier, Lubricant, Solubilizer
Sodium hydroxide	1310-73-2	INORGANIC, ALKALi	Acid neutralizer; caustic agent
Sodium hydroxide (1%)	1310-73-2	ALKALI	Caustic agent
Sodium hydroxide (10%)	1310-73-2	ALKALI	Caustic agent
Sodium hydroxide (F31)	1310-73-2	ALKALI	Caustic agent
Sodium lauryl sulfate (15 %)	151-21-3	SALT, ORGANIC, CARBOXYLIC ACID, SALT	Surfactant (anionic), Detergent
Sodium lauryl sulfate (3 %)	151-21-3	SALT, ORGANIC, CARBOXYLIC ACID, SALT	Surfactant (anionic), Detergent
Sodium oxalate	62-76-0	SALT, ORGANIC, CARBOXYLIC ACID, SALT	Textile finishing; Pyrotechnic, Industrial byproduct
Sodium perborate tetrahydrate	10486-00-7	SALT, INORGANIC, BORON COMPOUND	Household cleaner; Detergent
4,4`-Sulfonylbisbenzenamine (F25)	80-08-0	SULFUR COMPOUND, ORGANIC	Active pharmaceutical ingredient
Toluene	108-88-3	CYCLIC HYDROCARBON	Solvent; Gasoline additive; Manufacture of benzene derivatives, medicines, dyes, perfumes
Toluene	108-88-3	CYCLIC HYDROCARBON	Solvent; Gasoline additive; Manufacture of benzene derivatives, medicines, dyes, perfumes
Toluene (F40)	108-88-3	CYCLIC HYDROCARBON	Solvent; Gasoline additive; Manufacture of benzene derivatives, medicines, dyes, perfumes
Triacetin	102-76-1	ESTER	Solvent, Fixative
Tributyltin chloride	688-73-3	ORGANOTIN COMPOUND	Molluscicide, Slime control in paper mills, Wood preservative, disinfectant, Biocide in cooling systems, Leather and textile processing
Trichloroacetic acid (3%)	76-03-9	CARBOXYLIC ACID	Caustic agent; Fixative; Herbicide
Trichloroacetic acid (30%)	76-03-9	CARBOXYLIC ACID	Caustic agent; Fixative; Herbicide
Trichloroacetic acid (F32)	76-03-9	CARBOXYLIC ACID	Caustic agent; Fixative; Herbicide
Triethanolamine	102-71-6	AMINE, ALCOHOL	Chemical intermediate, Cosmetic ingredient, Vulcanization acelerator
Triton X-100 (10 %)	9002-93-1	ETHER	Surfactant (nonionic), Detergent, Emulsifier
Triton X-100 (5 %)	9002-93-1	ETHER	Surfactant (nonionic), Detergent, Emulsifier

Table of Chemical and Product Class Information

Substance	CASRN	Chemical Class	Product Class
Tween 20	9005-64-5	ESTER, ETHER	Surfactant (nonionic), Detergent

Appendix B2

Table of Formulation Components in Gettings et al. (1996)

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Formulation	Formulation Components	% (w/w)
HZA-Shampoo No. 7[a]	Water	53.86
	Sodium lauryl sulfate (30%)	25.00
	Disodium laureth sulfocuccinate (40%)	15.00
	Lauramide DEA	0.50
	Butylene glucol	5.00
	Methyl and propylparabens	0.25
	Carageenan	0.35
	Methyl and methylchloroisothiazolinone	0.04
HZB-Liquid Soap No. 1	Water and volatiles	65-85
	Ammonium lauryl sulfate	1-10
	Sodium laureth sulfate	1-10
	Lauramide DEA	1-10
	Glycerine	1-10
	Isostearamidopropyl morpholine lactate	0.1-1.0
	Disodium ricinoleamido MEA-sulfosuccinate	0.1-1.0
	DMDM hydantoin	0.1-1.0
	Citric acid	0.1-1.0
	Triclosan	0.1-1.0
	Tetrasodium EDTA	<0.1
	FD&C Yellow No. 5	<0.1
	FD&C Red No. 4	<0.1
HZC-Shampoo No. 1	Water	14.037
	Laurylamidopropyl betaine (30%)	60.000
	Cetrimonium chloride	16.000
	PEG-3 cocamide	4.500
	Citric acid	3.500
	Sodium chloride	1.000
	Ditallowdimonium chloride (73%)	0.700
	Lauryl alcohol	0.250
	Methyl and chloroisothiazolinone (1.5%)	0.033

Formulation	Formulation Components	% (w/w)
HZD-Shampoo No. 5[a]	Water	54.120
	Sodium laureth sulfate (26%)	38.00
	Cocamide DEA	3.000
	Cocamide propyl betaine (37%)	1.750
	Disodium EDTA	0.050
	Methylparaben	0.150
	Propylparaben	0.100
	Citric acid	0.250
	FD&C Yellow No. 5 (1%)	0.050
	D&C Red No. 33 (0.5%)	0.015
	DMDM hydantoin (54%)	0.300
	BHT	0.050
	Sodium glutamate	2.000
	Sodium choride	0.170
HZE-Gel Cleanser[a]	Water	59.974
	Acylglutamate CT-12 (30%)	15.000
	Cocoamphodiacetate (50%)	15.000
	Sodium nonoxynol-6 phosphate (88.5%)	6.000
	Quaternium-26 (58%)	1.500
	PEG-120-methyl glucose dioleate	1.500
	Citric acid	0.100
	Sodium citrate	0.500
	Disodium EDTA	0.050
	Methylparaben	0.150
	DMDM hydentoin (55%)	0.200
	FD&C Yellow No. 10 (1%)	0.001
	D&C Blue No. 1 (0.746%)	0.025
HZF-Baby Shampoo No. 2[a]	Water	57.653
	Sodium laureth (2EO) sulfate (28%)	21.430
	Disodium laureth-3-sulfosuccinate (40%)	9.090
	Cocamidopropyl betaine (30%)	10.000
	Lauramide DEA	1.500
	Kathon CG (1.5%)	0.067
	Tetrasodium EDTA (30%)	0.260

Formulation	Formulation Components	% (w/w)
HZG-Shampoo No. 8	Water	48.43
	Sodium laureth sulfate (28%)	20.00
	Sodium lauryl sulfate (30%)	25.00
	Lauramide-DEA	5.00
	Hydroxyethyl tallow glycinate	1.00
	Citric acid	0.20
	PEG-45M	0.20
	Methyl and propylparabens	0.13
	Methyl and chloromethyl-isothiazolinone	0.04
HZH-Eye Make-Up Remover[a]	Water	96.242
	Sodium laureth sulfate (21%)	0.900
	Cocoamphocarboxyglycinate (40%)	1.100
	Hexylene glycol	1.000
	Dipotassium phosphate	0.394
	Potassium phosphate	0.102
	Allantoin	0.050
	Methyl paraben	0.150
	EDTA	0.150
	Rose water	0.008
	Thimerosal	0.003
HZI-Skin Cleanser	Water	44.0
	Sodium laureth sulfate (30%)	50.0
	Cocamide MEA	5.0
	Sodium chloride	0.4
	Disodium EDTA	0.2
	Imidizolidinyl urea	0.2
	Methylparaben	0.2
	Benzoic acid	0.1

Formulation	Formulation Components	% (w/w)
HZJ-Mild Shampoo[a]	Water	52.09
	Tween 20	12.63
	Cocoamphodiacetate (24%)	21.25
	PEG 6000	2.60
	Cedepal TD403 (75%)	6.53
	Hydrochloric acid (15%)	1.68
	Arlacel 20	0.92
	Benzyl alcohol	0.10
	Dowicil 200	0.10
	D&C Yellow No. 10 (0.2%)	1.70
	D&C Orange No. 4 (0.2%)	0.20
HZK-Bubble Bath[a]	Water	68.75
	Sodium laureth sulfate (60%)	25.00
	Lauramide DEA	4.50
	SD Alcohol 3-A	3.75
	Sodium chloride	0.80
	Triethanolamine	0.40
	Phosphoric acid (86.5%)	0.35
	Sorbic acid	0.20
HZL-Foam Bath	Water	47.760
	Sodium laureth sulfate (26%)	46.000
	Cocamido propyl betaine (30%)	2.500
	Sodium chloride	2.400
	Glycol monostearate	0.400
	Color solution	0.300
	DMDM hydantoin (54%)	0.250
	Methylparaben	0.200
	Propylparaben	0.100
	BHT	0.050
	Aloe vera gel	0.015
	Citric acid	0.016
	Tetrasodium EDTA	0.010

Formulation	Formulation Components	% (w/w)
HZM-Shampoo No. 3	Water	80-90
	Ammonium lauryl sulfate	5-10
	Lauramide DEA	1-5
	Cocamidopropyl sultaine	1-5
	Citric acid	<1.0
	Ammonium chloride	<1.0
	DMDM Hydantoin	<1.0
	Tetrasodium EDTA	<1.0
	Methylparaben	<1.0
	FD&C Yellow No. 5	<0.1
	D&C Yellow No. 10	<0.1
	FD&C Red No. 4	<0.1
	PPG-9	q.s.
HZN-Shampoo No. 6[a]	Water	44.381
	Sodium laureth (2EO) sulfate (28%)	43.634
	cocamidopropyl betaine (30%)	11.760
	Tetrasodium EDTA	0.125
	Formalin	0.100
HZP-Baby Shampoo No. 1[a]	Water	49.54
	PEG-80 sorbitan laurate (50%)	23.60
	Sodium trideceth sulfate (50%)	17.40
	Lauroamphocarboxyglycinate (50%)	5.40
	PEG-150 distearate (50%)	5.00
	Cocamidoroyl hydroxysultane (50%)	4.00
	Sodium laureth-13 carboxylate (50%)	1.00
	Quaternium 15	0.03
	Benzyl alcohol	0.05
	FD&C Yellow No. 5 (1.0%)	0.25
	FD&C Yellow No. 6 (1.0%)	0.05
	Citric acid	0.08

Formulation	Formulation Components	% (w/w)
HZQ-Cleansing Gel[a]	Water	68.93
	Lauramphocarboxyglycinate (25%)	10.40
	Sodium trideceth sulfate (16%)	10.60
	TEA-lauryl sulfate (40%)	3.50
	Lauramide DEA	0.50
	PEG-150 distearate	2.80
	Propylene glycol	1.40
	Hexylene glycol	1.05
	Citric acid	0.28
	Diazolidinyl urea	0.20
	Methylparaben	0.20
	Sodium citrate	0.14
HZR-Facial Cleansing Foam	Water	32.97
	Sodium cocoyl isethionate	20.00
	Sodium lauroyl sarcosinate (30%)	25.00
	PPG-5-ceteth-10 phosphate	4.00
	Linoleamide DEA	2.00
	Sorbitol (70%)	2.75
	Glycol stearate	5.50
	Glycerin	2.00
	Diglycerol	2.00
	Cetearyl alcohol	2.75
	Mineral oil	0.50
	Methylparaben	0.15
	Propylparaben	0.10
	Trisodium EDTA	0.10
	Beeswax	0.10
	Ceresin	0.06
	Sodium borate	0.02

Formulation	Formulation Components	% (w/w)
HZS-Shower Gel	Water	27.567
	Sodium lauroyl sarcosinate (30%)	25.000
	Laurimidopropyl betaine (30%)	25.000
	Cocamidopropyl hydroxysultaine (50%)	15.000
	Linoleamide DEA	4.500
	Glycol stearate	1.000
	Polyquaternium-2	1.000
	Phosphoric acid (86.5%)	0.600
	Tetrasodium EDTA	0.200
	BHT	0.050
	PPG-12-buteth-16	0.050
	Methyl and chlorosothiazolinone (1.5%)	0.033
HZT-Polishing Scrub	Water	33.85
	Mineral oil	10.00
	Lauroamphocarboxyglycinate (25%)	8.80
	Sodium trideceth sulfate (16%)	9.40
	Petrolatum	6.60
	Isopropyl palmitate	6.60
	Propylene glycol	5.00
	Cetyl palmitate	4.40
	Glyceryl stearate and PEG-100 stearate	4.40
	Aluminum silicate	3.00
	Cetyl alcohol	2.50
	Polypropylene	2.50
	Magnesium aluminum silicate	1.00
	Titanium dioxide	0.50
	Hexylene glycol	0.40
	Imidazolidinyl urea	0.30
	Methylparaben	0.30
	Lactic acid	0.25
	Propylparaben	0.20

Formulation	Formulation Components	% (w/w)
HZU-Hand Soap	Water	37.95
	Sodium C14-16 olefin sulfonate (36%)	20.25
	Sodium lauroyl sarcosinate	20.00
	Cocamidopropyl hydroxysultaine	8.00
	Propylene glycol	3.00
	Glycerol stearate	3.00
	PPG-12-PEG-50 lanolin	3.00
	Polyquaternium-7	2.00
	Citric acid	1.00
	Hydrolysed animal protein	1.00
	Polyquaternium-10	0.50
	Quaternium-15	0.20
	Aloe vera gel	0.10
HZV-Shampoo No. 4	Water	80-90
	Ammonium lauryl sulfate	5-10
	Lauramide DEA	1-5
	Cocamidopropyl sultaine	<1.0
	Ammonium chloride USP	<1.0
	Citric acid	<1.0
	DMDM hydantoin	<1.0
	Tetrasodium EDTA	<1.0
	Methylparaben	<1.0
	FD&C Yellow No. 5	<1.0
	D&C Yellow No. 10	<1.0
	FD&C Red No. 4	<1.0
	PPG-9	q.s.
HZW-Liquid Soap No. 2	Water and volatiles	60-80
	TEA-lauryl sulfate	1-10
	Sodium laureth sulfate	1-10
	Sodium lauroyl sarcosinate	1-10
	Lauramide DEA	1-10
	Glycol distearate	1-10
	Isostearamideopropyl morpholine lactate	0.1-1.0
	Disodium ricinoleamido MEA-sulfosuccinate	0.1-1.0
	DMDM hydantoin	0.1-1.0
	Citric acid	0.1-1.0
	Tetrasodium EDTA	<0.1

Formulation	Formulation Components	% (w/w)
HZX-Shampoo No. 2	Water	69.1895
	Ammonium lauryl sulfate (25%)	25.0000
	Cocamide DEA	3.0000
	Hydroxypropyl methylcellulose	1.4500
	EDTA	0.6000
	Formaldehyde	0.2000
	Benzyl alcohol	0.2000
	Benzophenone-4 sodium hydroxide	0.0400
	Citric acid	0.0100
	Ammonium chloride	0.0100
	FD&C Blue No. 1	0.0005
HZY-Anti-Dandruff Shampoo	Water	27.13
	Sodium lauroyl sarcosinate (30%)	15.00
	Lauramide DEA	4.50
	TEA-lauryl sulfate (40%)	45.00
	Glycol distearate	3.00
	Zinc pyrithione	2.10
	Sodium chloride	1.20
	Citric acid	0.90
	Imidazolidinyl urea	0.50
	Methylparaben	0.30
	Propylparaben	0.10
	Xanthan gum	0.27

Formulation	Formulation Components	% (w/w)
HZZ-Facial Cleanser[a]	Water	32.55
	Mineral oil	40.00
	Beeswax	2.30
	PEG-16 soya sterol	5.00
	PEG-8 dilaurate	2.00
	Cetearyl alcohol (70%)	0.80
	Ceteareth 20 (30%)	0.80
	Beheme acid	0.80
	Sodium borate	0.75
	Ceresin	0.50
	Carbopol dispersion (25%)	15.00
	Methylparaben	0.15
	Propylparaben	0.10
	Disodium EDTA	0.05

Abbreviations: 2EO = diethoxylated; BHT = Butylated hydroxytoluene; D&C = Drug and Cosmetic; DEA = Diethanolamide; EDTA = ethylenediaminetetraacetic acid; FD&C = Food, Drug, and Cosmetic; MEA = Monoethanolamide; PEG = Polyethylene glycol; PPG = Polypropylene glycol; TEA = Triethanolamine; USP = U.S. Pharmacopoeia; w/w = weight to weight ratio.

[a]Noted formulations are included in the proposed recommended list of substances in **Section 12.0**.

Appendix C

In Vitro Data for Substances Tested in the IRE Test Method

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Appendix C1

Balls et al. (1995) Data Sorted by Reference

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Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
Acetone	67-64-1	Liquid	IREA 26	3	2	10	25	Severe	Severe
Acetone	67-64-1	Liquid	IREA 23	3	2	32.6	76.3	Severe	
Acetone	67-64-1	Liquid	IREA 28	3.7	0.2	5.5	11.3	Nonsevere	
Acetone	67-64-1	Liquid	IREA 29	2	0	13	15	Nonsevere	
Ammonium nitrate	6484-52-2	Solid	IREA 26	0	0	10	25	Severe	Nonsevere
Ammonium nitrate	6484-52-2	Solid	IREA 23	0	0	3	3	Nonsevere	
Ammonium nitrate	6484-52-2	Solid	IREA 28	0	0	9.1	12.7	Nonsevere	
Ammonium nitrate	6484-52-2	Solid	IREA 29	0	0	7	0	Nonsevere	
L-Aspartic acid	70-47-3	Solid	IREA 26	0	0	5	5	Nonsevere	Nonsevere
L-Aspartic acid	70-47-3	Solid	IREA 23	0	0	0	0	Nonsevere	
L-Aspartic acid	70-47-3	Solid	IREA 28	0	0	9.2	15.3	Nonsevere	
L-Aspartic acid	70-47-3	Solid	IREA 29	1	1	6	4	Nonsevere	
Benzalkonium chloride (10%)	8001-54-5	Solution	IREA 26	2	3	20	80	Severe	Severe
Benzalkonium chloride (10%)	8001-54-5	Solution	IREA 23	2	3	41.7	62.9	Severe	
Benzalkonium chloride (10%)	8001-54-5	Solution	IREA 28	1	2	24	37.4	Severe	
Benzalkonium chloride (10%)	8001-54-5	Solution	IREA 29	1.7	2	60	112	Severe	
Benzalkonium chloride (5%)	8001-54-5	Solution	IREA 26	2	2	29.2	66.4	Severe	Severe
Benzalkonium chloride (5%)	8001-54-5	Solution	IREA 23	2	2.7	21.5	115.6	Severe	
Benzalkonium chloride (5%)	8001-54-5	Solution	IREA 28	0	1.3	27.6	114.9	Severe	
Benzalkonium chloride (5%)	8001-54-5	Solution	IREA 29	1.3	1	51	100	Severe	
Benzalkonium chloride (1 %)	8001-54-5	Solution	IREA 26	1	0	20.8	20.8	Severe	Severe
Benzalkonium chloride (1 %)	8001-54-5	Solution	IREA 23	2	0	18.8	61.7	Severe	
Benzalkonium chloride (1 %)	8001-54-5	Solution	IREA 28	0.7	0	24.8	47.8	Severe	
Benzalkonium chloride (1 %)	8001-54-5	Solution	IREA 29	0	0	31	81	Severe	
Butyl acetate	123-86-4	Liquid	IREA 26	0	0	0	5	Nonsevere	Nonsevere
Butyl acetate	123-86-4	Liquid	IREA 23	0	0	9.4	29.1	Severe	
Butyl acetate	123-86-4	Liquid	IREA 28	0	0.7	5.8	7.8	Nonsevere	
Butyl acetate	123-86-4	Liquid	IREA 29	0	0.7	11	17	Nonsevere	
g-Butyrolactone	96-48-0	Liquid	IREA 26	1	3	19.3	29.1	Severe	Severe
g-Butyrolactone	96-48-0	Liquid	IREA 23	0	2	27.4	49.6	Severe	
g-Butyrolactone	96-48-0	Liquid	IREA 28	0	1	20	30.6	Severe	
g-Butyrolactone	96-48-0	Liquid	IREA 29	0	0.7	19	44	Severe	
Captan 90 concentrate	133-06-2	Solid	IREA 26	2	2	0	10	Nonsevere	Nonsevere
Captan 90 concentrate	133-06-2	Solid	IREA 23	0	1.7	12	31.6	Severe	
Captan 90 concentrate	133-06-2	Solid	IREA 28	0	0	8.9	13.4	Nonsevere	
Captan 90 concentrate	133-06-2	Solid	IREA 29	1	0.3	5	20	Nonsevere	
4-Carboxybenzaldehyde	619-66-9	Solid	IREA 26	0	0	5	5	Nonsevere	Nonsevere

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
4-Carboxybenzaldehyde	619-66-9	Solid	IREA 23	0	0	11.1	25.6	Severe	
4-Carboxybenzaldehyde	619-66-9	Solid	IREA 28	0	0.7	2.8	13.2	Nonsevere	
4-Carboxybenzaldehyde	619-66-9	Solid	IREA 29	1	1	6	8	Nonsevere	
Cetylpyridinium bromide (10%)	140-72-7	Solution	IREA 26	1	2	23.7	31.6	Severe	Severe
Cetylpyridinium bromide (10%)	140-72-7	Solution	IREA 23	2	3	23	87.4	Severe	
Cetylpyridinium bromide (10%)	140-72-7	Solution	IREA 28	0	1	11.7	30.1	Severe	
Cetylpyridinium bromide (10%)	140-72-7	Solution	IREA 29	0.33	0.7	13	25	Severe	
Cetylpyridinium bromide (6%)	140-72-7	Solution	IREA 26	1	2	30	30	Severe	Severe
Cetylpyridinium bromide (6%)	140-72-7	Solution	IREA 23	0	2.7	28.1	46.7	Severe	
Cetylpyridinium bromide (6%)	140-72-7	Solution	IREA 28	0.3	1.3	13.6	26.3	Severe	
Cetylpyridinium bromide (6%)	140-72-7	Solution	IREA 29	1	1	14	25	Severe	
Cetylpyridinium bromide (0.1%)	140-72-7	Solution	IREA 26	0	0	24.2	25	Severe	Severe
Cetylpyridinium bromide (0.1%)	140-72-7	Solution	IREA 23	0	0	14.9	30.6	Severe	
Cetylpyridinium bromide (0.1%)	140-72-7	Solution	IREA 28	0	0	8.6	8.6	Nonsevere	
Cetylpyridinium bromide (0.1%)	140-72-7	Solution	IREA 29	0	0	11	15	Nonsevere	
Chlorhexidine	55-56-1	Solid	IREA 26	3	4	*	*	Severe	Severe
Chlorhexidine	55-56-1	Solid	IREA 23	0	2	19.4	37.8	Severe	
Chlorhexidine	55-56-1	Solid	IREA 28	1	2.7	17	54.7	Severe	
Chlorhexidine	55-56-1	Solid	IREA 29	1	2	44	115	Severe	
Cyclohexanol	108-93-0	Liquid	IREA 26	2	3	13.8	68.1	Severe	Severe
Cyclohexanol	108-93-0	Liquid	IREA 23	0	2	18.4	60.3	Severe	
Cyclohexanol	108-93-0	Liquid	IREA 28	1	2	29	103	Severe	
Cyclohexanol	108-93-0	Liquid	IREA 29	1.33	3	36	97	Severe	
Dibenzoyl-L-tartaric acid	2743-38-6	Solid	IREA 26	3	3	50	50	Severe	Nonsevere
Dibenzoyl-L-tartaric acid	2743-38-6	Solid	IREA 23	0	2.3	5.9	13.3	Nonsevere	
Dibenzoyl-L-tartaric acid	2743-38-6	Solid	IREA 28	0	0.3	11.8	16.7	Nonsevere	
Dibenzoyl-L-tartaric acid	2743-38-6	Solid	IREA 29	1	2	5	18	Nonsevere	
Dibenzyl phosphate	1623-08-1	Solid	IREA 26	1	1	5.1	5.1	Nonsevere	Nonsevere
Dibenzyl phosphate	1623-08-1	Solid	IREA 23	0	2	7.4	25.9	Severe	
Dibenzyl phosphate	1623-08-1	Solid	IREA 28	0	1	14.6	20.8	Nonsevere	
Dibenzyl phosphate	1623-08-1	Solid	IREA 29	1	0.3	11	14	Nonsevere	
2,6-Dichlorobenzoyl chloride	4659-45-4	Liquid	IREA 26	1	2.3	3.3	9.9	Nonsevere	Nonsevere
2,6-Dichlorobenzoyl chloride	4659-45-4	Liquid	IREA 23	0	2	21.6	36.3	Severe	
2,6-Dichlorobenzoyl chloride	4659-45-4	Liquid	IREA 28	1	2.3	5.2	24.2	Nonsevere	
2,6-Dichlorobenzoyl chloride	4659-45-4	Liquid	IREA 29	1	1	4	14	Nonsevere	
2,2-Dimethylbutanoic acid	595-37-9	Liquid	IREA 26	3	3	29.8	45.6	Severe	Severe
2,2-Dimethylbutanoic acid	595-37-9	Liquid	IREA 23	3	3	41.5	72.6	Severe	

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
2,2-Dimethylbutanoic acid	595-37-9	Liquid	IREA 28	3	2.3	34	76	Severe	
2,2-Dimethylbutanoic acid	595-37-9	Liquid	IREA 29	2	2.7	29	78	Severe	
2,5-Dimethylhexanediol	110-03-2	Solid	IREA 26	1	1	35	35	Severe	Nonsevere
2,5-Dimethylhexanediol	110-03-2	Solid	IREA 23	0	0	10.3	14	Nonsevere	
2,5-Dimethylhexanediol	110-03-2	Solid	IREA 28	0	0	5.9	11.8	Nonsevere	
2,5-Dimethylhexanediol	110-03-2	Solid	IREA 29	0.33	0.7	10	5	Nonsevere	
Ethanol	64-17-5	Liquid	IREA 26	2	3	50	60.8	Severe	Severe
Ethanol	64-17-5	Liquid	IREA 23	1	3	14.2	48.5	Severe	
Ethanol	64-17-5	Liquid	IREA 28	1.2	2.3	17.8	41	Severe	
Ethanol	64-17-5	Liquid	IREA 29	2.67	2	25	60	Severe	
Ethyl acetate	141-78-6	Liquid	IREA 26	0	2	6.5	13.7	Nonsevere	Severe
Ethyl acetate	141-78-6	Liquid	IREA 23	0	2	18	45.1	Severe	
Ethyl acetate	141-78-6	Liquid	IREA 28	0	0.7	19.8	38.7	Severe	
Ethyl acetate	141-78-6	Liquid	IREA 29	0	1	14	25	Severe	
2-Ethyl-1-hexanol	104-76-7	Liquid	IREA 26	1	1	16.7	16.7	Nonsevere	Nonsevere
2-Ethyl-1-hexanol	104-76-7	Liquid	IREA 23	0	1	5.9	20.7	Nonsevere	
2-Ethyl-1-hexanol	104-76-7	Liquid	IREA 28	0	1.7	8.3	24.7	Nonsevere	
2-Ethyl-1-hexanol	104-76-7	Liquid	IREA 29	0	2	12	19	Nonsevere	
Ethyl-2-methylacetoacetate	609-14-3	Liquid	IREA 26	0	1	2.5	5	Nonsevere	Severe
Ethyl-2-methylacetoacetate	609-14-3	Liquid	IREA 23	1	2.7	25.2	29.4	Severe	
Ethyl-2-methylacetoacetate	609-14-3	Liquid	IREA 28	0	2	25.5	36.5	Severe	
Ethyl-2-methylacetoacetate	609-14-3	Liquid	IREA 29	0.67	1	12	14	Nonsevere	
Ethyl trimethyl acetate	3938-95-2	Liquid	IREA 26	0	1	5.3	10.5	Nonsevere	Nonsevere
Ethyl trimethyl acetate	3938-95-2	Liquid	IREA 23	0	2	14.3	19.3	Nonsevere	
Ethyl trimethyl acetate	3938-95-2	Liquid	IREA 28	0	0.3	2.9	13.3	Nonsevere	
Ethyl trimethyl acetate	3938-95-2	Liquid	IREA 29	0	0	4	5	Nonsevere	
Fomesafen	72128-02-0	Solid	IREA 26	3	3	25	25	Severe	Severe
Fomesafen	72128-02-0	Solid	IREA 23	0	1.7	2.3	29.3	Severe	
Fomesafen	72128-02-0	Solid	IREA 28	0	0	5.5	12	Nonsevere	
Fomesafen	72128-02-0	Solid	IREA 29	0.33	0	4	-1	Nonsevere	
Glycerol	56-81-5	Liquid	IREA 26	0	1	9.2	10	Nonsevere	Nonsevere
Glycerol	56-81-5	Liquid	IREA 23	0	0	5.2	5.2	Nonsevere	
Glycerol	56-81-5	Liquid	IREA 28	0	0.3	11.3	11.3	Nonsevere	
Glycerol	56-81-5	Liquid	IREA 29	0	0	5	4	Nonsevere	
n-Hexanol	111-27-3	Liquid	IREA 26	1.3	2	20.5	38.5	Severe	Severe
n-Hexanol	111-27-3	Liquid	IREA 23	0	3	13.7	43.6	Severe	
n-Hexanol	111-27-3	Liquid	IREA 28	0	2.7	19.1	62.1	Severe	

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
n-Hexanol	111-27-3	Liquid	IREA 29	1.33	3	21	49	Severe	
Imidazole	288-32-4	Solid	IREA 26	2	3	50	80	Severe	Severe
Imidazole	288-32-4	Solid	IREA 23	3	3	45.5	75.8	Severe	
Imidazole	288-32-4	Solid	IREA 28	3	3	45.8	63.2	Severe	
Imidazole	288-32-4	Solid	IREA 29	2	2	38	80	Severe	
iso-Butanol	78-83-1	Liquid	IREA 26	2	3	20	85	Severe	Severe
iso-Butanol	78-83-1	Liquid	IREA 23	0	2	14.5	47	Severe	
iso-Butanol	78-83-1	Liquid	IREA 28	2	3	26	78.9	Severe	
iso-Butanol	78-83-1	Liquid	IREA 29	1.33	2	40	91	Severe	
iso-Propanol	67-63-0	Liquid	IREA 26	1.7	3	4	22.4	Severe	Severe
iso-Propanol	67-63-0	Liquid	IREA 23	2	2.3	30.9	65.5	Severe	
iso-Propanol	67-63-0	Liquid	IREA 28	0	0.7	13.1	23.4	Nonsevere	
iso-Propanol	67-63-0	Liquid	IREA 29	1.67	1.7	16	32	Severe	
Maneb	12427-38-2	Solid	IREA 26	3	2	49.2	52.5	Severe	Severe
Maneb	12427-38-2	Solid	IREA 23	0	2	29.9	37.3	Severe	
Maneb	12427-38-2	Solid	IREA 28	1	0	10.8	16.5	Nonsevere	
Maneb	12427-38-2	Solid	IREA 29	0	0	6	0	Nonsevere	
Methyl acetate	79-20-9	Liquid	IREA 26	1	3	21	49.6	Severe	Severe
Methyl acetate	79-20-9	Liquid	IREA 23	0	0	12	18.8	Nonsevere	
Methyl acetate	79-20-9	Liquid	IREA 28	1	1.7	12.2	28.1	Severe	
Methyl acetate	79-20-9	Liquid	IREA 29	0	1.7	15	26	Severe	
Methyl cyanoacetate	105-34-0	Liquid	IREA 26	0	0	5.1	5.1	Nonsevere	Nonsevere
Methyl cyanoacetate	105-34-0	Liquid	IREA 23	0	2	5.8	8.7	Nonsevere	
Methyl cyanoacetate	105-34-0	Liquid	IREA 28	0	0.3	2.9	6.8	Nonsevere	
Methyl cyanoacetate	105-34-0	Liquid	IREA 29	0.33	0.3	6	7	Nonsevere	
Methylcyclopentane	96-37-7	Liquid	IREA 26	0	0	7.4	7.4	Nonsevere	Nonsevere
Methylcyclopentane	96-37-7	Liquid	IREA 23	0	0	17.6	21	Nonsevere	
Methylcyclopentane	96-37-7	Liquid	IREA 28	0	0	2.8	5.6	Nonsevere	
Methylcyclopentane	96-37-7	Liquid	IREA 29	0	0	5	4	Nonsevere	
Methyl ethyl ketone	78-93-3	Liquid	IREA 26	2	3	20.8	91.7	Severe	Severe
Methyl ethyl ketone	78-93-3	Liquid	IREA 23	1	2	20.7	56.3	Severe	
Methyl ethyl ketone	78-93-3	Liquid	IREA 28	0	2.3	29.4	51	Severe	
Methyl ethyl ketone	78-93-3	Liquid	IREA 29	0.67	2.3	14	46	Severe	
Methyl isobutyl ketone	108-10-1	Liquid	IREA 26	1	2	34.5	40.4	Severe	Severe
Methyl isobutyl ketone	108-10-1	Liquid	IREA 23	0	3	29.3	64.6	Severe	
Methyl isobutyl ketone	108-10-1	Liquid	IREA 28	0	1.3	0	8.7	Nonsevere	
Methyl isobutyl ketone	108-10-1	Liquid	IREA 29	0	0	9	23	Nonsevere	

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
1-Naphthalene acetic acid	86-87-3	Solid	IREA 26	0	0.3	15	15	Nonsevere	Nonsevere
1-Naphthalene acetic acid	86-87-3	Solid	IREA 23	0	2.3	21.9	24.1	Nonsevere	
1-Naphthalene acetic acid	86-87-3	Solid	IREA 28	0	0	1	2.8	Nonsevere	
1-Naphthalene acetic acid	86-87-3	Solid	IREA 29	1	1	10	13	Nonsevere	
1-Naphthalene acetic acid, Na salt	61-31-4	Solid	IREA 26	2	3	67	108.7	Severe	Severe
1-Naphthalene acetic acid, Na salt	61-31-4	Solid	IREA 23	0	2.7	16.1	41.6	Severe	
1-Naphthalene acetic acid, Na salt	61-31-4	Solid	IREA 28	0	3	61	125.1	Severe	
1-Naphthalene acetic acid, Na salt	61-31-4	Solid	IREA 29	2	2	84	155	Severe	
n-Octanol	111-87-5	Liquid	IREA 26	0	1	4.4	14.8	Nonsevere	Severe
n-Octanol	111-87-5	Liquid	IREA 23	0	2	17.6	26.1	Severe	
n-Octanol	111-87-5	Liquid	IREA 28	0	1.8	14.2	29.9	Severe	
n-Octanol	111-87-5	Liquid	IREA 29	0	1	11	16	Nonsevere	
p-Fluoraniline	371-40-4	Liquid	IREA 26	2	2	25	55	Severe	Severe
p-Fluoraniline	371-40-4	Liquid	IREA 23	0	2	27.4	63.7	Severe	
p-Fluoraniline	371-40-4	Liquid	IREA 28	1.3	3	33.7	68.7	Severe	
p-Fluoraniline	371-40-4	Liquid	IREA 29	1.67	2.2	25	70	Severe	
Polyethylene glycol 400	25322-68-3	Liquid	IREA 26	1	1	29.4	31.1	Severe	Severe
Polyethylene glycol 400	25322-68-3	Liquid	IREA 23	0	1	20.7	27.4	Severe	
Polyethylene glycol 400	25322-68-3	Liquid	IREA 28	0	0	6	12.8	Nonsevere	
Polyethylene glycol 400	25322-68-3	Liquid	IREA 29	0	0	4	-1	Nonsevere	
Potassium cyanate	590-28-3	Solid	IREA 26	0	0	9.1	9.1	Nonsevere	Nonsevere
Potassium cyanate	590-28-3	Solid	IREA 23	0	0	1.5	1.5	Nonsevere	
Potassium cyanate	590-28-3	Solid	IREA 28	0	0	5.3	11.5	Nonsevere	
Potassium cyanate	590-28-3	Solid	IREA 29	0	0	5	-1	Nonsevere	
Promethazine HCl	58-33-3	Solid	IREA 26	2	2	17.5	45	Severe	Severe
Promethazine HCl	58-33-3	Solid	IREA 23	1	3	76.1	117.1	Severe	
Promethazine HCl	58-33-3	Solid	IREA 28	1	2.3	20.8	86.9	Severe	
Promethazine HCl	58-33-3	Solid	IREA 29	2	2	62	110	Severe	
Pyridine	110-86-1	Liquid	IREA 26	1	3	18.2	53.7	Severe	Severe
Pyridine	110-86-1	Liquid	IREA 23	2	3	10.3	48.5	Severe	
Pyridine	110-86-1	Liquid	IREA 28	2	3	34.9	42.4	Severe	
Pyridine	110-86-1	Liquid	IREA 29	2.33	2.3	40	75	Severe	
Quinacrine	69-05-6	Solid	IREA 26	0	0	10.5	10.5	Nonsevere	Nonsevere
Quinacrine	69-05-6	Solid	IREA 23	0	0	0	0.7	Nonsevere	
Quinacrine	69-05-6	Solid	IREA 28	0	0.7	13	16.7	Nonsevere	
Quinacrine	69-05-6	Solid	IREA 29	0	0	5	4	Nonsevere	
Sodium hydroxide (10%)	1310-73-2	Solution	IREA 26	3	4	86	110.5	Severe	Severe

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
Sodium hydroxide (10%)	1310-73-2	Solution	IREA 23	3	4	107.8	144.3	Severe	
Sodium hydroxide (10%)	1310-73-2	Solution	IREA 28	3.7	4	*	*	Severe	
Sodium hydroxide (10%)	1310-73-2	Solution	IREA 29	2	4	111	160	Severe	
Sodium hydroxide (1%)	1310-73-2	Solution	IREA 26	1.3	2	40	85	Severe	Severe
Sodium hydroxide (1%)	1310-73-2	Solution	IREA 23	0	3	41.7	65.2	Severe	
Sodium hydroxide (1%)	1310-73-2	Solution	IREA 28	1	3	57.1	121.9	Severe	
Sodium hydroxide (1%)	1310-73-2	Solution	IREA 29	1.67	3	62	102	Severe	
Sodium lauryl sulfate (15 %)	151-21-3	Solution	IREA 26	0	1	21.1	21.1	Nonsevere	Nonsevere
Sodium lauryl sulfate (15 %)	151-21-3	Solution	IREA 23	0	2	13.5	26.3	Severe	
Sodium lauryl sulfate (15 %)	151-21-3	Solution	IREA 28	0	0.3	14.5	22.3	Nonsevere	
Sodium lauryl sulfate (15 %)	151-21-3	Solution	IREA 29	0.33	2	16	24	Nonsevere	
Sodium lauryl sulfate (3 %)	151-21-3	Solution	IREA 26	0	1	5.3	15.8	Nonsevere	Nonsevere
Sodium lauryl sulfate (3 %)	151-21-3	Solution	IREA 23	0	0	13.4	22.4	Nonsevere	
Sodium lauryl sulfate (3 %)	151-21-3	Solution	IREA 28	0	0	8.4	9.3	Nonsevere	
Sodium lauryl sulfate (3 %)	151-21-3	Solution	IREA 29	0	1	12	14	Nonsevere	
Sodium oxalate	62-76-0	Solid	IREA 26	0	0	5	5	Nonsevere	Nonsevere
Sodium oxalate	62-76-0	Solid	IREA 23	0	0	0.8	6.7	Nonsevere	
Sodium oxalate	62-76-0	Solid	IREA 28	0	0	17.3	22	Nonsevere	
Sodium oxalate	62-76-0	Solid	IREA 29	0	0	6	5	Nonsevere	
Sodium perborate tetrahydrate	10486-00-7	Solid	IREA 26	0	0	5	5	Nonsevere	Nonsevere
Sodium perborate tetrahydrate	10486-00-7	Solid	IREA 23	0	0	0.8	2.3	Nonsevere	
Sodium perborate tetrahydrate	10486-00-7	Solid	IREA 28	0	0	2.9	14.7	Nonsevere	
Sodium perborate tetrahydrate	10486-00-7	Solid	IREA 29	0	0	4	0	Nonsevere	
tetra-Aminopyrimidine sulfate	5392-28-9	Solid	IREA 26	2	1	12.5	25	Severe	Nonsevere
tetra-Aminopyrimidine sulfate	5392-28-9	Solid	IREA 23	0	0	0.7	4.4	Nonsevere	
tetra-Aminopyrimidine sulfate	5392-28-9	Solid	IREA 28	0	0	1	2.9	Nonsevere	
tetra-Aminopyrimidine sulfate	5392-28-9	Solid	IREA 29	1	2	3	9	Nonsevere	
Thiourea	62-56-6	Solid	IREA 26	0	0	10	10	Nonsevere	Nonsevere
Thiourea	62-56-6	Solid	IREA 23	0	0	5.1	5.1	Nonsevere	
Thiourea	62-56-6	Solid	IREA 28	0	0	5.7	5.7	Nonsevere	
Thiourea	62-56-6	Solid	IREA 29	0	0.7	7	4	Nonsevere	
Toluene	108-88-3	Liquid	IREA 26	1	1	28.3	30	Severe	Severe
Toluene	108-88-3	Liquid	IREA 23	0	0	11.1	37.6	Severe	
Toluene	108-88-3	Liquid	IREA 28	0.7	1	10.1	17.5	Nonsevere	
Toluene	108-88-3	Liquid	IREA 29	1	0	8	6	Nonsevere	
Trichloroacetic acid (30%)	76-03-9	Solution	IREA 26	4	4	*	*	Severe	Severe
Trichloroacetic acid (30%)	76-03-9	Solution	IREA 23	3	4	44	100.8	Severe	

Substance	CASRN	Form Tested	Testing Lab	Opacity		Swelling		In Vitro Irritancy Classification	Overall In Vitro Irritancy Classification
				1 Hr	4 Hr	1 Hr	4 Hr		
Trichloroacetic acid (30%)	76-03-9	Solution	IREA 28	3.7	3.7	*	*	Severe	
Trichloroacetic acid (30%)	76-03-9	Solution	IREA 29	3	3	4	54	Severe	
Trichloroacetic acid (3%)	76-03-9	Solution	IREA 26	0	1	6	6	Nonsevere	Nonsevere
Trichloroacetic acid (3%)	76-03-9	Solution	IREA 23	1	2	12.1	36.2	Severe	
Trichloroacetic acid (3%)	76-03-9	Solution	IREA 28	0.7	0	7.2	20.6	Nonsevere	
Trichloroacetic acid (3%)	76-03-9	Solution	IREA 29	1	0	7	11	Nonsevere	
Triton X-100 (10 %)	9002-93-1	Solution	IREA 26	2	3	8.8	19.2	Severe	Severe
Triton X-100 (10 %)	9002-93-1	Solution	IREA 23	0	2	24.2	32.6	Severe	
Triton X-100 (10 %)	9002-93-1	Solution	IREA 28	0	2	36.2	95.3	Severe	
Triton X-100 (10 %)	9002-93-1	Solution	IREA 29	0.67	2.3	39	80	Severe	
Triton X-100 (5 %)	9002-93-1	Solution	IREA 26	2	3	25.4	31.6	Severe	Severe
Triton X-100 (5 %)	9002-93-1	Solution	IREA 23	0	2	23.3	30.1	Severe	
Triton X-100 (5 %)	9002-93-1	Solution	IREA 28	0	1.3	10	25.2	Severe	
Triton X-100 (5 %)	9002-93-1	Solution	IREA 29	0.33	1.5	20	45	Severe	
Tween 20	9005-64-5	Liquid	IREA 26	0	0	12.6	15.1	Nonsevere	Nonsevere
Tween 20	9005-64-5	Liquid	IREA 23	0	1	28.1	29.6	Severe	
Tween 20	9005-64-5	Liquid	IREA 28	0	0	6.4	14.7	Nonsevere	
Tween 20	9005-64-5	Liquid	IREA 29	0	0	7	4	Nonsevere	

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Appendix C2

Guerriero et al. (2004) Data Sorted by Substance

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Substance	CASRN	Form Tested	Testing Lab	Maximum Corneal Opacity		Maximum Fluorescein Uptake	
				Cloudiness	Area	Intensity	Area
Acetanilide (F22)	NA	Solid	GSK	0	0	0	0
Acetone (F33)	67-64-1	Liquid	GSK	2	3	3	4
Acetophenone (F27)	98-86-2	Solid	GSK	0	0	0	0
Dimethyl carbonate (F30)	616-38-6	Solid	GSK	0	0	0	0
Allyl alcohol (F34)	107-18-6	Liquid	GSK	3	3	4	4
Ammonium nitrate (F36)	6484-52-2	Solid	GSK	0	0	0	0
di-Isopropyl aminoethyldiphenyl acetamide (F15)	NA	Solid	GSK	0	0	0	0
3,4-Dimethoxybenzaldehyde (F19)	NA	Solid	GSK	0	0	0	0
5-Chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[B]thiophene-2-sulfonamide monohydrochloride (F4)	NA	Solid	GSK	2	3	2	1
2,6-Dichlorobenzenesulfonyl chloride (F1)	NA	Solid	GSK	3	4	2	3
(S)-1-Phenyl-N-propylamine (F13)	NA	Liquid	GSK	2	4	2	4
n-Butanol (F35)	71-36-3	Liquid	GSK	3	4	3	4
n-Butyl acetate (F39)	123-86-4	Liquid	GSK	1	3	2	1
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methylethyl)-gamma-phenyl-, iodide (F14)	NA	Solid	GSK	0	0	0	0
Cetylpyridium bromide (F37)	140-72-7	Solid	GSK	2	3	3	3
3,4-Dichloroaniline hydrochloride (F11)	NA	Solid	GSK	4	4	3	4
Sodium dicyanamide (F3)	NA	Solid	GSK	2	3	2	2
Isopropyl dicyanamide (F9)	35695-36-4	Solid	GSK	3	4	3	4
Glycerol (F41)	56-81-5	Liquid	GSK	0	0	0	0
1-(3,4-Dichlorophenyl)-5-isopropylbiguanide HCl (F6)	537-21-3	Solid	GSK	2	3	4	2

Substance	CASRN	Form Tested	Testing Lab	Maximum Corneal Opacity		Maximum Fluorescein Uptake	
				Cloudiness	Area	Intensity	Area
2-(4-Oxopentyl)-1H-isoindole-1,3 (2H)-dione (F26)	3197-25-9	Solid	GSK	0	0	1	3
4,4'-Methylenebis-(2,6-di-tert-butylphenol) (F43)	118-82-3	Solid	GSK	0	0	0	0
1H-Indole-2,3-dione (F28)	91-56-5	Solid	GSK	0	0	0	0
Methyl ethyl ketone (F38)	78-93-3	Liquid	GSK	3	4	4	3
1-(5-Amino-2-methoxyphenyl) piperazine hydrochloride (F20)	NA	Solid	GSK	2	2	2	2
1-(2-Methoxyphenyl)piperazine hydrogen sulfate (F2)	NA	Solid	GSK	1	4	1	4
Polyethylene glycol 400 (F44)	25322-68-3	Liquid	GSK	0	0	0	0
Propylene glycol (F42)	57-55-6	Liquid	GSK	0	0	0	0
Iodine chloride with pyridine (1:1) (F10)	6443-90-9	Solid	GSK	2	2	2	3
tetra-N-Octylammonium bromide (F8)	14866-33-2	Solid	GSK	2	4	4	4
3-Hydroxy-2-phenyl-4-quinolinecarboxylic acid (F24)	485-89-2	Solid	GSK	0	0	0	0
Sodium hydroxide (F31)	1310-73-2	Liquid	GSK	4	4	4	4
2-Nitro-4-thio-N-propylaniline (F21)	54393-89-4	Solid	GSK	0	0	1	4
2-Nitro-4-propoxyaniline (F23)	NA	Solid	GSK	0	0	1	3
2-Nitro-4-thiocyanoaniline (F7)	54029-45-7	Solid	GSK	0	0	2	2
Mebrophen hydramine HCl (F5)	13977-28-1	Solid	GSK	2	4	3	4
4,4'-Pyridylpiperidine (F12)	NA	Solid	GSK	4	4	4	3

Substance	CASRN	Form Tested	Testing Lab	Maximum Corneal Opacity		Maximum Fluorescein Uptake	
				Cloudiness	Area	Intensity	Area
2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (F16)	96568-04-6	Solid	GSK	0	0	0	0
6-(Methylamino)-2-pyridine ethanol formate (1:1) (salt) (F17)	NA	Solid	GSK	1	4	0	0
3-((Benzylthio)methyl)-6-chloro-,1,1-dioxide (F29)	NA	Solid	GSK	0	0	0	0
4,4`-Sulfonylbisbenzenamine (F25)	NA	Solid	GSK	0	0	0	0
Sulfonyl chloride #18	80-08-0	Solid	GSK	1	2	1	2
Toluene (F40)	108-88-3	Liquid	GSK	0	0	0	0
Trichloroacetic acid (F32)	76-03-9	Liquid	GSK	4	4	4	4

NA = Not Applicable; GSK = GlaxoSmithKline

Substance	Mean Corneal Swelling (Hr)				Corneal Epithelial Observations	In Vitro Irritancy Classification
	1	2	3	4		
Acetanilide (F22)	3.2	1.4	1.5	2.6	Normal	Nonsevere Irritant
Acetone (F33)	19.3	24.5	39.0	49.6	Loosening/roughening of epithelium	Severe Irritant
Acetophenone (F27)	6.1	3.4	2.0	2.5	Residual test material present	Nonsevere Irritant
Dimethyl carbonate (F30)	16.6	22.8	30.7	39.4	Slight pitting	Severe Irritant
Allyl alcohol (F34)	41.0	56.1	68.5	77.2	Pitting, sloughing	Severe Irritant
Ammonium nitrate (F36)	10.5	12.2	NA	15.0	Normal	Nonsevere Irritant
di-Isopropyl aminoethyldiphenyl acetamide (F15)	6.3	6.9	6.6	7.6	Normal	Nonsevere Irritant
3,4-Dimethoxybenzaldehyde (F19)	7.2	13.0	16.5	17.4	Normal	Nonsevere Irritant
5-Chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[B]thiophene-2-sulfonamide monohydrochloride (F4)	3.2	4.8	4.0	7.6	Residual test material present	Severe Irritant
2,6-Dichlorobenzenesulfonyl chloride (F1)	21.8	30.4	39.5	45.4	Residual test material present	Severe Irritant
(S)-1-Phenyl-N-propylamine (F13)	20.3	75.4	80.5	98.2	Cloudy, pitted	Severe Irritant
n-Butanol (F35)	54.6	68.3	81.4	91.8	Pitting, loosening of epithelium	Severe Irritant
n-Butyl acetate (F39)	18.5	17.2	21.5	30.1	Normal	Severe Irritant
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methylethyl)-gamma-phenyl-, iodide (F14)	7.3	4.8	5.2	5.9	Normal	Nonsevere Irritant
Cetylpyridium bromide (F37)	49.0	48.8	61.0	31.1+	Loosening of epithelium	Severe Irritant
3,4-Dichloroaniline hydrochloride (F11)	51.4	98.0	112.6	118.2	Mottled, residual test material, smooth cloudy appearance	Severe Irritant
Sodium dicyanamide (F3)	40.7	58.7	64.3	71.0	Sloughing	Severe Irritant
Isopropyl dicyanamide (F9)	31.8	43.6	53.0	62.2	Mottled, sloughing	Severe Irritant
Glycerol (F41)	13.1	15.5	16.5	21.4	Normal	Nonsevere Irritant
1-(3,4-Dichlorophenyl)-5-isopropylbiguanide HCl (F6)	13.2	21.7	27.0	27.7	Sloughing	Severe Irritant

Substance	Mean Corneal Swelling (Hr)				Corneal Epithelial Observations	In Vitro Irritancy Classification
	1	2	3	4		
2-(4-Oxopentyl)-1H-isoindole-1,3 (2H)-dione (F26)	5.9	9.8	10.9	14.8	Residual test material present	Nonsevere Irritant
4,4'-Methylenebis-(2,6-di-tert-butylphenol) (F43)	9.4	12.3	12.8	15.1	Normal	Nonsevere Irritant
1H-Indole-2,3-dione (F28)	1.3	0.2	0.4	2.0	Normal	Nonsevere Irritant
Methyl ethyl ketone (F38)	34.8	62.6	85.6	104.7	Loosening of epithelium	Severe Irritant
1-(5-Amino-2-methoxyphenyl) piperazine hydrochloride (F20)	12.4	12.0	19.1	22.4	Mottled Appearance	Severe Irritant
1-(2-Methoxyphenyl)piperazine hydrogen sulfate (F2)	9.3	14.1	15.7	20.0	Normal	Severe Irritant
Polyethylene glycol 400 (F44)	12.7	13.4	16.9	17.8	Normal	Nonsevere Irritant
Propylene glycol (F42)	10.1	10.2	10.8	16.3	Normal	Nonsevere Irritant
Iodine chloride with pyridine (1:1) (F10)	16.6	23.6	28.4	25.4	Normal	Severe Irritant
tetra-N-Octylammonium bromide (F8)	22.2	74.7	83.9	109.9	Pitting/Sloughing	Severe Irritant
3-Hydroxy-2-phenyl-4-quinolinecarboxylic acid (F24)	2.4	3.7	5.0	7.1	Residual test material present	Nonsevere Irritant
Sodium hydroxide (F31)	110.5	NA	NA	NA	Rippling, loosening of epithelium	Severe Irritant
2-Nitro-4-thio-N-propylaniline (F21)	17.6	22.5	28.3	31.5	Normal	Severe Irritant
2-Nitro-4-propoxyaniline (F23)	4.8	5.6	3.4	6.8	Residual test material adhered to cornea	Nonsevere Irritant
2-Nitro-4-thiocyanoaniline (F7)	6.6	8.1	9.3	7.4	Residual test material adhered to cornea, pitting	Severe Irritant
Mebrophen hydramine HCl (F5)	58.8	79.6	99.2	113.0	Mottled Appearance	Severe Irritant
4,4'-Pyridylpiperidine (F12)	111.5	117.5	83.9	101.1	Sloughing/mottled	Severe Irritant

Substance	Mean Corneal Swelling (Hr)				Corneal Epithelial Observations	In Vitro Irritancy Classification
	1	2	3	4		
2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (F16)	4.6	4.9	4.7	2.7	Normal	Nonsevere Irritant
6-(Methylamino)-2-pyridine ethanol formate (1:1) (salt) (F17)	4.5	4.9	4.3	2.5	Normal	Severe Irritant
3-((Benzylthio)methyl)-6-chloro-,1,1-dioxide (F29)	1.5	1.4	1.5	2.0	Normal	Nonsevere Irritant
4,4`-Sulfonylbisbenzenamine (F25)	2.9	2.7	3.2	4.4	Normal	Nonsevere Irritant
Sulfonyl chloride #18	8.6	12.9	14.2	14.9	Residual test material present	Nonsevere Irritant
Toluene (F40)	7.4	9.8	13.4	14.6	Normal	Nonsevere Irritant
Trichloroacetic acid (F32)	12.2	21.1	38.2	54.1	Roughening of epithelium	Severe Irritant

NA = Not Applicable; GSK = (

Appendix C3

Gettings et al. (1996) Data Sorted by Substance

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Substance	CASRN	Form Tested	Testing Lab	REET I Corneal Thickness Measurement	REET 2 Corneal Thickness Score (1-4)	Overall In Vitro Irritancy Classification
HZA-1	NA	Liquid formulation	Unilever	32.1	2.3	Severe irritant
HZB-1	NA	Liquid formulation	Unilever	24.8	1.7	Nonsevere irritant
HZC-1	NA	Liquid formulation	Unilever	21.2	1.0	Nonsevere irritant
HZD-1	NA	Liquid formulation	Unilever	20.3	1.0	Nonsevere irritant
HZE-1	NA	Liquid formulation	Unilever	6.4	0.0	Nonsevere irritant
HZF-1	NA	Liquid formulation	Unilever	14.3	1.0	Nonsevere irritant
HZG-1	NA	Liquid formulation	Unilever	29.5	2.0	Severe irritant
HZH-1	NA	Liquid formulation	Unilever	7.7	0.0	Nonsevere irritant
HZI-1	NA	Liquid formulation	Unilever	28.6	1.7	Severe irritant
HZJ-1	NA	Liquid formulation	Unilever	16.3	1.0	Nonsevere irritant
HZK-1	NA	Liquid formulation	Unilever	36.4	2.7	Severe irritant
HZL-1	NA	Liquid formulation	Unilever	36.2	2.7	Severe irritant

Substance	CASRN	Form Tested	Testing Lab	REET I Corneal Thickness Measurement	REET 2 Corneal Thickness Score (1-4)	Overall In Vitro Irritancy Classification
HZM-1	NA	Liquid formulation	Unilever	26.0	1.7	Severe irritant
HZN-1	NA	Liquid formulation	Unilever	37.7	2.7	Severe irritant
HZP-1	NA	Liquid formulation	Unilever	25.0	1.7	Severe irritant
HZQ-1	NA	Liquid formulation	Unilever	7.5	0.3	Nonsevere irritant
HZR-1	NA	Liquid formulation	Unilever	13.4	1.0	Nonsevere irritant
HZS-1	NA	Liquid formulation	Unilever	33.3	2.3	Severe irritant
HZT-1	NA	Liquid formulation	Unilever	2.5	0.0	Nonsevere irritant
HZU-1	NA	Liquid formulation	Unilever	36.7	3.0	Severe irritant
HZV-1	NA	Liquid formulation	Unilever	25.6	1.3	Severe irritant
HZW-1	NA	Liquid formulation	Unilever	23.9	1.7	Nonsevere irritant
HZX-1	NA	Liquid formulation	Unilever	20.9	1.3	Nonsevere irritant
HZY-1	NA	Liquid formulation	Unilever	18.6	1.0	Nonsevere irritant

Substance	CASRN	Form Tested	Testing Lab	REET I Corneal Thickness Measurement	REET 2 Corneal Thickness Score (1-4)	Overall In Vitro Irritancy Classification
HZZ-1	NA	Liquid formulation	Unilever	2.8	0.0	Nonsevere irritant

NA = Not Applicable; REET = Rabbit Enucleated Eye Test

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Appendix C4

CEC (1991) Data Sorted by Substance

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Substance	CASRN	Form Tested	Testing Lab	Mean Corneal Swelling (%) - Minutes				
				30	75	120	180	240
Acetic acid	64-19-7	Liquid	TNO-CIVO	2	8	16	22	27
Acetic acid	64-19-7	Liquid	I.H.E.	16	23	31	40	50
Acetic acid	64-19-7	Liquid	SHELL	-	31	34	42	45
Brij 35	9002-92-0	Solution	TNO-CIVO	7	11	12	13	15
Brij 35	9002-92-0	Solution	I.H.E.	2	6	9	11	9
Brij 35	9002-92-0	Solution	SHELL	-	6	11	14	13
Benzalkonium chloride	8001-54-5	Solution	TNO-CIVO	14	26	37	51	52
Benzalkonium chloride	8001-54-5	Solution	I.H.E.	28	50	65	75	81
Benzalkonium chloride	8001-54-5	Solution	SHELL	-	45	83	107	115
Dimethyl sulfoxide	67-68-5	Solution	TNO-CIVO	11	14	16	17.0	20.0
Dimethyl sulfoxide	67-68-5	Solution	I.H.E.	4	6	6	3.0	-1.0
Dimethyl sulfoxide	67-68-5	Solution	SHELL	-	4	11	15.0	16.0
Sodium fluorecein	518-47-8	Solution	TNO-CIVO	1	0	0	-1	-1
Sodium fluorecein	518-47-8	Solution	I.H.E.	-1	1	3	3	1
Sodium fluorecein	518-47-8	Solution	SHELL	-	6	9	11	14
Glycerol	56-81-5	Solution	TNO-CIVO	6	6	5	5	4
Glycerol	56-81-5	Solution	I.H.E.	2	4	6	6	4
Glycerol	56-81-5	Solution	SHELL	-	0	1	4	8
Triacetin	102-76-1	Solution	TNO-CIVO	3	2	1	0	0
Triacetin	102-76-1	Solution	I.H.E.	4	4	5	5	0
Triacetin	102-76-1	Solution	SHELL	-	1	1	2	3
Mercury chloride	7546-30-7	Solution	TNO-CIVO	7	23	38	52	57
Mercury chloride	7546-30-7	Solution	I.H.E.	5	15	35	65	95
Mercury chloride	7546-30-7	Solution	SHELL	-	-	NT	NT	NT
Silver nitrate	7761-88-8	Solution	TNO-CIVO	15	13	8	9	9
Silver nitrate	7761-88-8	Solution	I.H.E.	9	14	18	21	20
Silver nitrate	7761-88-8	Solution	SHELL	-	15	17	20	21
Sodium hydroxide	1310-73-2	Solution	TNO-CIVO	27	34	35	44	52
Sodium hydroxide	1310-73-2	Solution	I.H.E.	37	47	64	69	81
Sodium hydroxide	1310-73-2	Solution	SHELL	-	35	49	60	69
Toluene	108-88-3	Liquid	TNO-CIVO	3	11	13	13	13
Toluene	108-88-3	Liquid	I.H.E.	4	7	8	9	7
Toluene	108-88-3	Liquid	SHELL	-	9	11	11	12
Triethanolamine	102-71-6	Solution	TNO-CIVO	1	0	0	1	1
Triethanolamine	102-71-6	Solution	I.H.E.	4	9	12	14	13
Triethanolamine	102-71-6	Solution	SHELL	-	1	2	4	5

Substance	CASRN	Form Tested	Testing Lab	Mean Corneal Swelling (%) - Minutes				
				30	75	120	180	240
n-Hexane	110-54-3	Liquid	TNO-CIVO	2	2	2	0	0
n-Hexane	110-54-3	Liquid	I.H.E.	9	10	14	17	15
n-Hexane	110-54-3	Liquid	SHELL	-	0	1	3	6
Chloroform	67-66-3	Liquid	TNO-CIVO	14	19	22	29	29
Chloroform	67-66-3	Liquid	I.H.E.	16	26	34	30	46
Chloroform	67-66-3	Liquid	SHELL	-	6	10	13	17
2-Methoxyethanol	109-86-4	Liquid	TNO-CIVO	8	15	21	32	42
2-Methoxyethanol	109-86-4	Liquid	I.H.E.	15	25	30	39	46
2-Methoxyethanol	109-86-4	Liquid	SHELL	-	18	30	34	40
Butanol	71-36-3	Liquid	TNO-CIVO	22	29	35	44	49
Butanol	71-36-3	Liquid	I.H.E.	23	32	46	59	67
Butanol	71-36-3	Liquid	SHELL	-	33	42	53	65
Acetaldehyde	75-07-0	Liquid	TNO-CIVO	12	15	17	18	27
Acetaldehyde	75-07-0	Liquid	I.H.E.	7	10	17	29	36
Acetaldehyde	75-07-0	Liquid	SHELL	-	12	31	36	41
2-Butoxyethyl acetate	112-07-2	Liquid	TNO-CIVO	7	8	10	12	12
2-Butoxyethyl acetate	112-07-2	Liquid	I.H.E.	6	10	13	14	16
2-Butoxyethyl acetate	112-07-2	Liquid	SHELL	-	12	31	36	41
Sodium dodecyl sulfate	151-21-3	Solution	TNO-CIVO	8	13	17	19	21
Sodium dodecyl sulfate	151-21-3	Solution	I.H.E.	8	10	12	12	11
Sodium dodecyl sulfate	151-21-3	Solution	SHELL	-	24	31	34	40
Dibutyltin	683-18-1	Solution	TNO-CIVO	NT	NT	NT	NT	NT
Dibutyltin	683-18-1	Solution	I.H.E.	6	13	20	31	38
Dibutyltin	683-18-1	Solution	SHELL	-	9	11	10	21
Tributyltin	688-73-3	Solution	TNO-CIVO	7	10	16	43	58
Tributyltin	688-73-3	Solution	I.H.E.	4	17	29	57	109
Tributyltin	688-73-3	Solution	SHELL	32	40	75	95	124

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; NT = Not Tested; TNO = Institute CIVO-Toxicology and Nutrition; I.H.E. = Institute for Hygiene and Epidemiology; Shell = Shell Oil Company

Substance	Mean Corneal Opacity - Minutes					Mean Corneal Swelling (%) - Minutes			
	30	75	120	180	240	30	75	120	180
Acetic acid	1.8	2.0	2.0	2.0	2.0	2.0	2.0	Severe	Severe
Acetic acid	1.6	-	-	-	1.4	1.6	3.0	Severe	
Acetic acid	-	-	-	-	-	-	2.0	Severe	
Brij 35	1.0	1.0	1.0	1.0	1.0	1.0	1.0	Nonsevere	Nonsevere
Brij 35	0.0	-	-	-	0.0	3.0	1.6	Severe	
Brij 35	-	-	-	-	-	-	0.0	Nonsevere	
Benzalkonium chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	Severe	Severe
Benzalkonium chloride	1.2	-	-	-	3.0	3.0	3.0	Severe	
Benzalkonium chloride	-	-	-	-	-	-	2.0	Severe	
Dimethyl sulfoxide	1.0	1.0	1.0	1.0	1.0	2.0	2.0	Nonsevere	Nonsevere
Dimethyl sulfoxide	0.0	-	-	-	0.0	0.0	0.0	Nonsevere	
Dimethyl sulfoxide	-	-	-	-	-	-	2.0	Nonsevere	
Sodium fluorecein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Nonsevere	Nonsevere
Sodium fluorecein	0.0	-	-	-	0.0	0.0	0.0	Nonsevere	
Sodium fluorecein	-	-	-	-	-	-	1.0	Nonsevere	
Glycerol	0.8	0.8	0.8	0.8	0.8	1.0	1.0	Nonsevere	Nonsevere
Glycerol	0.0	-	-	-	0.0	0.0	0.0	Nonsevere	
Glycerol	-	-	-	-	-	-	0.0	Nonsevere	
Triacetin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Nonsevere	Nonsevere
Triacetin	0.0	-	-	-	0.0	0.0	0.0	Nonsevere	
Triacetin	-	-	-	-	-	-	0.0	Nonsevere	
Mercury chloride	NM	3.0	3.0	2.7	2.0	NM	3.0	Severe	Severe
Mercury chloride	3.0	-	-	-	2.8	1.0	2.0	Severe	
Mercury chloride	NT	NT	NT	NT	NT	NT	NT	NT	
Silver nitrate	1.0	1.0	1.0	1.5	1.5	1.0	1.0	Nonsevere	Nonsevere
Silver nitrate	2.0	-	-	-	2.0	0.0	0.0	Nonsevere	
Silver nitrate	-	-	-	-	-	-	2.0	Nonsevere	
Sodium hydroxide	3.0	3.0	3.0	3.0	3.0	2.0	3.0	Severe	Severe
Sodium hydroxide	3.0	-	-	-	3.0	3.0	3.0	Severe	
Sodium hydroxide	-	-	-	-	-	-	3.0	Severe	
Toluene	1.2	1.2	1.2	1.2	1.2	2.0	2.2	Nonsevere	Nonsevere
Toluene	0.0	-	-	-	0.0	1.8	2.0	Nonsevere	
Toluene	-	-	-	-	-	-	1.0	Nonsevere	
Triethanolamine	0.0	0.0	0.0	0.0	0.0	0.2	0.2	Nonsevere	Nonsevere
Triethanolamine	0.0	-	-	-	0.0	0.0	0.0	Nonsevere	
Triethanolamine	-	-	-	-	-	-	0.0	Nonsevere	

Substance	Mean Corneal Opacity - Minutes					Mean Corneal Swelling (%) - Minutes			
	30	75	120	180	240	30	75	120	180
n-Hexane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Nonsevere	Nonsevere
n-Hexane	0.0	-	-	-	0.0	2.0	1.0	Nonsevere	
n-Hexane	-	-	-	-	-	-	0.0	Nonsevere	
Chloroform	1.5	2.0	2.0	2.0	2.0	3.0	3.0	Severe	Severe
Chloroform	0.0	-	-	-	1.4	2.8	3.0	Severe	
Chloroform	-	-	-	-	-	-	3.0	Severe	
2-Methoxyethanol	1.0	1.5	2.0	2.0	2.0	3.0	3.0	Severe	Severe
2-Methoxyethanol	0.0	-	-	-	2.8	2.0	2.0	Severe	
2-Methoxyethanol	-	-	-	-	-	-	3.0	Severe	
Butanol	3.0	3.0	3.0	3.0	3.0	3.0	3.0	Severe	Severe
Butanol	1.0	-	-	-	2.0	2.4	3.0	Severe	
Butanol	-	-	-	-	-	-	3.0	Severe	
Acetaldehyde	1.4	1.5	1.5	1.5	1.5	2.8	2.8	Severe	Severe
Acetaldehyde	2.0	-	-	-	2.0	3.0	3.0	Severe	
Acetaldehyde	-	-	-	-	-	-	3.0	Severe	
2-Butoxyethyl acetate	0.5	0.9	0.9	0.9	0.9	2.0	2.0	Nonsevere	Nonsevere
2-Butoxyethyl acetate	1.0	-	-	-	1.0	2.0	0.0	Nonsevere	
2-Butoxyethyl acetate	-	-	-	-	-	-	2.0	Severe	
Sodium dodecyl sulfate	0.8	1.0	1.8	1.8	1.8	3.0	3.0	Severe	Severe
Sodium dodecyl sulfate	0.0	-	-	-	1.0	0.0	0.2	Nonsevere	
Sodium dodecyl sulfate	-	-	-	-	-	-	3.0	Severe	
Dibutyltin	NT	NT	NT	NT	NT	NT	NT	NT	Severe
Dibutyltin	1.0	-	-	-	1.0	1.0	2.0	Severe	
Dibutyltin	-	-	-	-	-	-	2.0	Nonsevere	
Tributyltin	2.0	2.0	2.0	2.5	2.0	3.0	3.0	Severe	Severe
Tributyltin	1.0	-	-	-	2.2	2.0	2.6	Severe	
Tributyltin	-	-	-	-	-	-	2.0	Severe	

Appendix D

In Vivo and In Vitro Comparison of Ocular Irritancy Classification

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Appendix D1

Data Sorted by Reference

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In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Acetaldehyde	75-07-0	100%	-	-	Review Data	Severe irritant	CEC (1991)
Acetic acid	**64-19-7**	**10%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Benzalkonium chloride	8001-54-5	100%	-	-	R41	Severe Irritant	CEC (1991)
Brij 35	9002-92-0	100%	-	-	nonirritant	Nonsevere Irritant	CEC (1991)
Butanol	71-36-3	100%	-	-	nonirritant	Severe Irritant	CEC (1991)
2-Butoxyethyl acetate	112-07-2	100%	-	-	nonirritant	Nonsevere Irritant	CEC (1991)
Chloroform	67-66-3	100%	-	-	Review Data	Nonsevere irritant	CEC (1991)
Dibutyltin chloride	**683-18-1**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Dimethyl sulfoxide	67-68-5	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Fluorescein, sodium	518-47-8	100%	-	-	Review Data	Nonsevere irritant	CEC (1991)
Glycerol	56-81-5	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
n-Hexane	110-54-3	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Mercuric chloride	7546-30-7	100%	-	-	RD	Severe irritant	CEC (1991)
2-Methoxyethanol	**109-86-4**	**100%**	**-**	**-**	**Nonirritant**	**Severe Irritant**	**CEC (1991)**
Silver nitrate	7761-88-8	3% in water	-	-	Review Data	Nonsevere irritant	CEC (1991)
Sodium dodecyl sulfate	**151-21-3**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Sodium hydroxide	1310-73-2	1% in water	-	-	Review Data	Severe irritant	CEC (1991)
Toluene	108-88-3	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Triacetin	102-76-1	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Tributyltin chloride	**688-73-3**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Triethanolamine	102-71-6	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Acetone	**67-64-1**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
tetra-Aminopyrimidine sulfate	5392-28-9	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Ammonium nitrate	6484-52-2	100%	Category 2B	Category III	R36	Nonsevere Irritant	Balls et al. (1995)
L-Aspartic acid	70-47-3	100%	SCNM	SCNM	Review Data	Nonsevere Irritant	Balls et al. (1995)
Benzalkonium chloride (1 %)	**8001-54-5**	**1%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Benzalkonium chloride (10%)	**8001-54-5**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Benzalkonium chloride (5%)	**8001-54-5**	**5%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
iso-Butanol	**78-83-1**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
N-Butyl acetate	123-86-4	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
g-Butyrolactone	**96-48-0**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Captan 90 concentrate	133-06-2	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)

In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Cetylpyridinium bromide (0.1%)	**140-72-7**	**0.1%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Cetylpyridinium bromide (10%)	**140-72-7**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Cetylpyridinium bromide (6%)	**140-72-7**	**6%**	**Category 1**	**SCNM**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Chlorhexidine	**55-56-1**	**100%**	**Category 1**	**SCNM**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Cyclohexanol	**108-93-0**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Dibenzoyl-L-tartaric acid	2743-38-6	100%	Category 1	SCNM	R41	Nonsevere Irritant	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	100%	Category 2A	Category II	Review Data	Nonsevere Irritant	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	100%	SCNM	Category I	Review Data	Severe Irritant	Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Ethanol	**64-17-5**	**100%**	**Category 2A**	**Category III**	**nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Ethyl acetate	**141-78-6**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
2-Ethyl-1-hexanol	104-76-7	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Ethyl-2-methylacetoacetate	**609-14-3**	**100%**	**Category 2B**	**Category III**	**nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Ethyl trimethyl acetate	3938-95-2	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
p-Fluoraniline	**371-40-4**	**100%**	**SCNM**	**SCNM**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Fomesafen, acid form (solid)	**72128-02-0**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Glycerol	56-81-5	100%	Nonirritant	Category IV	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
***n*-Hexanol**	**111-27-3**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Imidazole	**288-32-4**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Maneb	**12427-38-2**	**100%**	**SCNM**	**Category III**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Methyl acetate	**79-20-9**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Methyl cyanoacetate	105-34-0	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Methylcyclopentane	96-37-7	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	100%	Category 2A	Category III	R36	Severe Irritant	Balls et al. (1995)
Methyl isobutyl ketone	**108-10-1**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
1-Naphthalene acetic acid	86-87-3	100%	Category 1	Category I	Review Data	Nonsevere Irritant	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	**61-31-4**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
***n*-Octanol**	**111-87-5**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Polyethylene glycol 400	25322-68-3	100%	Nonirritant	Category IV	Nonirritant	Severe Irritant	Balls et al. (1995)
Potassium cyanate	590-28-3	100%	SCNM	SCNM	Review Data	Nonsevere Irritant	Balls et al. (1995)
Promethazine HCl	**58-33-3**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
iso-Propanol	**67-63-0**	**100%**	**Category 2A**	**Category III**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**

In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Pyridine	**110-86-1**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Quinacrine	69-05-6	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Sodium hydroxide (1%)	**1310-73-2**	**1%**	**Category 2B**	**Category III**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Sodium hydroxide (10%)	1310-73-2	10%	Category 1	Category I	R41	Severe Irritant	Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	15%	Category 1	Category I	Review Data	Nonsevere Irritant	Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	3%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Sodium oxalate	62-76-0	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Sodium perborate tetrahydrate	10486-00-7	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Toluene	108-88-3	100%	Nonirritant	Category III	Nonirritant	Severe Irritant	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	3%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	Category 1	Category I	R41	Severe Irritant	Balls et al. (1995)
Triton X-100 (10 %)	**9002-93-1**	**10%**	**Category 1**	**Category II**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Triton X-100 (5 %)	**9002-93-1**	**5%**	**Category 2A**	**Category III**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Tween 20	9005-64-5	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
HZA-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZB-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZC-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZD-1	-	25%	Category 2B	Category III	nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZE-1	-	Undiluted	SCNM	Category I	SCNM	Nonsevere irritant	Gettings et al. (1996)
HZF-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZG-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZH-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZI-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZJ-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZK-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZL-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZM-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZN-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZP-1	**-**	**Undiluted**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe irritant**	**Gettings et al. (1996)**
HZQ-1	-	Undiluted	Nonirritant	Category III	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZR-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZS-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZT-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZU-1	**-**	**25%**	**Category 2B**	**Category III**	**R36**	**Severe irritant**	**Gettings et al. (1996)**
HZV-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZW-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)

In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
HZX-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZY-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZZ-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
Acetone (F33)	67-64-1	100%	Category 2A	Category II	R36	Severe Irritant	Guerriero et al. (2004)
2-(Acetyloxy)-1-phenylethanone (F27)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Allyl alcohol (F34)	107-18-6	100%	NT	NT	NT	Severe Irritant	Guerriero et al. (2004)
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methylethyl)-gamma-phenyl-, iodide (F14)	-	100%	Category 2A	Category II	R36	Nonsevere Irritant	Guerriero et al. (2004)
1-(5-Amino-2-methoxyphenyl) piperazine hydrochloride (F20)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
Ammonium nitrate (F36)	**6484-52-2**	**100%**	**Category 2B**	**Category III**	**R36**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
3-((Benzylthio)methyl)-6-chloro-,1,1-dioxide (F29)	**-**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
n-Butanol (F35)	**71-36-3**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Guerriero et al. (2004)**
n-Butyl acetate (F39)	**123-86-4**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
Cetylpyridium bromide (F37)	**140-72-7**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
5-Chloro-2,4-disulfamoyl chloroacetanilide (F22)	**-**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
5-Chloro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (F18)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
5-Chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[B]thiophene-2-sulfonamide monohydrochloride (F4)	**-**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
3,4-Dichloroaniline hydrochloride (F11)	**-**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
2,6-Dichlorobenzenesulfonyl chloride (F1)	**6579-54-0**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**

In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (F16)	96568-04-6	100%	Category 2B	Category III	nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
1-(3,4-Dichlorophenyl)-5-isopropylbiguanide HCl (F6)	537-21-3	100%	Category 1	Category I	R41	Severe Irritant	Guerriero et al. (2004)
3,4-Dimethoxybenzaldehyde (F19)	120-14-9	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
Dimethyl carbonate (F30)	616-38-6	100%	Nonirritant	Category III	Nonirritant	Severe Irritant	Guerriero et al. (2004)
Glycerol (F41)	56-81-5	100%	Nonirritant	Category IV	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
3-Hydroxy-2-phenyl-4-quinolinecarboxylic acid (F24)	485-89-2	100%	Nonirritant	Category II	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
1H-Indole-2,3-dione (F28)	91-56-5	100%	Nonirritant	Category IV	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
Iodine chloride with pyridine (1:1) (F10)	6443-90-9	100%	Category 1	Category I	R41	Severe Irritant	Guerriero et al. (2004)
di-Isopropyl aminoethyldiphenyl acetamide (F15)	-	100%	Category 2B	Category III	R36	Nonsevere Irritant	Guerriero et al. (2004)
Isopropyl dicyanamide (F9)	35695-36-4	100%	Category 1	Category I	R41	Severe Irritant	Guerriero et al. (2004)
Mebrophen hydramine HCl (F5)	13977-28-1	100%	Category 1	Category I	R41	Severe Irritant	Guerriero et al. (2004)
1-(2-Methoxyphenyl)piperazine hydrogen sulfate (F2)	-	100%	SCNM	SCNM	SCNM	Severe Irritant	Guerriero et al. (2004)
6-(Methylamino)-2-pyridine ethanol formate (1:1) (salt) (F17)	-	100%	Nonirritant	Category III	Nonirritant	Severe Irritant	Guerriero et al. (2004)
4,4'-Methylenebis-(2,6-di-tert-butylphenol) (F43)	118-82-3	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)
Methyl ethyl ketone (F38)	78-93-3	100%	Nonirritant	Category III	Nonirritant	Severe Irritant	Guerriero et al. (2004)
2-Nitro-4-propoxyaniline (F23)	-	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Guerriero et al. (2004)

In Vivo and In Vitro
Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
2-Nitro-4-thiocyanoaniline (F7)	**54029-45-7**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
2-Nitro-4-thio-N-propylaniline (F21)	**54393-89-4**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
tetra-N-Octylammonium bromide (F8)	**14866-33-2**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
2-(4-Oxopentyl)-1H-isoindole-1,3 (2H)-dione (F26)	**3197-25-9**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
(S)-1-Phenyl-N-propylamine (F13)	**3789-59-1**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
Polyethylene glycol 400 (F44)	**25322-68-3**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Propylene glycol (F42)	**57-55-6**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
4,4'-Pyridylpiperidine (F12)	**-**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
Sodium dicyanamide (F3)	**-**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Sodium hydroxide (F31)	**1310-73-2**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
4,4`-Sulfonylbisbenzenamine (F25)	**80-08-0**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Toluene (F40)	**108-88-3**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Trichloroacetic acid (F32)	**76-03-9**	**30%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**

Abbreviations: SCNM = study criteria not met to allow for a classification
Bolded Substances are Included in the Expanded Data Set

Appendix D2

Data Sorted by Substance

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In Vivo and In Vitro Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Acetaldehyde	75-07-0	100%	-	-	Review Data	Severe irritant	CEC (1991)
Acetic acid	**64-19-7**	**10%**	-	-	**R41**	**Severe Irritant**	**CEC (1991)**
Acetone	**67-64-1**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Acetone (F33)	67-64-1	100%	Category 2A	Category II	R36	Severe Irritant	Guerriero et al. (2004)
2-(Acetyloxy)-1-phenylethanone (F27)	-	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Allyl alcohol (F34)	107-18-6	100%	NT	NT	NT	Severe Irritant	Guerriero et al. (2004)
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methylethyl)-gamma-phenyl-, iodide (F14)	-	100%	Category 2A	Category II	R36	Nonsevere Irritant	Guerriero et al. (2004)
1-(5-Amino-2-methoxyphenyl) piperazine hydrochloride (F20)	-	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
tetra-Aminopyrimidine sulfate	5392-28-9	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Ammonium nitrate	6484-52-2	100%	Category 2B	Category III	R36	Nonsevere Irritant	Balls et al. (1995)
Ammonium nitrate (F36)	**6484-52-2**	**100%**	**Category 2B**	**Category III**	**R36**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
L-Aspartic acid	70-47-3	100%	SCNM	SCNM	Review Data	Nonsevere Irritant	Balls et al. (1995)
Benzalkonium chloride	8001-54-5	100%	-	-	R41	Severe Irritant	CEC (1991)
Benzalkonium chloride (1 %)	**8001-54-5**	**1%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Benzalkonium chloride (10%)	**8001-54-5**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Benzalkonium chloride (5%)	**8001-54-5**	**5%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
3-((Benzylthio)methyl)-6-chloro-,1,1-dioxide (F29)	-	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Brij 35	9002-92-0	100%	-	-	nonirritant	Nonsevere Irritant	CEC (1991)
Butanol	71-36-3	100%	-	-	nonirritant	Severe Irritant	CEC (1991)
iso-Butanol	**78-83-1**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
n-Butanol (F35)	**71-36-3**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Guerriero et al. (2004)**
2-Butoxyethyl acetate	112-07-2	100%	-	-	nonirritant	Nonsevere Irritant	CEC (1991)
N-Butyl acetate	123-86-4	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
n-Butyl acetate (F39)	**123-86-4**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
g-Butyrolactone	**96-48-0**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**

In Vivo and In Vitro
Comparison of Irritancy
Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Captan 90 concentrate	133-06-2	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Cetylpyridium bromide (F37)	**140-72-7**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Cetylpyridinium bromide (0.1%)	**140-72-7**	**0.1%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Cetylpyridinium bromide (10%)	**140-72-7**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Cetylpyridinium bromide (6%)	**140-72-7**	**6%**	**Category 1**	**SCNM**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
5-Chloro-2,4-disulfamoyl chloroacetanilide (F22)	**-**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Chloroform	67-66-3	100%	**-**	**-**	Review Data	Nonsevere irritant	CEC (1991)
5-Chloro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (F18)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
5-Chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[B]thiophene-2-sulfonamide monohydrochloride (F4)	**-**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Chlorhexidine	**55-56-1**	**100%**	**Category 1**	**SCNM**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Cyclohexanol	**108-93-0**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Dibenzoyl-L-tartaric acid	2743-38-6	100%	Category 1	SCNM	R41	Nonsevere Irritant	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Dibutyltin chloride	**683-18-1**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
3,4-Dichloroaniline hydrochloride (F11)	**-**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
2,6-Dichlorobenzenesulfonyl chloride (F1)	**6579-54-0**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
2,6-Dichlorobenzoyl chloride	4659-45-4	100%	Category 2A	Category II	Review Data	Nonsevere Irritant	Balls et al. (1995)
2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (F16)	**96568-04-6**	**100%**	**Category 2B**	**Category III**	**nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
1-(3,4-Dichlorophenyl)-5-isopropylbiguanide HCl (F6)	**537-21-3**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
3,4-Dimethoxybenzaldehyde (F19)	**120-14-9**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**

In Vivo and In Vitro
Comparison of Irritancy
Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
2,2-Dimethylbutanoic acid	595-37-9	100%	SCNM	Category I	Review Data	Severe Irritant	Balls et al. (1995)
Dimethyl carbonate (F30)	**616-38-6**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
2,5-Dimethylhexanediol	110-03-2	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Dimethyl sulfoxide	67-68-5	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Ethanol	**64-17-5**	**100%**	**Category 2A**	**Category III**	**nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Ethyl acetate	**141-78-6**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
2-Ethyl-1-hexanol	104-76-7	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Ethyl-2-methylacetoacetate	**609-14-3**	**100%**	**Category 2B**	**Category III**	**nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Ethyl trimethyl acetate	3938-95-2	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Fluorescein, sodium	518-47-8	100%	-	-	Review Data	Nonsevere irritant	CEC (1991)
p-Fluoraniline	**371-40-4**	**100%**	**SCNM**	**SCNM**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Fomesafen, acid form (solid)	**72128-02-0**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
Glycerol	56-81-5	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Glycerol	56-81-5	100%	Nonirritant	Category IV	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Glycerol (F41)	**56-81-5**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
n-Hexane	110-54-3	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
***n*-Hexanol**	**111-27-3**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
3-Hydroxy-2-phenyl-4-quinolinecarboxylic acid (F24)	**485-89-2**	**100%**	**Nonirritant**	**Category II**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
HZA-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZB-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZC-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZD-1	-	25%	Category 2B	Category III	nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZE-1	-	Undiluted	SCNM	Category I	SCNM	Nonsevere irritant	Gettings et al. (1996)
HZF-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZG-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZH-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZI-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZJ-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZK-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZL-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZM-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZN-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**

In Vivo and In Vitro Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
HZP-1	**-**	**Undiluted**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe irritant**	**Gettings et al. (1996)**
HZQ-1	-	Undiluted	Nonirritant	Category III	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZR-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZS-1	**-**	**Undiluted**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZT-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
HZU-1	**-**	**25%**	**Category 2B**	**Category III**	**R36**	**Severe irritant**	**Gettings et al. (1996)**
HZV-1	**-**	**25%**	**Category 1**	**Category I**	**R41**	**Severe irritant**	**Gettings et al. (1996)**
HZW-1	-	25%	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZX-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZY-1	-	Undiluted	Category 1	Category I	R41	Nonsevere irritant	Gettings et al. (1996)
HZZ-1	-	Undiluted	Nonirritant	Category IV	Nonirritant	Nonsevere irritant	Gettings et al. (1996)
Imidazole	**288-32-4**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
1H-Indole-2,3-dione (F28)	**91-56-5**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Iodine chloride with pyridine (1:1) (F10)	**6443-90-9**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
di-Isopropyl aminoethyldiphenyl acetamide (F15)	**-**	**100%**	**Category 2B**	**Category III**	**R36**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Isopropyl dicyanamide (F9)	**35695-36-4**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Maneb	**12427-38-2**	**100%**	**SCNM**	**Category III**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Mebrophen hydramine HCl (F5)	**13977-28-1**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Mercuric chloride	7546-30-7	100%	**-**	**-**	RD	Severe irritant	CEC (1991)
2-Methoxyethanol	**109-86-4**	**100%**	**-**	**-**	**Nonirritant**	**Severe Irritant**	**CEC (1991)**
1-(2-Methoxyphenyl)piperazine hydrogen sulfate (F2)	**-**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
Methyl acetate	**79-20-9**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
6-(Methylamino)-2-pyridine ethanol formate (1:1) (salt) (F17)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
Methyl cyanoacetate	105-34-0	100%	Category 2A	Category II	R36	Nonsevere Irritant	Balls et al. (1995)
Methylcyclopentane	96-37-7	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)

In Vivo and In Vitro Comparison of Irritancy Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
4,4'-Methylenebis-(2,6-di-tert-butylphenol) (F43)	**118-82-3**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Methyl ethyl ketone	78-93-3	100%	Category 2A	Category III	R36	Severe Irritant	Balls et al. (1995)
Methyl ethyl ketone (F38)	**78-93-3**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
Methyl isobutyl ketone	**108-10-1**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Balls et al. (1995)**
1-Naphthalene acetic acid	86-87-3	100%	Category 1	Category I	Review Data	Nonsevere Irritant	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	**61-31-4**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
2-Nitro-4-propoxyaniline (F23)	**-**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
2-Nitro-4-thiocyanoaniline (F7)	**54029-45-7**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
2-Nitro-4-thio-N-propylaniline (F21)	**54393-89-4**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Severe Irritant**	**Guerriero et al. (2004)**
***n*-Octanol**	**111-87-5**	**100%**	**Category 2A**	**Category II**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
tetra-N-Octylammonium bromide (F8)	**14866-33-2**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
2-(4-Oxopentyl)-1H-isoindole-1,3 (2H)-dione (F26)	**3197-25-9**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
(S)-1-Phenyl-N-propylamine (F13)	**3789-59-1**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
Polyethylene glycol 400	25322-68-3	100%	Nonirritant	Category IV	Nonirritant	Severe Irritant	Balls et al. (1995)
Polyethylene glycol 400 (F44)	**25322-68-3**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Potassium cyanate	590-28-3	100%	SCNM	SCNM	Review Data	Nonsevere Irritant	Balls et al. (1995)
Promethazine HCl	**58-33-3**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
iso-Propanol	**67-63-0**	**100%**	**Category 2A**	**Category III**	**Review Data**	**Severe Irritant**	**Balls et al. (1995)**
Propylene glycol (F42)	**57-55-6**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Pyridine	**110-86-1**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
4,4'-Pyridylpiperidine (F12)	**-**	**100%**	**SCNM**	**SCNM**	**SCNM**	**Severe Irritant**	**Guerriero et al. (2004)**
Quinacrine	69-05-6	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Silver nitrate	7761-88-8	3% in water	**-**	**-**	Review Data	Nonsevere irritant	CEC (1991)

In Vivo and In Vitro
Comparison of Irritancy
Classification

Substance	CASRN	Concentration Tested	*In Vivo* Classification (GHS)	*In Vivo* Classification (EPA)	*In Vivo* Classification (EU)	*In Vitro* Classification	Reference
Sodium dicyanamide (F3)	**-**	**100%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Sodium dodecyl sulfate	**151-21-3**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Sodium hydroxide	1310-73-2	1% in water	-	-	Review Data	Severe irritant	CEC (1991)
Sodium hydroxide (1%)	**1310-73-2**	**1%**	**Category 2B**	**Category III**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Sodium hydroxide (10%)	1310-73-2	10%	Category 1	Category I	R41	Severe Irritant	Balls et al. (1995)
Sodium hydroxide (F31)	**1310-73-2**	**10%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Sodium lauryl sulfate (15 %)	151-21-3	15%	Category 1	Category I	Review Data	Nonsevere Irritant	Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	3%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Sodium oxalate	62-76-0	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
Sodium perborate tetrahydrate	10486-00-7	100%	Category 1	Category I	R41	Nonsevere Irritant	Balls et al. (1995)
4,4`-Sulfonylbisbenzenamine (F25)	**80-08-0**	**100%**	**Nonirritant**	**Category IV**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Toluene	108-88-3	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Toluene	108-88-3	100%	Nonirritant	Category III	Nonirritant	Severe Irritant	Balls et al. (1995)
Toluene (F40)	**108-88-3**	**100%**	**Nonirritant**	**Category III**	**Nonirritant**	**Nonsevere Irritant**	**Guerriero et al. (2004)**
Triacetin	102-76-1	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Tributyltin chloride	**688-73-3**	**100%**	**-**	**-**	**R41**	**Severe Irritant**	**CEC (1991)**
Trichloroacetic acid (3%)	76-03-9	3%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	Category 1	Category I	R41	Severe Irritant	Balls et al. (1995)
Trichloroacetic acid (F32)	**76-03-9**	**30%**	**Category 1**	**Category I**	**R41**	**Severe Irritant**	**Guerriero et al. (2004)**
Triethanolamine	102-71-6	100%	-	-	Nonirritant	Nonsevere Irritant	CEC (1991)
Triton X-100 (10 %)	**9002-93-1**	**10%**	**Category 1**	**Category II**	**R41**	**Severe Irritant**	**Balls et al. (1995)**
Triton X-100 (5 %)	**9002-93-1**	**5%**	**Category 2A**	**Category III**	**R36**	**Severe Irritant**	**Balls et al. (1995)**
Tween 20	9005-64-5	100%	Nonirritant	Category III	Nonirritant	Nonsevere Irritant	Balls et al. (1995)

Abbreviations: SCNM = study criteria not met to allow for a classification
Bolded substances are included in the Expanded Data Set

Appendix E

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

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Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
A	BCOPP9	60	a	1.000				
A	BCOPP10	60	b	0.777	1.000			
A	BCOPP11	60	c	0.886	0.862	1.000		
A	BCOPP12	60	d	0.797	0.683	0.859	1.000	
A	BCOPP13	60	e	0.856	0.788	0.906	0.892	1.000
A	BCOPO9	60	a	1.000				
A	BCOPO10	60	b	0.924	1.000			
A	BCOPO11	60	c	0.934	0.898	1.000		
A	BCOPO12	60	d	0.946	0.905	0.978	1.000	
A	BCOPO13	60	e	0.970	0.936	0.953	0.955	1.000
A	BCOPI9	60	a	1.000				
A	BCOPI10	60	b	0.894	1.000			
A	BCOPI11	60	c	0.922	0.896	1.000		
A	BCOPI12	60	d	0.924	0.867	0.957	1.000	
A	BCOPI13	60	e	0.955	0.901	0.947	0.958	1.000
A	BCOPI9b	60	a	1.000				
A	BCOPI10b	60	b	0.898	1.000			
A	BCOPI11b	60	c	0.913	0.913	1.000		
A	BCOPI12b	60	d	0.908	0.848	0.916	1.000	
A	BCOPP13b	60	e	0.939	0.885	0.938	0.938	1.000
A	HETQ14	49	a	1.000				
A	HETQ15	40	b	0.790	1.000			
A	HETQ16	47	c	0.473	0.521	1.000		
A	HETQ17	41	d	0.550	0.734	0.664	1.000	
A	HETS14	11	a	1.000				
A	HETS15	13	b	0.174	1.000			
A	HETS16	13	c	-0.171	-0.171	1.000		
A	HETS17	17	d	-0.103	0.808	0.031	1.000	
A	HETQ14b	49	a	1.000				
A	HETQ15b	40	b	0.627	1.000			
A	HETQ16b	47	c	0.709	0.638	1.000		
A	HETQ17b	41	d	0.449	0.814	0.528	1.000	
A	HETS14b	11	a	1.000				
A	HETS15b	13	b	*	1.000			
A	HETS16b	13	c	-0.043	-0.316	1.000		
A	HETS17b	41	d	*	*	*	*	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
A	ICES 22	60	a	1.000				
A	ICES 27	60	b	0.721	1.000			
A	ICES 24	59	c	0.750	0.715	1.000		
A	ICES 25	58	d	0.627	0.668	0.734	1.000	
A	ICEO 22	60	a	1.000				
A	ICEO 27	60	b	0.700	1.000			
A	ICEO 24	60	c	0.759	0.716	1.000		
A	ICEO 25	60	d	0.752	0.679	0.732	1.000	
A	ICEF 22	60	a	1.000				
A	ICEF 27	60	b	0.693	1.000			
A	ICEF 24	59	c	0.768	0.525	1.000		
A	ICEF 25	60	d	0.719	0.654	0.690	1.000	
A	ICEC 22	60	a	1.000				
A	ICEC 27	60	b	0.829	1.000			
A	ICEC 24	60	c	0.849	0.759	1.000		
A	ICEC 25	60	d	0.844	0.801	0.853	1.000	
A	IREA 26	60	a	1.000				
A	IREA 23	60	b	0.441	1.000			
A	IREA 28	60	c	0.585	0.695	1.000		
A	IREA 29	60	d	0.619	0.587	0.677	1.000	
A	IREB 26	60	a	1.000				
A	IREB 23	60	b	0.728	1.000			
A	IREB 28	60	c	0.714	0.688	1.000		
A	IREB 29	60	d	0.688	0.617	0.808	1.000	
A	IREC 26	58	a	1.000				
A	IREC 23	60	b	0.524	1.000			
A	IREC 28	58	c	0.485	0.414	1.000		
A	IREC 29	60	d	0.625	0.681	0.819	1.000	
A	IRED 26	58	a	1.000				
A	IRED 23	60	b	0.623	1.000			
A	IRED 28	58	c	0.707	0.618	1.000		
A	IRED 29	60	d	0.813	0.698	0.882	1.000	
A	IRESUM 26	60	a	1.000				
A	IRESUM 23	59	b	0.502	1.000			
A	IRESUM 28	60	c	0.574	0.834	1.000		
A	IRESUM 29	54	d	0.689	0.709	0.798	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
B	BCOPP9	30	a	1.000				
B	BCOPP10	30	b	0.733	1.000			
B	BCOPP11	30	c	0.864	0.818	1.000		
B	BCOPP12	30	d	0.760	0.521	0.807	1.000	
B	BCOPP13	30	e	0.880	0.666	0.870	0.840	1.000
B	BCOPO9	30	a	1.000				
B	BCOPO10	30	b	0.945	1.000			
B	BCOPO11	30	c	0.971	0.932	1.000		
B	BCOPO12	30	d	0.962	0.927	0.964	1.000	
B	BCOPO13	30	e	0.959	0.938	0.946	0.928	1.000
B	BCOPI9	30	a	1.000				
B	BCOPI10	30	b	0.906	1.000			
B	BCOPI11	30	c	0.952	0.936	1.000		
B	BCOPI12	30	d	0.929	0.855	0.944	1.000	
B	BCOPI13	30	e	0.950	0.864	0.949	0.948	1.000
B	BCOPI9b	30	a	1.000				
B	BCOPI10b	30	b	0.888	1.000			
B	BCOPI11b	30	c	0.936	0.938	1.000		
B	BCOPI12b	30	d	0.892	0.823	0.916	1.000	
B	BCOPP13b	30	e	0.930	0.850	0.952	0.926	1.000
B	HETQ14	25	a	1.000				
B	HETQ15	17	b	0.711	1.000			
B	HETQ16	23	c	0.355	0.387	1.000		
B	HETQ17	18	d	0.456	0.760	0.679	1.000	
B	HETS14	5	a	*				
B	HETSd15	9	b	*	1.000			
B	HETS16	7	c	*	0.949	1.000		
B	HETS17	11	d	*	0.831	0.420	1.000	
B	HETQ14b	25	a	1.000				
B	HETQ15b	17	b	0.727	1.000			
B	HETQ16b	23	c	0.645	0.594	1.000		
B	HETQ17b	18	d	0.927	0.470	0.535	1.000	
B	ICES 22	30	a	1.000				
B	ICES 27	30	b	0.808	1.000			
B	ICES 24	29	c	0.722	0.789	1.000		
B	ICES 25	29	d	0.691	0.795	0.789	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
B	ICEO 22	30	a	1.000				
B	ICEO 27	30	b	0.775	1.000			
B	ICEO 24	30	c	0.775	0.821	1.000		
B	ICEO 25	30	d	0.847	0.812	0.771	1.000	
B	ICEF 22	30	a	1.000				
B	ICEF 27	30	b	0.803	1.000			
B	ICEF 24	29	c	0.846	0.692	1.000		
B	ICEF 25	30	d	0.676	0.727	0.704	1.000	
B	ICEC 22	30	a	1.000				
B	ICEC 27	30	b	0.892	1.000			
B	ICEC 24	30	c	0.881	0.860	1.000		
B	ICEC 25	30	d	0.881	0.896	0.858	1.000	
B	IREA 26	30	a	1.000				
B	IREA 23	30	b	0.503	1.000			
B	IREA 28	30	c	0.624	0.814	1.000		
B	IREA 29	30	d	0.608	0.706	0.701	1.000	
B	IREB 26	30	a	1.000				
B	IREB 23	30	b	0.754	1.000			
B	IREB 28	30	c	0.699	0.746	1.000		
B	IREB 29	30	d	0.690	0.674	0.912	1.000	
B	IREC 26	29	a	1.000				
B	IREC 23	30	b	0.606	1.000			
B	IREC 28	28	c	0.655	0.439	1.000		
B	IREC 29	30	d	0.777	0.733	0.855	1.000	
B	IRED 26	29	a	1.000				
B	IRED 23	30	b	0.663	1.000			
B	IRED 28	28	c	0.799	0.598	1.000		
B	IRED 29	30	d	0.855	0.747	0.939	1.000	
B	IRESUM 26	30	a	1.000				
B	IRESUM 23	29	b	0.568	1.000			
B	IRESUM 28	30	c	0.595	0.955	1.000		
B	IRESUM 29	25	d	0.835	0.749	0.799	1.000	
C	BCOPP9	18	a	1.000				
C	BCOPP10	18	b	0.915	1.000			
C	BCOPP11	18	c	0.932	0.893	1.000		
C	BCOPP12	18	d	0.785	0.688	0.894	1.000	
C	BCOPP13	18	e	0.901	0.889	0.963	0.922	1.000

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
C	BCOPO9	18	a	1.000				
C	BCOPO10	18	b	0.959	1.000			
C	BCOPO11	18	c	0.913	0.896	1.000		
C	BCOPO12	18	d	0.942	0.928	0.991	1.000	
C	BCOPO13	18	e	0.982	0.972	0.961	0.978	1.000
C	BCOPI9	18	a	1.000				
C	BCOPI10	18	b	0.946	1.000			
C	BCOPI11	18	c	0.898	0.879	1.000		
C	BCOPI12	18	d	0.937	0.915	0.980	1.000	
C	BCOPI13	18	e	0.981	0.964	0.947	0.978	1.000
C	BCOPI9b	18	a	1.000				
C	BCOPI10b	18	b	0.943	1.000			
C	BCOPI11b	18	c	0.864	0.877	1.000		
C	BCOPI12b	18	d	0.949	0.916	0.923	1.000	
C	BCOPP13b	18	e	0.971	0.954	0.905	0.968	1.000
C	HETQ14	12	a	1.000				
C	HETQ15	11	b	0.944	1.000			
C	HETQ16	12	c	0.809	0.745	1.000		
C	HETQ17	11	d	0.621	0.580	0.782	1.000	
C	HETS14	6	a	1.000				
C	HETS15	4	b	0.096	1.000			
C	HETS16	6	c	-0.159	-0.910	1.000		
C	HETS17	4	d	-0.288	0.852	-0.094	1.000	
C	HETQ14b	12	a	1.000				
C	HETQ15b	11	b	0.692	1.000			
C	HETQ16b	12	c	0.816	0.642	1.000		
C	HETQ17b	11	d	0.626	0.830	0.562	1.000	
C	ICES 22	18	a	1.000				
C	ICES 27	18	b	0.671	1.000			
C	ICES 24	18	c	0.757	0.599	1.000		
C	ICES 25	17	d	0.514	0.210	0.732	1.000	
C	ICEO 22	18	a	1.000				
C	ICEO 27	18	b	0.498	1.000			
C	ICEO 24	18	c	0.704	0.414	1.000		
C	ICEO 25	18	d	0.786	0.442	0.851	1.000	
C	ICEF 22	18	a	1.000				
C	ICEF 27	18	b	0.433	1.000			
C	ICEF 24	18	c	0.847	0.371	1.000		
C	ICEF 25	18	d	0.745	0.517	0.763	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
C	ICEC 22	18	a	1.000				
C	ICEC 27	18	b	0.705	1.000			
C	ICEC 24	18	c	0.844	0.569	1.000		
C	ICEC 25	18	d	0.763	0.595	0.905	1.000	
C	IREA 26	18	a	1.000				
C	IREA 23	18	b	0.413	1.000			
C	IREA 28	18	c	0.599	0.722	1.000		
C	IREA 29	18	d	0.656	0.480	0.634	1.000	
C	IREB 26	18	a	1.000				
C	IREB 23	18	b	0.629	1.000			
C	IREB 28	18	c	0.683	0.552	1.000		
C	IREB 29	18	d	0.607	0.409	0.575	1.000	
C	IREC 26	17	a	1.000				
C	IREC 23	18	b	0.169	1.000			
C	IREC 28	18	c	0.276	0.456	1.000		
C	IREC 29	18	d	0.210	0.392	0.748	1.000	
C	IRED 26	17	a	1.000				
C	IRED 23	18	b	0.490	1.000			
C	IRED 28	18	c	0.704	0.689	1.000		
C	IRED 29	18	d	0.790	0.615	0.874	1.000	
C	IRESUM 26	18	a	1.000				
C	IRESUM 23	18	b	0.481	1.000			
C	IRESUM 28	18	c	0.555	0.861	1.000		
C	IRESUM 29	18	d	0.628	0.964	0.896	1.000	
D	BCOPP9	12	a	1.000				
D	BCOPP10	12	b	0.835	1.000			
D	BCOPP11	12	c	0.932	0.912	1.000		
D	BCOPP12	12	d	0.843	0.966	0.922	1.000	
D	BCOPP13	12	e	0.766	0.924	0.921	0.958	1.000
D	BCOPO9	12	a	1.000				
D	BCOPO10	12	b	0.957	1.000			
D	BCOPO11	12	c	0.971	0.981	1.000		
D	BCOPO12	12	d	0.947	0.972	0.957	1.000	
D	BCOPO13	12	e	0.967	0.995	0.985	0.973	1.000
D	BCOPI9	12	a	1.000				
D	BCOPI10	12	b	0.914	1.000			
D	BCOPI11	12	c	0.951	0.952	1.000		
D	BCOPI12	12	d	0.915	0.989	0.936	1.000	
D	BCOPI13	12	e	0.915	0.959	0.947	0.966	1.000

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
D	BCOPI9b	12	a	1.000				
D	BCOPI10b	12	b	0.914	1.000			
D	BCOPI11b	12	c	0.951	0.952	1.000		
D	BCOPI12b	12	d	0.915	0.989	0.936	1.000	
D	BCOPP13b	12	e	0.915	0.959	0.947	0.966	1.000
D	HETQ14	12	a	1.000				
D	HETQ15	12	b	0.793	1.000			
D	HETQ16	12	c	0.438	0.779	1.000		
D	HETQ17	12	d	0.816	0.876	0.579	1.000	
D	HETQ14b	12	a	1.000				
D	HETQ15b	12	b	0.721	1.000			
D	HETQ16b	12	c	0.670	0.768	1.000		
D	HETQ17b	12	d	0.420	0.966	0.721	1.000	
D	ICES 22	12	a	1.000				
D	ICES 27	12	b	0.741	1.000			
D	ICES 24	12	c	0.920	0.696	1.000		
D	ICES 25	12	d	0.641	0.392	0.543	1.000	
D	ICEO 22	12	a	1.000				
D	ICEO 27	12	b	0.618	1.000			
D	ICEO 24	12	c	0.719	0.759	1.000		
D	ICEO 25	12	d	0.438	0.834	0.483	1.000	
D	ICEF 22	12	a	1.000				
D	ICEF 27	12	b	0.663	1.000			
D	ICEF 24	12	c	0.636	0.546	1.000		
D	ICEF 25	12	d	0.950	0.748	0.664	1.000	
D	ICEC 22	12	a	1.000				
D	ICEC 27	12	b	0.827	1.000			
D	ICEC 24	12	c	0.854	0.805	1.000		
D	ICEC 25	12	d	0.870	0.759	0.724	1.000	
D	IREA 26	12	a	1.000				
D	IREA 23	12	b	0.433	1.000			
D	IREA 28	12	c	0.317	0.567	1.000		
D	IREA 29	12	d	0.678	0.462	0.480	1.000	
D	IREB 26	12	a	1.000				
D	IREB 23	12	b	0.786	1.000			
D	IREB 28	12	c	0.894	0.789	I.000		
D	IREB 29	12	d	0.814	0.736	0.845	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
D	IREC 26	12	a	1.000				
D	IREC 23	12	b	0.091	1.000			
D	IREC 28	12	c	-0.148	0.269	1.000		
D	IREC 29	12	d	-0.010	0.527	0.835	1.000	
D	IRED 26	12	a	1.000				
D	IRED 23	12	b	0.647	1.000			
D	IRED 28	12	c	0.405	0.635	1.000		
D	IRED 29	12	d	0.686	0.589	0.758	1.000	
D	IRESUM 26	12	a	1.000				
D	IRESUM 23	12	b	0.363	1.000			
D	IRESUM 28	12	c	0.769	0.498	1.000		
D	IRESUM 29	11	d	0.665	0.614	0.872	1.000	
E	BCOPP9	20	a	1.000				
E	BCOPP10	20	b	0.773	1.000			
E	BCOPP11	20	c	0.926	0.843	1.000		
E	BCOPP12	20	d	0.878	0.563	0.889	1.000	
E	BCOPP13	20	e	0.932	0.670	0.934	0.886	1.000
E	BCOPO9	20	a	1.000				
E	BCOPO10	20	b	0.941	1.000			
E	BCOPO11	20	c	0.908	0.887	1.000		
E	BCOPO12	20	d	0.912	0.903	0.977	1.000	
E	BCOPO13	20	e	0.966	0.930	0.952	0.942	1.000
E	BCOPI9	20	a	1.000				
E	BCOPI10	20	b	0.902	1.000			
E	BCOPI11	20	c	0.897	0.872	1.000		
E	BCOPI12	20	d	0.880	0.852	0.960	1.000	
E	BCOPI13	20	e	0.945	0.884	0.943	0.942	1.000
E	BCOPI9b	20	a	1.000				
E	BCOPI10b	20	b	0.881	1.000			
E	BCOPI11b	20	c	0.887	0.869	1.000		
E	BCOPI12b	20	d	0.870	0.776	0.889	1.000	
E	BCOPP13b	20	e	0.921	0.824	0.925	0.930	1.000
E	HETQ14	9	a	1.000				
E	HETQ15	0	b	*	*			
E	HETQ16	7	c	0.500	*	1.000		
E	HETQ17	1	d	*	*	*	*	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
E	HETS14	11	a	1.000				
E	HETS15	13	b	0.174	1.000			
E	HETS16	13	c	-0.171	-0.171	1.000		
E	HETS17	17	d	-0.103	0.808	0.031	1.000	
E	HETQ14b	9	a	1.000				
E	HETQ15b	0	b	*	*			
E	HETQ16b	7	c	0.985	*	1.000		
E	HETQ17b	1	d	*	*	*	*	
E	ICES 22	20	a	1.000				
E	ICES 27	20	b	0.869	1.000			
E	ICES 24	20	c	0.847	0.734	1.000		
E	ICES 25	19	d	0.778	0.722	0.811	1.000	
E	ICEO 22	20	a	1.000				
E	ICEO 27	20	b	0.595	1.000			
E	ICEO 24	20	c	0.752	0.602	1.000		
E	ICEO 25	20	d	0.868	0.649	0.752	1.000	
E	ICEF 22	20	a	1.000				
E	ICEF 27	20	b	0.729	1.000			
E	ICEF 24	20	c	0.864	0.678	1.000		
E	ICEF 25	20	d	0.739	0.869	0.674	1.000	
E	ICEC 22	20	a	1.000				
E	ICEC 27	20	b	0.806	1.000			
E	ICEC 24	20	c	0.874	0.752	1.000		
E	ICEC 25	20	d	0.883	0.816	0.880	1.000	
E	IREA 26	20	a	1.000				
E	IREA 23	20	b	0.195	1.000			
E	IREA 28	20	c	0.394	0.908	1.000		
E	IREA 29	20	d	0.405	0.543	0.468	1.000	
E	IREB 26	20	a	1.000				
E	IREB 23	20	b	0.782	1.000			
E	IREB 28	20	c	0.629	0.649	1.000		
E	IREB 29	20	d	0.569	0.524	0.672	1.000	
E	IREC 26	19	a	1.000				
E	IREC 23	20	b	0.335	1.000			
E	IREC 28	20	c	0.670	0.404	1.000		
E	IREC 29	20	d	0.559	0.628	0.829	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
E	IRED 26	19	a	1.000				
E	IRED 23	20	b	0.540	1.000			
E	IRED 28	20	c	0.791	0.685	1.000		
E	IRED 29	20	d	0.798	0.689	0.949	1.000	
E	IRESUM 26	20	a	1.000				
E	IRESUM 23	19	b	0.199	1.000			
E	IRESUM 28	20	c	0.191	0.991	1.000		
E	IRESUM 29	15	d	0.432	0.606	0.635	1.000	
F	BCOPP9	14	a	1.000				
F	BCOPP10	14	b	0.731	1.000			
F	BCOPP11	14	c	0.901	0.864	1.000		
F	BCOPP12	14	d	0.795	0.903	0.896	1.000	
F	BCOPP13	14	e	0.699	0.846	0.875	0.933	1.000
F	BCOPO9	14	a	1.000				
F	BCOPO10	14	b	0.984	1.000			
F	BCOPO11	14	c	0.985	0.959	1.000		
F	BCOPO12	14	d	0.989	0.968	0.987	1.000	
F	BCOPO13	14	e	0.984	0.988	0.955	0.976	1.000
F	BCOPI9	14	a	1.000				
F	BCOPI10	14	b	0.917	1.000			
F	BCOPI11	14	c	0.975	0.920	1.000		
F	BCOPI12	14	d	0.974	0.914	0.974	1.000	
F	BCOPI13	14	e	0.969	0.926	0.954	0.980	1.000
F	BCOPI9b	14	a	1.000				
F	BCOPI10b	14	b	0.899	1.000			
F	BCOPI11b	14	c	0.970	0.928	1.000		
F	BCOPI12b	14	d	0.955	0.921	0.962	1.000	
F	BCOPP13b	14	e	0.946	0.918	0.976	0.972	1.000
F	HETQ14	14	a	1.000				
F	HETQ15	14	b	0.880	1.000			
F	HETQ16	14	c	0.776	0.730	1.000		
F	HETQ17	14	d	0.712	0.842	0.765	1.000	
F	HETQ14b	14	a	*				
F	HETQ15b	14	b	*	1.000			
F	HETQ16b	14	c	*	0.591	1.000		
F	HETQ17b	14	d	*	0.974	0.590	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
F	ICES 22	14	a	1.000				
F	ICES 27	14	b	0.617	1.000			
F	ICES 24	13	c	0.757	0.856	1.000		
F	ICES 25	13	d	0.539	0.889	0.821	1.000	
F	ICEO 22	14	a	1.000				
F	ICEO 27	14	b	0.797	1.000			
F	ICEO 24	14	c	0.796	0.907	1.000		
F	ICEO 25	14	d	0.794	0.868	0.717	1.000	
F	ICEF 22	14	a	1.000				
F	ICEF 27	14	b	0.781	1.000			
F	ICEF 24	13	c	0.604	0.543	1.000		
F	ICEF 25	14	d	0.901	0.689	0.772	1.000	
F	ICEC 22	14	a	1.000				
F	ICEC 27	14	b	0.873	1.000			
F	ICEC 24	14	c	0.877	0.905	1.000		
F	ICEC 25	14	d	0.907	0.913	0.868	1.000	
F	IREA 26	14	a	1.000				
F	IREA 23	14	b	0.648	1.000			
F	IREA 28	14	c	0.733	0.712	1.000		
F	IREA 29	14	d	0.789	0.596	0.817	1.000	
F	IREB 26	14	a	1.000				
F	IREB 23	14	b	0.808	1.000			
F	IREB 28	14	c	0.862	0.812	1.000		
F	IREB 29	14	d	0.789	0.746	0.906	1.000	
F	IREC 26	13	a	1.000				
F	IREC 23	14	b	0.914	1.000			
F	IREC 28	12	c	0.464	0.682	1.000		
F	IREC 29	14	d	0.805	0.815	0.845	1.000	
F	IRED 26	13	a	1.000				
F	IRED 23	14	b	0.776	1.000			
F	IRED 28	12	c	0.613	0.575	1.000		
F	IRED 29	14	d	0.868	0.696	0.781	1.000	
F	IRESUM 26	14	a	1.000				
F	IRESUM 23	14	b	0.770	1.000			
F	IRESUM 28	14	c	0.863	0.811	1.000		
F	IRESUM 29	14	d	0.884	0.800	0.957	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
G	BCOPP9	26	a	1.000				
G	BCOPP10	26	b	0.733	1.000			
G	BCOPP11	26	c	0.801	0.856	1.000		
G	BCOPP12	26	d	0.781	0.612	0.801	1.000	
G	BCOPP13	26	e	0.893	0.794	0.858	0.845	1.000
G	BCOPO9	26	a	1.000				
G	BCOPO10	26	b	0.961	1.000			
G	BCOPO11	26	c	0.935	0.955	1.000		
G	BCOPO12	26	d	0.949	0.961	0.967	1.000	
G	BCOPO13	26	e	0.961	0.964	0.913	0.940	1.000
G	BCOPI9	26	a	1.000				
G	BCOPI10	26	b	0.873	1.000			
G	BCOPI11	26	c	0.875	0.939	1.000		
G	BCOPI12	26	d	0.897	0.851	0.902	1.000	
G	BCOPI13	26	e	0.953	0.891	0.898	0.956	1.000
G	BCOPI9b	26	a	1.000				
G	BCOPI10b	26	b	0.873	1.000			
G	BCOPI11b	26	c	0.875	0.939	1.000		
G	BCOPI12b	26	d	0.897	0.851	0.902	1.000	
G	BCOPP13b	26	e	0.953	0.891	0.898	0.956	1.000
G	HETQ14	26	a	1.000				
G	HETQ15	26	b	0.755	1.000			
G	HETQ16	26	c	0.221	0.450	1.000		
G	HETQ17	26	d	0.492	0.692	0.704	1.000	
G	HETQ14b	26	a	1.000				
G	HETQ15b	26	b	0.721	1.000			
G	HETQ16b	26	c	0.771	0.638	1.000		
G	HETQ17b	26	d	0.675	0.765	0.591	1.000	
G	ICES 22	26	a	1.000				
G	ICES 27	26	b	0.779	1.000			
G	ICES 24	26	c	0.690	0.736	1.000		
G	ICES 25	26	d	0.626	0.461	0.560	1.000	
G	ICEO 22	26	a	1.000				
G	ICEO 27	26	b	0.757	1.000			
G	ICEO 24	26	c	0.770	0.695	1.000		
G	ICEO 25	26	d	0.719	0.692	0.764	1.000	

Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995)

Chemical Category[1]	*In Vitro* Endpoint	No. samples tested *In Vitro*	Interlaboratory correlation of *in vitro* data					
			Lab	Lab a	Lab b	Lab c	Lab d	Lab e
G	ICEF 22	26	a	1.000				
G	ICEF 27	26	b	0.607	1.000			
G	ICEF 24	26	c	0.748	0.394	1.000		
G	ICEF 25	26	d	0.594	0.494	0.654	1.000	
G	ICEC 22	26	a	1.000				
G	ICEC 27	26	b	0.856	1.000			
G	ICEC 24	26	c	0.830	0.745	1.000		
G	ICEC 25	26	d	0.778	0.751	0.803	1.000	
G	IREA 26	26	a	1.000				
G	IREA 23	26	b	0.496	1.000			
G	IREA 28	26	c	0.685	0.518	1.000		
G	IREA 29	26	d	0.709	0.625	0.704	1.000	
G	IREB 26	26	a	1.000				
G	IREB 23	26	b	0.525	1.000			
G	IREB 28	26	c	0.628	0.526	1.000		
G	IREB 29	26	d	0.664	0.470	0.824	1.000	
G	IREC 26	26	a	1.000				
G	IREC 23	26	b	0.137	1.000			
G	IREC 28	26	c	0.245	0.214	1.000		
G	IREC 29	26	d	0.342	0.101	0.808	1.000	
G	IRED 26	26	a	1.000				
G	IRED 23	26	b	0.539	1.000			
G	IRED 28	26	c	0.712	0.507	1.000		
G	IRED 29	26	d	0.790	0.613	0.906	1.000	
G	IRESUM 26	26	a	1.000				
G	IRESUM 23	26	b	0.527	1.000			
G	IRESUM 28	26	c	0.693	0.793	1.000		
G	IRESUM 29	25	d	0.626	0.696	0.716	1.000	

Abbreviations: BCOPI = Index; BCOPIb = Index, cut-off at 200; BCOPO = Opacity; BCOPP = Permeability; ICEC = Irritation Index; ICEF = Fluorescein retention; ICEO = Opacity; ICES = Swelling; IREA = Opacity (1 hr); IREB = Opacity (4 hr); IREC = Swelling (1 hr); IRED = Swelling (4 hr); IRESUM = Summary score; HETQ = Q Score; HETQB = Q Score, cutoff at 2; HETS = S Score; HETSB = S Score, cutoff at 2.

[1]A = Full set of chemicals, B= Water soluble, C = Water insoluble, D = Surfactants, E = Solids, F = Solutions, G = Liquids

The numbers 1-38 against each endpoint in the Table refer to the laboratories which conducted each particular test. Laboratory 36 left the study without submitting any results

* = No data

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