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Safety Evaluation of Enzyme Detergents. Oral and Cutaneous Toxicity, Irritancy and Skin Sensitization Studies

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(Received 29 August 1969)

Abstract—Toxicological studies in man and animals are reported for laundry detergents and presoak products containing commercial preparations of the proteolytic enzymes, subtilisin BPN and subtilisin Carlsberg. On acute oral administration to rats, the enzyme materials *per se* displayed a low order of oral toxicity and did not affect toxicity when incorporated in detergent and presoak products. The products showed no toxic effects in dogs and the enzyme ingredients did not alter the emetic properties of the laundry products. There was no evidence of toxicity in 4-wk or 13-wk applications of enzyme-detergent mixtures to the abraded or intact skin of rabbits, though skin irritation was greater than for controls at the highest level of application of the enzyme preparation. Extensive eye irritation studies with rabbits showed no effect of any consequence when the enzyme preparations were added to the products.

Laboratory tests on more than 700 human subjects of a variety of enzyme-containing detergents showed no adverse effect on mildness from the enzyme ingredient except under conditions of high concentration, occlusion of the skin and prolonged contact. Clinical mildness tests on 5943 housewives, diaper rash studies on 360 infants, and repeated insult patch tests of 1478 men and women revealed no untoward effect from products containing the enzyme ingredients.

INTRODUCTION

Few recent innovations have brought such clear-cut improvements in the performance of laundry products as has the incorporation of proteolytic enzymes. The first known commercial application of enzymes in consumer products began with the marketing of a laundry presoaking product in Germany in 1913. Attempts to conserve fatty acids during World War II resulted in renewed interest in enzyme washing products in Europe, but it was not until 1963 in Holland that the first commercially successful product was introduced. With the development of relatively active, heat- and alkali-stable enzymes, these materials became attractive to the American detergent industry.

The first enzyme washing products were introduced in the United States early in 1967. At the present time there are several nationally distributed brands of enzyme products in which soiled clothing is soaked prior to washing, as well as several major brands of general-purpose laundry detergents that contain enzymes. Consequently, it can be assumed that a

major proportion of the population of the USA is now using one or more of these products. The purpose of this report is to describe the toxicological and dermatological studies that have been performed in our laboratories to assess the safety of enzyme-containing laundry products.

EXPERIMENTAL

Materials

Proteolytic enzymes. The enzymes studied were commercial raw materials that were typical of those currently used in laundry products. They were obtained by large-scale fermentation using selected strains of *Bacillus subtilis*. Two types were involved, both alkaline proteases, namely subtilisin BPN from *B. subtilis* BPN and subtilisin Carlsberg from *B. subtilis* Carlsberg (Smith, DeLange, Evans, Landon & Markland, 1968). The differences between these sources have been discussed by Keay & Moser (1969). The commercial materials contained 5–15% of actual proteolytic enzyme, the balance being other protein, inorganic salts and carbohydrates. The BPN type also contained some amylase. Part of the inorganic salt present had been added by the suppliers in order to standardize the enzymatic activity of the commercial material. The subtilisin BPN tested contained 25–30% protein and 8–25% inorganic salts, and the Carlsberg type, 8% protein and 60% inorganic salts. In both types of enzyme preparation, the inorganic salts were primarily sodium chloride and sodium and calcium sulphates.

Detergent formulations. Except for the presence of the enzyme ingredient, the detergent formulations studied were conventional, general-purpose household detergent formulations containing 15–20% linear alkylate sulphonate (LAS), 40–50% sodium tripolyphosphate builder, 10–15% sodium sulphate, 5–10% sodium silicate ($\text{SiO}_2:\text{Na}_2\text{O} = 1.3:1-2:1$) and minor ingredients such as perfume, colourings and optical brighteners. The commercial enzyme materials were incorporated at levels of 0.08–1.0% as indicated.

Presoak formulations. These formulations contained 2–10% nonionic surfactant (fatty alcohol-ethylene oxide condensate) and 60–75% builder (sodium tripolyphosphate). They also contained 20–30% sodium perborate, an oxygen bleaching agent. Enzyme preparation levels were 1.2–5.0%.

Oral toxicity studies

Acute oral toxicity in rats. Test materials were administered as concentrated aqueous solutions or slurries to groups of ten rats (body weight 200–300 g) that had been fasted overnight. The LD_{50} was calculated by the method of Weil (1952) from the number of animals surviving in each group at the end of 14 days. Screening tests to give an estimated lethal dose utilized the technique of Deichmann & LeBlanc (1943).

Acute oral toxicity in dogs. Test materials in a dose of 2.5 g/kg, as a 40% aqueous slurry, were given by stomach tube to groups of one male and one female beagle. The dogs were starved overnight and water was withheld for approximately 3 hr prior to dosing. Each animal was subjected to gastroscopy under anaesthesia before dosing and 2 or 4 wk afterwards, to determine the condition of the gastro-intestinal mucosa. Other observations included gross physical examination, routine blood analyses (haematocrit and total and differential white cell counts) and blood urea-nitrogen and serum glutamic-pyruvic transaminase determinations, together in some cases with the bromsulphophthalein test for liver function.

Emetic studies in dogs. Tests were performed to detect any alteration in the emetic properties of detergents (Weaver & Griffith, 1969) as a result of the addition of enzyme material to the formulations. The emetic dose₅₀ of products was determined (Weil, 1952) by intra-gastric administration of graded doses of 40% aqueous slurries to groups of two male and two female beagles. Each animal was observed for time of onset and duration of emesis. Each test series included a control group that received only water.

Subacute oral toxicity in rats. Enzyme-containing detergents were administered by incorporating 1% of each formulation in the Purina Laboratory Chow diet of a group of 20 male and 20 female albino rats for 4 wk. Similar groups of control animals received the diet without added detergent. Food consumption, growth rate and feed efficiency were recorded for all animals. At the end of the experiment, five male and five female rats were selected at random from each group for haematological studies (red cell count, haemoglobin estimation, haematocrit and total and differential white cell counts) and autopsy. Liver and kidney weights relative to terminal body weight were determined and sections of the adrenals, urinary bladder, bone marrow, gonads, heart, intestine, kidney, liver, lung, lymph nodes, pancreas, spleen, stomach, thyroid and skin were examined histologically.

Skin and eye tests in rabbits

Subacute dermal toxicity in rabbits. Aqueous solutions containing 2.5% detergent and 2.5% enzyme material or 10% solutions of detergent containing 0.15–5% enzyme material were applied in doses of 2.0 ml/kg body weight to the skin of groups of albino rabbits, according to the method of Draize (1959). Applications were made daily for 4 wk on abraded skin or for 13 wk on intact skin. At the termination of the experiments haematological and histological studies, as listed in the previous paragraph, were carried out on all surviving animals.

Eye irritation in rabbits. Enzymes and enzyme-containing products were tested in the eyes of albino rabbits according to the method of Draize (1959), either as solids or as aqueous solutions. In some experiments, the corneas were first abraded with a cotton tip applicator after instillation of 5% procaine hydrochloride. One eye of each animal was left untreated as a control. Each test involved nine rabbits, three eyes being treated with 3 mg powdered material and left unrinsed, three with powdered material followed by rinsing with water, and three with an aqueous solution of material but not rinsed. The treated eyes were examined and a quantitative assessment of changes was made daily with the aid of a CRC Stereoptic Binocular Magnifier until all signs of irritation had disappeared. The scale for scoring ocular lesions was essentially that of Draize (1959) except that any effect on the cornea other than superficial dulling or stippling was assigned a score of 2 or more.

Skin irritation in rabbits. Single occluded patches containing 0.5 ml of an aqueous solution of the test material were applied to the clipped backs of groups of albino rabbits for 24 hr. On removal of the patches and again after 72 hr, skin irritation at the sites of application was graded according to the scoring system of Draize (1959).

Tests in man

Skin irritation in man. A variety of laboratory tests for skin irritancy of enzyme-product formulations were carried out with human volunteers. Briefly these tests, which have been described elsewhere (Carter & Griffith, 1965; Johnson, Kile, Fliegelman & Fix, 1951; Johnson, Kile, Kooyman, Whitehouse & Brod, 1953; Kooyman & Snyder, 1942), consisted of washing, immersion and patch exposures. Skin-washing and hand-immersion studies

were carried out on groups of six to ten volunteers using high concentrations (up to 10%) of product in solutions heated to 41°C. Two materials were tested simultaneously on each subject, one of them usually being an enzyme-free control formulation. The washings or immersions were done three times daily on five consecutive days. Skin reactions were scored on a 10 (normal) to 1 (extreme irritation) scale. The patch tests consisted of single or three consecutive applications every other day of 0.4 ml of 0.25–1.0% aqueous solutions of detergent. Patches were applied on either side of the midline of the back. Any irritant reactions that developed were graded on a four-point scale of intensity of erythema and results were compared with those for control materials tested simultaneously.

Clinical and usage studies. Large panels of housewife-volunteers were given enzyme-containing products and non-enzyme control products to use in hand dishwashing for periods of 2 wk each. The tests followed a double-blind, cross-over design in which the participants' hands were graded by a dermatologist for skin condition before and after the use of each product (Carter & Griffith, 1965; Johnson *et al.* 1953). In a given test, each product was used by approximately 300 women. Dermatologists' scores (100 = normal; 10 = severe irritation) were analysed statistically by analysis of variance to determine whether or not there were differences between products in regard to mildness.

Detergents and presoak products containing enzymes were supplied for use in diaper laundering to mothers of infants 24 months old and younger in double-blind, cross-over studies each involving 120 infants. Control products were the mother's regular presoak product, or, in the case of detergents, a non-enzyme formulation. The skin in each baby's diaper area was examined and graded by a registered nurse under the supervision of a paediatrician. Scores so obtained were analysed for statistical significance by analysis of variance.

Other usage studies involved paired comparison of washing products with and without enzymes. In studies of this type, no examination of the users' skin was involved, but participants were interviewed following the use of each product to record impressions of the products, including any comments on effects on the skin.

Cutaneous sensitization. Groups of 16–133 human volunteers were employed in the repeated insult patch test for cutaneous sensitization. The test design, similar to those of Draize (1959) and Voss (1958), involved nine applications, each lasting 24 hr, of 0.5 ml of each test solution to the outer aspect of the upper arm of the subject during a 3-wk 'insult' period. The patches were placed on the same site at each application. Following a 'rest' period for 10–14 days, duplicate 'challenge' applications were made, one to the site to which a patch had previously been applied, the other to a fresh site on the contralateral arm. Patches consisted of Webril padding and were held in place with Blenderm adhesive. Heightening of the skin reaction following the challenge application, as compared to the response to the original exposure, was considered suggestive of cutaneous sensitization.

RESULTS

Oral toxicity studies

Acute oral toxicity in rats. Results with the two types of commercial enzyme preparation are presented in Table 1. The LD₅₀ values of 20% aqueous solutions ranged from 3.7 to more than 10.3 g/kg body weight. The lower value in this range represents a higher degree of toxicity than would be expected for an enzyme. Based upon chemical analyses of the enzyme preparations tested, it has been tentatively concluded that their acute oral LD₅₀s are

directly related to the levels of inorganic salts present as diluents, and are essentially independent of the levels of organic constituents.

Table 1. *Typical acute oral toxicities of enzyme preparations and detergent and presoak products in rats*

Material tested	Level of enzyme preparation (%)	Test concn (% w/w)	Oral LD ₅₀ (with 95% confidence limits) (g/kg)
Enzyme preparation			
BPN type	100	20	c.9* ; >10.3† (-)
Carlsberg type	100	20	3.7 (3.3-4.1) *
Laundry detergent	0.0	40	4.0 (3.6-4.6)
	0.3	40	4.8 (4.3-5.3)
	1.0	40	3.8 (3.3-4.3)
Presoak product	0.0	40	c.2.9‡ (-)
	1.2	40	2.6 (2.3-3.0)
	5.0	40	c.2.9‡ (-)

* No deaths at 7.7 g/kg; eight of ten rats died at 10.0 g/kg.

† No deaths at 10.3 g/kg or lower doses.

‡ Estimated lethal dose test. Confidence limits not determinable.

Five general-purpose detergents containing from 0.08 to 1.0% enzyme material were compared with the same formulations without enzyme. Though the LD₅₀ of individual detergent formulations ranged from 2.8 to 4.8 g/kg, there was no significant difference between formulations with enzyme and those without enzyme, all other conditions being the same. Similarly, an enzyme presoak product, containing from 1.2 to 5.0% enzyme material and having LD₅₀s or estimated LDs of 2.3-2.9 g/kg, did not differ in toxicity from the same formulation without enzyme and having an estimated LD of 2.9 g/kg (Table 1).

Acute oral toxicity in dogs. Enzyme-containing detergents and presoak products, in single oral doses of 2.5 g/kg, were administered as 40% aqueous slurries by intragastric intubation to a total of seven groups each of one male and one female beagle dog. Typical results for each type of product are shown in Table 2. Emesis occurred promptly. All animals appeared grossly normal when emesis had ceased. Haematological indices, results of liver function tests and gastroscopic findings were the same 2 or 4 wk after dosing as they were prior to treatment.

Table 2. *Typical results of acute oral toxicity tests of detergent and presoak products in beagles*

Material tested	Level of enzyme preparation (%)	Test concn (% w/w)	Dose (g/kg)	Emesis		No. of emetic episodes
				Onset (min after dosing)	Duration (min)	
Laundry detergent	0.3	40	2.5	1	45	20
Presoak product	1.2	40	2.5	1	15	8

Emetic studies in dogs. These studies gave results consistent with earlier tests on washing products (Snyder, Opdyke, Griffith, Rubenkoenig, Tusing & Paynter, 1964). Emetic dose₅₀

values ranged from less than 6 mg/kg to approximately 25 mg/kg (Table 3). There was no correlation with the level of enzyme material (0.08–1.2%). Onset of emesis consistently occurred within 1 or 2 min and continued for 1–4 min. There were no fatalities or residual toxic effects among any of the dogs used in these tests. All dogs appeared grossly normal when emesis had ceased.

Table 3. *Typical emetic activity of enzyme laundry products in beagles*

Material tested	Level of enzyme preparation (%)	Test concn (% w/w)	ED ₅₀ * (g/kg)	Emesis	
				Mean onset (min after dosing)	Mean duration (min)
Laundry detergent	0.1	40	<0.025	1.5	2.3
	0.2	40	c.0.016	1.4	4.2
	0.25	40	<0.013	1.1	1.8
Presoak product	1.2	40	c.0.006	2.2	1.9

* Emetic dose₅₀. Because of the small dosage volume involved relative to the dosing apparatus employed, it was not possible to administer doses sufficiently small to permit calculation of confidence limits for these tests.

Subacute oral toxicity in rats. General purpose detergents containing 0.15 or 0.25% enzyme material fed as 1% of the diet had no adverse effect on rats in two separate 4-wk studies. All gross and histological findings were normal.

Skin and eye tests in rabbits

Subacute dermal toxicity in rabbits. Six 4-wk tests on abraded skin and four 13-wk tests on intact skin were performed. In either type of test, exposure of the skin of albino rabbits to 5% aqueous solutions of 1:1 enzyme-detergent mixtures resulted in irritation that was greater than that from the equivalent amount of detergent without enzyme. The observed blood effects comprised increased amphophile and decreased lymphocyte counts, which were considered to have resulted from the inflammatory response in the skin. The mean lymphocyte level for the control group was slightly higher than the normal range for control animals. Both the detergent control and the enzyme-detergent test groups displayed haemoglobin levels marginally higher than the normal range.

With lower levels of enzyme material (0.15–5.0%) only mild to moderate irritation was seen, which was essentially equivalent to that seen from non-enzyme formulations. There was no treatment-related pathological change in any of the rabbits following the 4- or 13-wk exposures, suggesting that percutaneous absorption either did not occur or, if it did take place, was of no toxicological significance.

Eye irritation in rabbits. Typical data from ten tests involving 90 rabbits are shown in Table 4. Several additional tests gave comparable results. Instillation of 3 mg powdered enzyme material, *per se*, into the eye produced moderate conjunctival irritation and transient corneal haziness, which cleared in 2–4 days. Rinsing the eye with water reduced the intensity of this irritation and prevented corneal involvement in most of the eyes. Enzyme preparations in 1 or 10% solutions were comparable in irritancy to the powders. Abrasion of the cornea

Table 4. *Typical results of rabbit eye irritation tests with enzyme preparations and formulations*

Material tested	Initial state of cornea	Level of enzyme preparation (%)	Treatment	Maximum average score*	Rate of eye clearance†
Enzyme preparation BPN type	Intact	100	3 mg (NR)	34.6	3 (4)
			3 mg (R)	12.6	1 (2); 2 (3)
			10% aq. (NR)	23.0	1 (2); 2 (3)
	Abraded		3 mg (NR)	31.3	2 (3); 1 (4)
			3 mg (R)	23.6	2 (3); 1 (4)
			10% aq. (NR)	35.0	3 (3)
Enzyme preparation Carlsberg type	Intact	100	3 mg (NR)	19.3	2 (2); 1 (4)
			3 mg (R)	5.3	3 (1)
			1% aq. (NR)	16.3	1 (1); 1 (2); 1 (3)
	Abraded		3 mg (NR)	28.3	1 (2); 2 (4)
			3 mg (R)	11.3	2 (1); 1 (3)
			1% aq. (NR)	27.0	3 (2)
Laundry detergent	Intact	1.0	3 mg (NR)	20.6	2 (2); 1 (3)
			3 mg (R)	5.3	2 (1); 1 (2)
			1% aq. (NR)	8.6	3 (1)
		0.2	3 mg (NR)	24.0	1 (2); 2 (3)
			3 mg (R)	3.3	2 (1); 1 (2)
			10% aq. (NR)	14.3	1 (3); 1 (4); 1 (7)
		0.0	3 mg (NR)	21.7	3 (4)
			3 mg (R)	3.3	2 (1); 1 (2)
			10 aq. (NR)	20.6	1 (2); 1 (3); 1 (4)
	Intact	5.0	3 mg (NR)	6.0	3 (2)
			3 mg (R)	4.6	3 (2)
			10% aq. (NR)	4.6	2 (2); 1 (3)
		1.2	3 mg (NR)	9.6	1 (1); 2 (2)
			3 mg (R)	2.3	2 (1 hr); 1 (2)
			10% aq. (NR)	7.6	3 (1)
		0.0	3 mg (NR)	6.0	3 (2)
			3 mg (R)	2.0	3 (1)
			10% aq. (NR)	3.3	1 (1); 1 (2); 1 (3)

R = rinsed eyes NR = non-rinsed eyes

* Maximum average score is the highest daily mean score for the three eyes tested in each group.

† Expressed as the number of eyes clear within the number of days indicated in parentheses.

prior to instillation of the enzyme preparation resulted in some increase in intensity of irritation but did not significantly lengthen the time required for the eye to return to normal.

Detergent and presoak-product formulations were each tested with two levels of added enzyme preparation and without enzyme. Although the detergent formulation was more irritating than the presoak product, addition of enzyme had no effect of any consequence on eye irritancy. Data from tests with abraded corneas are not shown in Table 4. Though abrasion did increase the intensity of irritation from the slight to moderate range in most cases, only four of 45 eyes so tested required more than 1-4 days to clear; these four were clear within 7 days.

Skin irritation in rabbits. Enzyme-containing detergent formulations in 1% aqueous solution produced moderate primary skin irritation under the occlusive conditions of the test (Table 5). Applications of non-enzyme controls were only mildly irritating.

Table 5. *Typical results of rabbit skin irritation tests with 1% aqueous solutions of enzyme detergents*

Material tested	Level of enzyme preparation (%)	Primary irritation index*	Degree of irritation
Laundry detergent	0.0	1.3	Mild
	0.2	4.8	Moderate
	0.3	3.7	Moderate

* Calculated according to Draize (1959).

Tests in man

Skin irritation in man. Laboratory tests of several types have been carried out on a total of 739 volunteer subjects in order to compare the effects on skin, i.e. the mildness, of various washing products with and without added enzyme preparations. All of these tests constituted exaggerated exposure compared with that encountered in actual usage.

In eight arm-washing studies involving a total of 80 people, the cubital fossa of one arm was washed with a 10% solution of enzyme-containing detergent and that of the other arm with a similar solution of non-enzyme detergent (Table 6). General-purpose detergent and presoak formulations having levels of enzyme preparations of 0.1–1.2% were found to be equal in their effect on the skin to like formulations that did not contain enzyme. None of the products so tested produced visible redness of the skin until after repeated washing had been carried out, using a procedure in which the wash solution was blotted from the skin and not rinsed from it.

Table 6. *Typical results of arm washing tests* on detergent and presoak formulations*

Material tested	Level of enzyme preparation (%)	Mean grade†
Control detergent	0.0	9.1
Enzyme detergent	0.2	8.7
	0.2	8.4
	0.25	7.3
Enzyme presoak product	1.2	8.6

* Involving three 1-min exposures daily to a 10% aqueous solution at 41°C on five consecutive days.

† Based on a scale ranging from 10 to 1, where 10 represents no erythema and 7 represents moderate erythema. The grades decrease as erythema increases. Values are means of groups of ten subjects.

Nineteen tests involving a total of 136 subjects were conducted in which the subjects immersed each hand in a different detergent solution maintained at 41°C. Detergents and presoak products containing 0.1–1.2% enzyme proved to be equivalent in mildness to control products without enzyme (Table 7). In one test involving six subjects, a product containing 5.0% enzyme preparation gave the impression of being milder than the control product, but this was attributed to the smoothing effect of the protease on the skin through removal of superficial scales of stratum corneum.

Table 7. *Typical results of hand immersion tests* on detergent and presoak formulations*

Material tested	Level of enzyme preparation (%)	Mean grades	
		Scaling†	Erythema‡
Control detergent	0.0	8.3	8.5
Enzyme detergent	0.2	9.0	8.3
	1.0	9.3	9.0
Enzyme presoak product	1.2	9.0	8.5

* Involving three 15-min immersions daily in a 2% aqueous solution at 41°C on five consecutive days.

† Based on a scale ranging from no scaling (10) to cracking and fissuring (1).

‡ Based on a scale ranging from 10 to 1, in which the grades decrease as erythema increases.

Panels of volunteers participated in either single or triple application patch tests of products containing 1.2% or less of enzyme preparation (Table 8). Forty-four were single application patch tests involving 413 subjects and 11 were triple patch tests involving a total of 110 subjects. In addition, there were ten applications of a solution of enzyme preparation

Table 8. *Typical results of primary irritation patch tests of an enzyme preparation and detergent and presoak products*

Material tested	Level of enzyme preparation (%)	Test concn (%)	Mean grade* of erythema with patch application no.		
			1	2	3
Control detergent	0.0	4	0.1		
Enzyme detergent	0.175	4	2.0		
	0.2	4	2.0		
Control detergent	0.0	1	0.1	0.1	0.7
Enzyme detergent	0.175	1	0.3	0.7	1.1
	0.2	1	0.2	0.8	1.2
Enzyme presoak product	1.2	4	0	0	0
Enzyme preparation, Carlsberg type	100	0.2	0.1		

* Erythema grading scale: 0 = none; 1 = faint; 2 = moderate; 3 = marked.

alone, and 30 applications of a laundry-detergent formulation containing enzyme. Following the design of the test, these patches were removed at intervals shorter than 24 hr. Under the occlusive and otherwise exaggerated conditions of the patch test, formulations containing enzyme were found to be more irritating to the skin than the same formulations without enzyme. In the one test of enzyme material alone, essentially no irritation was seen at the concentration used.

Clinical and usage studies. Eleven clinical mildness tests of enzyme detergents were conducted on a total of 5943 housewives (Table 9). In all cases the grades assigned by the dermatologists for the condition of the housewives' hands indicated that there were essentially no differences in mildness between the products containing enzyme preparations and the non-enzyme control formulations.

Table 9. *Clinical mildness tests of general-purpose enzyme detergents used for hand dishwashing*

Test	Level of enzyme preparation (%)	No. of subjects	Dermatologists' mean grade*	LSD†
2-66A	0.1	34	83.8 (84.6)	2.9
2-66B	1.0	32	87.5 (82.8)	2.4
5-66	0.2	51	91.8 (94.9)	3.5
8-66	0.2	1113	92.3 (92.1)	0.6
10-66	0.2	303	80.9 (79.3)	1.5
2-67	0.6	502	74.9 (73.8)	0.8
3-67A	1.0	268	81.8 (82.9)	
		262	82.3 (82.9)	1.3
3-67B	0.1	266	84.3 (83.0)	
	0.17	269	82.6 (83.0)	
	0.2	266	83.7 (83.0)	1.7
10-67	0.1	270	83.1 (82.9)	
	0.2	273	84.3 (83.1)	
		272	84.4 (84.1)	1.7
1-68	0.2	250	77.0 (76.8)	
	1.0	241	78.2 (76.8)	1.8
11-68	0.2	259	81.5 (81.7)	
		251	82.7 (81.7)	
	0.4	249	83.9 (82.5)	
		251	82.3 (82.5)	1.6

* Based on a scale ranging from 100 (perfect) to 10 (extremely poor). Grades in parentheses indicate dermatologists' mean grade for the non-enzyme counterpart.

† Least significant difference between means at $P = 0.95$.

Three clinical studies on diapers, involving 360 infants, have been conducted on enzyme-containing presoak products or detergent formulations. It was found that there was no difference in the average incidence or severity of diaper rash between groups of infants wearing diapers laundered in the two types of product.

Cutaneous sensitization in man. Twenty-two repeated insult patch tests involving 1478

human volunteers have been completed (Table 10). There were no signs of cutaneous sensitization. Application concentrations of the products ranged from 0.25 to 1.0% with the levels of enzyme preparation in the formulations ranging from 0.1 to 1.2%. Under the occlusive conditions of this test, enzyme-containing formulations were more irritating than the control formulations.

Table 10. *Repeated insult patch test for cutaneous sensitization in man*

Material tested	Level of enzyme preparation (%)	Test concn (%)	No. of tests	No. of persons	
				Tested	Sensitized
Laundry detergent	0.1	0.25	1	75*	0
	0.2	0.5	4	284	0
	0.2	1.0	1	63	0
	0.3	0.25	1	74	0
	0.3	0.5	4	233	0
	0.4	0.25	1	133	0
	0.5	0.5	2	81	0
	0.5	1.0	1	59	0
	0.6	0.25	1	63	0
	0.6	0.5	2	141	0
	0.6	1.0	1	64	0
	0.7	0.25	1	64	0
	0.7	1.0	1	71	0
	0.8	0.5	1	73	0
			—	—	—
		Sub-total. . .	22	1478	0
Presoak	1.2	0.25	1	75*	0
			—	—	—
		Sub-total. . .	1	75	0
			Total. . .	1478	0

* These two tests were performed simultaneously on the same panel of subjects. The number of subjects tested is included once only in the final total.

DISCUSSION

Animal and human tests of enzyme-containing synthetic detergent and presoak formulations have shown them to be equivalent to comparable non-enzyme formulations in all respects except for primary irritancy of the skin, and then only under severe conditions of exposure such as those involved in a patch test. As would be expected from the inclusion of proteolytic-enzyme preparations into these formulations, solutions of high concentration in prolonged contact with the skin caused appreciably more primary irritation than that produced by control formulations. Under circumstances of normal use in which product concentrations are typically 0.1–0.2% and exposures are measured in minutes rather than hours, the enzyme ingredients have no adverse effect on the mildness of the products. This is consistent with the findings of Loomans & Hannan (1969), who showed that the intact cells of stratum corneum are resistant to proteolytic attack by subtilisin and that this resistance is not affected by the presence of surfactant. It has been shown repeatedly in extensive testing of enzyme laundry-product formulations under moderately exaggerated or normal conditions of use that no problems of primary irritation would be expected to arise from them in

the course of routine laundry and cleaning applications. Marketing experience with enzyme-containing products, acquired during the course of nearly 3 yr, has confirmed that mildness to the skin has not been impaired by their use. Likewise, the development of allergic contact sensitization does not appear to be a likelihood, judging from the hundreds of patch-test exposures and thousands of usage exposures that have been carried out without incident.

There have recently been reports from England (Flindt, 1969; Pepys, Hargreave, Longbottom & Faux, 1969) of respiratory symptoms among workers in the detergent industry exposed to proteolytic enzyme preparations similar to those used in the present studies. These industrial exposures to the enzyme in the form of respirable dust resulted in breathlessness, wheezing, cough and other asthmatic symptoms. Some months before these publications it had been recognized in the detergent industry that industrial exposures to enzyme dust could result in a range of respiratory allergic manifestations in susceptible individuals not unlike those seen in other industrial exposures to dusts with antigenic properties. A basic investigation of the situation is continuing. In the meantime, the improved dust control measures adopted have essentially eliminated the problem.

Apart from the question of industrial hygiene, the significance of these inhalation effects for the consumer must also be considered. In the opinion of the authors, the hazard to the consumer is negligible. Air sampling has shown that exposures to enzyme-containing dust in detergent manufacturing operations are greatly in excess of those encountered in the home. This is true for dust concentration, duration of exposure and the physical and chemical characteristics of the dust. Also, for more than 2 yr, all cases of consumers reporting symptoms suggestive of allergy have been carefully followed up, including clinical investigations by physicians whenever possible. Similar investigations were carried out as part of pre- and post-marketing consumer-usage tests of the products. During this entire period the enzyme products have been used by many millions of people, yet this continuing programme of surveillance has revealed no case of allergy or other respiratory problem traceable to their use. This experience supports the conclusion that the enzyme-containing washing products are safe for consumer use.

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Évaluation de l'innocuité des détergents contenant des enzymes. Études de la toxicité orale et cutanée, du pouvoir d'irritation et de sensibilisation de la peau

Résumé—On signale des études toxicologiques chez l'homme et les animaux pour les détergents de blanchissage et les produits de pré-trempe renfermant des préparations commerciales d'enzymes protéolytiques: subtilisine BPN et subtilisine Carlsberg. En administration orale à dose aiguë chez le rat, les préparations enzymatiques *per se* ont une toxicité orale de faible intensité et n'affectent pas la toxicité quand on les incorpore dans les produits détersifs et de pré-trempe. Les produits ne sont pas toxiques pour les chiens et les ingrédients enzymatiques ne modifient pas les propriétés émétiques des produits de lavage. Il n'y a pas eu de signe de toxicité au cours d'applications de 4 semaines ou de 13 semaines des mélanges enzyme-détergent sur la peau écorchée ou intacte des lapins, bien que l'irritation de la peau ait été plus grande que pour les témoins, à la dose la plus élevée d'application du matériel enzymatique. Des études étendues de l'irritation des yeux sur des lapins n'ont pas montré d'effet important dû aux préparations enzymatiques ajoutées aux produits.

Des essais au laboratoire sur plus de 700 sujets avec divers détergents contenant des enzymes n'ont pas montré d'effet d'irritation cutanée due aux enzymes, sauf dans des conditions de concentration élevée, d'occlusion de la peau et de contact prolongé. Des essais cliniques de tolérance cutanée sur 5943 ménagères, des études d'éruptions occasionnées par des couches, portant sur 360 bébés, et des essais répétés d'application sous timbre, portant sur 1478 hommes et femmes, n'ont pas révélé d'effets fâcheux causés par les produits renfermant les ingrédients enzymatiques.

Unschädlichkeitsuntersuchungen von Enzymwaschmitteln. Untersuchungen zur oralen und Haut-Toxizität, Reizwirkung und Hautsensibilisierung

Zusammenfassung—Toxikologische Untersuchungen am Menschen und am Tier mit Wasch- und Einweichmitteln, die handelsübliche proteolytische Enzyme, wie Subtilisin BPN und Subtilisin Carlsberg, enthielten, werden beschrieben. Die Enzyme *per se* hatten bei akuter oraler Verabreichung an Ratten geringe Toxizität und zeigten keine zusätzliche Wirkung nach Vermischen mit Wasch- und Einweichmitteln. Die Produkte waren für Hunde nicht toxisch und die emetische Wirkung der Reinigungsmittel wurde durch die Enzymbestandteile nicht beeinflusst. Keine Andeutung von Toxizität wurde bei vierwöchiger und dreizehnwöchiger Verabreichung von Enzym-Waschmittel-Mischungen auf die aufgerauhte und intakte Haut von Kaninchen festgestellt, jedoch war die Hautreizung im Falle der stärksten Enzymkonzentrationen grösser als bei Kontrollversuchen. Ausgedehnte Untersuchungen auf Augenreizung am Kaninchen zeigten keine nennenswerten durch die in den Reinigungsmitteln enthaltenen Enzyme hervorgerufenen Effekte.

Laboratoriumsteste, die mit einer Reihe von enzymhaltigen Waschmitteln an über 700 Versuchspersonen durchgeführt wurden, ergaben keine nachteiligen, durch die Enzyme verursachten Hautunverträglichkeiten, ausser bei hohen Enzymkonzentrationen unter Abschluss der Haut und überlangem Kontakt. Klinische Hautverträglichkeitsversuche an 5934 Hausfrauen, Beobachtungen hinsichtlich des Wundseins an 360 Windelkindern und wiederholte "Läppchenteste" an 1478 Männern und Frauen ergaben, dass keine ungünstigen Effekte von den enzymhaltigen Produkten verursacht wurden.