Estimation of the Underprediction Rates for the *In Vivo* Rabbit Dermal Corrosion Assay

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Original Abstract

Alternative *in vitro* test methods proposed to substitute or replace an *in vivo* assay should provide equivalent or improved protection of human or animal health to gain regulatory and general acceptance. ICCVAM evaluated four *in vitro* dermal corrosivity assays as potential replacements for the *in vivo* dermal corrosivity assay. ICCVAM recommended that these assays be used in accordance with the globally harmonized tiered testing scheme in a weight-of-evidence approach. In this approach positive substances could be classified and labeled as corrosives and negative substances are further evaluated in accordance with an internationally accepted testing scheme. This recommendation was based largely on the 12-17% false negative rates of the *in vitro* assays in identifying corrosive substances. ICCVAM concluded that these false negative rates likely exceeded that of the currently used *in vivo* assay and would not provide adequate public health protection. To estimate the likelihood of a false negative result in the *in vivo* assay, the available data was reviewed. Relevant *in vivo* dermal corrosivity data were obtained from federal agencies and the published literature. The database consisted of 50 corrosive substances. Since the "true" likelihood of a corrosive response for each of the substances in the database was unknown, the sample rate was considered the best estimate of the true positive response rate. Initial analysis of the database indicated that the current *in vivo* dermal corrosivity test has an estimated false negative rate of 5.5%. The analysis also suggests that underclassification of a substance would most likely occur only for weak corrosives. NICEATM continues to seek additional high-quality *in vivo* corrosivity data to refine the estimated *in vivo* assay false negative rate. This evaluation emphasizes the need for high quality *in vivo* dermal corrosivity data that can be used to evaluate the performance of proposed alternative assays. ILS staff supported by NIEHS contract N01-ES-35504.

Introduction

For almost 60 years, the Draize *in vivo* rabbit skin irritation/corrosivity assay has been used to predict the ability of test substances to induce skin irritation and/or corrosion in humans (Draize et al., 1944). However, to date, only one study has been conducted to assess the reliability of this test method. In 1971, Weil and Scala reported on a study that evaluated the reproducibility of the Draize *in vivo* rabbit skin test method within and among twenty-four laboratories for ten reference substances. The resulting analysis indicated that there was moderate intra-laboratory reproducibility but low inter-laboratory reproducibility. Weil and Scala (1971) concluded that subjective classification of the skin response was the primary reason for the low reproducibility among the participating laboratories. Despite the limited number of substances tested and the use of a 24-hour exposure (compared to the currently accepted test method protocol of no greater than a 4-hour exposure), the results of this study have frequently been used to support the opinion that the *in vivo* rabbit skin irritation/corrosivity test is unreliable. Recently, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) initiated a study to estimate the underprediction rate of a positive response in the current version of the *in vivo* rabbit skin irritation test. The results of this analysis will be used to help establish the performance characteristics that alternative *in vitro* test methods would need to exhibit to replace the traditional *in vivo* rabbit skin test method.

Materials and Methods

Database

Data compiled for this analysis are from corrosivity studies using the *in vivo* rabbit skin test method recommended by U.S. Federal agencies (EPA 1998) and Organisation for Economic Co-operation and Development (OECD). Data were received from InVitro International, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) (See **Table 1**). Data from appropriate studies were extracted and entered into Excel spreadsheets. Information compiled for each entry included test substance name or unique identifier, source of data, number of rabbits tested, and the number of animals that exhibited a corrosive reaction to the test substance, and if the study was conducted in compliance with GLP guidelines. The database consists of 171 substances tested in 185 separate *in vivo* rabbit skin studies (**Table 3**). Several of the substances tested represented commercial products, which were identified by a unique identifier and whose formulation and chemical composition were unknown.

Table 1. Distribution of Tests Provided by Each Data Source

Source #	Source	GLP?	# Tests	# of Times Substance Tested 1	2	3	4	5
1	InVitro Internat. (Bio-Technics)	Yes	117	92	9	1	1	0
2	ECETOC	Yes	23	23	0	0	0	0
3	EPA (Data submissions)	Unknown	26	26	0	0	0	0
4	FDA (Internal testing)	No	19	19	0	0	0	0
	Total		185	160	9	1	1	0

Table 2. Distribution of Animals Used per Study by Source

Source	# of Tests Conducted with 1 Animal	2 Animals	3 Animals	4 Animals	5 Animals	6 Animals
1	53	50	11		3	
2	3	1	10	4		5
3			6			20
4			2			17
Total	56	51	29	4	3	42

In Vivo Rabbit Skin Corrosivity Protocol

Since 1981, the *in vivo* rabbit skin corrosivity test method has been typically carried out according to OECD Test Guideline (TG) 404 and following GLP guidelines. According to TG 404, the test substance can be tested on a single animal if a strong corrosive response is suspected. The test substance is applied to intact skin for not more than four hours. The duration of observation period is sufficient to evaluate the reversibility of the effects. A corrosive response (i.e., necrosis, hemorrhage, hemorrhagic necrosis, eschar) in at least one animal leads to classification of the test substance as a corrosive. Thus, to reduce the potential for animal pain and suffering, treatment of animals is often sequential. If a corrosive response is not observed in the first animal, the negative response is confirmed in two animals. In those cases where a single animal is not tested initially, two or three animals may be treated with the test substance. If both animals exhibit a positive response, no further testing is required. Otherwise, a third animal is tested. Equivocal responses using three animals may require evaluation using additional animals.

Statistical Analysis

The positive response rate (i.e., number of animals displaying a positive response among the total number of animals tested) was calculated for each study. For each positive response rate, the likelihood that 0 of 3 animals would exhibit a corrosive response was calculated. This likelihood was calculated using the equation (1-positive response rate)^3 and a range of likelihood values from 0 (for a 100% positive response rate) to 0.5787 (for a 16.7% positive response rate) was calculated. Next, the total number of studies for each positive response rate was multiplied by the corresponding likelihood rate to provide a value that represented the total contribution to the underprediction for each positive response rate. The contributions for each positive response rate was totaled and then divided by the total number of studies to yield the overall underprediction of a positive corrosive response rate.

Assumptions

The following assumptions were used in the statistical analysis (described above):

(1) All studies in the database are independent.

(2) The *in vivo* rabbit skin corrosivity test method protocol used (except for the number of animals) for all chemicals followed OECD TG 404.

(3) Only corrosive substances induced a positive corrosive response (i.e., there were no false positive or negative responses).

(4) For each corrosive chemical in the database, the observed positive response rate is accurate.

(5) The distribution of chemicals in the database, in terms of corrosivity (i.e., the proportion of responding rabbits), is representative of the "real world" of corrosive substances.

(6) For the analysis, the basis for classifying a substance as corrosive is based on at least one of three animals exhibiting a "positive" response (i.e., TG 404).

Calculations Performed

Due to the nature of the database (i.e., few substances were tested multiple times, the number of animals tested ranged from one to nine), several different calculations were conducted to develop a range of underprediction rates for corrosive substances. First, data for substances tested in more than one study were either pooled or not pooled prior to analysis. Next, test substances that were tested using only one animal or only one or two animals were excluded from the database. In a third approach (i.e., Average), the total number of animals that exhibited a corrosive response was divided by the total number of animals that were tested to provide an overall average positive response rate. This average positive response rate was then used to calculate the average underprediction rate. After the initial "Average" analysis, two additional analyses were conducted where substances tested using only one or only one or two animals were excluded.

Table 3

List of Test Substances Used in Analysis

Source #	Test #	Chemical or Mixture Name	# Animals Tested (N)	# Animals with Corrosion	% Positive Response	Packing Group Classification*
1	1	2,2-Aminoethoxyethanol	1	1	100	I,II
2	1	2-Mercaptoethanol#	1	1	100	II,III
1	1	Butylamine (40% in ethanol/ethylene glycol 1:1)	1	1	100	C
1	1	Butyric acid	1	1	100	I,II
1	1	Cellusolve 20%/SMS 10%	1	1	100	I,II
1	1	Chemifax Gin	1	1	100	I,II
1	1	Chemifax IBC	1	1	100	I,II
1	1	Chemifax MA	1	1	100	I,II
1	1	Chemifax SHO	1	1	100	I,II
1	1	Chemifax APL	1	1	100	I,II
1	1	Chemtool LMC RK	1	1	100	I,II
1	1	Citric acid 20%/SDS 10%	1	1	100	C
1	1	Citric acid 50%	1	1	100	I,II
1	1	Cyclohexamine 99.9%	1	1	100	I,II
1	1	Dicyclohexylamine +97%	1	1	100	C
2	1	Diethylaminopropylamine	1	1	100	I,II
1	1	Dimethylbenzylamine 99.9%	1	1	100	C
1	1	Ethanol 20%/SDS 10%	1	1	100	I,II
1	1	Ethanolamine 50%	1	1	100	I,II
2	1	Glycolbromoacetate	1	1	100	I,II
1	1	Hexanoic acid#	1	1	100	II,III
1	1	Hydrochloric acid 1%/Sulfuric acid 1%/Citric acid 1%	1	1	100	I,II
1	1	Hydrochloric acid 1%/Sulfuric acid 1%/Citric acid 1%/SDS 10%	1	1	100	I,II
1	1	Hydrochloric acid 14.4%#	1	1	100	III,III
1	1	Hydrochloric acid 18%	1	1	100	I,II
1	1	Hydrochloric acid 2%/ Oxalic acid 1.6%/Sulfuric acid 2%	1	1	100	I,II
1	1	Hydrochloric acid 5%/ Sulfuric acid 5%/Citric acid 5%	1	1	100	I,II
1	1	Knight Boat Bottom Cleaner	1	1	100	I,II
1	1	Knight Super 50	1	1	100	C
1	1	Mobil-91439	2	1	50	III,III
1	1	Potassium bisulfate	1	1	100	I,II
1	1	Propionic acid	1	1	100	I,II
1	1	SCJ COR 11	1	1	100	C
1	1	SCJ COR 6	1	1	100	I,II
1	1	SCJ COR 9	1	1	100	C
1	1	SCJ COR20	1	1	100	C
1	1	Sodium carbonate	1	1	100	I,II
1	1	Sodium hydroxide 1%/BAC 10%	1	1	100	C
1	1	Sodium hydroxide 1%/Cellusolve 20%	1	1	100	C
1	1	Sodium hydroxide 1%/SDS 10%	1	1	100	C
1	1	Sodium hydroxide 1%/SMS 10%	1	1	100	I,II
1	1	Sodium hydroxide 2%/SMS 3%	1	1	100	I,II
1	1	Sodium hydroxide 3%/SMS 3%	1	1	100	I,II
1	1	Sodium hydroxide 5%/TX100 5%	1	1	100	I,II
1	1	Sulfuric acid 10%#	1	1	100	C
1	1	Sulfuric acid 5%/Cellusolve 20%	1	1	100	I,II
1	1	Sulfuric acid 5%/FeCl3 2%	1	1	100	I,II
1	1	Sulfuric acid 5%/TX100 10%	1	1	100	I,II
1	1	Sulfuric acid/SDS 10%	1	1	100	I,II
1	1	Thioglycolic acid 10%	1	1	100	C
1	1	Thioglycolic acid 50%	1	1	100	I,II
2	1	Allyl bromide	2	1	50	C
1	1	Sodium hydroxide 2%/ Sodium metasilicate 3%/SDS 5%	2	1	50	C
1	1	Sodium hydroxide 5%/Sodium metasilicate 3%	2	1	50	C
1	1	127	2	2	100	C
1	1	289	2	2	100	I,II
1	1	315	2	2	100	I,II
1	1	485	2	2	100	I,II
1	1	880	2	2	100	I,II
1	1	885	2	2	100	C
1	1	3000	2	2	100	I,II
1	1	4000	2	2	100	I,II
1	1	122 B Powder	2	2	100	C
1	1	122B	2	2	100	I,II
1	1	1702 BR	2	2	100	I,II
1	1	1703 CR	2	2	100	I,II
1	1	1709 B	2	2	100	I,II
1	1	Calcium chloride LCS	2	2	100	C
1	1	Calcium chloride VCS	2	2	100	I,II
1	1	Calcium chloride, anhydrous	2	2	100	I,II
1	1	Calgon LPHSE	2	2	100	C
1	1	Cell Clean 90	2	2	100	C
1	1	Formula #100-016	2	2	100	C
1	1	Formula #100-088	2	2	100	C
1	1	FT 451	2	2	100	C
1	1	Knight TTC-2000	2	2	100	C
1	1	PD 100	2	2	100	C
1	1	PD 101	2	2	100	C
1	1	Phosphorous tribromide#	2	2	100	I
1	1	RAM 8519	2	2	100	C
1	1	Super 50	2	2	100	C
1	1	ZWS 9352	2	2	100	C
1	1	Fluoboric acid	3	1	33.3	III
1	1	Maleic anhydride	3	1	33.3	III
4	1	Prod-00259	3	1	33.3	C
1	1	1-(2-AE) piperazine#	3	2	66.7	II
2	2	2-Methoxyethyl acrylate	3	2	66.7	III
2	1	60/40 Caprylic/Capric acid#	3	2	66.7	II,III
1	1	Boron trifluoride-Acetic acid complex	3	2	66.7	II
1	1	Ethanolamine	3	2	66.7	II
1	1	Ferric chloride	3	2	66.7	II
1	1	Hydrogen bromide	3	2	66.7	II
3	1	P98-0399	3	2	66.7	C
1	1	Phosphoric acid#	3	2	66.7	II
2	1	Tallow amine#	3	2	66.7	II
2	1	55/45 Caprylic/Capric acid#	3	3	100	II,III
2	1	65/35 Caprylic/Capric acid#	3	3	100	III,III
1	1	Boron trifluoride-dihydrate#	3	3	100	I
2	1	Caprylic acid#	3	3	100	III,III
2	1	Diamylamine	3	3	100	I,II
2	1	Dimethyldipropylene-triamine#	3	3	100	I
2	1	L92-0203	3	3	100	C
2	1	Methacrolein#	3	3	100	III,III
2	1	N,N-dimethylbenzylamine	3	3	100	I,II
3	1	P88-0045	3	3	100	C
3	1	P90-1537	3	3	100	C
3	1	P93-0988	3	3	100	C
3	1	P94-0626	3	3	100	C
1	1	Phosphorous pentachloride	3	3	100	C
2	1	Potassium hydroxide 10%#	3	3	100	C
4	1	Prod-00254	3	3	100	C
2	1	2-Methyl butyric acid#	4	2	50	II,III
2	1	Origanum oil	4	2	50	III
2	1	Beechwood creosote	4	4	100	III
2	1	Cavacrol#	4	4	100	II,III
1	1	887	5	2	40	C
1	1	Formula # 100-057B	5	3	60	C
1	1	Rm-B-110 Barquat 4280Z	5	5	100	C
6	1	IPBC	3	1	16.7	C
3	1	Kathon	6	1	16.7	C
4	1	No Name (FDA Group 4)	6	1	16.7	C
4	1	Prod-00245	6	1	16.7	C
4	1	Prod-00282	6	1	16.7	C
3	1	Tetrachlorvinphos/Dichlorvos	6	1	16.7	C
4	1	CPSC1	6	2	33.3	C
4	1	DLK2	6	2	33.3	C
3	1	IPBC/Hexazine-2,4-diamine	6	2	33.3	C
3	1	P83-1328	6	2	33.3	C
3	1	P99-0301	6	2	33.3	C
4	1	Prod-00246	6	2	33.3	C
4	1	Prod-00281	6	2	33.3	C
4	1	CPSC17	6	3	50	C
4	1	CPSC2	6	3	50	C
4	1	DLK1	6	3	50	C
4	1	Prod-00241	6	3	50	C
4	1	CPSC11	6	4	66.7	C
4	1	CPSC12	6	4	66.7	C
3	1	P92-1373	6	4	66.7	C
3	1	Sodium cyanide	6	4	66.7	C
4	1	CPSC13	6	5	83.3	C
4	1	CPSC20	6	5	83.3	C
3	1	P88-0013	6	5	83.3	C
3	1	P89-0579	6	5	83.3	C
2	1	2-Tertiary butyl phenol#	6	6	100	II,III
3	1	Alkyl imidazolines	6	6	100	C
4	1	CPSC16	6	6	100	C
4	1	CPSC23	6	6	100	C
2	1	Dimethylisopropylamine#	6	6	100	II,III
2	1	Dimethyl-n-butylamine#	6	6	100	C
3	1	Imazaquin/Imazethapyr/Pendimethalin	6	6	100	C
2	1	Methoxy-3-propylamine#	6	6	100	C
3	1	n-Heptylamine#	6	6	100	II,III
3	1	P84-1226	6	6	100	C
3	1	P86-0831	6	6	100	C
3	1	P86-1346	6	6	100	C
3	1	P88-1771	6	6	100	C
3	1	P90-0464	6	6	100	C
3	1	P90-1356	6	6	100	C
3	1	P94-1669	6	6	100	C
3	1	Trifluralin	6	6	100	C
1	1	Calgon Coverage Plus	1	1	100	C
1	2	Calgon Coverage Plus	2	2	100	C
1	1	Seal remover BSS	1	1	100	C
1	2	Seal remover BSS	2	1	100	I,II
1	1	Sodium hydroxide 2%/SDS 5%	1	1	100	C
1	2	Sodium hydroxide 2%/SDS 5%	2	2	100	I,II
1	1	Sodium hydroxide 3%/SDS 5%	1	1	100	C
1	2	Sodium hydroxide 3%/SDS 5%	2	2	100	I,II
1	1	Sodium hydroxide 3%/Sodium metasilicate 3%/ SDS 5%	2	1	50	II,III
1	2	Sodium hydroxide 3%/Sodium metasilicate 3%/ SDS 5%	2	2	100	C
1	1	Sodium hydroxide 5%/Triton 5%	2	1	50	C
1	2	Sodium hydroxide 5%/Triton 5%	2	2	100	C
1	1	COR 19	2	1	50	I,II
1	2	COR 19	2	2	100	C
1	1	COR 3	2	1	50	I,II
1	2	COR 3	2	2	100	C
1	1	Sodium hydroxide 10%	2	1	50	I,II
1	2	Sodium hydroxide 10%	2	2	100	C
1	3	Sodium hydroxide 10%	2	2	100	C

* Packing Group Classifications according to InVitro International or Fentem et al. (2001).
C Corrosive; test substance produced a corrosive response after an exposure not greater than 4 hours but packing group classification could not be determined.
Test substances used in prevalidation study on *in vitro* tests for acute skin irritation by ECVAM (Fentem et al. 2001).

Table 4

Distribution of Test Results Among the Positive Response Rates, Based on the Total Number of Animals Tested, When Test Substances Tested Multiple Times are Not Pooled (A) or Pooled (B).

A. Data Not Pooled

Positive Response Rate	# Positive/Total Animals Tested	# of Tests	Positive Response Rate	# Positive/Total Animals Tested	# of Tests
100%	1/1	56	50%	1/2	9
	2/2	42		2/4	2
	3/3	16		3/6	4
	4/4	2			
	5/5	1			
	6/6	17			
83.3%	5/6	4	40%	2/5	1
66.7%	2/3	10	33.3%	1/3	3
	4/6	4		2/6	7
60%	3/5	1	16.7%	1/6	6

B. Pooled Data

Positive Response Rate	# Positive/Total Animals Tested	# of Tests	Positive Response Rate	# Positive/Total Animals Tested	# of Tests
100%	1/1	50	60%	3/5	1
	2/2	29			
	3/3	19			
	4/4	4			
	5/5	1			
	6/6	18			
83.3%	5/6	4	50%	1/2	4
				2/4	2
				3/6	4
75%	3/4	3	40%	2/5	1
71.4%	5/7	1	33.3%	1/3	3
				2/6	7
66.7%	2/3	10	16.7%	1/6	6
	4/6	4			

Tables 5 and 6

Table 5. Distribution of Test Substances within the Database when Using Different Exclusion Criteria

Positive Response Rate	No Pooling Across Studies All Data	Excluding 1 Animal	Excluding 1 & 2 Animal Studies	Pooling Across Studies All Data	Excluding 1 Animal	Excluding 1 & 2 Animal Studies
100%	134	78	36	121	71	42
83.3%	4	4	4	4	4	4
75%	0	0	0	3	3	3
71.4%	0	0	0	1	1	1
66.7%	14	14	14	14	14	14
60%	1	1	1	1	1	1
58.3%	0	0	0	0	0	0
50%	15	15	6	10	10	6
40%	1	1	1	1	1	1
33.3%	10	10	10	10	10	10
16.7%	6	6	6	6	6	6
Total Test Substances	**185**	**129**	**78**	**171**	**121**	**88**

Table 6. Distribution of Total Animals Tested and Number of Animals with a Corrosive Response in the Database Using Different Exclusion Criteria

	Total Number of Animals Tested in Database	Number of Animals with a Corrosive Response	% Incidence
All Data Used	528	412	78.0%
Excluding 1 Animal Studies	472	356	75.4%
Excluding 1 & 2 Animal Studies	370	263	71.1%

Table 7

Calculated Likelihoods of Obtaining a Negative Response Based on the Probability of a Positive Response. Negative response data were calculated from the formula (1-probability of a positive response)^3

Positive Response Rate	Likelihood of a Negative Response in a 3-Animal Test
100%	0.0000
83.3%	0.0046
75%	0.0156
71.4%	0.0234
66.7%	0.0370
60%	0.0640
58.3%	0.0723
50%	0.1250
40%	0.2160
33.3%	0.2963
16.7%	0.5787

Table 8

Example Calculation of the Underprediction Rate of the *In Vivo* Rabbit Dermal Corrosivity Test Method, when Repeat Study Data are Not Pooled and No Studies are Excluded

Probability of a Positive Response	Frequency	Likelihood of a Negative Response in a 3-Animal Test	Contribution to the Under-Prediction Rate
100%	134	0.0000	0.0000
83.3%	4	0.0046	0.0184
75%	0	0.0156	0.0000
71.4%	0	0.0234	0.0000
66.7%	14	0.0370	0.5180
60%	1	0.0640	0.0640
58.3%	0	0.0723	0.0000
50%	15	0.1250	1.8750
40%	1	0.2160	0.2160
33.3%	10	0.2963	2.9630
16.7%	6	0.5787	3.4722
Total	**185**		**9.1266**

Under prediction of a corrosive response: (9.1266/185) * 100 = 4.9%

Example

Example Calculation of Underestimation Rate of *In Vivo* Rabbit Dermal Corrosivity Test Method when Based on the Average Database Response Rate

Positive Response Rate when all the Data are Used:

(412 animals with a positive response/528 tested animals) * 100 = 78.0% overall response rate

Underprediction Calculation

$(1\text{-}0.780)^3 = 0.0106 * 100 = 1.1\%$

Results

Table 9. Summary of Underprediction Rates of the *In Vivo* Rabbit Skin Corrosion Test Method

	No Pooling Before Exclusion of Data	Pooling Before Exclusion of Data	Average Approach
All Data Used	4.9%	5.0%	1.1%
Excluding 1 Animal Studies	7.1%	7.1%	1.5%
Excluding 1 & 2 Animal Studies	10.3%	9.2%	2.4%

More information on ICCVAM and NICEATM can be accessed at:

http://iccvam.niehs.nih.gov

Conclusions/Discussion

Conclusions

- Within the limits of the assumptions noted above, the underestimation rate of a positive response within the database ranged from 1.1% to 10.3%.
- The underprediction rate most likely to be representative of this group of corrosive substances is from 5 to 7%.

Discussion

- The highest underestimation rate (10.3%) of the database was observed when studies where 1 or 2 animals were tested were excluded from the analysis. Such an analysis significantly decreases the total number of test substances with a 100% positive response rate and is likely not representative of the actual distribution of corrosive test substances.
- Based on the calculations used to develop the likelihood of a negative response in three animals, underclassification of a substance would not occur for substances that induced a corrosive response in 100% of the animals (in a 3-animal test) and would occur only 3% of the time for substances that induced a positive response in 2 of 3 animals.
- The calculated underprediction rates are highly dependent upon the distribution of corrosive substances (in terms of incidence) in the database. In other words, the more test substances that produce a weak corrosive response (e.g., 1 of 3 tested animals has a corrosive response) the greater the underprediction rate.

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