

number of test animals. The method of testing the toxic substances referred to in §1500.3(c)(1)(ii)(C) and (c)(2)(iii) is as follows:

(a) *Acute dermal toxicity (single exposure)*. In the acute exposures, the agent is held in contact with the skin by means of a sleeve for periods varying up to 24 hours. The sleeve, made of rubber dam or other impervious material, is so constructed that the ends are reinforced with additional strips and should fit snugly around the trunk of the animal. The ends of the sleeve are tucked, permitting the central portion to “balloon” and furnish a reservoir for the dose. The reservoir must have sufficient capacity to contain the dose without pressure. In the following table are given the dimensions of

sleeves and the approximate body surface exposed to the test substance. The sleeves may vary in size to accommodate smaller or larger subjects. In the testing of unctuous materials that adhere readily to the skin, mesh wire screen may be employed instead of the sleeve. The screen is padded and raised approximately 2 centimeters from the exposed skin. In the case of dry powder preparations, the skin and substance are moistened with physiological saline prior to exposure. The sleeve or screen is then slipped over the gauze that holds the dose applied to the skin. In the case of finely divided powders, the measured dose is evenly distributed on cotton gauze which is then secured to the area of exposure.

DIMENSIONS OF SLEEVES FOR ACUTE DERMAL TOXICITY TEST  
[Test animal—Rabbits]

Measurements in centimeters		Range of weight of animals (grams)	Average area of exposure (square centimeters)	Average percentage of total body surface
Diameter at ends	Overall length			
7.0	12.5	2,500–3,500	240	10.7

(b) *Preparation of test animal*. The animals are prepared by clipping the skin of the trunk free of hair. Approximately one-half of the animals are further prepared by making epidermal abrasions every 2 or 3 centimeters longitudinally over the area of exposure. The abrasions are sufficiently deep to penetrate the stratum corneum (horny layer of the epidermis) but not to disturb the derma; that is, not to obtain bleeding.

(c) *Procedures for testing*. The sleeve is slipped onto the animal which is then placed in a comfortable but immobilized position in a multiple animal holder. Selected doses of liquids and solutions are introduced under the sleeve. If there is slight leakage from the sleeve, which may occur during the first few hours of exposure, it is collected and reapplied. Dosage levels are adjusted in subsequent exposures (if necessary) to enable a calculation of a dose that would be fatal to 50 percent of the animals. This can be determined from mortality ratios obtained at various doses employed. At the end of 24 hours the sleeves or screens are removed, the volume of unabsorbed ma-

terial (if any) is measured, and the skin reactions are noted. The subjects are cleaned by thorough wiping, observed for gross symptoms of poisoning, and then observed for 2 weeks.

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**§ 1500.41 Method of testing primary irritant substances.**

Guidelines for testing the dermal irritation and corrosivity properties 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 all of the following that are available: human and animal data, structure activity relationships, physicochemical properties, and dermal toxicity. If *in vivo* testing is conducted, a sequential testing strategy is recommended to reduce the number of test animals. The method of testing

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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 <sup>1</sup>
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

<sup>1</sup>The "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-

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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