

§ 1500.42

the dermal corrosivity and primary irritation of substances referred to in §1500.3(c)(3) and (4), respectively, is a patch-test technique on the abraded and intact skin of the albino rabbit, clipped free of hair. Primary irritation to the skin is measured by a patch-test technique on the abraded and intact skin of the albino rabbit, clipped free of hair. A minimum of six subjects are used in abraded and intact skin tests. Introduce under a square patch, such as surgical gauze measuring 1 inch by 1 inch and two single layers thick, 0.5 milliliter (in the case of liquids) or 0.5 gram (in the case of solids and semisolids) of the test substance. Dissolve solids in an appropriate solvent and apply the solution as for liquids. The animals are immobilized with patches secured in place by adhesive tape. The entire trunk of the animal is then wrapped with an impervious material, such as rubberized cloth, for the 24-hour period of exposure. This material aids in maintaining the test patches in position and retards the evaporation of volatile substances. After 24 hours of exposure, the patches are removed and the resulting reactions are evaluated on the basis of the designated values in the following table:

Skin reaction	Value ¹
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

¹The "value" recorded for each reading is the average value of the six or more animals subject to the test.

Readings are again made at the end of a total of 72 hours (48 hours after the first reading). An equal number of exposures are made on areas of skin that have been previously abraded. The abrasions are minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. Evaluate the reac-

16 CFR Ch. II (1-1-14 Edition)

tions of the abraded skin at 24 hours and 72 hours, as described in this paragraph. Add the values for erythema and eschar formation at 24 hours and at 72 hours for intact skin to the values on abraded skin at 24 hours and at 72 hours (four values). Similarly, add the values for edema formation at 24 hours and at 72 hours for intact and abraded skin (four values). The total of the eight values is divided by four to give the primary irritation score; for example:

Skin reaction	Exposure time (hours)	Evaluation value
Erythema and eschar formation:		
Intact skin	24	2
Do	72	1
Abraded skin	24	3
Do	72	2
Subtotal		8
Edema formation:		
Intact skin	24	0
Do	72	1
Abraded skin	24	1
Do	72	2
Subtotal		4
Total		12

Thus, the primary irritation score is $12 \div 4 = 3$.

[38 FR 27012, Sept. 27, 1973, as amended at 77 FR 73294, Dec. 10, 2012]

§ 1500.42 Test for eye irritants.

Guidelines for *in vivo* and *in vitro* testing of ocular irritation of substances, including testing that does not require animals, are presented in the CPSC's animal testing policy set forth in 16 CFR 1500.232. A weight-of-evidence analysis or a validated *in vitro* test method is recommended to evaluate existing information before *in vivo* tests are considered. This analysis should include any of the following: Existing human and animal data on ocular or dermal irritation, structure activity relationships, physicochemical properties, and chemical reactivity. If *in vivo* testing is conducted, a sequential testing strategy is recommended to reduce the number of test animals. Additionally, the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize

pain and distress in ocular safety testing is recommended.

(a)(1) In the method of testing the ocular irritation of a substance referred to in §1500.3(c)(4), six albino rabbits are used for each test substance. Six albino rabbits are used for each test substance. Animal facilities for such procedures shall be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal in the test group shall be examined before testing, and only those animals without eye defects or irritation shall be used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. For testing liquids, 0.1 milliliter is used. For solids or pastes, 100 milligrams of the test substance is used, except that for substances in flake, granule, powder, or other particulate form the amount that has a volume of 0.1 milliliter (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) shall be used whenever this volume weighs less than 100 milligrams. In such a case, the weight of the 0.1 milliliter test dose should be recorded. The eyes are not washed following instillation of test material except as noted below.

(2) The eyes are examined and the grade of ocular reaction is recorded at 24, 48, and 72 hours. Reading of reactions is facilitated by use of a binocular loupe, hand slit-lamp, or other expert means. After the recording of observations at 24 hours, any or all eyes may be further examined after applying fluorescein. For this optional test, one drop of fluorescein sodium ophthalmic solution U.S.P. or equivalent is dropped directly on the cornea. After flushing out the excess fluorescein with sodium chloride solution U.S.P. or equivalent, injured areas of the cornea appear yellow; this is best visualized in a darkened room under ultraviolet illumination. Any or all eyes

may be washed with sodium chloride solution U.S.P. or equivalent after the 24-hour reading.

(b)(1) An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster), or inflammation of the iris (other than a slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible.

(2) The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall be regarded as negative. If two or three animals a positive reaction, the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

(c) To assist testing laboratories and others interested in interpreting ocular irritation test results, the CPSC animal testing policy Web page at <http://www.cpsc.gov/library/animaltesting.html> will contain the scoring system defined in the U.S. EPA's Test Guideline, OPPTS 870.2400: Acute Eye Irritation¹ or the OECD Test Guideline 405: Acute Eye Irritation/Corrosion.²

[38 FR 27012, Sept. 27, 1973; 38 FR 30105, Nov. 1, 1973; 62 FR 46667, Sept. 4, 1997; 77 FR 73294, Dec. 10, 2012]

¹EPA. 1998. Health Effects Test Guidelines, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195. Washington, DC: U.S. Environmental Protection Agency. (Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/EPA/EPA_870_2400.pdf)

²OECD. 2002. OECD Guideline for the Testing of Chemicals 405: Acute Eye Irritation/

Continued