A SIMPLE METHOD FOR SCREENING ASSESSMENT OF SKIN AND EYE IRRITATION

Jun SEKIZAWA, Kazuo YASUHARA, Yuji SUYAMA*,
Sumie YAMANAKA*, Masuo TOBE**, and Masao NISHIMURA*

National Institute of Health Sciences, 1-18-1,

Kamiyoga, Setagaya, Tokyo 158, Japan,

**Department of Hygiene, Tokyo Dental College,*

1-2-2, Masago, Mihama, Chiba 261, Japan,

***Society of Japanese Pharmacopoeia, 2-12-15, Shibuya,*

Shibuya, Tokyo 150, Japan

(Received October 15, 1993 ; accepted December 27, 1993)

ABSTRACT — A stepwise, simple screening test for skin and eye irritations, suitable for industrial chemicals or pesticides which are not required to be examined for their exact potential irritancy levels, was developed. The efficacy of the test was evaluated using 15 chemicals including typical irritants (acetic acid, ammonia, chloroacetic acid, dioxane, ethanolamine, formaldehyde, formic acid, hydrogen peroxide, phenol, phosphoric acid, propionic acid, sodium hydroxide, sodium hypochlorite, sulfuric acid, and trichloroacetic acid). Chemicals were chosen so as to represent irritants which act by different mechanisms (i. e., strongly acidic, alkaline, reactive to protein, oxidizing etc.). The method consisted of physicochemical tests and animal tests using rats, mice or guinea pigs, namely, a skin irritation test, an intradermal reaction test and an eye irritation test in a sequential manner such that further tests are not required if a positive result is obtained in earlier steps. Results obtained between two laboratories using this method were very similar. Comparison of our results with the data obtained by the conventional method using rabbits showed fairly good coincidence. The method was shown to be useful in assessing skin and eye irritation of chemicals and causes minimal suffering to animals.

KEY WORDS : Skin, Eye, Irritants, Screening assessment Industrial chemicals, Pesticides

INTRODUCTION

Recent mass production and global trade of industrial chemicals, pesticides, and other chemical products urge both protection of people from potential hazards and international harmonization of regulatory mechanisms.

There is an international movement toward

minimization of animal use in assessing irritation by chemicals and harmonization of test methods. We proposed a simple method for screening assessment of acute oral, and dermal toxicity using only minimum animals (Yamanaka *et al.*, 1990). Use of alternative methods, such as testing physicochemical properties and use of *in vitro* assays was recommended in the screening steps (Anonymous 1985). For example, there is no need to test for irritation by strong alkalis ($\text{pH} \geq 11.5$) or strong acids ($\text{pH} \leq 2$) (OECD

Correspondence : Jun SEKIZAWA at the above address.

1981). Corrosion of steel or aluminum plate at 55°C for 1 year is used as a criterion for classification of dangerous goods in transport (United Nations, 1991). *In vitro* tests, such as using hen's eggs or cell cultures, were proposed as alternative methods for testing eye irritation (ECETOC, 1988) and using keratinocytes or skin slices was proposed as an alternative method for testing skin irritation (ECETOC, 1990). It was further recommended that alternative methods should give critical information on the nature of toxicity and elucidate the mechanism of toxicity (Anonymous, 1985).

Attempts have been made in the OECD meeting of experts on updating testing guidelines for skin and eye irritation to consider acute dermal toxicity or *in vitro* data and also structure-activity relationship or physicochemical data, before starting tests (OECD, 1991). A proposal for regulatory consensus was made in line with the above attempts in updating eye irritation test methods in a workshop hosted by the regulatory agencies in the USA by incorporating recent advances in assessment methods (Hurley *et al.*, 1993; Scala, 1993). However, it is the same that the rabbit is the animal recommended to be used in the skin and eye irritation test and the scoring system of irritancy levels are rather complex

(Chambers *et al.*, 1993).

We present here, a systematic, simple screening assessment method for skin and eye irritation, suitable for industrial chemicals or pesticides which are not applied to human skin or eyes intentionally and hence not required to be examined for their exact potential irritancy levels, based on cooperative work between two laboratories. Our method is a combination of a physicochemical test and stepwise animal tests using rodents arranged to obtain required information using a minimal number of animals. Any information, such as on irritating effects of structurally related compounds, and data from *in vitro* studies related to irritation effects, must be taken into account before starting screening tests.

MATERIALS AND METHODS

1. Materials

Chemicals which are known to be irritating to the skin, but by different mechanisms (strongly acidic, alkaline, reactive to protein, oxidizing) were selected for validation of the method (Table 1, see discussion). Chemicals (reagent grade from Wako Pure Chemical Industries) were dissolved in water or in saline to prepare test solutions.

Table 1. Chemicals used in the screening test.

Chemical	CAS No.*	NIOSH No.**
Acetic acid	64-19-7	AF1225000
Ammonia	7664-41-7	BO0875000
Chloroacetic acid	79-11-8	AF8575000
Dioxane	123-91-1	JG8225000
Ethanolamine	141-43-5	KJ5775000
Formaldehyde	50-00-0	LP8925000
Formic acid	64-18-6	LO4900000
Hydrogen peroxide	7722-84-1	MX0899000
Phenol	108-95-2	SJ3325000
Phosphoric acid	7664-38-2	TB6300000
Propionic acid	79-09-4	UE5950000
Sodium hydroxide	1310-73-2	WI3490000
Sodium hypochlorite	7681-52-9	NH3486300
Sulfuric acid	7664-93-9	WS5600000
Trichloroacetic acid	76-03-9	AJ7875000

* Chemical Abstracts Service registry number.

** NIOSH (1992).

2. Methods

The stepwise screening procedure developed by us is shown in Fig. 1. Although every test in the procedure was performed in this work, further tests need not be performed in practice, if appreciable effects are observed in any one step. Details of the steps are described below.

Fig. 1 Test procedure in the screening assessment of skin and eye irritation.

*see discussion for not including this step in our proposal of the screening test, after we examined results described in this paper.

Step 1 Physicochemical properties of chemicals were tested first by measuring the pHs of test solutions and then by examining the effects of chemicals on aluminum plates.

Step 1-1 pHs of test solutions were measured with a pH meter.

Step 1-2 Aluminum plates (No. 80202-30 obtained from Rare Metallic Co., 99.99% purity, 200×80 mm, 0.3 mm thick) were half immersed in an upright position in test solutions heated to 55°C for one week. The appearance of the surfaces of the aluminum plates was compared between the immersed and not immersed parts. The aluminum plates were weighed before and after immersion.

Step 2 Primary skin irritation tests were performed as follows. Three male and 3 female

Wistar rats, and 3 male and 3 female ddY mice, all 5-6 weeks of age, and 3 female Hartley guinea pigs (400-450 g) were used. Test solutions (1 ml/kg or 1 g/kg) were applied once, unoccluded (3×4 cm for rats, 1×2 cm for mice) to the electric-razor shaved skin of the backs of the rats and mice using an intubation feeding apparatus. Animals were equipped with collars and caged separately to prevent licking of test solutions. For guinea pigs (and rats for comparison), test solutions (0.01 ml) were applied as four unoccluded circles (each 1.5 cm diameter) on the shaved skin of the back. Distilled water was used as control in all cases. Inflammatory reactions were observed for one week after application.

Step 3 An intradermal test was performed as a screening step for the eye irritation test. Each test solution (0.01 ml) was injected intradermally at one spot on shaved back skin of rats and mice. For guinea pigs (and rats for comparison), test solutions (0.01 ml) were injected intradermally at four spots on the shaved back skin. Saline was used as control. Skin reactions were observed for one week after application.

Step 4 Eye irritation tests were performed at concentrations in which no or inappreciable effects were observed in Step 3. Test solutions (0.01 ml) of various concentrations were applied to one eye of each male and female rat and mouse whose eyes were kept open for 30 seconds. Saline was used as control and was applied to the other eye. Reactions on the eye were observed with a slit-lamp for one week after application.

Evaluation of effects:

Criteria for evaluation of the effects in each step except step 1-1, are shown in Table 2.

Laboratories: Studies were done both at the National Institute of Health Sciences and at the Tokyo Dental College using the above test procedure and the same test chemicals.

RESULTS

The results of the screening tests for formaldehyde, phenol, and sulfuric acid are shown as examples (Tables 3, 4, and 5). Good coincidence between the results obtained in the two laboratories was obvious with minor differences in scoring at certain ranges. Dose dependency

Table 2. Criteria for rating.

Step	Criteria used in classifying effects		
Grade	Mild ($\pm$)	Moderate (+)	Severe (++)
1-2	transition	forming on the surface	dissolution
2	rubor, edema	erosion	necrosis
3	rubor	erosion	necrosis
4	cornea diffuse, iris swelling	opaque cornea	no reaction to light

Table 3. Test results for formaldehyde solutions*.

Step	Animal or pH	Sex	Results for solutions of (%)						
			(37)	(15-18)	(7-9)	(3)	(0.75)	(0.15-0.38)	(0.09)
1-1	pH		3.6						
1-2			- (-)						
2	Rat	Male	+	+(+)	+($\pm$)	(-)			
		Female	+	+(+)	+($\pm$)	(-)			
	Mouse	Male	+	+(+)	+(+)				
		Female	+	+(+)	- (-)				
	Guinea pig	Male		+(+)	-				
3	Rat	Male		++(++)	++(++)	++(+)	++($\pm$)	++(-)	
		Female		++(++)	++(++)	++(+)	++($\pm$)	++(-)	
	Mouse	Male		++(++)	++(++)	++(+)	++($\pm$)	++(-)	
		Female		++(++)	++(++)	++(+)	++($\pm$)	++(-)	
	Guinea pig	Male					+	+	-
4	Rat	Male			+	++(-)			
	Mouse	Male			+	- (-)			

* Results obtained in two laboratories are shown with and without parentheses.

++ severe effects, + moderate effects, $\pm$ mild effects, - no effects.

Table 4. Test results for phenol solutions*.

Step	Animal or pH	Sex	Results for solutions of (%)				
			(50)	(20)	(5)	(2)	(0.5)
1-1	pH				5.1	5.7	5.9
1-2			(5.3)	(5.3)	(5.5)	(5.8)	(6.0)
			+ (±)	+ (+)	+ (+)	+ (±)	+ (±)
2	Rat	Male		+ (+)	- (-)		
		Female		+ (+)	- (-)		
	Mouse	Male		+ (+)	± (-)	-	
		Female		+ (+)	± (-)	-	
3	Rat	Male			+ (+)	+ (+)	± (-)
		Female			+ (+)	+ (+)	+ (-)
	Mouse	Male			(+)	(+)	+ (±)
		Female			(+)	(+)	+ (±)
	Guinea pig	Male			(+)	(+)	+ (±)
		Female			(+)	(+)	+ (±)

* Results obtained in two laboratories are shown with and without parentheses.

++ severe effects, + moderate effects, ± mild effects, - no effects.

Table 5. Test results for sulfuric acid solutions*.

Step	Animal or pH	Sex	Results for solutions of (%)					
			(10)	(5)	(2.5)	(1-1.25)	(0.5-0.6)	(0.1-0.15)
1-1	pH						<2.0(0.1%)	2.5(0.01%)
1-2			+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
2	Rat	Male	+ (++)	+ (+)	- (-)			
	Mouse	Male	+ (++)	+ (+)	- (-)			
3	Rat	Male		(++)	(+)	+ (+)	+ (+)	- (-)
	Mouse	Male			(+)	+ (+)	+ (+)	-
	Guinea pig	Female			(+)	(+)	+ (+)	+
4	Rat	Male			(+)	- (-)	-	
	Mouse	Male			(+)	- (-)	-	

* Results obtained in two laboratories are shown with and without parentheses.

++ severe effects, + moderate effects, ± mild effects, - no effects.

of the effects in animal tests was clear and the stepwise method was effective in detecting irritating concentrations. Intradermal effects were seen even at lower concentrations than those which produced eye irritation. Differences in the effects among animal species and between males and females were not noticeable, although some species were slightly more sensitive than others in regard to certain chemicals.

The results for 15 chemicals are summarized in Table 6. Among test chemicals, acetic acid (20%), chloroacetic acid (2%), formic acid (10%), phosphoric acid (0.2%), propionic acid (80%), sulfuric acid (>0.05%), and trichloroacetic acid (0.5%) had a pH of 2 or lower. Ammonia (2%), ethanolamine (2%), sodium hydroxide (0.1%), and sodium hypochlorite (2%) had a pH of 11.5 or greater. Ammonia, ethanolamine,

Table 6. Minimum concentrations or amounts at which chemicals showed positive effects ^{*1}.

Chemical	Step	Animal	Concentration (pH)	Amount ^{*2}
Acetic acid	1-1		20% (<2.0) ^{*3}	
	1-2		0.2% ^{*4}	
	2	Rat	5%	50 mg/kg
		Mouse	5%	50 mg/kg
	3	Rat	0.5%	250 µg/kg
		Mouse	0.2%	1 mg/kg
		Guinea pig	0.2%	50 µg/kg
Ammonia	1-1		2% (11.5-11.7) ^{*3}	
	1-2		<0.02% ^{*5}	
	2	Rat	>25%	>250 mg/kg
		Mouse	25%	250 mg/kg
	3	Rat	0.05%	25 µg/kg
		Mouse	0.05%	250 µg/kg
		Guinea pig	0.05%	12.5 µg/kg
Chloroacetic acid	1-1		2% (<2.0) ^{*3}	
	1-2		<0.05% ^{*4}	
	2	Rat	5%	50 mg/kg
		Mouse	5-20%	50-200 mg/kg
	3	Rat	0.2-0.5%	100-250 µg/kg
		Mouse	0.2%	1 mg/kg
		Guinea pig	0.2-0.5%	50-125 µg/kg
Dioxane	1-1		80% (6.8 or 8.0) ^{*6}	
	1-2		<0.2% ^{*5}	
	2	Rat	>80%	>800 mg/kg
		Mouse	>80%	>800 mg/kg
	3	Rat	80%	40 mg/kg
		Mouse	20%	100 mg/kg
		Guinea pig	80%	20 mg/kg
Ethanolamine	1-1		2% (11.2-11.6) ^{*3}	
	1-2		<0.05% ^{*5}	
	2	Rat	20%	200 mg/kg
		Mouse	5-20%	50-200 mg/kg
	3	Rat	0.5%	250 µg/kg
		Mouse	0.2%	1 mg/kg
		Guinea pig	0.2%	50 µg/kg
Formaldehyde	1-1		37% (3.6) ^{*6}	
	1-2		>37% ^{*5}	
	2	Rat	7-9%	70-90 mg/kg
		Mouse	7-18%	70-180 mg/kg
	3	Rat	0.75%	375 µg/kg
		Mouse	0.75%	3.75 mg/kg
	4	Mouse	3-9%	15-45 mg/kg

Table 6. (continued-1)

Chemical	Step	Animal	Concentration (pH)	Amount * ²
Formic acid	1-1		10% (<2.0) * ³	
	1-2		5-6% * ⁵	
	2	Rat	10-12%	100-120 mg/kg
		Mouse	10-12%	100-120 mg/kg
	3	Rat	2-3%	1-1.5 mg/kg
		Mouse	2-3%	10-15 mg/kg
	4	Rat	5-6%	2.5-3 mg/kg
		Mouse	5-6%	25-30 mg/kg
Hydrogen peroxide	1-1		30% (4.0) * ⁶	
	1-2		>30% * ⁵	
	2	Rat	15%	150 mg/kg
		Mouse	30%	300 mg/kg
	3	Rat	1-1.5%	500-750 µg/kg
		Mouse	1-1.5%	5-7.5 mg/kg
		Guinea pig	1-1.5%	250-375 µg/kg
	4	Rat	7.5%	3.75 mg/kg
Phenol	1-1		50% (5.3) * ⁶	
	1-2		<0.5% * ⁵	
	2	Rat	20%	200 mg/kg
		Mouse	20%	200 mg/kg
	3	Rat	0.5-2%	0.25-1 mg/kg
		Mouse	0.5	2.5 mg/kg
		Guinea pig	0.5%	125 µg/kg
Phosphoric acid	1-1		0.2% (<2.0) * ³	
	1-2		<0.2% * ⁵	
	2	Rat	5%	50 mg/kg
		Mouse	5%	50 mg/kg
	3	Rat	2%	1 mg/kg
		Mouse	2%	10 mg/kg
		Guinea pig	2%	500 µg/kg
Propionic acid	1-1		80% (<2.0) * ³	
	1-2		0.2% * ^{4,5}	
	2	Rat	>20%	>200 mg/kg
		Mouse	20%	200 mg/kg
	3	Rat	2%	1 mg/kg
		Mouse	0.5%	2.5 mg/kg
		Guinea pig	0.5%	125 µg/kg
Sodium hydroxide	1-1		0.1% (12.3) * ³	
	1-2		<0.075% * ⁵	
	2	Rat	5%	50 mg/kg
		Mouse	5%	50 mg/kg
	3	Guinea pig	5%	1.25 mg/kg
		Rat	0.25-0.3%	125-150 µg/kg
	4	Mouse	0.25-0.3%	1.25-1.5 mg/kg
		Guinea pig	0.075-0.1%	18.8-25 µg/kg
		Rat	1.25%	625 µg/kg

Table 6. (continued-2)

Chemical	Step	Animal	Concentration (pH)	Amount ^{*2}
Sodium hypochlorite	1-1		2% (11.5-12.0) ^{*3}	
	1-2		<0.02% ^{*5}	
	2	Rat	5%	50 mg/kg
		Mouse	2-5%	20-50 mg/kg
	3	Rat	0.5%	250 μ g/kg
		Mouse	0.5%	2.5 mg/kg
		Guinea pig	0.2%	50 μ g/kg
Sulfuric acid	1-1		0.1% (<2.0) ^{*3}	
	1-2		<0.01% ^{*5}	
	2	Rat	5%	50 mg/kg
		Mouse	5%	50 mg/kg
	3	Rat	0.5-0.6%	250-300 μ g/kg
		Mouse	0.5-0.6%	2.5-3 mg/kg
		Guinea pig	0.01%	2.5 μ g/kg
	4	Rat	2.5%	1.25 mg/kg
		Mouse	2.5%	12.5 mg/kg
Trichloroacetic acid	1-1		0.5% (<2.0) ^{*3}	
	1-2		<0.05% ^{*5}	
	2	Rat	20%	200 mg/kg
		Mouse	20%	200 mg/kg
	3	Rat	2%	1 mg/kg
		Mouse	0.2%	1 mg/kg
		Guinea pig	0.2%	50 μ g/kg

^{*1} Minimum concentrations at which at least one of the laboratories detected moderate or severe effects.

^{*2} Amounts calculated from the concentrations, volumes of the chemicals applied, and the weight of animals.

^{*3} Concentration at which the solution showed pH of lower than 2 or higher than 11.5.

^{*4} Rate of aluminum corrosion was inversely related to the concentration of test chemicals.

^{*5} Minimum concentration at which corrosion of aluminum plate was observed.

^{*6} pH of the test chemical at the highest concentration measured or pH of the lowest concentration at which test chemical showed pH of below 2.0 or above 11.5.

formic acid, phosphoric acid, sodium hydroxide, sodium hypochlorite and sulfuric acid corroded aluminum plates even at concentrations at which no reactions were observed in skin irritation tests. Intradermal skin reaction was detected at the same concentrations at which eye irritation was demonstrated or at lower concentrations. This means that the intradermal test is more sensitive than the eye irritation test.

DISCUSSION

Most chemicals chosen in this study are typical industrial chemicals which are known to cause some irritation to skin or eyes. The

reason is that the screening test is designed for early and simple discrimination of irritants and mostly applies to industrial chemicals, for which no detailed examination of effects to the skin or eyes are not required as in the case of some pharmaceuticals or cosmetics that are intentionally applied to skin or eyes.

The results of the new stepwise screening tests for skin and eye irritations were very similar between the two laboratories. Some minor differences observed were considered individual differences in weak reactions to dilute test solutions. This shows usefulness of the test in assessing skin and eye irritation for many chemicals. Although rabbit is a recommended species for skin and eye

irritation test by OECD, we used rodents to examine usefulness of these animals in screening tests for skin and eye irritations. Very little difference was observed in responses among mice, rats, and guinea pigs, which means any of these species can be used as test animals. The differences in dose levels causing skin irritation and those inducing intradermal inflammation, which in turn were somewhat lower than doses causing eye irritation, validated the efficacy of the present stepwise screening method. Since chemicals can react with body components without the interference of epidermal barrier in the intradermal reaction test, it appeared to be useful as a sensitive detection method for irritating potential of chemicals. Damages to eyes can be irreversible and also critical to animals and the intradermal test being somewhat more sensitive in detecting irritating potential than eye irritation test (see Tables 3 and 5), people may not need to test eye irritation for practical purposes, when positive responses were found in the intradermal test.

The use of pH for screening helps to minimize the use of animals. In spite of the results from pH measurement, tests for primary irritation, intradermal reaction and eye irritation were performed in this study for validation purposes. For example, negative results in the primary irritation test were obtained for sulfuric acid at a rather high concentration (2.5%) which had a pH lower than 2 (Table 4). This could be because the skin of the mouse or rat is less vulnerable or more impermeable to chemicals than that of humans. Therefore, use of pH measurement for screening irritants is still valid for the protection of humans. However, formaldehyde and phenol, which are not strong acid or strong alkali, gave positive effects of skin and eye irritation owing to their reactions to proteins. Therefore, although pH measurement is recommended in the screening steps of the OECD guideline, it is not always useful in screening irritation potential of all chemicals.

We used changes in surface appearances and weights of aluminum plates by chemicals as another physicochemical test, for detecting skin irritating potential of chemicals. Corrosion of aluminum is used in the special recommendation for allocating corrosive substances to class 8,

considering their potential to cause corrosion in metal surfaces of containers or machines by the United Nations Committee of Experts on the Transport of Dangerous Goods (United Nations, 1991). Aluminum reacts with oxygen in air and water to form a thin layer of aluminum oxide of various degrees of hydration on its surface. Aluminum as an amphoteric ion reacts with acids and alkalis. These reactions of aluminum are influenced by temperature, amount of dissolved oxygen in water, pH, and the kinds and amounts of coexisting anions or cations. Therefore, the effects of test solutions on the aluminum plates varied according to these factors. High concentrations of test solutions sometimes had almost no effect, whereas low concentrations of the same chemicals solutions gave positive effects (formic acid, formaldehyde). Phenol (solution of < 0.2%), which did not cause intradermal effects at all, gave positive effects on aluminum plates. Therefore, the corrosion of aluminum plates, does not only have relevance to biological effects, but also is not an appropriate means for examining the irritating potential of chemicals. Therefore, this step is not needed in the screening procedure.

All chemicals which are assigned as class 8 corrosives by the UN recommendations on the transport of dangerous goods (United Nations, 1991) were identified as irritants by our method. Sulfuric acid, sodium hydroxide, and phenol are used as typical examples of deleterious substances in the Poisonous and Deleterious Substances Control Law in Japan. Reproducibility in the results for these chemicals between the two laboratories shows the usefulness of the test in identifying deleterious substances for regulatory purposes.

Correlation between the data of this report and those of the standard Draize test regarding present test chemicals was examined, since the Draize tests using rabbits has been recommended as a standard test for skin and eye irritation. Table 7 summarizes the data of standard Draize test found in the Registry of Toxic Effects of Chemical Substances (RTECS), in which they list the lowest doses of chemicals to show positive effects in the tests according to their rule (NIOSH, 1992). When the minimum effective doses in skin and eye irritation tests using mice or

Table 7. Results of standard Draize test using rabbits (NIOSH 1992).

Chemical	Organ	Amount ^{*1} /Period ^{*2}	Condition	Result
Acetic acid	Skin	10 mg/kg/24h	Open ^{*4}	Moderate
	〃	263 mg/kg		Severe
	〃	25 mg/kg/24h		Mild
	Eye	25 µg/kg	Open ^{*4}	Severe
	〃	2.5 mg/kg/30sec	Rinse	Mild
Dioxane	Skin	256 mg/kg	Open ^{*4}	Mild
	Eye	50 mg/kg		Severe
	〃	50 mg/kg/24h		Moderate
Ethanolamine	Skin	253 mg/kg	Open ^{*4}	Moderate
	Eye	125 µg/kg		Severe
Formaldehyde	Skin	1 mg/kg/24h	Open ^{*4}	Severe
	〃	270 mg/kg		Mild
	Eye	375 µg/kg/24h		Severe
	〃	375 µg/kg		Severe
	〃	5 mg/kg		Severe
Formic acid	Skin	305 mg/kg	Open ^{*4}	Mild
	Eye	61 mg/kg		Severe
Phenol	Skin	250 mg/kg/24h	Open ^{*4}	Severe
	〃	268 mg/kg		Severe
	〃	50 mg/kg		Mild
	Eye	2.5 mg/kg	Rinse	Severe
	〃	2.5 mg/kg/30sec		Mild
Phosphoric acid	Skin	298 mg/kg/24h		Severe
	Eye	59 mg/kg		Severe
Propionic acid	Skin	248 mg/kg	Open ^{*4}	Severe
	Eye	495 µg/kg		Severe
Sodium hydroxide	Skin	250 mg/kg/24h		Severe
	Eye	200 µg/kg		Mild
	〃	1% ^{*3}		Severe
	〃	25 µg/kg/24h		Severe
	〃	0.5 mg/kg/24h		Severe
	〃	0.5 mg/kg/30sec	Rinse	Severe
Sodium hypochlorite	Eye	5 mg/kg		Moderate
Sulfuric acid	Eye	125 µg/kg	Rinse	Severe
	〃	2.5 mg/kg/30sec		Severe
Trichloroacetic acid	Skin	105 µg/kg		Mild
	Eye	1750 µg/kg/5sec		Severe

^{*1} Amounts calculated assuming the weight of a rabbit as 2 kg.

^{*2} Exposure period.

^{*3} Results shown in concentration only.

^{*4} Open patch.

rats in our method (Table 6) were compared with the data of Draize tests, differences in the amounts which caused minimal effects to animals are within 5 fold between two methods. Considering that there are variations in the choice of concentrations tested, the application methods used, and the data selection principle used in the RTECS (see above), this difference can be seen fairly small. This suggests that rats or mice may be used in skin and eye irritation tests, although OECD guidelines recommend to use albino rabbits (OECD, 1991). In conclusion, our stepwise and simple method with pH measurement, skin irritation, skin reaction, and eye irritation, will be an appropriate test for screening assessment of eye and skin irritation potential of industrial chemicals or pesticides for which detailed information on irritating potential may not be needed as in the cases of pharmaceuticals or cosmetics which may be directly applied to skin or eyes. It also gives minimal suffering to animals. We stress that every consideration must be given to data of *in vitro* tests, information on structure-activity relationship, and acute dermal toxicity, etc., before applying our screening test. We used typical irritants for the most part to show efficacy of our method in screening irritating potential of chemicals according to different dose levels. Since we did not use mild irritants, such as some detergents in our tests, applicability of our methods to these kinds of chemicals needs to be examined in the near future. Validation of our method by the third parties is also requested to establish it as one of the standard methods.

ACKNOWLEDGMENTS

We thank Dr. K. Naito, Dr. O. Uchida and Dr. K. Takada of the National Institute of Health Sciences for help in performing animal tests. We also thank Dr. M. Takahashi and Dr. J. Monma of the National Institute of Health Sciences for supplying information on recent progress in testing guidelines. Technical assistance of Ms. T. Kondo in preparing the manuscript is gratefully acknowledged. This work was supported by the grant from Ministry of Health and Welfare in Japan.

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