Corrositex®: An *In Vitro* Test Method for Assessing Dermal Corrosivity Potential of Chemicals

The Results of an Independent Peer Review Evaluation
Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
and the
National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

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ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BAC	*N*,*N*'-bis(acrylyl)cystamine
CDS	Chemical Detection System
CFR	Code of Federal Regulations
CPSC	Consumer Product Safety Commission
CV	Coefficient of Variation
DOD	Department of Defense
DOE	Department of Energy
DOI	Department of the Interior
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EG	Ethylene glycol
FDA	Food and Drug Administration
FW	Formula Weight
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IVI	InVitro International, Inc.
NA	Not Applicable
NaOH	Sodium Hydroxide
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health Sciences
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NTP	National Toxicology Program
OECD	Organisation for Economic Co-Operation and Development
OSHA	Occupational Safety and Health Administration
PRP	Peer Review Panel
QAU	Quality Assurance Unit
SAR	Structure-Activity Relationships
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SLS	Sodium Lauryl Sulfate
SOP	Standard Operating Procedures
Sponsor	InVitro International, Inc.
Submission	Corrositex® Submission as provided by InVitro International, Inc.
TER	Transcutaneous Electrical Resistance
UK	United Kingdom

UN	United Nations
US	United States
US DOT	US Department of Transportation
US EPA	US Environmental Protection Agency

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Preface

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) with support from the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) recently sponsored the independent scientific peer review of Corrositex®, a new test method proposed for assessing the dermal corrosivity potential of chemicals. Such reviews are critical components in the ICCVAM process that culminates in achieving regulatory acceptance and implementation of new scientifically validated toxicological testing methods. These methods are generally more predictive of adverse human health effects than current methods, and they may be alternative methods that provide for improved animal well-being, that use phylogenetically lower species, or that reduce or eliminate the need for animals. The peer review was conducted in accordance with public health directives of Public Law 103-43, which directed the National Institute of Environmental Health Sciences (NIEHS) to develop and validate improved alternative toxicological testing methods, and to develop a process for the regulatory acceptance of such methods (see: *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods*, NIH publication 97-3981; http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/iccvam.html).

The ICCVAM was established as a standing collaborative effort by NIEHS and 13 other regulatory and research agencies and programs. ICCVAM coordinates issues within the Federal government that relate to the development, validation, acceptance, and national/international harmonization of toxicological test methods. The Committee's functions include the coordination of interagency scientific reviews of toxicological test methods and communication with stakeholders throughout the process of test method development and validation. The following Federal regulatory and research agencies and organizations are participating in this effort:

Consumer Product Safety Commission
Department of Defense
Department of Energy
Department of Health and Human Services
- Agency for Toxic Substances and Disease Registry
- Food and Drug Administration
- National Institute for Occupational Safety and Health/Centers for Disease Control
- National Institutes of Health
- National Cancer Institute
- National Institute of Environmental Health Sciences
- National Library of Medicine

Department of the Interior
Department of Labor
- Occupational Safety and Health Administration

Department of Transportation
- Research and Special Programs Administration

Environmental Protection Agency

Corrositex® was proposed to ICCVAM by InVitro International, Inc. (IVI, Sponsor), as an *in vitro* alternative test method to *in vivo* methods for assessing the potential of chemicals to cause skin corrosion. NICEATM and an ICCVAM Corrosivity Working Group (CWG) composed of Federal employees, through interactions with the Sponsor, requested and assembled information for an independent scientific peer review of the method. Suggested experts for the peer review panel (PRP) were solicited from Federal agencies and national and international professional societies and organizations. The CWG recommended a panel composition that would represent a broad range of experience and expertise, including general and *in vitro* toxicology, clinical dermatology, and

biostatistics. PRP members were from industry, academia, and government, and included scientists from the United States (US), Canada, Japan, and the United Kingdom (UK).

The PRP was charged with developing a scientific consensus on the usefulness and limitations of Corrositex® for assessing the skin corrosivity potential of chemicals. In reaching this determination, the PRP was requested to evaluate all available information and data on the Corrositex® assay, and to assess the extent to which each of the criteria for validation and regulatory acceptance developed in the ICCVAM Report were addressed. The PRP was provided with guidance for their evaluation, which included questions from the CWG to ensure that the assessment provided adequate information to facilitate ICCVAM and agency decisions on the method.

A request for information regarding the usefulness of the Corrositex® assay, including information about completed, ongoing, or planned studies and other data was made via a *Federal Register* notice (July 28, 1998, Vol. 63, No. 144, 40303.) The availability of the test method submission materials, a request for public comments, and the planned public peer review meeting were announced in October, 1998 (*Federal Register*, October 27, 1998, Vol. 63, No. 207, page 57303). All comments and information submitted in response to the requests and announcements were provided to the PRP in advance of the review meeting.

The PRP met in public session on January 21, 1999, at the National Institutes of Health (NIH) Natcher Conference Center in Bethesda, Maryland. During the meeting, PRP members presented their evaluations and proposed conclusions and recommendations on each of the major sections and the PRP subsequently reached a consensus for each section. The opportunity for public comment was provided during the meeting. Following the meeting, the written evaluations, conclusions, and recommendations were consolidated into the PRP Report, which follows. The PRP concurred in a public meeting via teleconference on April 22, 1999, that the Report accurately reflects the conclusions and recommendations of the January 21 meeting. Additional data analyses prepared for the PRP by NICEATM and the test method submission are also included as appendices in this document.

Following the peer review meeting, the CWG reviewed the PRP Report, and provided recommendations to ICCVAM. This entire report has been reviewed and endorsed by the CWG and ICCVAM. The Report, along with recommendations on the usefulness of the method, will be forwarded by ICCVAM to Federal agencies for their consideration. Agencies will determine the regulatory acceptability and applicability of this method according to their statutory mandates, and as deemed appropriate, issue guidelines, guidance documents, or proposed changes in regulations.

The work of the PRP was truly a team effort, and their thoughtful and unselfish contributions are gratefully acknowledged. While all members contributed to this evaluation, the exceptional efforts of Dr. Robert Scala, who served as the PRP chair, deserves special recognition. The efforts of the CWG were instrumental in assuring a meaningful and comprehensive review that would address regulatory needs, and for evaluation of the recommendations of the PRP. Finally, the efforts of the NICEATM staff to ensure accurate analyses and timely distribution of information for the review are acknowledged, particularly the efforts of Dr. Thomas Goldsworthy, Dr. Raymond Tice, and Ms. Karen Haneke. On behalf of ICCVAM, we thank the many individuals who contributed to this report.

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EXECUTIVE SUMMARY

Corrosive substances are defined as chemicals that cause visible destruction of, or irreversible alterations in, living tissue by chemical action at the site of contact (29 CFR [Code of Federal Regulations] 1917.28, 1998). Dermal corrosivity testing is conducted to identify chemicals that potentially pose this hazard to humans. Test results are used to classify and label chemicals with regard to this potential hazard so that consumers and workers can take appropriate precautions to prevent injury. Test results are also used to determine appropriate packaging that will minimize hazardous spills during transport. US Federal agencies that have regulations related to corrosivity testing include the Consumer Product Safety Commission (CPSC), Occupational Safety and Health Administration (OSHA), US Department of Transportation (US DOT), and the Environmental Protection Agency (EPA) (Appendix Q). Regulations and guidelines include testing methods for assessing dermal corrosivity, appropriate chemical packaging and labeling, appropriate transport and/or storage methods, and awareness education programs for workers in industrial settings.

For regulatory purposes, identification of corrosive chemicals and chemical mixtures has been based on the ability of a chemical or chemical mixture to produce visible destruction or irreversible alterations of the skin at the site of contact. The commonly used *in vivo* test method involves application of chemicals or chemical mixtures on the intact skin of a rabbit. The skin is visually evaluated for corrosion within three minutes, and at one or four hours after application. Animal welfare considerations have led to efforts to develop *in vitro* alternative test methods.

In May 1998, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) received a Corrositex® Submission (Submission) from InVitro International, Inc. (IVI, Sponsor) for peer review. The Submission included additional information requested by ICCVAM following previous communications between ICCVAM and IVI. ICCVAM determined that the Submission was sufficiently complete to undergo peer review. An independent Peer Review Panel (PRP) was assembled to evaluate the extent to which each of the ICCVAM validation and acceptance criteria had been addressed, and to determine the usefulness and limitations of Corrositex® for the identification of potential human corrosive chemicals or chemical mixtures.

The PRP evaluation of the Corrositex® Submission addressed seven topics, with two to five PRP members assigned as primary reviewers for each topic. This report is organized by these topics, as follows: (1) Test Method Description; (2) Test Method Data Quality; (3) Test Method Performance; (4) Test Method Reliability (Repeatability/Reproducibility); (5) Other Scientific Reviews; (6) Other Considerations; and (7) Related Issues. Thesetopics are followed by a Summary Conclusions and Recommendations section. This report focuses on the performance of Corrositex® as compared to the *in vivo* rabbit skin corrosivity test. The validity of the *in vivo* rabbit skin corrosivity test was not evaluated.

Corrositex® is an *in vitro* method used to determine the dermal corrosive potential of chemicals and chemical mixtures. Corrositex® is based on the ability of a corrosive chemical or chemical mixture to pass through, by diffusion and/or destruction/erosion, a biobarrier and to elicit a color change in the underlying liquid Chemical Detection System (CDS). The biobarrier is composed of a hydrated collagen matrix in a supporting filter membrane, while

the CDS is composed of water and pH indicator dyes. Test chemicals and chemical mixtures, including solids and liquids, are applied directly to the biobarrier. The time it takes for a test chemical or chemical mixture to penetrate the biobarrier and produce a color change in the CDS is compared to a classification chart to determine corrosivity/noncorrosivity and to identify the appropriate US DOT packing group. Chemicals are prescreened for compatibility with the assay by directly applying the test chemical or chemical mixture to the CDS; if a color change is not induced, then the test chemical or chemical mixture does not qualify for testing with this assay. The US DOT currently accepts the use of Corrositex® to assign subcategories of corrosivity (packing groups) for labeling purposes according to United Nations (UN) Committee of Experts on the Transport of Dangerous Goods guidelines. However, the US DOT limits the use of Corrositex® to specific chemical classes, including acids, acid derivatives, acyl halides, alkylamines and polyalkylamines, bases, chlorosilanes, metal halides, and oxyhalides (Appendix Q).

The database used in this evaluation consisted of Corrositex® data on 163 chemicals and chemical mixtures for which there were corresponding *in vivo* rabbit corrosivity data. Data on 118 chemicals and chemical mixtures were provided by IVI, while data on an additional 45 chemicals and chemical mixtures were obtained from two peer-reviewed publications that evaluated the validity of Corrositex® (Botham et al., 1995; Fentem et al., 1998). These two studies involved a total of 77 chemicals and chemical mixtures, of which 32 chemicals and chemical mixtures were also included in the IVI Submission.

A public meeting of the PRP took place on January 21, 1999, in Bethesda, MD, to reach conclusions and make recommendations regarding the usefulness of Corrositex® for assessing the corrosivity potential of chemicals or chemical mixtures. The PRP addressed the following two major questions:

1. *Has Corrositex® been evaluated sufficiently and is its performance satisfactory to support its proposed use for assessing the corrosivity potential of chemicals or chemical mixtures?*

2. *Does Corrositex® offer advantages with respect to animal welfare considerations (refinement[1], reduction[2], and replacement[3] alternatives)?*

In response to the first question, Corrositex® performance indicates that, in specific testing circumstances such as that required by US DOT, Corrositex® is useful as a stand-alone assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives. The current US DOT exemption allows the use of Corrositex® for assigning packing groups for acids, acid derivatives, acyl halides, alkylamines and polyalkylamines, bases, chlorosilanes, metal halides, and oxyhalides. However, the Corrositex® database evaluated by the PRP did not include acyl halides, chlorosilanes, metal halides, or oxyhalides; thus no statement was made by the PRP for these chemical classes. In other testing circumstances, and for other chemical and product classes, Corrositex® may be used as part of a tiered assessment strategy. In this approach, negative responses must be followed

[1] Refinement alternative: A new or revised test method that refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being (NIEHS, 1997).

[2] Reduction alternative: A new or revised test method that reduces the number of animals required (NIEHS, 1997).

[3] Replacement alternative: A new or revised test method that replaces animals with non-animal systems or one animal species with a phylogenetically lower one (NIEHS, 1997).

by dermal irritation testing, and positive responses require no further testing unless the investigator is concerned about potential false positive responses. In either testing strategy, an investigator may conclude that confirmation testing is necessary based on consideration of supplemental information, such as pH, structure-activity relationships, and other chemical and/or testing information. As additional test results with Corrositex® are obtained, the utility of the assay may need to be reconsidered. Corrositex® is limited in its universal utility because of the proportion of chemicals that are not compatible with the CDS and thus cannot be evaluated.

The following specific changes to the protocol were recommended:

(1) It should be explicitly stated that the biobarrier should be allowed to harden on a level surface and to cool overnight before use.

(2) Even though replicate variability has been shown to be very low, guidance should be provided on how to evaluate an aberrant value.

(3) The IVI Corrositex® Data Sheets provided with the test kit should contain a provision for recording the performance of the positive and negative controls. This information should be used to determine the suitability of the test results .

(4) Description of the test protocol would benefit from the addition of a flow diagram illustrating the steps in the procedure.

In future studies, compliance with Good Laboratory Practice (GLP) Guidelines and inclusion of quality control procedures, would improve data quality and credibility. Positive and negative control values should be reported concurrently with each assay to demonstrate that the test is working properly.

The PRP also suggested that laboratories unfamiliar with conducting the test should obtain appropriate training and conduct tests with test reference chemicals before undertaking any testing of unknown chemicals and chemical mixtures.

Given the ease and cost effectiveness of conducting a pH test, the PRP recommended that pH testing be conducted prior to use of Corrositex®. Such information could be used in the future to re-evaluate the agreement between pH and Corrositex® in identifying corrosivity.

Compared to *in vivo* rabbit skin corrosivity test results, Corrositex® had an overall sensitivity[4] of 85% (76/89), specificity[5] of 70% (52/74), and accuracy[6] of 79% (128/163) for the chemicals and chemical mixtures evaluated. The three data sets reviewed (Submission [Appendix D]; Botham et al., 1995; Fentem et al., 1998) generally showed a similar degree of sensitivity, specificity, and accuracy.

The sensitivity, specificity, and accuracy of Corrositex® by chemical or product class are provided in Table 1.

The PRP concluded that the protocol supplied by IVI for conducting Corrositex® was complete and provided the necessary details for a user to

[4] Sensitivity is defined as the proportion of all positive chemicals or chemical mixtures that are correctly classified as positive in a test (NIEHS, 1997).

[5] Specificity is defined as the proportion of all negative chemicals or chemical mixtures that are correctly classified as negative in a test (NIEHS, 1997).

[6] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)

Table 1. Performance of Corrositex® by Chemical or Product Class

Chemical/Product Class	Sensitivity	Specificity	Accuracy
Overall	85% (76/89)	70% (52/74)	79% (128/163)
Inorganic and organic acids plus acid mixtures	79% (22/28)	63% (5/8)	75% (27/36)
Acid derivatives	100% (7/7)	86% (6/7)	93% (13/14)
Amines	84% (16/19)	67% (4/6)	80% (20/25)
Inorganic and organic bases plus base mixtures	89% (25/28)	57% (4/7)	83% (29/35)
Organic and inorganic acids and bases	86% (54/63)	68% (15/22)	81% (69/85)
Cleaners and detergents	90% (9/10)	29% (4/14)	54% (13/24)
Undefined industrial Chemicals	87% (13/15)	62% (8/13)	75% (21/28)
Surfactants	*	100% (22/22)	100% (22/22)

The numbers in parentheses provide the data on which the percentages are based.
*No corrosive surfactants tested.

conduct the assay correctly. The transferability of the method between laboratories and the simplicity of the assay were considered to be attractive features of Corrositex®. Similarly, methods for data analysis and the decision criteria were straightforward. Instructions on how to convert the breakthrough time to the determination of corrosivity were considered to be comprehensive and easy to follow.

The PRP concluded that an attractive feature of Corrositex® is that no animals are used. In addition, the test is rapid and less expensive than the comparable rabbit corrosivity test (see Section 6.2). In addition to the available information on inter- and intra-laboratory variability, the *in vitro* nature of the test allows for the development of an expanded database on coded compounds tested over time and in several laboratories to provide additional information on inter- and intra-laboratory variability in assay response. No such formal studies have been conducted with the *in vivo* rabbit corrosivity test. Additionally, Corrositex® includes concurrent positive and negative controls to determine whether each test trial is performing correctly; no controls are included in the *in vivo* assay, which limits an assessment of precision[7].

A limitation of the method is that many noncorrosive chemicals and chemical mixtures and some corrosive chemicals and chemical mixtures do not qualify for testing by Corrositex®. Test chemicals and chemical mixtures are considered nonqualifying if they do not cause a color change in the CDS; the CDS changes color when a chemical or chemical mixture changes the pH of the solution to less than 5 or greater than 8.5. The majority of nonqualifying chemicals and chemical mixtures (49 of 50) in the IVI and Validation Study (Botham et al., 1995; Fentem et al., 1998) database for which pH data were also available had a pH between three and

[7] Precision is defined as the extent to which a measurement procedure gives the same results when repeated under identical conditions; the inverse of variance (Dorland's Illustrated Medical Dictionary, 1994)

ten. Of the 75 nonqualifying chemicals and chemical mixtures in the database for which *in vivo* data were available, 85% (64/75) were classified as noncorrosive in the *in vivo* test[8]. The remaining 11 of 75 nonqualifying chemicals and chemical mixtures (15%) were classified as corrosive in the *in vivo* test. Thus, it appears that the qualification step eliminates primarily chemicals that are noncorrosive. Other *in vitro* methods (e.g., rat skin transcutaneous electrical resistance [TER] and EPISKIN™) for corrosivity have not shown this same limitation (Fentem et al., 1998).

Information on the composition of the complex mixtures (generally industrial chemicals, cleaners and detergents, and surfactants) tested using Corrositex® and the *in vivo* rabbit skin assay was not available. The accuracy was 54% (13/24) for cleaners and detergents, 75% (21/28) for unspecified industrial chemicals, and 100% (22/22) for surfactants. However, the PRP did not consider it appropriate to reach conclusions on the usefulness of Corrositex® for these product classes without knowing the composition of these materials.

The PRP concluded that a limitation in the evaluation of the Corrositex® database was the relatively small number of chemicals and/or the unbalanced distribution of corrosive and noncorrosive chemicals and chemical mixtures evaluated in some chemical/product classes. As a consequence, accurate conclusions about the performance of Corrositex® for some chemical classes could not be made.

In response to the second question, the PRP concluded that Corrositex® offers several advantages with respect to animal welfare considerations, including refinement, reduction, and replacement of animal use. Corrositex®, when used as a stand-alone assay in some testing situations, replaces the use of animals for corrosivity testing. Similarly, the use of Corrositex® as part of a tiered approach reduces and refines the use of animals in testing by providing a basis for decisions on further testing. Follow-up tests using *in vivo* methods, when deemed necessary, could employ fewer animals and test agent dilution schemes to minimize possible pain in any individual animal.

[8] In some instances, this analysis includes results from several concentrations of the same chemical.

1.0 TEST METHOD DESCRIPTION

1.1 Sufficiency of test method and protocol description

1.1.1 Adequacy and completeness of the test method

The Submission contained a thorough protocol. The scientific and empirical basis of the test was described in sufficient detail. Corrositex® measures the time required for a chemical or chemical mixture to pass through a hydrated collagen matrix (biobarrier) and supporting filter membrane. Passage through the biobarrier is observed by a color change in the CDS, an underlying aqueous solution of two pH indicator dyes. The time required to pass through the biobarrier, referred to as the breakthrough time, is used as a measure of the corrosive potential of the chemical or chemical mixture under test. The Sponsors propose that the time required to pass through the biobarrier depends on: (1) the strength of the acid or base; (2) the rate of diffusion of the test chemical or chemical mixture; and (3) for more corrosive substances, the rate of destruction of the biobarrier. However, one member of the PRP noted that the composition of the biobarrier, which is IVI proprietary information, could not be compared directly with skin.

The destruction/erosion of the biobarrier is easily observed with strong acids and bases. IVI suggests that the breakthrough time for dilute acids or bases depends on diffusion through the biobarrier, rather than on destruction of the biobarrier itself. However, if the assay were simply measuring the rate of diffusion of the chemical or chemical mixture through the biobarrier, noncorrosive chemicals and chemical mixtures (e.g., buffered solutions of organic acids) would be expected to diffuse through the biobarrier, on a molar basis, as quickly as unbuffered mild corrosives. Certainly, there is diffusion through the collagen matrix and, with time, even noncorrosive chemicals and chemical mixtures will reach the CDS. For that reason, four hours is used as the upper limit for the assay. Some Category 1[9] noncorrosive chemicals and chemical mixtures (e.g., dithiopropionic acid) react with the CDS, yet the chemical or chemical mixture does not reach the CDS within the required four hours of exposure. The Submission contains data on the breakthrough times for various chemicals and chemical mixtures at different concentrations. When the breakthrough times for acetic (FW [Formula Weight] 60.5), propionic (FW 74), butyric (FW 88), and citric (FW 192) acids as a function of molar concentration are compared, very different breakthrough times are observed (Table 2). These data suggest that the interaction between a weak acid and the biobarrier is more complex than simple diffusion.

Corrositex® is correlative in nature, rather than mechanistic. The matrix was engineered to block passage of certain chemicals and chemical mixtures for a period of time similar to the time allowed for that chemical or chemical mixture to stay in contact with rabbit skin without causing "necrosis or ulceration." However, while an acellular matrix might ulcerate, it cannot undergo necrosis.

In the Submission, the range of applications (i.e., types of test chemicals and chemical mixtures) was insufficiently described. The Sponsors pro-

[9] The instruction manual provides instructions for determining the category (1 or 2) of a material based on the degree of color change observed in the qualification step, which is indicative of the degree of the acid/alkaline reserve. This categorization determines the cut-off times used to determine the corrosivity/noncorrosivity of a sample. See the Corrositex® Instruction Manual in Appendix D for a complete description of this determination.

Table 2. Comparison of Breakthrough Times for Acids as a Function of Concentration

Acid	Concentration (M)	Breakthrough Time (min)	Lowest Concentration (M) Yielding Breakthrough in Less than 60 Min.	Breakthrough Time (min)
Acetic	0.50	53	0.50	53
Propionic	0.54	86	1.35	59
Butyric	0.57	88	11.24	56
Citric	0.52	66	0.76	59

posed that Corrositex® is comparable to the *in vivo* rabbit skin corrosivity test with regard to its ability to assess the corrosivity of well-defined inorganic and organic acids and bases, and complex mixtures of inorganic and organic acids and bases. The Sponsors also stated that the test was suitable for assessing US DOT Class 8 corrosive materials, which are defined either as liquids or solids that cause full thickness destruction of human skin at the site of contact within a specified period of time, or as liquids that have a severe corrosion rate on steel or aluminum based on specified criteria (Appendix Q). However, the Submission does not address such broad applicability. For example, the chlorosilanes are not represented in the provided database. In general, the chemical/product classes as defined in the Submission are broad and generic (e.g., amines and acid derivatives). It would have been useful if the chemical and product classes were more specific. Amines could have been classified as primary, secondary, or tertiary, and acid derivatives could have been classified as anhydrides, haloacids, salts, etc., as appropriate. For the database evaluated, all test chemicals and chemical mixtures were assigned to the same chemical or product classes used in the Submission. The categories were as follows: inorganic and organic acids, acid mixtures, acid derivatives, amines, inorganic bases, base mixtures, cleaners, detergents, undefined industrial chemicals, and surfactants. It was not possible to determine whether chemicals and chemical mixtures tested in each chemical or product class are representative of the variety of chemicals in that class.

1.1.2 Adequacy of agreement between the validation protocol and proposed protocol

The protocol used to generate the supporting Submission data is consistent with the protocol currently proposed by IVI. Only three differences were noted. First, the stability limit of the biobarrier was changed from ten to seven days. Second, the time for classification of Category 2 chemicals and chemical mixtures for Packing Group III[10] was changed from "greater than 30 to 45 minutes" to "greater than 30 to 60 minutes." Third, the Corrositex® cut-off time for Category 2 noncorrosive chemicals and chemical mixtures was increased from greater than 45 minutes to greater than 60 minutes. The impact, if any, of the first change is not known.

[10] US DOT assigns packing groups to corrosives based on the severity of the corrosive response. Packing Group I corrosives are defined as materials that cause full thickness destruction of intact skin tissue within an observation period of up to 60 minutes starting after the exposure time of three minutes or less. Packing Group II corrosives are materials not meeting Packing Group I criteria and causing full thickness destruction of intact skin tissue within 14 days after an exposure time of more than three minutes but less than 60 minutes. Packing Group III corrosives are materials not meeting Packing Group I or II criteria but causing full thickness destruction of intact skin tissue within 14 days after an exposure time of more than 60 minutes but less than four hours (49 CFR 173.137).

The other changes were mandated by US DOT in granting the second exemption (US DOT, 1996). The data provided in the current Submission are based on the revised cut-off times for Category 2 chemicals and chemical mixtures. The two latter changes do not affect the performance evaluation of Corrositex® versus the *in vivo* corrosivity test.

The Corrositex® protocol used in the Prevalidation Study (Botham et al., 1995) and European Centre for the Validation of Alternative Methods (ECVAM) Validation Study (Fentem et al., 1998) was similar to the one used by IVI, with three exceptions. First, the IVI protocol required that the biobarrier be refrigerated for a minimum of two hours prior to use, while the protocol for both validation studies required an overnight cooling period. The longer cooling period was used to decrease breakthrough time variability when sodium hydroxide (NaOH) pellets were used as the positive control. Second, the positive and negative controls differed between the IVI and the two validation study protocols. The validation study protocols used a positive control consisting of a single pellet of NaOH and a "blank" as a negative control. The IVI protocol suggests the use of 68 to 73% nitric acid or 95 to 98% sulfuric acid as a positive control, and 10% citric acid or 6% propionic acid as a negative control. Third, the Prevalidation Study protocol did not include the categorization step used in the IVI and the ECVAM Validation Study protocols. The categorization step is used to enable a test chemical to be measured against a revised scoring scale, based on the acid/alkaline reserve of the sample. No impact on the quality of the data is expected based on these differences.

1.1.3 Appropriateness of dose and breakthrough time selection procedure

The dose and breakthrough time selection procedures were considered to be appropriate. Both were based on an extensive evaluation of corrosive and noncorrosive chemicals and chemical mixtures and of knowledge gained through the Prevalidation and Validation Studies (Botham et al., 1995; Fentem et al., 1998). The rate of false positive and false negative responses for various chemical classes suggests some value in attempting to further refine the dose and breakthrough times if the assay is to be used as a stand-alone assay for additional chemical classes.

The amount of test chemical or chemical mixture used in Corrositex® is the same as that specified for the rabbit studies, although rationale for this dose selection in Corrositex® was not considered by the PRP. However, the ratio of test chemical or chemical mixture to unit area of the biobarrier (0.5 g or 0.5 mL to 0.71 cm^2) exceeds the expected ratio of test chemical or chemical mixture to skin area in the rabbit study by 8.5-fold.

Selection of the maximum exposure time and interpretation of the breakthrough time are based on the categorization screen (Category 1 or 2). The categorization screen determinations are reproducible within and across laboratories. In the ECVAM Validation Study (Fentem et al., 1998), 60 chemicals were evaluated by three laboratories in two independent trials. Of the 40 chemicals that qualified, the categorization screen results were in disagreement among the laboratories only twice. Thus, this aspect of the assay seems appropriate.

The qualification test with the CDS provides a number of benefits to the assay. It immediately eliminates those chemicals and chemical mixtures that cannot be evaluated using Corrositex® and it provides the operator with a clear indication of the expected color change that the test chemical or chemical mixture would induce upon breakthrough of the biobarrier.

1.2 Adequacy and completeness of the test method protocol

1.2.1 Test method material and equipment, animal usage

The protocol for the assay is complete and provides the necessary detail for the user to apply the assay correctly. The manual gives a detailed description of the steps to conduct the assay, the materials and equipment needed, and special precautions for ensuring a successful test. The transferability of the method between laboratories, as defined by the low interlaboratory variability in the validation studies (Botham et al., 1995; Fentem et al., 1998), supports the conclusion that the manual provides sufficient detail. No animal use is involved.

Additional detail to the IVI protocol, such as allowing the biobarrier to harden on a level surface, may be useful in helping to eliminate potential sources of error. This type of information was provided previously by IVI technical service personnel. Such additions to the protocol would not alter its fundamental properties.

1.2.2 Data collection

Submission information on the test method clearly stated that data sheets are included in the test kit, along with instructions on what is to be measured and how the data are to be interpreted. The simplicity of the procedure is one of the attractive features of Corrositex®. The same may be said of the data analysis and decision criteria. At least three types of experimental data are collected on each chemical or chemical mixture tested: (1) the presence or absence of a color change in the CDS in the qualification screen; (2) the presence or absence of a color change in the categorization screen; and (3) the time required, up to the limits of the exposure time, to induce a color change in the CDS after addition of the test chemical or chemical mixture to the biobarrier. In addition, the breakthrough time(s) of the positive and negative controls would be recorded for each trial. The usual ancillary data associated with any assay (test article identification, number, descriptions, lot numbers, etc.) would also be recorded. The time required to breakthrough the biobarrier is used to determine whether the test chemical or chemical mixture would be classified as a corrosive according to the cut-off times provided in the appropriate category table.

A topic that is not discussed in the protocol or in the Submission is the acceptable variability among replicate tests for the same chemical or chemical mixture (i.e., expected coefficient of variation). While the results of the validation studies (Botham et al., 1995; Fentem et al., 1998) suggest that the replicate determinations are very close, guidance on how to evaluate an aberrant value would be valuable.

1.2.3 Data analysis, evaluation, and decision criteria

The descriptions of data collection, data evaluation, and the decision criteria used to identify chemicals and chemical mixtures as qualifying or nonqualifying, Category 1 or 2, and corrosive or noncorrosive are well documented. The performance of the qualification test with the CDS is well described, and the prescreen prepares the operator for the color change that will occur in the CDS when the chemical or chemical mixture is tested. As the color change can be subtle, there is the potential for operator subjectivity. Such differences were sporadically noted in the ECVAM Validation Study (Fentem et al., 1998). The categorization screen is well documented and the manufacturer provides sufficient color plates to facilitate interpretation. Instructions on how to convert the breakthrough time to the determination of corrosivity are complete and easy to comprehend. The protocol also includes information on how to make a

decision when the breakthrough values are very close to the cut-off between classes.

1.2.4 Number of replicates per test article

The Prevalidation and ECVAM Validation Studies (Botham et al., 1995; Fentem et al., 1998) found a lack of appreciable variability in the replicate data. The Corrositex® Instruction Manual (Appendix D) recommends the use of four replicates. The current test method kit contains four replicate vials to be used for each chemical assay.

1.3 Positive, negative, and irritation control chemicals

Corrositex® provides for both positive and negative controls. The role of these controls is to assure that the test system is working properly during each trial. To achieve this goal, the controls should be able to detect over and under prediction (relative to the controls) and should be the basis for accepting a test. Thus, the control(s) must be included with each trial. The negative control serves as a color control for the CDS so that the operator has immediate reference to the "normal" appearance of the CDS under the test conditions. This is very helpful when a subtle change in color upon breakthrough of the test chemical or chemical mixture is expected. The positive control evaluates the proper function of the assay system (biobarrier, CDS, etc.) and the operator.

The standard Corrositex® kit provides sufficient test system components to test both positive and negative controls as well as four replicates of the sample. Positive and negative control chemicals do not necessarily need to be those suggested by IVI. Very strong acids are unlikely to show appreciable underprediction, especially if a relatively broad range of breakthrough times compared to the full range of the Packing Group I limits is used. In the validation studies (Botham et al., 1995; Fentem et al., 1998), a NaOH pellet was used as a positive control based on the desire to have a breakthrough time in the Packing Group II category. This allowed for readily observable differences in breakthrough times. The addition of a true negative control to the protocol by IVI is an excellent amendment. A chemical or chemical mixture that has a breakthrough time closer to the cut-off of 60 minutes might be more appropriate, or it might be useful to extend the duration of the assay to determine a breakthrough time for the concurrent negative control. In the Submission, the discussion of controls applies only to Corrositex® since the current *in vivo* assays do not include controls.

A letter dated November 3, 1998 from Dr. Rosalind Wei of IVI indicated that the control values must be within a specified range for the data to be accepted. However, no provision is made for the performance of the controls to be recorded on the IVI Corrositex® Data Sheet (7/98 Rev.3). Such information should be included.

Corrositex® does not make provision for determining the irritancy of noncorrosives nor does it make any such claim.

1.4 Strengths and/or limitations

Corrositex® is a non-animal means of estimating the dermal corrosivity potential of a test chemical or chemical mixture. The test is rapid, relatively simple, and appears to be less expensive than the comparable *in vivo* rabbit test. A clear and detailed protocol is available; concurrent positive and negative controls are included to determine the fitness of each trial. The data on coded compounds tested over time and in several laboratories indicates excellent performance with regard to inter- and intra-laboratory reproducibility. However, one member of the PRP felt that additional interlaboratory valida-

tion studies using laboratories with no previous experience in conducting the assay were needed before an accurate assessment of reproducibility can be made.

One advantage of this assay compared to the *in vivo* rabbit skin corrosivity assay is the potential for developing a comprehensive database on coded compounds tested over time in several laboratories to provide a clear measure of inter- and intra-laboratory variations in assay responses. Since controls are not incorporated into the *in vivo* assay, the only measures of precision are animal-to-animal differences in response within an assay and inter- and intra-laboratory reproducibility. Such measures of *in vivo* precision were not available in the Submission. However, a limited measure of animal-to-animal variability is available for the ECVAM Validation Study (Fentem et al., 1998) (Table 3). This *in vivo* reference data, compiled by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1995), shows that variation exists in the numbers of animals tested and in the response observed.

Table 3. *In Vivo* Reference Data for 17 Corrosive Chemicals Evaluated in the ECVAM Validation Study (ECETOC, 1995)

ECVAM (#) and Name	ECVAMChem Class	P I I	Exposure	N	Response
(15) dimethyldipropylenetriamine	Org. Base	NPC	4 hr	1	Ne @ 1 hr
(13) 3-methoxypropylamine	Org. Base	6.7	4 hr	6	Br 6/6 @ 1 hr
(17) dimethylisopropylamine	Org. Base	5.6	4 hr	6	Br 6/6 @ 1 hr
(45) heptylamine	Org. Base	6.7	4 hr	6	Br 6/6 @ 1 hr
(48) glycol bromoacetate (85%)	Electrophile	7.7	4 hr	1	Ne 1/1 @ 1 hr
(23) 2-tert-butylphenol	Phenol	5.7	4 hr	6	Ne 5/6 @ 1 hr
(18) KOH (10%)	Inorg. Base	NPC	4 hr	3	Ne 3/3 @ D 1
(42) 2-mercaptoethanol (Na)	Inorg. Base	NPC	3 min	3	Ne 1/3 @ D 1 2/3 neg @ D 7
(47) 60/40 octanoic/decanoic acid	Org. Acid	NPC	4 hr	3	Ne 2/3 @ D 1 Ne 3/3 @ D 7
(29) 65/35 octanoic/decanoic acid	Org. Acid	NPC	4 hr	3	Es 2/3 @ D 1 Es 3/3 @ D 2
(40) octanoic acid	Org. Acid	4.4	4 hr	3	Ne 3/3 @ D 2
(50) 55/45 octanoic/decanoic acid	Org. Acid	5.1	4 hr	3	Ne 3/3 @ D 2
(3) carvacrol	Phenol	>4.0	4 hr	4	Es 3/4 @ D 1 Es 4/4 @ D 2
(14) allyl bromide	Electrophile	7.2	4 hr	2	Ne 2/2 @ D 7
(38) tallow amine	Org. Base	NPC	4 hr	3	Ne 2/3 @ D 7
(36) 2-methylbutyric acid	Org. Acid	>4.0	4 hr	4	Es 2/4 @ D 7
(5) methacrolein	Electrophile	4.1	4 hr	3	Ne 3/3 @ D 9

Abbreviations: Br = Burned, Es = Eschar, Inorg. = Inorganic, N = Number of rabbits tested, Ne = Necrotic, neg = negative (for corrosion), NPC = Not possible to calculate, Org. = Organic, PII = Primary Irritation Index

A request was made to the appropriate Federal regulatory agencies for data on the inter- and intra-laboratory reproducibility of the *in vivo* corrosivity assay; the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was informed by agency representatives that these data were either not available or not readily available. However, Weil and Scala (1971) examined intra- and inter-laboratory variability in the results of eye and skin irritation tests. Standard protocols were used by 22 laboratories to test 12 reference chemicals. For the skin test, eight rabbits were used for each agent and 0.5 mL was applied for 24 hours under semiocclusive bindings. A portion of the evaluation dealt with how laboratories ranked samples, a measure of interlaboratory variability. Stated differently, did laboratories rate agents of low irritancy differently from those of high irritancy?

The laboratories, as a whole, ranked the agents much more consistently than would be expected by chance. The intralaboratory variability was less than the interlaboratory variability, and only 2/22 laboratories lacked significant correlation with the intralaboratory ranking of samples. Of particular interest with respect to Corrositex® was the frequency and consistency with which skin necrosis was reported in these *in vivo* studies. There was considerable scatter in the number of animals with necrosis (i.e., visible destruction or irreversible alteration of the tissue [49 CFR 173 App. A, 1992]) for some of the agents tested (Table 4). For example, chemical E induced necrosis in four out of eight rabbits in one laboratory, one of eight in a second laboratory, and zero out of eight in the remaining 20 laboratories. This study was conducted in 1968-1970 using a defined protocol, but prior to the introduction of the FDA/EPA GLP guidelines. More recent interlaboratory performance evaluation studies addressing the *in vivo* dermal irritation assay have not been conducted. The PRP suggests that it may be useful to compile inter- and intra-laboratory *in vivo* corrosivity data for reference chemicals.

Table 4. Necrosis Observed in the Weil and Scala (1971) Study

Agent	Number of Laboratories Reporting Necrosis in Eight Rabbits								
	0/8	**1/8**	**2/8**	**3/8**	**4/8**	**5/8**	**6/8**	**7/8**	**8/8**
E	20	1			1				
F	12				1	1	2	1*	5
G	19	1	2*						
I	18	2	2*						
J	14	2		2*		1	3		
J-1**	13	2		2*		1	3	1	
K	15		2			1*		1	3
L	8		1		1	1		3***	8
M	14	2	1	4	1				
M-1**	13	2	1	4	1			1	
N	21		1						
O	21	1							

*1/8 rabbits died in one laboratory.

**Laboratory 31 reported two sets of data for this sample. 0/8 with necrosis in one run and 7/8 in the other.

***1/8 rabbits died in two laboratories.

Based on the available data, there is uncertainty as to the precision of the reference data against which Corrositex® is compared. With this limitation of the reference data in mind, the only limitation of Corrositex® that can be stated quantitatively is the fraction of the test chemicals and chemical mixtures within each test set that did not qualify for testing. Estimates of specificity and sensitivity of the Corrositex® data against the *in vivo* data might be viewed as "worst case" since they presume 100% precision for the *in vivo* test.

Corrositex® is correlative in nature and is not based on mechanistic principles. A major limitation of the method is the large proportion of chemicals and chemical mixtures that do not qualify for testing by Corrositex®. Based on an analysis by NICEATM (Appendix G), the majority (49) of nonqualifying chemicals and chemical mixtures for which pH data were also available (N=50) had a pH between three and ten. Of the 75 nonqualifying chemicals and chemical mixtures for which *in vivo* data were available, 85% were classified as noncorrosive in the *in vivo* test, suggesting that there is only a 15% likelihood that a nonqualifying chemical or chemical mixture will be corrosive. Other *in vitro* methods for corrosivity (e.g., rat skin TER assay and EPISKIN™) have not shown this same limitation (Fentem et al., 1998).

Another limitation of Corrositex® is the relatively high number of false positive and false negatives in some chemical classes (see Section 3.6). Also, the lack of composition information for the undefined complex mixtures tested using Corrositex® precluded an analysis of the predictive value of this assay for untested complex mixtures.

1.5 Editorial/technical corrections

No specific editorial/technical corrections were identified other than those already indicated above.

1.6 Recommendations

The following specific changes to the protocol were recommended:

(1) It should be explicitly stated that the biobarrier should be allowed to harden on a level surface and to cool overnight before use.

(2) Even though replicate variability has been shown to be very low, guidance should be provided on how to evaluate an aberrant value.

(3) The IVI Corrositex® Data Sheets provided with the test kit should have a provision for recording the performance of the positive and negative controls. These controls should be used to determine the suitability of the test results.

(4) Description of the test protocol would benefit from the addition of a flow diagram illustrating the steps in the procedure.

2.0 TEST METHOD DATA QUALITY

2.1 Quality control and quality assurance procedures including data audits

The *in vitro* and *in vivo* experiments conducted by IVI were not in accordance with GLP guidelines. However, audits were conducted by NICEATM and by the NTP Quality Assurance Unit (QAU) that compared the data provided in the Submission against original study records to verify accuracy and completeness (Appendix N). Although the audits identified missing GLP-required information (e.g., data, laboratory/facility records, Standard Operating Procedures (SOPs), protocols, written amendments and deviations), the auditors concluded that the errors and omissions found did not alter the credibility of the IVI database and that the data could be evaluated with confidence. The PRP agreed with this conclusion.

Corrositex® tests in the Prevalidation and ECVAM Validation Studies (Botham et al., 1995; Fentem et al., 1998) were stated to have been conducted in the "spirit" of GLP. Thus, one of the two laboratories involved in the Prevalidation Study and the three laboratories involved in the ECVAM Validation Study had experience in conducting GLP studies for submission to regulatory authorities. A formal audit of the ECVAM data by a QAU was not conducted; however, all data submitted by the participating laboratories were verified against the original data sheets by ECVAM staff on at least three separate occasions. Similarly, rigorous checks of all calculations, data analyses, etc., were conducted independently by ECVAM staff and the Management Team representatives of the lead laboratories. *In vivo* data for 49 of the 60 chemicals tested in the validation studies were obtained from ECETOC (1995). Data for the remaining 11 chemicals were from data sheets that were provided in confidence to ECVAM by an industrial company. The criteria used in the selection of test chemicals are detailed in Barratt et al. (1998) and the criteria adopted during the ECETOC review are provided in the report by the Working Party (Bagley et al., 1996). All *in vivo* studies are stated to have been carried out in accordance with the Organisation for Economic Co-Operation and Development (OECD) Guideline for Testing of Chemicals: Acute Dermal Irritation/Corrosion (OECD, 1992).

2.2 Protocol consistency during validation

IVI used the January 1995 version of the Corrositex® protocol in generating all IVI submitted data. Audits of the study-specific information provided to support the Submission indicate that there were no chemical-specific protocol amendments or deviations. The data resulting from the Prevalidation and ECVAM Validation Studies (Botham et al., 1995, Fentem et al., 1998) followed an established protocol. As discussed in Section 1.1.2, the IVI and validation study protocols differed in regard to the length of the refrigeration cooling period before the biobarriers were to be used; in the selection of positive and negative controls; and in the use of a categorization step. These differences do not invalidate the studies conducted by either organization. It is suggested that IVI include the reporting of positive/negative control values in the protocol in order for an objective assessment to be made as to whether the controls meet the specified acceptance criteria and whether the test is working properly.

In the Submission, IVI provided an abbreviated protocol for the animal studies conducted by a

contract laboratory for the validation assessment; there was no assurance that this protocol was rigorously followed throughout the studies. This was a weakness of the Submission, along with the associated failure to identify where each chemical or chemical mixture was tested and the date of testing. With respect to ECVAM, the *in vivo* studies generally used three to four animals treated at several sites to provide time course data.

2.3 Recommendations

In future studies, compliance with GLP guidelines and inclusion of quality control procedures, would improve data quality and credibility. Positive and negative control values should be reported concurrently with each assay to determine if the test is working properly.

3.0 TEST METHOD PERFORMANCE

3.1 Data presentation

The Submission provided *in vitro* and *in vivo* data on 118 chemicals and chemical mixtures; all data were supported by paper documentation. Fifty-five of the chemicals and chemical mixtures consisted of mixtures and proprietary products whose compositions were not provided. The remaining 63 defined chemicals and chemical mixtures in the Submission and the data in the Prevalidation Study (Botham et al., 1995) and ECVAM Validation Study (Fentem et al., 1998) were grouped into four chemical classes. Five of the 63 IVI defined chemicals and chemical mixtures were unclassified. The four chemical classes were (1) inorganic and organic acids and acid mixtures, (2) acid derivatives; (3) amines (organic bases), and (4) inorganic bases and base mixtures. The 55 chemicals and chemical mixtures with unknown composition in the IVI database and similar chemicals and chemical mixtures in the validation studies were grouped into three product classes: (1) cleaners and detergents; (2) undefined industrial chemicals; and (3) surfactants, with one chemical or chemical mixture remaining unclassified. The chemicals evaluated in the validation studies were classified as inorganic acids, organic acids, inorganic bases, organic bases, neutral organics, phenols, electrophiles, inorganic salts, and surfactants. For the PRP evaluations, all chemicals and chemical mixtures were classified according to the chemical class scheme used by IVI. The chemical classes provided in the US DOT Exemption were similar to the chemical classification scheme used in the Submission.

The criterion used by IVI and ECVAM to select the chemicals and chemical mixtures for evaluation was reasonable, where stated, but potentially biased. That is, the database may not represent the entire chemical population of interest for corrosivity testing. The Submission did not provide sufficient detail to permit a comprehensive evaluation of the total data of 118 chemicals and chemical mixtures. The areas that confounded evaluation were:

- The lack of composition information for 55 chemicals and chemical mixtures. Such information is proprietary and was not provided to IVI by the manufacturer. Although positive results by qualified chemicals and chemical mixtures would yield useful corrosivity results for a particular complex mixture, the lack of composition information precluded an analysis of the predictive value of Corrositex® for untested complex mixtures.

- With the exception of the ECVAM Validation Study (Barratt et al., 1998), primary irritation scores for each substance were not provided. This information would have been helpful for understanding the irritancy potential of the chemicals and chemical mixtures evaluated. The lack of this information ultimately affected the PRP's ability to comment on the ability of the assay to distinguish between highly irritating versus corrosive chemicals and chemical mixtures.

- Some chemical classes were too broadly defined to make specific conclusions about the utility of Corrositex® for that class. For example, a possible subclassification alternative for acid derivatives would have been to classify these chemicals based on structure, pH, and reserve acidity. More specific class designations such as anhydrides, haloacids, salts, etc., would have been helpful.

- For some chemical classes, the number of

chemicals in the database was too few or the distribution of corrosives versus noncorrosives too unbalanced to ensure an adequate evaluation of performance.

At the request of ICCVAM, nonqualifying chemicals and chemical mixtures were not included by IVI in the 1998 Submission. However, the proportion of nonqualifying chemicals and chemical mixtures can be estimated from the validation studies, the 1996 IVI Submission, and other relevant databases:

- In the Prevalidation Study (Botham et al., 1995), testing was conducted in two laboratories; the proportion of nonqualifying chemicals in the two laboratories were 24% (12/50, Laboratory A) and 27% (13/48, Laboratory B). For noncorrosive chemicals, 32% (8/25) were nonqualifying in both laboratories. For corrosive chemicals, 16% (4/25) and 22% (5/23) were nonqualifying in Laboratories A and B, respectively.

- In the ECVAM Validation Study (Fentem et al., 1998), the proportion of nonqualifying chemicals was 37% (22/60). For noncorrosive chemicals, 58% were nonqualifying, but only 10% of the corrosive test samples were nonqualifying.

- The proportions of nonqualifying chemicals and chemical mixtures observed in three industrial studies by Gordon et al. (1994) were 8.1% (3/37), 31.7% (13/41), and 0% (0/47) for chemicals/agrochemicals, petrochemicals, and cleansers/surfactants, respectively.

- In the 1996 IVI Submission to ICCVAM, the proportion of nonqualifying chemicals and chemical mixtures was 16.5% (83/502).

The overall rate of nonqualifying chemicals and chemical mixtures for all databases combined was 18% (133/733).

3.2 Adequacy of prediction of endpoint of interest

The demonstrated linkage between Corrositex® and the *in vivo* rabbit skin corrosivity test is the ability of the assay to correctly identify *in vivo* rabbit corrosive and noncorrosive compounds. The generally favorable accuracy analysis of the *in vitro* data with the *in vivo* test results supports such a linkage. Presumably, this linkage is also predicated on the biological relevance of the *in vitro* model; the diffusion through and/or the destruction of a collagen-based barrier is suggested to be analogous to chemical-induced corrosion of rabbit skin. The assay is highly pH and reserve acidity and alkalinity sensitive (i.e., the more acidic or basic the chemical or chemical mixture, the more likely it is to give an accurate prediction). This is consistent with what is known about the *in vivo* dermal corrosion potential of a chemical or chemical mixture. However, based on the databases evaluated, the assay had false positive (30-100%; Tables 5 and 9, respectively) and false negative (15-21%; Tables 5 and 6, respectively) rates that were considered to be excessive for some chemical and product classes. For the database evaluated (Submission plus both validation studies), the false positive rate was considered by the PRP to be excessive for all chemical and product classes except surfactants (Tables 5-14). The false negative rates were also considered by the PRP to be excessive in the overall data set (Table 5), and for amines (Table 8) and inorganic and organic acids and acid mixtures (Table 6). As indicated by the generally high false positive rates, Corrositex® tends to overpredict corrosivity potential as compared to *in vivo* data. Among the 163 chemicals and chemical mixtures that qualified, the accuracy of Corrositex® with the *in vivo* test was 79%.

3.3 Adequacy of test method performance evaluation

In support of the PRP, the performance of Corrositex® was evaluated by NICEATM using the data submitted by IVI and from the two validation studies (Botham et al., 1995; Fentem et al., 1998). Accuracy, sensitivity, specificity, positive predictivity[11], negative predictivity[12], false positive rate[13], and false negative rate[14] were determined for the total database and for each of the three data sets. Corrositex® data published by Gordon et al. (1994) were excluded from the performance analysis since the reference data were obtained from a US DOT Table (49 CFR 172.101, 1992) and actual *in vivo* results were not available. The sensitivity varied from 71 to 92% for the three studies (Table 6). The remaining performance calculations among the three data sets appear to be comparable.

The Submission and the validation studies presented not only the results of tests conducted with individual chemicals and chemical mixtures but also included several of the usual statistical parameters to evaluate the validity of a new method. Absent from the evaluations was a discussion of how predictive value varies with prevalence. As the prevalence of corrosive chemicals and chemical mixtures in the test population decreases, the predictive value decreases strikingly, even with high values for specificity and sensitivity (Purchase, 1982).

3.4 Adequacy of test method performance data

It would have been helpful if the Submission had included a comprehensive evaluation of the variability in breakthrough times among replicate tests for the 118 chemicals and chemical mixtures tested. Inter- and intra-laboratory reproducibility of Corrositex® was evaluated formally within the ECVAM Validation Study (Fentem et al., 1998) using analysis of variance (ANOVA) methods. The results of these analyses indicate that the within and between laboratory reproducibility for Corrositex® was excellent and that the variability in assay results was acceptable.

3.5 The adequacy of the chemicals/products (numbers/types) selected to evaluate the performance of the method for each chemical/product class

IVI provided data on 118 chemicals and chemical mixtures. However, the number of chemical classes represented by these 118 chemicals and chemical mixtures is limited, and generalizations on performance should not be made beyond these chemicals and chemical classes. Furthermore, of the 118 separate chemicals and chemical mixtures whose data were provided by IVI, 63 were defined chemicals and 55 were chemicals and chemical mixtures of unknown composition. While analysis of the 55 chemicals and chemical mixtures of unknown composition can provide supporting information on performance, the formal performance analysis focused on the defined test samples. The Submission included samples from seven chemical/product classes: inorganic and organic acids and acid mixtures (n=22), acid derivatives (n=14), amines (n=14), inorganic bases and base

[11] Positive predictivity is defined as the proportion of correct positive responses among materials testing positive (NIEHS, 1997). The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested.

[12] Negative predictivity is defined as the proportion of correct negative responses among materials testing negative (NIEHS, 1997). The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested.

[13] False positive rate is defined as the proportion of all negative (noncorrosive) substances that are falsely identified as positive (NIEHS, 1997).

[14] False negative rate is defined as the proportion of all positive (corrosive) substances that are falsely identified as negative (NIEHS, 1997)

mixtures (n=8), cleaners and detergents (n=18), industrial chemicals (n=22), and surfactants (n=14). This chemical classification scheme is similar to that provided in the US DOT Exemption. The Prevalidation Study (Botham et al., 1995) classified chemicals as inorganics (n=6), organic acids (n=13), organic bases (n=11), neutral organics (n=10), amine oxides (n=2), anionic surfactants (n=3), and cationic surfactants (n=5). The ECVAM Validation Study (Barratt et al., 1998; Fentem et al., 1998) classified chemicals as inorganic acids (n=7), organic acids (n=11), inorganic bases (n=4), organic bases (n=10), neutral organics (n=9), phenols (n=5), electrophiles (n=8), inorganic salts (n=3), and surfactants (n=3). After reclassifying the chemicals to meet the IVI chemical and chemical mixture classification scheme, the number of chemicals and chemical mixtures per class were: inorganic and organic acids and acid mixtures (n=36), acid derivatives (n=14), amines (n=25), inorganic bases and base mixtures (n=10), cleaners and detergents (n=24), industrial chemicals (n=28), and surfactants (n=22). In addition, organic and inorganic acids and bases were combined into one class for evaluation (n=85).

Because of the relatively small numbers of chemicals evaluated in some chemical classes and the unbalanced nature of corrosive versus noncorrosive chemicals (corrosive >>noncorrosive), definitive conclusions as to the adequacy of Corrositex® for some chemical classes were difficult to make with confidence.

3.6 Accuracy, sensitivity, specificity, positive predictivity, negative predictivity, false positive rate, and false negative rate and chemical classes

For the purposes of this evaluation, the formal performance evaluation was limited to the chemicals and chemical mixtures evaluated in the Submission and the two validation studies (Fentem et al., 1998; Botham et al., 1995). The Gordon et al. (1994) data set was not included since the Corrositex® responses were compared against the US DOT-assigned packing group designations specified in the Hazardous Materials Table (49 CFR 172.101, 1992) instead of available *in vivo* data. However, consideration of this data set provides value from a weight-of-evidence perspective, and is therefore included in the performance analysis tables (Tables 5-14). Performance characteristics of the total data set, including the Gordon et al. (1994), are also provided in the tables for the same reason. Unless otherwise specified, the values provided in the following subsections represent the data set including the Submission and the two validation studies only.

3.6.1 Total chemical and chemical mixtures (Table 5)

Based on 163 chemicals and chemical mixtures, Corrositex® has an accuracy of 79% (128/163), a sensitivity of 85% (76/89), a specificity of 70% (52/74), a positive predictivity of 78% (76/98), a negative predictivity of 80% (52/65), a false positive rate of 30% (22/74), and a false negative rate of 15% (13/89).

3.6.2 Inorganic and organic acids and acid mixtures (Table 6)

Based on the 36 chemicals and chemical mixtures in this data set, Corrositex® has an accuracy of 75% (27/36), a sensitivity of 79% (22/28), a specificity of 63% (5/8), a positive predictivity of 88% (22/25), a negative predictivity of 45% (5/11), a false positive rate of 38% (3/8), and a false negative rate of 21% (6/28).

Table 5. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Overall)[15]

Data Source	Number of Chemicals	Accuracy[16]		Sensitivity[17]		Specificity[18]		Positive Predictivity[19]		Negative Predictivity[20]		False Positive Rate[21]		False Negative Rate[22]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[23]	118	82	(97/118)	92	(59/64)	70	(38/54)	79	(59/75)	88	(38/43)	30	(16/54)	8	(5/64)
Prevalidation Study[24]	37	76	(28/37)	81	(17/21)	69	(11/16)	81	(17/21)	73	(11/15)	31	(5/16)	19	(4/21)
ECVAM Validation Study[25]	40	75	(30/40)	71	(17/24)	81	(13/16)	85	(17/20)	65	(13/20)	19	(3/16)	29	(7/24)
Submission Plus Validation Studies[26]	163	79	(128/163)	85	(76/89)	70	(52/74)	78	(76/98)	80	(52/65)	30	(22/74)	15	(13/89)
Gordon et al. (1994)	75	97	(73/75)	97	(58/60)	100	(15/15)	100	(58/58)	88	(15/17)	0	(0/15)	3	(2/60)
Entire Data Set[27]	221	84	(185/221)	89	(118/132)	75	(67/89)	84	(118/140)	83	(67/81)	25	(22/89)	11	(14/132)

Table 6. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Inorganic and Organic Acids and Acid Mixtures)[15]

Data Source	Number of Chemicals	Accuracy[16]		Sensitivity[17]		Specificity[18]		Positive Predictivity[19]		Negative Predictivity[20]		False Positive Rate[21]		False Negative Rate[22]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[23]	22	82	(18/22)	88	(15/17)	60	(3/5)	88	(15/17)	60	(3/5)	40	(2/5)	12	(2/17)
Prevalidation Study[24]	12	75	(9/12)	80	(8/10)	50	(1/2)	89	(8/9)	50	(1/2)	50	(1/2)	20	(2/10)
ECVAM Validation Study[25]	13	62	(8/13)	50	(4/8)	80	(4/5)	80	(4/5)	50	(4/8)	20	(1/5)	50	(4/8)
Submission Plus Validation Studies[26]	36	75	(27/36)	79	(22/28)	63	(5/8)	88	(22/25)	45	(5/11)	38	(3/8)	21	(6/28)
Gordon et al. (1994)	21	100	(21/21)	100	(20/20)	100	(1/1)	100	(20/20)	100	(1/1)	0	(0/1)	0	(0/20)
Entire Data Set[27]	47	81	(38/47)	84	(32/38)	67	(6/9)	91	(32/35)	50	(6/12)	33	(3/9)	16	(6/38)

[15] This table was created by NICEATM for use by the PRP during their review. It is based on the list of chemicals in Appendix A of this report

[16] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)

[17] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test (NIEHS, 1997)

[18] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test (NIEHS, 1997)

[19] Positive predictivity is defined as the proportion of correct positive responses among chemicals or chemical mixtures testing positive. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested (NIEHS, 1997)

[20] Negative predictivity is defined as the proportion of correct negative responses among chemicals or chemical mixtures testing negative. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested (NIEHS, 1997)

[21] False positive rate is defined as the proportion of all negative (noncorrosive) chemicals or chemical mixtures that are falsely identified as positive (NIEHS, 1997)

[22] False negative rate is defined as the proportion of all positive (corrosive) chemicals or chemical mixtures that are falsely identified as negative (NIEHS, 1997)

[23] Submission to ICCVAM by InVitro International, Inc. (IVI)

[24] Botham et al. (1995)

[25] Fentem et al. (1998)

[26] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998)

[27] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), ECVAM Validation Study (Fentem et al., 1998), and Gordon et al. (1994).

3.6.3 Acid derivatives (Table 7)

"Acid derivative" is a non-specific class designation and is broadly defined as an acid produced from a chemical substance either directly or by modification or partial substitution. This class includes anhydrides, haloacids, salts, and other types of chemicals. A more precise classification of these materials is needed to make a definitive statement about the utility of Corrositex® for these chemicals.

Based on the chemicals assigned to the acid derivatives class (N=14), Corrositex® has an accuracy of 93% (13/14), a sensitivity of 100% (7/7), a specificity of 86% (6/7), a positive predictivity of 88% (7/8), a negative predictivity of 100% (6/6), a false positive rate of 14% (1/7), and a false negative rate of 0% (0/7). No acid derivatives were included in the Prevalidation Study (Botham et al., 1995), so these values reflect the chemicals provided in the Submission and the ECVAM Validation Study (Fentem et al., 1998).

3.6.4 Amines (organic bases) (Table 8)

This data set includes primary, secondary, and tertiary amines. These chemicals are also classified as organic bases when test results are combined across chemical classes. Based on the 25 chemicals and chemical mixtures in this data set, Corrositex® has an accuracy of 80% (20/25), a sensitivity of 84% (16/19), a specificity of 67% (4/6), a positive predictivity of 89% (16/18), a negative predictivity of 57% (4/7), a false positive rate of 33% (2/6), and a false negative rate of 16% (3/19).

3.6.5 Inorganic bases and base mixtures (Table 9)

Based on the ten chemicals and chemical mixtures in this data set, Corrositex® has an accuracy of 90% (9/10), a sensitivity of 100% (9/9), a specificity of 0% (0/1), a positive predictivity of 90% (9/10), a false positive rate of 100% (1/1), and a false negative rate of 0% (0/9). The analysis was confounded by the limited number of chemicals and chemical mixtures tested. Additionally, more corrosive chemicals and chemical mixtures than noncorrosive chemicals and chemical mixtures have been tested, resulting in very low specificity (0%) and a high false positive rate (100%).

3.6.6 Inorganic and organic bases and base mixtures (Table 10)

Pooling the chemical classes containing inorganic and organic bases and base mixtures resulted in a data set of 35 chemicals. For this class, Corrositex® has an accuracy of 83% (29/35), a sensitivity of 89% (25/28), a specificity of 57% (4/7), a positive predictivity of 89% (25/28), a negative predictivity of 57% (4/7), a false positive rate of 43% (3/7), and a false negative rate of 11% (3/28).

3.6.7 Organic and inorganic acids and bases (Table 11)

Pooling the chemical classes containing organic and inorganic acids and bases resulted in a relatively large data set of 85 chemicals, with the distribution of chemicals weighted toward corrosives (63 corrosives versus 22 noncorrosives). For this class, Corrositex® has an accuracy of 81% (69/85), a sensitivity of 86% (54/63), a specificity of 68% (15/22), a positive predictivity of 89% (54/61), a negative predictivity of 63% (15/24), a false positive rate of 32% (7/22), and a false negative rate of 14% (9/63).

3.6.8 Cleaners and Detergents (Table 12)

Based on the 24 chemicals and chemical mixtures in this data set, Corrositex® has an accu-

Table 7. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Acid Derivatives)[28]

Data Source	Number of Chemicals	Accuracy[29]		Sensitivity[30]		Specificity[31]		Positive Predictivity[32]		Negative Predictivity[33]		False Positive Rate[34]		False Negative Rate[35]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[36]	14	93	(13/14)	100	(7/7)	86	(6/7)	88	(7/8)	100	(6/6)	14	(1/7)	0	(0/7)
Prevalidation Study[37]	0														
ECVAM Validation Study[38]	7	100	(7/7)	100	(5/5)	100	(2/2)	100	(5/5)	100	(2/2)	0	(0/2)	0	(0/5)
Submission Plus Validation Studies[39]	14	93	(13/14)	100	(7/7)	86	(6/7)	88	(7/8)	100	(6/6)	14	(1/7)	0	(0/7)
Gordon et al. (1994)	27	96	(26/27)	96	(26/27)	NA		100	(27/27)	0	(0/1)	NA		4	(1/27)
Entire Data Set[40]	39	95	(37/39)	97	(31/32)	86	(6/7)	97	(31/32)	86	(6/7)	14	(1/7)	3	(1/32)

NA = Not applicable

Table 8. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Amines)[28]

Data Source	Number of Chemicals	Accuracy[29]		Sensitivity[30]		Specificity[31]		Positive Predictivity[32]		Negative Predictivity[33]		False Positive Rate[34]		False Negative Rate[35]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[36]	14	93	(13/14)	91	(10/11)	100	(3/3)	100	(10/10)	75	(3/4)	0	(0/3)	9	(1/11)
Prevalidation Study[37]	11	73	(8/11)	75	(6/8)	67	(2/3)	86	(6/7)	50	(2/4)	33	(1/3)	25	(2/8)
ECVAM Validation Study[38]	8	88	(7/8)	86	(6/7)	100	(1/1)	100	(6/6)	50	(1/2)	0	(0/1)	14	(1/7)
Submission Plus Validation Studies[39]	25	80	(20/25)	84	(16/19)	67	(4/6)	89	(16/18)	57	(4/7)	33	(2/6)	16	(3/19)
Gordon et al. (1994)	8	100	(8/8)	100	(8/8)	NA		100	(8/8)	NA		NA		0	(0/8)
Entire Data Set[40]	31	87	(27/31)	88	(23/26)	80	(4/5)	96	(23/24)	57	(4/7)	20	(1/5)	12	(3/26)

NA = Not applicable

[28] This table was created by NICEATM for use by the PRP during their review. It is based on the list of chemicals in Appendix A of this report
[29] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)
[30] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test (NIEHS, 1997)
[31] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test (NIEHS, 1997)
[32] Positive predictivity is defined as the proportion of correct positive responses among chemicals or chemical mixtures testing positive. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested (NIEHS, 1997)
[33] Negative predictivity is defined as the proportion of correct negative responses among chemicals or chemical mixtures testing negative. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested (NIEHS, 1997)
[34] False positive rate is defined as the proportion of all negative (noncorrosive) chemicals or chemical mixtures that are falsely identified as positive (NIEHS, 1997)
[35] False negative rate is defined as the proportion of all positive (corrosive) chemicals or chemical mixtures that are falsely identified as negative (NIEHS, 1997)
[36] Submission to ICCVAM by InVitro International, Inc. (IVI)
[37] Botham et al. (1995)
[38] Fentem et al. (1998)
[39] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998)
[40] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), ECVAM Validation Study (Fentem et al., 1998), and Gordon et al. (1994).

Table 9. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Inorganic Bases and Base Mixtures)[41]

Data Source	Number of Chemicals	Accuracy[42]		Sensitivity[43]		Specificity[44]		Positive Predictivity[45]		Negative Predictivity[46]		False Positive Rate[47]		False Negative Rate[48]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[49]	8	88	(7/8)	100	(7/7)	0	(0/1)	88	(7/8)	NA		100	(1/1)	0	(0/7)
Prevalidation Study[50]	1	100	(1/1)	100	(1/1)	NA		100	(1/1)	NA		NA		0	(0/1)
ECVAM Validation Study[51]	4	50	(2/4)	100	(2/2)	0	(0/2)	50	(2/4)	NA		100	(2/2)	0	(0/2)
Submission Plus Validation Studies[52]	10	90	(9/10)	100	(9/9)	0	(0/1)	90	(9/10)	NA		100	(1/1)	0	(0/9)
Gordon et al. (1994)	3	100	(3/3)	100	(3/3)	NA		100	(3/3)	NA		NA		0	(0/3)
Entire Data Set[53]	11	82	(9/11)	100	(9/9)	0	(0/2)	82	(9/11)	NA		100	(2/2)	0	(0/9)

NA = Not applicable

Table 10 . Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to In Vivo Findings (Inorganic and Organic Bases and Base Mixtures)[41]

Data Source	Number of Chemicals	Accuracy[42]		Sensitivity[43]		Specificity[44]		Positive Predictivity[45]		Negative Predictivity[46]		False Positive Rate[47]		False Negative Rate[48]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[49]	22	91	(20/22)	94	(17/18)	75	(3/4)	94	(17/18)	75	(3/4)	25	(1/4)	6	(1/18)
Prevalidation Study[50]	12	75	(9/12)	78	(7/9)	67	(2/3)	88	(7/8)	50	(2/4)	33	(1/3)	22	(2/9)
ECVAM Validation Study[51]	12	75	(9/12)	89	(8/9)	33	(1/3)	80	(8/10)	50	(1/2)	67	(2/3)	11	(1/9)
Submission Plus Validation Studies[52]	35	83	(29/35)	89	(25/28)	57	(4/7)	89	(25/28)	57	(4/7)	43	(3/7)	11	(3/28)
Gordon et al. (1994)	11	100	(11/11)	100	(11/11)	NA		100	(11/11)	NA		NA		0	(0/11)
Entire Data Set[53]	42	86	(36/42)	91	(32/35)	57	(4/7)	91	(32/35)	57	(4/7)	43	(3/7)	9	(3/35)

NA = Not applicable

[41] This table was created by NICEATM for use by the PRP during their review. It is based on the list of chemicals in Appendix A of this report
[42] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)
[43] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test (NIEHS, 1997)
[44] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test (NIEHS, 1997)
[45] Positive predictivity is defined as the proportion of correct positive responses among chemicals or chemical mixtures testing positive. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested (NIEHS, 1997)
[46] Negative predictivity is defined as the proportion of correct negative responses among chemicals or chemical mixtures testing negative. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested (NIEHS, 1997)
[47] False positive rate is defined as the proportion of all negative (noncorrosive) chemicals or chemical mixtures that are falsely identified as positive (NIEHS, 1997)
[48] False negative rate is defined as the proportion of all positive (corrosive) chemicals or chemical mixtures that are falsely identified as negative (NIEHS, 1997)
[49] Submission to ICCVAM by InVitro International, Inc. (IVI)
[50] Botham et al. (1995)
[51] Fentem et al. (1998)
[52] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998)
[53] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), ECVAM Validation Study (Fentem et al., 1998), and Gordon et al. (1994).

Table 11. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Organic and Inorganic Acids and Bases)[54]

Data Source	Number of Chemicals	Accuracy[55]		Sensitivity[56]		Specificity[57]		Positive Predictivity[58]		Negative Predictivity[59]		False Positive Rate[60]		False Negative Rate[61]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[62]	58	88	(51/58)	93	(39/42)	75	(12/16)	91	(39/43)	80	(12/15)	25	(4/16)	7	(3/42)
Prevalidation Study[63]	24	75	(18/24)	79	(15/19)	60	(3/5)	88	(15/17)	43	(3/7)	40	(2/5)	21	(4/19)
ECVAM Validation Study[64]	32	75	(24/32)	77	(17/22)	70	(7/10)	85	(17/20)	58	(7/12)	30	(3/10)	23	(5/22)
Submission Plus Validation Studies[65]	85	81	(69/85)	86	(54/63)	68	(15/22)	89	(54/61)	63	(15/24)	32	(7/22)	14	(9/63)
Gordon et al. (1994)	58	98	(57/58)	98	(55/56)	100	(2/2)	100	(55/55)	67	(2/3)	0	(0/2)	2	(1/56)
Entire Data Set[66]	127	87	(110/127)	90	(94/104)	70	(16/23)	93	(94/101)	62	(16/26)	30	(7/23)	10	(10/104)

Table 12. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Cleaners and Detergents)[54]

Data Source	Number of Chemicals	Accuracy[55]		Sensitivity[56]		Specificity[57]		Positive Predictivity[58]		Negative Predictivity[59]		False Positive Rate[60]		False Negative Rate[61]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[62]	18	56	(10/18)	88	(7/8)	30	(3/10)	50	(7/14)	75	(3/4)	70	(7/10)	13	(1/8)
Prevalidation Study[63]	5	40	(2/5)	100	(2/2)	0	(0/3)	40	(2/5)	NA		100	(3/3)	0	(0/2)
ECVAM Validation Study[64]	1	100	(1/1)	NA		100	(1/1)	NA		100	(1/1)	0	(0/1)	NA	
Submission Plus Validation Studies[65]	24	54	(13/24)	90	(9/10)	29	(4/14)	47	(9/19)	80	(4/5)	71	(10/14)	10	(1/10)
Gordon et al. (1994)	3	100	(3/3)	NA		100	(3/3)	NA		100	(3/3)	0	(0/3)	NA	
Entire Data Set[66]	27	59	(16/27)	90	(9/10)	41	(7/17)	47	(9/19)	88	(7/8)	59	(10/17)	10	(1/10)

NA = Not applicable

[54] This table was created by NICEATM for use by the PRP during their review. It is based on the list of chemicals in Appendix A of this report
[55] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)
[56] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test (NIEHS, 1997)
[57] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test (NIEHS, 1997)
[58] Positive predictivity is defined as the proportion of correct positive responses among chemicals or chemical mixtures testing positive. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested (NIEHS, 1997)
[59] Negative predictivity is defined as the proportion of correct negative responses among chemicals or chemical mixtures testing negative. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested (NIEHS, 1997)
[60] False positive rate is defined as the proportion of all negative (noncorrosive) chemicals or chemical mixtures that are falsely identified as positive (NIEHS, 1997)
[61] False negative rate is defined as the proportion of all positive (corrosive) chemicals or chemical mixtures that are falsely identified as negative (NIEHS, 1997)
[62] Submission to ICCVAM by InVitro International, Inc. (IVI)
[63] Botham et al. (1995)
[64] Fentem et al. (1998)
[65] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998)
[66] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), ECVAM Validation Study (Fentem et al., 1998), and Gordon et al. (1994).

racy of 54% (13/24), a sensitivity of 90% (9/10), a specificity of 29% (4/14), a positive predictivity of 47% (9/19), a negative predictivity of 80% (4/5), a false positive rate of 71% (10/14), and a false negative rate of 10% (1/10). No conclusive statement can be made about the predictive value of Corrositex® for cleaners and detergents because of the lack of information about the identity of the ingredients in each product.

3.6.9 Undefined industrial chemicals (Table 13)

Based on the 28 unidentified chemicals and chemical mixtures in this data set, Corrositex® has an accuracy of 75% (21/28), a sensitivity of 87% (13/15), a specificity of 62% (8/13), a positive predictivity of 72% (13/18), a negative predictivity of 80% (8/10), a false positive rate of 38% (5/13), and a false negative rate of 13% (2/15). No conclusive statements can be made about the predictive value of Corrositex® for undefined industrial chemicals because of the lack of information about the identity of the ingredients in each product.

3.6.10 Surfactants (Table 14)

Based on the 22 noncorrosive chemicals and chemical mixtures in this data set, Corrositex® has an accuracy of 100% (22/22), a specificity of 100% (22/22), a negative predictivity of 100% (22/22), and a false positive rate of 0% (0/22). No conclusive statements can be said about this class of chemicals and chemical mixtures since the ingredients in the chemicals and chemical mixtures are not known and the only chemicals and chemical mixtures evaluated were noncorrosive.

3.7 UN Packing Group Classification

To ensure safety in the transport of hazardous materials, US DOT requires additional information on the severity of the corrosive response of a chemical or chemical mixture so that appropriate packing groups may be assigned. US DOT currently accepts the use of Corrositex® for assigning such packing groups (Appendix Q), which are determined by assessing the breakthrough times of a chemical or chemical mixture. Information on the ability of Corrositex® to accurately assign packing groups was provided in the IVI Submission (Table 23 of Appendix C). However, the PRP did not investigate the use of Corrositex® for this purpose.

3.8 Clinical relevance and human predictivity

The overall accuracy and the positive and negative predictivity indicate that Corrositex® results are generally similar to those obtained using the *in vivo* test. Information on which chemicals and chemical mixtures in the Submission and validation studies are human corrosives and noncorrosives is needed before an assessment can be made on the performance of this assay for predicting human response. Such information is not available.

3.9 Regulatory utility of the method

Corrositex® is limited in its universal utility because of the proportion of chemicals that are not compatible with the CDS and thus cannot be evaluated. Corrositex® performance indicates that, in specific testing circumstances such as that required by US DOT, Corrositex® is useful as a stand-alone assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives. The current US DOT exemption allows the use of Corrositex® for assigning packing groups for acids, acid derivatives, acyl halides, alkylamines and polyalkylamines, bases, chlorosilanes, metal halides, and oxyhalides. However, the database evaluated by the PRP did not include acyl halides, chlorosilanes, metal halides, or oxyhalides; thus

Table 13. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Industrial Chemicals)[67]

Data Source	Number of Chemicals	Accuracy[68]		Sensitivity[69]		Specificity[70]		Positive Predictivity[71]		Negative Predictivity[72]		False Positive Rate[73]		False Negative Rate[61]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[75]	22	73	(16/22)	93	(13/14)	38	(3/8)	72	(13/18)	75	(3/4)	63	(5/8)	7	(1/14)
Prevalidation Study[76]	3	100	(3/3)	NA		100	(3/3)	NA		100	(3/3)	0	(0/3)	NA	
ECVAM Validation Study[77]	3	67	(2/3)	0	(0/1)	100	(2/2)	NA		67	(2/3)	0	(0/2)	100	(1/1)
Submission Plus Validation Studies[78]	28	75	(21/28)	87	(13/15)	62	(8/13)	72	(13/18)	80	(8/10)	38	(5/13)	13	(2/15)
Gordon et al. (1994)	2	100	(2/2)	NA		100	(2/2)	NA		100	(2/2)	0	(0/2)	NA	NA
Entire Data Set[79]	30	77	(23/30)	87	(13/15)	67	(10/15)	72	(13/18)	83	(10/12)	33	(5/15)	13	(2/15)

NA = Not applicable

Table 14. Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Surfactants)[67]

Data Source	Number of Chemicals	Accuracy[68]		Sensitivity[69]		Specificity[70]		Positive Predictivity[71]		Negative Predictivity[72]		False Positive Rate[73]		False Negative Rate[61]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Submission[75]	14	100	(14/14)	NA		100	(14/14)	NA		100	(14/14)	0	(0/14)	NA	
Prevalidation Study[76]	5	100	(5/5)	NA		100	(5/5)	NA		100	(5/5)	0	(0/5)	NA	
ECVAM Validation Study[77]	2	100	(2/2)	NA		100	(2/2)	NA		100	(2/2)	0	(0/2)	NA	
Submission Plus Validation Studies[78]	22	100	(22/22)	NA		100	(22/22)	NA		100	(22/22)	0	(0/22)	NA	
Gordon et al. (1994)	5	100	(5/5)	100	(1/1)	100	(4/4)	100	(1/1)	100	(4/4)	0	(0/4)	0	(0/1)
Entire Data Set[79]	27	100	(27/27)	100	(1/1)	100	(26/26)	100	(1/1)	100	(26/26)	0	(0/26)	0	(0/1)

NA = Not applicable

[67] This table was created by NICEATM for use by the PRP during their review. It is based on the list of chemicals in Appendix A of this report
[68] Accuracy (concordance) is defined as the proportion of correct outcomes of a method (NIEHS, 1997)
[69] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test (NIEHS, 1997)
[70] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test (NIEHS, 1997)
[71] Positive predictivity is defined as the proportion of correct positive responses among chemicals or chemical mixtures testing positive. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested (NIEHS, 1997)
[72] Negative predictivity is defined as the proportion of correct negative responses among chemicals or chemical mixtures testing negative. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested (NIEHS, 1997)
[73] False positive rate is defined as the proportion of all negative (noncorrosive) chemicals or chemical mixtures that are falsely identified as positive (NIEHS, 1997)
[74] False negative rate is defined as the proportion of all positive (corrosive) chemicals or chemical mixtures that are falsely identified as negative (NIEHS, 1997)
[75] Submission to ICCVAM by InVitro International, Inc. (IVI)
[76] Botham et al. (1995)
[77] Fentem et al. (1998)
[78] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998)
[79] This includes chemicals or chemical mixtures provided in the Submission, Prevalidation Study (Botham et al., 1995), ECVAM Validation Study (Fentem et al., 1998), and Gordon et al. (1994).

no statement can be made by the PRP for these chemical classes. In other testing circumstances, and for other chemical and product classes, Corrositex® may be more appropriately used as part of a tiered assessment, in which negative responses must be followed by dermal irritation testing, and positive responses require no further testing unless the investigator is concerned about potential false positive responses. In either testing strategy, an investigator may conclude that confirmation testing is necessary based on supplemental information. As additional test results with Corrositex® are obtained, the utility of the assay may need to be reconsidered.

4.0 TEST METHOD RELIABILITY[80] (REPEATABILITY[81]/REPRODUCIBILITY[82])

4.1 Adequacy of intralaboratory repeatability and reproducibility evaluations

The Submission addresses the issue of intralaboratory variability. Data points were provided for six reference chemicals (n > 200). The standard deviations (SDs) of the breakthrough times for two of the six chemicals were reported to be about 5% of the mean values; for three others they were reported to be approximately 10%, and for the sixth chemical or chemical mixture the SD was shown to be about 18% (of a very small number). These data were not part of the data set audited.

In the Submission, individual breakthrough times of less than 240 minutes were provided for 80 chemicals and chemical mixtures. The number of replicates for these 80 chemicals/products ranged from two to six. The coefficients of variation (CVs) for intralaboratory variability were calculated for these 80 chemicals and chemical mixtures. With few exceptions, the CVs for these chemicals and chemical mixtures were all less than 5%. The maximum CV was 15.6%, which occurred for two chemicals (hydrobromic acid and 10-undecenoic acid). However, a mean value based on two replicates is probably not reliable, particularly when the mean is in the range of the experimental variability of the cut-off value between packing group designations. For example, the breakthrough times for hydrobromic acid (Packing Group II assigned on the basis of the *in vivo* test) are 2.53 and 2.03 minutes, giving a mean of 2.28 minutes and a SD of 0.35. Since the mean is less than three minutes, hydrobromic acid is classified to be in Packing Group I, an over-prediction compared to the *in vivo* test results. The determination of optimal cut-off values is critical for Corrositex®, but was not addressed in the Submission. The cut-off value of three minutes for separating Packing Groups I and II is particularly important, since it represents a relatively short time interval. It is not clear whether the cut-off values assigned have taken into consideration the experimental variability as well as the number of replicates used.

There has been limited, but probably sufficient, assessment of the intralaboratory reproducibility of Corrositex®. This conclusion is based on an assessment of all data, including those from the Prevalidation and Validation Studies and the IVI submissions to US DOT. It also takes into account the evaluation of the interlaboratory reproducibility of Corrositex® (see Sections 4.2-4.4), assuming that if the test results are reproducible between laboratories, they are also likely to be reproducible when tested within the same laboratory.

4.2 Adequacy of interlaboratory reproducibility evaluations

For chemicals and chemical mixtures that can be tested using Corrositex®, two types of data are produced. One set that includes specific breakthrough times is continuous; the other set

[80] Reliability is defined as a measure of the degree to which a test can be performed reproducibly within and among laboratories over time (NIEHS, 1997).

[81] Repeatability is defined as the closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period (NIEHS, 1997).

[82] Reproducibility is defined as the variability between single test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test samples (NIEHS, 1997).

is categorical and includes the breakthrough times recorded as >240 minutes for Category 1 test chemicals and chemical mixtures or as >60 minutes for Category 2 test chemicals and chemical mixtures. The latter set consists of chemicals and chemical mixtures that did not penetrate the biobarrier within the timescale defined by the prediction model.

The Submission addressed the issue of the interlaboratory reproducibility of Corrositex®. Ten chemicals were tested in five laboratories with six replicates per test chemical or chemical mixture. The extent of interlaboratory agreement appears to be acceptable. An ANOVA approach for estimating within-laboratory and between-laboratory variability would have been preferred.

Two studies were performed to evaluate specifically interlaboratory reproducibility: (1) a double-blind study of 29 chemicals which demonstrated an interlaboratory reproducibility of 93%, and (2) an end-user evaluation of 20 chemicals which demonstrated a reproducibility of 95%.

In the ECVAM Validation Study (Fentem et al., 1998), the three laboratories differed in only three cases in obtaining continuous or categorical values (i.e., corrosive versus noncorrosive results). Statistical evaluation of the intra- and inter-laboratory variabilities of the continuous data (the results for 20 of the 60 chemicals) indicated no systematic bias. The between-laboratory reproducibility of the breakthrough times was acceptable. Within laboratories, there were no cases where the corrosivity classification changed from one run to the next, although there was one case where a chemical or chemical mixture was nonqualifying in one run and noncorrosive in the other. Interlaboratory differences in classification were also quite rare; there were only two cases where a chemical was classified as corrosive in one laboratory and noncorrosive in the others.

One PRP member felt that insufficient data were available from independent laboratories to assess fully interlaboratory reproducibility. However, the unanimous consensus of the other PRP members was that there has been adequate evaluation of the interlaboratory reproducibility of Corrositex®, although conducting additional studies with less-experienced laboratories and a larger number of test chemicals and chemical mixtures could be informative. The reported interlaboratory reproducibility reflects the training and expertise of the laboratories participating in the validation studies. It is suggested that laboratories unfamiliar with conducting the test should obtain similar training and conduct tests with test reference chemicals before undertaking any testing of unknown chemicals and chemical mixtures. The *in vitro* test appears to be precise (i.e., the data points were in close agreement).

4.3 Reproducibility of reference chemicals or products

Results specifically for reference chemicals or controls were not available for evaluation. However, data on appropriate test chemicals and chemical mixtures have been reported (see Section 4.2), which enable an adequate assessment of the reproducibility of Corrositex® both within and between laboratories.

4.4 Repeatability and reproducibility of results

The results appear to be sufficiently repeatable and reproducible, both within and between laboratories.

The ECVAM Validation Study (Fentem et al., 1998) involved the testing of 60 coded chemi-

cals in three laboratories. In this study, some differences were found between the three laboratories in relation to the qualification of the test chemicals and chemical mixtures. For 26 of the 60 chemicals (43% of the test chemicals and chemical mixtures in the validation study), one or more laboratories found the chemical to be non-qualifying. In 18 of these cases, all three laboratories were unable to qualify the chemical, while in the remaining eight cases at least one laboratory qualified the chemical or chemical mixture and obtained a predicted corrosivity classification. In all of these eight cases, the classification determined was noncorrosive. This problem of nonqualification was especially obvious for the neutral organics (all nine of the neutral organics were nonqualifying in at least two of the laboratories), phenols (all five were nonqualifying in at least two of the laboratories), and electrophiles (seven of the eight chemicals were reported as non-qualifying by at least one laboratory). However, there were only two cases where a chemical was classified as corrosive by one laboratory and noncorrosive by the others.

4.5 Reproducibility and reliability of Corrositex® versus standard *in vivo* assays

The reproducibility of Corrositex® is expected to be better than that of most biological tests, since Corrositex® involves a standardized physico-chemical test system rather than a biological endpoint. It is impossible to compare directly the reproducibility of the *in vitro* and animal tests due to the lack of objective information on the intra- and inter-laboratory reproducibility of the rabbit skin corrosivity test procedure.

5.0 OTHER SCIENTIFIC REVIEWS

5.1 Literature reviewed

Three major reports are available on Corrositex® in the open literature. Two of the reports (Botham et al., 1995; Fentem et al., 1998) published in peer-reviewed journals present results of validation studies. The Botham et al. (1995) paper presents the results of a Prevalidation Study conducted to determine if Corrositex® had sufficient merit to warrant a validation study. Based on the results, a validation study was subsequently performed and reported in the Fentem et al. (1998) paper. The publication by Gordon et al. (1994) describes results of Corrositex® evaluations conducted by or for IVI or reported to IVI. This paper was published as a chapter of a book and was not peer-reviewed.

The Prevalidation Study (Botham et al., 1995) conducted during 1993 and 1994 involved the evaluation of 50 chemicals, half of which were corrosive and half of which were noncorrosive. Selection of these chemicals for testing was based on the availability of sufficient *in vivo* data to allow the chemicals to be unambiguously classified. IVI was one of the two participating laboratories. The subsequent ECVAM Validation Study (Fentem et al., 1998) was conducted during 1996 and 1997 and involved the evaluation of 60 coded chemicals representing a diverse group of chemical classes, including organic acids, organic bases, neutral organics, inorganic acids, inorganic bases, inorganic salts, electrophiles, phenols, and soaps/detergents. The Validation Study was conducted in three laboratories. Both studies, with the exception of one laboratory in the Prevalidation Study, were conducted in the "spirit" of GLP. Well-defined criteria for the selection of the chemicals evaluated and clear objectives of the validations were established. The selection and distribution of the chemicals for the Validation Study are described in a companion paper by Barratt et al. (1998) published along with the Fentem et al. (1998) report. Based on the conditions under which the validation studies (Botham et al., 1995; Fentem et al., 1998) were conducted, the PRP placed considerable weight on the results obtained and conclusions drawn from these studies.

The study reported by Gordon et al. (1994) evaluated 75 chemicals that could be classified as to corrosivity based on published US DOT-assigned packing group designations (49 CFR 172.101, 1992). The report also summarizes the results of several previously conducted Corrositex® studies of agrochemicals, petrochemicals, and household cleaners. Specific details on the conduct of these studies with respect to laboratories used, GLP compliance, criteria for selection of chemicals and full identification of the chemical evaluated were not provided. Most importantly, corresponding *in vivo* data were not available to make the appropriate performance comparisons; thus, the PRP did not use these data in the evaluation of performance.

Test results on some of the chemicals and chemical mixtures evaluated in the above-mentioned papers were also submitted by IVI among the 118 chemicals comprising the Submission. This overlap with data reported in the validation studies consists of two of the 37 qualifying chemicals from the Prevalidation Study (Botham et al., 1995), and 32 of the 40 qualifying chemicals from the ECVAM Validation Study (Fentem et al., 1998); however, only nine of these 32 chemicals were independently tested by IVI. Nine of the 75 chemicals in the Gordon et al. (1994) report were also included in the Submission, along with the corresponding *in vivo* data compiled by IVI.

5.2 Comparison of the findings of the published studies and the Submission

Both the validation studies (Botham et al., 1995; Fentem et al., 1998) and the Submission agree with respect to repeatability. In the Prevalidation Study reported by Botham et al. (1995), the data were sufficiently reproducible, both in terms of individual breakthrough times and the corrosivity classification derived, for the two laboratories that performed Corrositex®. In the Validation Study reported by Fentem et al. (1998), the intra- and inter-laboratory reproducibility was sufficient.

For both of the laboratories in the Prevalidation Study (Botham et al, 1995), 76% and 73%, respectively, of the chemicals qualified for Corrositex®. Similarly, 60% of the chemicals and chemical mixtures in the Validation Study (Fentem et al., 1998) qualified for testing. A much higher percentage of noncorrosives (60%) failed to qualify than corrosives (10%). Overall, there is adequate agreement between the validation studies and the IVI Submission in results obtained for chemicals that qualified for evaluation with Corrositex®. Accuracy is similar between the validation studies (75-76%) and the Submission (82%). Gordon et al. (1994) reported a much higher accuracy of 97%, which may be due to the test chemicals and chemical mixtures selected for evaluation. Sensitivity was about 71% in the ECVAM Validation Study but, in the Prevalidation Study, it was closer (81%) to the 92% reported in the Submission. Specificity was similar for both the Prevalidation Study and the Submission at about 70%. Specificity was slightly higher for the ECVAM Validation Study (81%). Sensitivity and specificity in the Gordon et al. (1994) study were at 97% and 100%, respectively. The validation studies and the Submission were generally similar relative to false positive rates. However, false negative rates were much higher in the validation studies than for the Submission (19- 29% versus 8%). False positive and negative rates were 0% and 3%, respectively, in the Gordon et al. (1994) study.

With regard to the utilization/functionality of Corrositex® for specific classes of chemicals, agreement between the two validation studies (Botham et al., 1995; Fentem et al., 1998) and the Submission was variable with respect to accuracy due to differences in results obtained between the two validation studies. However, for the classes of chemicals and chemical mixtures tested, the performance values between the Submission and at least one of the two validation studies were relatively similar. Differences in accuracy between the studies are possibly the result of the relatively small number of chemicals that comprised a chemical class, particularly as this relates to noncorrosives. Because of the small numbers of chemicals and chemical mixtures evaluated, a single missed prediction greatly shifts the percent accuracy value. The small number of chemicals and chemical mixtures evaluated for certain classes of chemicals also weakens any comparison of sensitivity, specificity, and false positive and negative rates for specific classes of chemicals between the validation studies and the Submission. However, there appears to be sufficient information to conclude that the false negative rate for acids and bases was higher in the two validation (Botham et al., 1995; Fentem et al., 1998) studies than in the Submission (21-23% versus 7%). ECVAM concluded that Corrositex® may be valid for testing specific classes of chemicals such as organic bases and inorganic acids. There was insufficient chemical class information in the Gordon et al. (1994) study for a reliable comparison with the Submission.

Regarding the overall ability of Corrositex® to assign accurately chemicals to UN packing groups, Gordon et al. (1994) indicates an agree-

ment of 88%, which is relatively close to the IVI value of 80%. However, the Gordon et al. (1994) study shows about equal occurrence of overestimates and underestimates, while the Submission reported 20% overestimation and 2% underestimation. The ECVAM Validation Study (Fentem et al., 1998) agrees with the Submission in the limited ability of the method to assign correctly Packing Group I to the chemicals evaluated (about 50% and 60% for the ECVAM Validation Study and the Submission, respectively). However, only about 62% of the chemicals and chemical mixtures were correctly assigned to Packing Group II/Packing Group III in the ECVAM Validation Study versus approximately 90% for the Submission.

ECVAM reported concern for the large number of chemicals (40%) that did not qualify for evaluation with Corrositex®, considering this a major limitation of Corrositex® as compared to other *in vitro* corrosivity assays. The concern was especially significant for phenols, neutral organics and electrophiles. ECVAM concluded that Corrositex® is robust, readily available commercially, and compares relatively well with other *in vitro* tests for corrosivity, the method suffers because of its incompatibility with many chemicals. ECVAM further concluded that Corrositex® performs moderately well in its ability to distinguish between corrosives and noncorrosives for chemicals that qualify for this method.

6.0 OTHER CONSIDERATIONS

6.1. Test method transferability

One of the most attractive features of this *in vitro* test is its apparent ease of transfer among properly equipped and staffed laboratories. The operating directions are explicit; data sheets are included; and there is a decision algorithm as part of the test method. The test solutions and biomembrane components are provided so that the issue of reagent variation is greatly minimized. No information was provided on difficulties in reconstituting the biomembrane; this could be a source of variance.

6.1.1 Sensitivity to minor protocol changes

The method as described seems to have a number of steps at which changes in technique or conditions between laboratories might affect the outcome. However, considering the high level of reproducibility for tests conducted in different laboratories, this does not seem to be an issue. The results of testing to demonstrate the effect of minor protocol changes was not provided by IVI, nor was it addressed in either validation study.

6.1.2 Reasonableness of required training and expertise

The testing is straightforward and does not require extensive training. Indeed, the Corrositex® Submission noted that after a two-hour training session on the scientific background and directions for use of the test, a technician from an end-user organization produced results with 20 test chemicals that showed 95% reproducibility with results obtained in the Sponsor's own laboratory. Thus, based on the assay description, it would seem that a technician with a suitable experience in chemistry would, with modest training, be able to handle the analyses well.

6.1.3 Ease in obtaining necessary equipment and supplies

Equipment, chemicals and chemical mixtures, and supplies needed to conduct this *in vitro* test are readily available to end-users with a chemical laboratory, or could be readily obtained from existing commercial sources. The only limitation with respect to obtaining the needed equipment and supplies might be the ability of the Sponsor to provide the test kits. Use levels were not predicted. However, the Sponsor indicated that they have a greater than ten-year supply of biobarrier material.

6.2 Cost-effectiveness

At the Peer Review Meeting, the Sponsor quoted a price of $575 for a kit to test two chemicals/ chemical mixtures, not including technical time. Based on this price, two chemicals would be tested by Corrositex® at a small cost savings over an *in vivo* rabbit study. The commercial price for the *in vivo* test is in the range of $800 to $1,000. This savings may be lost, if Corrositex® is utilized in a tiered approach where it would be required that findings using Corrositex® need to be verified with a rabbit study. However, fewer animals may need to be tested following Corrositex® results.

6.3 Reasonableness of time needed to conduct the test

The time required was thought to be reasonable by the PRP. Dr. Wei (IVI) stated at the Peer Review Meeting that a reasonable workload for one technician for one day would be eight to ten chemicals. The only concern is whether a technician can start successive test vials on the kind of schedule that will also allow accurate reading of the breakthrough time.

6.4 Additional information

The work of the PRP was impeded by the lack of a complete and organized collection of information submitted to ICCVAM by IVI. This made it difficult for the PRP to evaluate efficiently the information provided. The Sponsor also failed to supply a clear and concise statement of the proposed regulatory use of the test. It must be noted that ICCVAM guidelines were not available until after the Submission had been submitted to ICCVAM. Future submissions to ICCVAM will benefit from detailed guidance for submission preparation (ICCVAM, 1998). The descriptions of the test protocol could benefit from the addition of a flow diagram to illustrate the steps in the procedure, to accompany but not to replace the description. The use of photographs to illustrate the procedure also would help the novice analyst to become adept at the assay. Information on the composition of the biobarrier, its source and stability, within bounds of proprietary information, would be helpful.

6.5 Refinement, reduction, and replacement considerations

Since the method is designed as a replacement for animals, the adoption of Corrositex® would clearly reduce the requirement for animal testing for corrosivity. Therefore, it has the potential to eliminate the use of animals for the determination of corrosivity for those chemicals that can qualify for detection by this method. However, Corrositex® does not meet criteria for replacement of *in vivo* test in all evaluations because of the limited number of chemical classes for which it is applicable as a stand-alone assay. If used in a tiered approach, Corrositex® provides for reduction and refinement of animal use. For chemicals that test negative or do not qualify, these agents have a low likelihood of causing corrosive lesions if tested in animals (refinement).

6.6 Comparison to current regulatory use of pH extremes

In the September 28, 1992 Application for Exemption to the US DOT (IVI, 1992), IVI compared Corrositex® with a physico-chemical method that uses pH and acid/alkali reserve to predict dermal corrosivity; 19 acids and bases diluted to different pH and acid/alkali reserve were tested. The Sponsor reported that the physico-chemical method underestimated the corrosivity of six chemicals and overestimated a seventh. Corrositex® did not underestimate any of the 19 chemicals used and overestimated the corrosivity of six. No other data were noted in the Submission regarding the use of pH extremes to classify chemicals. This sample is too small to draw any useful conclusions about the physico-chemical method.

If Corrositex® were to be endorsed as a valid alternative to the rabbit test solely for identifying corrosive inorganic and organic acid and bases, then the advantages of Corrositex® over simple pH determination must be clearly stated. In this respect, a comparison of the corrosive/noncorrosive predictions obtained with either Corrositex® or pH, for the 60 test chemicals used in the ECVAM Validation Study (Fentem et al., 1998), showed that there were only three chemicals which would have been classified differently using Corrositex®: 2-methylbutyric acid (corrosive *in vivo*, corrosive with Corrositex®, noncorrosive on the basis of pH), dimethyldipropylenetriamine (corrosive *in vivo*, corrosive with Corrositex®, noncorrosive on the basis of pH), and 50% sodium carbonate (noncorrosive *in vivo*, corrosive with Corrositex®, noncorrosive based on pH). In addition, 25 of the 60 test chemicals were nonqualifying in the Corrositex® assay and, of these, 21 would not have invoked correct classification correctly solely on the basis of pH.

A more detailed comparison of data for pH extremes and Corrositex® by NICEATM (Appendices I and K) showed both pH and Corrositex® are adequate methods for identifying the corrosivity of chemicals in the extreme pH ranges (i.e., pH < 2 or pH > 11.5). However, Corrositex® was slightly but consistently more accurate than pH for predicting corrosivity at the extreme pH ranges. Corrositex® correctly identifies several noncorrosive chemicals in the extreme pH ranges that would be false positive results if analyzed by pH only. A number of chemicals in non-extreme pH ranges (pH >2 and pH <11.5) were identified as corrosive based on *in vivo* results. Corrositex® correctly identified the majority of these agents as corrosive. Also, Corrositex®, in contrast to a pH analysis, can be used for specifying packing group designations. Thus, it would appear that Corrositex® offers advantages over determination of pH extremes for the identification of corrosives.

6.7 Effectiveness for assessing corrosivity/ noncorrosivity

The method appears to be reliable for those chemicals and chemical mixtures that qualify for the test. A major concern is that a high percentage of chemicals and chemical mixtures used in the validation studies did not qualify for the test method. However, as a test to classify those chemicals and chemical mixtures that do qualify, Corrositex® appears to be reasonably robust. The analysis of chemicals and chemical mixtures that do not qualify will, obviously, require another testing procedure.

This *in vitro* test is especially less effective for chemicals and chemical mixtures with a pH between 5.0 and 8.5; chemicals and chemical mixture in this range show a significant rate of nonqualification because the CDS does not change color in this pH range. Certain chemicals and chemical mixtures containing dilute alkali, dilute sodium metasilicate, and a surfactant showed elevated false positive rates. Industrial cleaners have a high false positive rate and lubricants, refinery streams and kerosene additives were usually nonqualifying (Gordon et al. 1994).

7.0 RELATED ISSUES

7.1 Other test methods for this endpoint, or other endpoints to be evaluated by ICCVAM

Other methods for consideration for this endpoint include the rat skin TER assay, and tests using human skin models such as EPISKIN™ and EPIDERM™. EPISKIN™ and the rat skin TER assay have been shown to be scientifically valid and have been recommended by ECVAM (Fentem et al., 1998).

In regard to validation assessments of other endpoints, several assays were suggested for consideration including: the neutral red uptake *in vitro* phototoxicity assay, eye irritation assessments that incorporate *in vitro* cytotoxicity tests, acute oral toxicity assessments using structure activity relationships and *in vitro* methods, and assessment of dermal penetration models.

7.2. Suggestions/recommendations for corrosivity-related workshops or validation efforts

It was suggested that ICCVAM concentrate its resources predominantly on endpoints other than skin corrosivity. However, a means of achieving US regulatory authority acceptance of the results of *in vitro* corrosivity studies conducted primarily in Europe could be to organize a workshop to discuss the rat skin TER and EPISKIN assays, both of which were considered to be validated in the ECVAM Validation Study (Fentem et al.,1998). Another possibility would be a workshop on how to assess laboratory-to-laboratory variation for the validation of assays.

8.0 SUMMARY CONCLUSIONS AND RECOMMENDATIONS

The PRP has evaluated the extensive body of information on Corrositex®, in support of a request in the Submission that this assay be evaluated as an *in vitro* alternative to *in vivo* methods employed to assess dermal corrosivity. Corrositex® has been subjected to a series of intra- and inter-laboratory validation studies in which its reliability and relevance have been examined. The data set from the Submission and the two validation studies included 163 primary chemicals and chemical mixtures for which there were corresponding *in vivo* rabbit corrosivity data. Data on 118 chemicals and chemical mixtures were provided by IVI, while data on 77 chemicals and chemical mixtures were obtained from two peer-reviewed publications that evaluated the validity of Corrositex® (Botham et al., 1995; Fentem et al., 1998). There was overlap for 32 chemicals and chemical mixtures between IVI and the two validation studies (Botham et al., 1995; Fentem et al., 1998). Corrositex® data provided in Gordon et al. (1994) were not included in the overall performance assessment since the Corrositex® responses were compared against US DOT-assigned packing group designations instead of *in vivo* data. However, these data were provided for comparative purposes.

This review includes a description of the test method and an evaluation of data quality, assay performance, and assay reliability, as well as other considerations. Also, the adequacy of the method description, limitations and conditions of the assay, and its relevance to *in vivo* studies have been addressed.

8.1 Test method description

Corrositex® measures the time required for a chemical or chemical mixture to pass a hydrated collagen matrix and supporting filter membrane. Passage through these layers is observed by a color change in the CDS, an underlying aqueous solution of two pH indicator dyes. The time required to pass through the matrix is used as a measure of the corrosive potential of the chemical or chemical mixture under test. The Submission contained a thorough protocol. The scientific basis of the test was generally described in sufficient detail. Corrositex® is correlative in nature, rather than mechanistic. The matrix was engineered to block passage of certain chemicals and chemical mixtures for a period of time similar to the time allowed for that chemical or chemical mixture to stay in contact with rabbit skin without causing "necrosis or ulceration." While an acellular matrix might ulcerate, it cannot undergo necrosis. The dose and breakthrough time selection procedures were considered to be appropriate. Both were established as a result of the extensive evaluation of corrosive and noncorrosive chemicals and chemical mixtures and of knowledge gained through Prevalidation and Validation Studies. The amount of test chemical or chemical mixture used in Corrositex® is the same as those specified for the animal studies, but the actual concentration per unit surface area is approximately 8.5-fold higher. The qualification test with the CDS provides a number of benefits to the assay. It immediately eliminates chemicals and chemical mixtures that do not qualify for the test. It also provides the operator with a clear indication of the expected color change that the test chemical or chemical mixture would produce upon breakthrough of the biobarrier. The descriptions of data collection, data evaluation, and the decision criteria used to identify chemicals and chemical mixtures as qualifying or nonqualifying, Category 1 or 2, and corrosive or noncorrosive are well documented. The Corrositex® protocol provides for both positive and negative controls. The role of these controls is to assure that the test system is working

properly during each trial. Corrositex® does not make provision for determining the irritancy of noncorrosives nor does it make any such claim.

The following specific changes to the protocol were recommended:

(1) It should be explicitly stated that the biobarrier should be allowed to harden on a level surface and to cool overnight before use.

(2) Even though replicate variability has been shown to be very low, guidance should be provided on how to evaluate an aberrant value.

(3) The IVI Corrositex® Data Sheets provided with the test kit should have a provision for recording the performance of the positive and negative controls. These data should be used to determine the suitability of the test results.

(4) Description of the test protocol would benefit from the addition of a flow diagram illustrating the steps in the procedure.

8.2 Test method data quality

The IVI *in vitro* and *in vivo* experiments were not conducted in accordance with GLP guidelines. However, audits were conducted by NICEATM and by the NTP QAU; the audits compared the data provided in the Submission against original study records to verify accuracy and completeness. The auditors concluded that the errors and omissions identified did not alter the credibility of the IVI database. A formal audit of the ECVAM data conducted by a QAU was not conducted; however, all data submitted by the participating laboratories were verified against the original data sheets by ECVAM staff on at least three separate occasions. Similarly, rigorous checks of all calculations, data analyses, etc, were conducted independently by ECVAM staff and the Management Team representatives of the lead laboratories.

IVI used a consistent protocol when generating the data included in the Submission. The data resulting from the validation studies (Botham et al., 1995; Fentem et al., 1998) followed an established protocol that differed only slightly. The differences noted did not invalidate the studies conducted by IVI or the laboratories participating in the validation studies. The PRP urged compliance with GLP guidelines in future studies to improve data quality and credibility.

The PRP recommended that positive and negative control values should be reported concurrently with each assay to determine if the test is working properly.

8.3 Test method performance

Compared to *in vivo* rabbit skin corrosivity test results, this *in vitro* test had a sensitivity of 85% (76/89), specificity of 70% (52/74), and accuracy of 79% (128/163) for the chemicals and chemical mixtures provided in the Submission and the two validation studies (Botham et al., 1995; Fentem et al., 1998). The three data sets reviewed (Submission; Botham et al., 1995; Fentem et al., 1998) showed a similar degree of sensitivity, specificity, and accuracy.

When considered by chemical or product class, the sensitivity, specificity, and accuracy of Corrositex® were 79% (22/28), 63% (5/8), and 75% (27/36), respectively, for inorganic and organic acids plus acid mixtures; 100% (7/7), 86% (6/7), and 93% (13/14), respectively, for acid derivatives; 84% (16/19), 67% (4/6), and 80% (20/25), respectively, for amines; 89% (25/28), 57% (4/7), and 83% (29/35), respectively, for

inorganic and organic bases plus base mixtures; 86% (54/63), 68% (15/22), and 81% (69/85), respectively, for organic and inorganic acids and bases; 90% (9/10), 29% (4/14), and 54% (13/24), respectively, for cleaners and detergents; and 87% (13/15), 62% (8/13), and 75% (21/28), respectively, for undefined industrial chemicals. The specificity of Corrositex® for surfactants was 100% (22/22); no corrosive surfactants were tested.

The demonstrated linkage between Corrositex® and the *in vivo* rabbit skin corrosivity test is the ability of the assay to correctly identify *in vivo* rabbit corrosive and noncorrosive compounds. The generally favorable accuracy analysis of the *in vitro* data with the *in vivo* test results supports such a linkage. The assay is highly pH and reserve acidity and alkalinity sensitive (i.e., the more acidic or basic the chemical or chemical mixture, the more likely it is to give an accurate prediction). This is consistent with what is known about the *in vivo* dermal corrosion potential of a chemical or chemical mixture. The overall accuracy and the positive and negative predictivity indicate that Corrositex® shows a fair degree of agreement with the *in vivo* test. Corrositex® appears to perform better with certain classes of chemicals than with other chemical classes.

Because of the relatively small numbers of chemicals evaluated in some chemical classes and the unbalanced nature of corrosive versus noncorrosive chemicals (corrosive>>noncorrosive), definitive conclusions as to the adequacy of Corrositex® for some classes of chemicals are difficult to make with a high degree of confidence.

8.4 Test method reliability

The results appear to be sufficiently repeatable and reproducible, both within and between laboratories.

The reproducibility of Corrositex® is expected to be better than that of most biological tests, since Corrositex® involves a standardized physico-chemical test system rather than a biological endpoint. It is impossible to compare directly the reproducibility of the *in vitro* test and animal tests due to the lack of objective information on the intra- and inter-laboratory reproducibility of the rabbit skin corrosivity test procedure.

The evaluations of intra- and inter-laboratory reproducibility are generally thought to be adequate. Further relevant information could be generated by analyzing existing data on positive and negative controls, and by conducting ANOVA. Using a weight-of-evidence approach, including data from the published validation studies (Botham et al., 1995; Fentem et al., 1998) as well as those reported in the Submission, it appears that the intra- and inter-laboratory reproducibilities are acceptable. However, one reviewer felt that insufficient data were available from independent laboratories to fully evaluate interlaboratory reproducibility.

The PRP suggested that laboratories unfamiliar with conducting the test should obtain appropriate training and conduct tests with test reference chemicals before undertaking any testing of unknown chemicals and chemical mixtures.

8.5 Other scientific reviews

The PRP believes that, for chemicals that qualified for Corrositex®, the similarity in performance of the data sets in the published evaluations of Corrositex®, particularly the validation studies (Botham et al., 1995; Fentem et al., 1998) and the Submission, provide an indication as to the overall ability of the assay to predict correctly the corrosive and noncorrosive potential of various chemical classes. However, ECVAM found a higher degree of false nega-

tive results for Corrositex® than indicated in the Submission.

8.6 Other considerations

One of the most attractive features of Corrositex® is its apparent ease of transfer among properly equipped and staffed laboratories. Furthermore, slight changes in technique or conditions do not appear to affect test outcome. Extensive training is not required, and equipment and supplies are readily accessible. The cost of the Corrositex® test kit is less than the usual cost of an *in vivo* assay. The PRP noted that the evaluation was impeded by the lack of a complete and organized collection of data submitted to ICCVAM by IVI.

A more detailed comparison of data for pH extremes and Corrositex® by NICEATM (Appendices I and K) showed both pH and Corrositex® are adequate methods for identifying the corrosivity of chemicals in the extreme pH ranges (i.e., pH < 2 or pH > 11.5). Corrositex® was slightly but consistently more accurate than pH for predicting corrosivity at the extreme pH ranges. Corrositex® correctly identified several noncorrosive chemicals in the extreme pH ranges that would be false positive results if analyzed only by pH. A number of chemicals in non-extreme pH ranges (pH >2 and pH <11.5) were identified as corrosive based on *in vivo* results. Corrositex® correctly identified the majority of these agents as corrosive. Also, Corrositex®, in contrast to the pH extreme, can be used for specifying packing group designations. Thus, it would appear that Corrositex® offers advantages over determination of pH extremes for the identification of corrosives.

Given the ease and cost effectiveness of conducting a pH test, the PRP recommended that pH testing be conducted prior to use of the Corrositex®. Such information could be used in the future to re-evaluate the agreement between pH and Corrositex® in identifying corrosivity.

8.7 Related issues

Other methods for consideration for this endpoint include the rat skin TER assay and tests using human skin models, such as EPISKIN™ and EPIDERM™.

A means of achieving US regulatory authority acceptance to the results of *in vitro* corrosivity studies conducted primarily in Europe could be to organize a workshop to discuss the rat skin TER assay and EPISKIN™ assay, both of which were considered validated in the ECVAM Validation Study (Fentem et al., 1998). Another possibility would be a workshop assessing how to evaluate laboratory-to-laboratory variation for the validation of assays.

In regard to validation assessments of other endpoints, several suggestions of other assays to consider were made including: the neutral red uptake *in vitro* phototoxicity assay, eye irritation assessments that incorporate *in vitro* cytotoxicity tests, acute oral toxicity assessments using structure activity relationships and *in vitro* methods, and assessment of dermal penetration models.

8.8 *Has Corrositex® been evaluated sufficiently and is its performance satisfactory to support its proposed use for assessing corrosivity potential of chemicals or chemical mixtures?*

Corrositex® is limited in its universal utility because of the proportion of chemicals that are not compatible with the CDS and thus cannot be evaluated. Corrositex® performance indicates that, in specific testing circumstances such as that required by US DOT, Corrositex® is use-

ful as a stand-alone assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives. The current US DOT exemption allows the use of Corrositex® for assigning packing groups for acids, acid derivatives, acyl halides, alkylamines and polyalkylamines, bases, chlorosilanes, metal halides, and oxyhalides. However, the database evaluated by the PRP did not include acyl halides, chlorosilanes, metal halides, or oxyhalides; thus no statement can be made by the PRP for these chemical classes. In other testing circumstances, and for other chemical and product classes, Corrositex® may be more appropriately used as part of a tiered assessment, in which negative responses must be followed by dermal irritation testing, and positive responses require no further testing unless the investigator is concerned about potential false positive responses. In either testing strategy, an investigator may conclude that confirmation testing is necessary based on supplemental information. As additional test results with Corrositex® are obtained, the utility of the assay may need to be reconsidered.

8.9 *Does Corrositex® offer advantages with respect to animal welfare considerations (refinement, reduction, and replacement alternatives)?*

Corrositex® was developed as a laboratory alternative to the *in vivo* methods that are commonly employed to assess dermal corrosivity. The method does not meet the criteria for a total replacement of the *in vivo* test because of the limited number of chemical classes for which it is applicable. The use of Corrositex® as a stand-alone assay for testing acids and bases reduces and replaces the use of animals in corrosivity testing. Similarly, use of Corrositex® as a component of a tiered approach for testing other chemical and product classes reduces and refines the use of animals in testing.

8.10 Advantages of Corrositex®

The advantage of the Corrositex® method is that it is a non-animal test that can be used to evaluate the skin corrosive potential of selected classes of chemicals and chemical mixtures and as a basis for setting priorities for further testing, if necessary. The method demonstrates sufficient inter- and intra-laboratory reproducibility. The method has the additional advantage that large numbers of chemicals and chemical mixtures can be tested quickly and relatively simply in a standard chemical laboratory setting.

8.11 Limitations of Corrositex®

The false positive rate for Corrositex® is higher than may be desirable for some classes of chemicals. The overall false positive rate for the database evaluated was 30% (22/74) and the corresponding false negative rate was 15% (13/89). The *in vivo* method and the corresponding reference data set also have inherent limitations. However, the *in vivo* method is accepted by the regulatory agencies and is the current standard against which all corrosivity alternative tests are measured.

The principal limitation of the method is the large proportion of test chemicals and chemical mixtures that do not qualify to be tested by Corrositex®. For the 1998 Submission, IVI was instructed to only provide data on qualified samples. The ECVAM Validation Study (Fentem et al., 1998) found that 37% of the chemicals (22 of 60) selected for evaluation did not qualify for Corrositex®. That is, the chemical or chemical mixture was not capable of inducing a color change in the CDS so that the breakthrough of the chemical or chemical mixture through the biobarrier could be detected. Of the total data set evaluated, including the 1996 Submission, a total of 92 nonqualifiers

were found. The majority of nonqualifiers have a pH between three and ten. Of the 75 nonqualifying chemicals for which *in vivo* data were available, 64 chemicals (85%) were classified as noncorrosive in the *in vivo* test. This would suggest that only 15% of nonqualifying chemicals or chemical mixtures would be expected to be corrosive. Other *in vitro* methods for corrosivity have not shown the same limitation with respect to the qualification of test chemicals and chemical mixtures that have been found with Corrositex® (Fentem et al., 1998). Examples of other *in vitro* methods for corrosivity include the rat skin TER assay and the EPISKIN™ assay.

In addition, no information was provided that would support the predictive value of Corrositex® for complex mixtures. Although the results for many such chemicals and chemical mixtures were comparable to the *in vivo* results, in the absence of composition data, no confidence could be placed in the predictive value of the test as compared with its correlative value.

Another limitation or condition related to the evaluation of Corrositex® is the relatively small number of chemicals evaluated in some chemical classes. The small number of chemicals and chemical mixtures and the unbalanced distribution of corrosive and noncorrosive chemicals and chemical mixtures evaluated in each of several chemical classes reduces the confidence associated with any conclusions regarding the utility of the method for all chemical classes.

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List of Chemicals Evaluated in Corrositex®

The list provided on the following pages is a tabulation of all the chemicals that were evaluated in the papers/documents referenced below.

- Barratt, M. D., P. G. Brantom, J. H. Fentem, I. Gerner, A. P. Walker, and A. P. Worth. 1998. The ECVAM international validation study for *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicol. In Vitro 12:471-482.

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- InVitro International, Inc. Corrositex® ICCVAM Submission, May, 1998

Chemical/product class information was determined based on the information provided in the papers/submission. Where such information was not available, chemical/product class designations were assigned by National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff member Bonnie Carson, M.S., Organic Chemistry.

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
315		industrial chemical	+	+	I, II	II	Submission	
485		industrial chemical	+	+	I, II	II	Submission	
880		industrial chemical	+	+	I, II	II	Submission	
3000		industrial chemical	+	+	I, II	II	Submission	
4000		industrial chemical	+	+	I, II	II	Submission	
91440		petrochemical	-	-	NC	NC	Submission	
122 B		industrial chemical	+	+	I, II	II	Submission	
1702 RR		industrial chemical	+	+	I, II	II	Submission	
1703 CR		industrial chemical	+	+	I, II	II	Submission	
1709 B		industrial chemical	+	+	I, II	II	Submission	
3-11-23x Undyed		industrial chemical	-	+	NC	III	Submission	
3-12-02P		industrial chemical	-	+	NC	III	Submission	
3-12-03K Undyed		industrial chemical	-	+	NC	II	Submission	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
acetic acid	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution; glacial acetic acid. Human reference data reported that exposure frequently causes burns and deep ulceration with ultimate scarring.
acetic anhydride	anhydride/acid derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
acrylic acid	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution
allyl bromide	electrophile/halogen derivative	industrial chemical	+	-	R34/II & III	NC	Barratt et al. (1998); Fentem et al. (1998)	NQ Corrositex® data also obtained
aluminum bromide, anhydrous	acid derivative/metal halide/Lewis acid		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
aluminum chloride	acid derivative/metal halide/Lewis acid		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
1-(2-aminoethyl)piperazine	amine/organic base		+	+	II, R34	II, R34	Submission; Barratt et al. (1998); Fentem et al. (1998)	Fentem et al. (1998) lists the Corrositex® packing group as R34, while the submission lists it as II.
4-amino-1,2,4-triazole	amine/organic base		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
ammonium hydrogen difluoride	acid derivative/halogen derivative/halide salt		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
ammonium hydrogen sulfate	acid derivative/inorganic salt		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
o -anisoyl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
antimony tribromide	acid derivative/metal halide		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
antimony trichloride	acid derivative/metal halide/halide salt		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
APL 9372		cleaner	-	+	NC	II	Submission	
Armeen 2C	amine/organic base		-	-			Botham et al. (1995)	
Armeen CD	amine/organic base		+	+			Botham et al. (1995)	
Armeen TD	amine/organic base		+	-			Botham et al. (1995)	
Aromox DMMCD-W	amine oxide	surfactant	-	-			Botham et al. (1995)	
Arquad 16-50		cationic surfactant	+	NQ			Botham et al. (1995)	
Arquad C-33-W		cationic surfactant	-	-			Botham et al (1995)	
Arquad DMMCB-50		cationic surfactant	+	NQ			Botham et al. (1995)	
benzalkonium chloride	quaternary ammonium compound	surfactant	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
benzene sulfonyl chloride	halogen derivative/acid derivative		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
benzylacetone	neutral organic/ketone	industrial chemical	-	-	NC	NC	Barratt et al. (1998); Fentem et al. (1998)	NQ Corrositex® data also obtained
benzyl chloroformate	acid derivative/ester/halogen derivative		+	-	I	NC	Gordon et al. (1994)	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
boat bottom cleaner		cleaner	+	+	I, II	II	Submission	
boron trifluoride dihydrate	inorganic acid/Lewis acid/acid derivative/halogen derivative		+	+	I, R35	I, R35	Submission; Barratt et al. (1998); Fentem et al. (1998)	Decomposes. Fentem et al. (1998) list R35 as the Corrositex® packing group designation, while the Submission lists it as I.
boron trifluoride-acetic acid complex	acid mixture		+	+	II	II	Submission; Gordon et al. (1994)	
bromoacetic acid (55.6%)	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	
bromoacetic acid (8%)	organic acid		+	+			Botham et al. (1995)	
2-bromobutane; butyl bromide	electrophile/alkyl halide	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
BSS 9487		industrial chemical	+	+	NP	II	Submission	
butylamine	amine/organic base		+	+			Botham et al. (1995)	
butylamine (in ethanol/ethylene glycol 1:1)	amine/organic base		+	+	NP	III	Submission	concentration wt.- 81.8%
butylbenzene	neutral organic/aromatic hydrocarbon	industrial chemical	-	NQ			Botham et al. (1995)	
n -butyl propionate	neutral organic/ester	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
butyric acid	organic acid		+	+	II	II	Submission	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
butyric anhydride	anhydride/acid derivative		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
calcium carbonate	acid derivative/inorganic salt		-	-	NC	NC	Submission	
capric/caprylic acid	organic acid		+	-			Botham et al. (1995)	
caprylic acid	organic acid		+	-			Botham et al. (1995)	1/2 laboratories found the response to be NQ
carvacrol	organic acid/phenol		+	NQ	R34/II & III		Barratt et al. (1998); Fentem et al. (1998)	Borderline C/NC chemical, as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
Cascade		dishwashing detergent	-	+	NC	III	Submission	
Cellosolve + TX100		surfactant blend	-	-	NC	NC	Submission	concentration wt - 20.0/10.0%
chloroacetic acid	organic acid/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution. Human reference data reported that chloroacetic acid caused epidermal and superficial dermal burns, in addition to other systemic poisoning effects.
chromium(III) fluoride	acid derivative/inorganic salt		-	-	NC	NC	Submission	pH tested on 10% solution
cinnamaldehyde	electrophile/aldehyde		-	-	NC	NC	Submission; Barratt et al. (1998);	
coconut/palm soap (20:80)		soaps/ surfactants	-	-	NC	NC	[illegible]	
concentrated organic cleaner		cleaner	-	-	NC	NC	[illegible]	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
copper(II) chloride	acid derivative/inorganic salt		-	+	NC	II	Submission	pH tested on 10% solution
COR 11		cleaner	+	+	NP	III	Submission	
COR 18		cleaner	-	+	NC	III	Submission	
COR 19		cleaner	+	+	I, II	II	Submission	
COR 20		cleaner	+	+	NP	III	Submission	
COR 3		cleaner	+	+	I, II	II	Submission	
COR 4		cleaner	-	+	NC	III	Submission	
COR 6		cleaner	-	+	NC	II	Submission	
COR 9		cleaner	+	+	NP	III	Submission	
Coverage Plus		cleaner	+	-	NP	NC	Submission	
crotonic acid	organic acid		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
cyanuric chloride	acid derivative/halogen derivative		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
cyclohexylamine	amine/organic base		+	+	II	II	Submission; Barratt et al. (1998); Fentem et al. (1998)	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
1,9-decadiene	neutral organic/alkene	industrial chemical	-	-	NC	NC	Barratt et al. (1998); Fentem et al. (1998)	NQ Corrositex® data also obtained
degreaser		degreaser	-	-	NC	NC	Gordon et al. (1994)	#870 in table; other degreasers in table as well
Genapol LRO		anionic surfactant	-	-			Botham et al. (1995)	
Dequest 2000	organic acid		-	+			Botham et al. (1995)	
1,4-diaminobutane	amine/organic base		+	+			Botham et al. (1995)	
1,2-diaminopropane	amine/organic base		+	+	I, R35	II, R34	Submission; Barratt et al. (1998); Fentem et al. (1998)	Fentem et al. (1998) lists the Corrositex® packing group as R34, while the submission lists it as II.
dichloroacetic acid	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution
2,2-dichloroacetyl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
dichlorophenylphosphine	organic acid/halogen derivative		+	+	II	I	Gordon et al. (1994)	pH tested on 10% solution
dicyclohexylamine	amine/organic base		+	+	III	III	Submission; Gordon et al. (1994)	pH tested on 10% solution
diethylamine	organic base/amine		+	+			Botham et al. (1995)	
diethylenetriamine	amine/organic base		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
dilutable cleaner		cleaner	-	-	NC	NC	Gordon et al. (1994)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
dilutable cleaner		cleaner	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
N,N -dimethylbenzylamine	amine/organic base		+	+	III	III	Submission	pH tested on 10% solution
dimethylcarbamyl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
dimethyldipropylene-triamine	organic base/amine		+	+	R34, R35/I	R34		Fentem et al. (1998) lists the in vivo Corrositex® packing group as R35/I, while the submission lists it as R34.
dimethylisopropylamine	organic base/amine		+	+	R34/II & III	R34/R35		Fentem et al. (1998) lists the Corrositex® packing group as R34 in 2/6 tests and R35 in 4/6 tests. The submission lists the Corrositex® packing group as R34.
3,3'-Dithiodipropionic acid	organic acid		-	-	NC	NC		
dithiodipropionic acid	organic acid		-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
dodecanoic acid; lauric acid	organic acid		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
dodecyltrichlorosilane	acid derivative/silane		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
Dowanol PNB	neutral organic	industrial chemical	-	NQ			Botham et al. (1995)	
Duoquad		cationic surfactant	+	NQ			Botham et al. (1995)	
Elfan OS46		anionic surfactant	-	NQ			Botham et al. (1995)	
Empicol LZPV/C		anionic surfactant	-	equiv			Botham et al. (1995)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
Empigen OB	amine oxide	surfactant	-	-			Botham et al. (1995)	
Emplan CME	neutral organic	industrial chemical	-	-			Botham et al. (1995)	
Emplan KB2	neutral organic	industrial chemical	-	-			Botham et al. (1995)	
ethanolamine	amine/organic base		+	+	II	II	Submission	pH tested on 10% solution
Ethomeen T/25	amine/organic base		-	-			Botham et al. (1995)	
2-ethoxyethyl methacrylate	electrophile/ester	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
ethylenediamine	silane/amine		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
2-ethylhexylamine	amine/organic base		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
ethyl triglycol methacrylate	acid ester	industrial chemical	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
eugenol	organic acid/phenol		-	-	NC	NC	Barratt et al. (1998); Fentem et al. (1998)	Borderline NC/C chemical as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis); NQ Corrositex® data also obtained
ferric chloride; iron (III) chloride	acid derivative/halide salt/inorganic salt		+	+	II, R34	II, R34/R35	Submission; Barratt et al. (1998); Fentem et al. (1998)	In the Fentem et al. (1998) study, 4/6 calls designated the packing group as R34, while 2/6 designated the packing group as R35. The submission lists the packing group as II.
ferrous chloride tetrahydrate	acid derivative/metal halide		+	+	III	II	Gordon et al. (1994)	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
fluoboric acid; hydrogen tetrafluoroborate	inorganic acid		+	+	II, III	I	Submission; Gordon et al. (1994)	pH tested on 10% solution; Gordon paper references the DOT designated packing group as II, while the submission lists the *in vivo* packing group designation as III.
fluorosulfonic acid	inorganic acid		+	+	I	I	Gordon et al. (1994)	pH tested on 10% solution
formic acid	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution
formula # 100-016		industrial chemical	+	+	NP	III	Submission	
formula # 100-057B		industrial chemical	+	-	NP	NC	Submission	
formula # 100-088		industrial chemical	+	+	NP	III	Submission	
fuel additive		additive	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
fumaryl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
Genamin KDM-F		cationic surfactant	-	-			Botham et al. (1995)	
GIN 8672		industrial chemical	-	+	NC	II	Submission	
glycol bromoacetate (85%)	electrophile/acid derivative		+	+	R34, II & III	R34	Submission; Barratt et al. (1998); Fentem et al. (1998)	
n- heptylamine	organic base/amine		+	+	R34/II & III	R34	Submission; Barratt et al. (1998); Fentem et al. (1998)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
hexanoic acid	organic acid		+	discordant	R34/II & III	III, R34, NC	Submission; Barratt et al. (1998); Fentem et al. (1998)	Submission lists response as C, and assigned a Corrositex®-designated packing group of III. Botham et al. (1995) lists response as C. Fentem et al. (1998) lists the Corrositex® packing group as R34 in 2/6 tests and NC in 4/6 tests. The submission does not list an *in vivo* packing group.
n -hexanol	neutral organic/alcohol	industrial chemical	-	NQ			Botham et al. (1995)	
Hostaphat KLD	neutral organic	industrial chemical	-	NQ			Botham et al. (1995)	
hydrobenzenesulfonic acid	organic acid		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
hydrochloric acid (14.4% wt.)	inorganic acid		+	+	R34/II & III	R34	Submission; Barratt et al. (1998);	Human reference data reported that exposure frequently causes burns and deep ulceration with ultimate scarring.
hydrochloric acid/sulfuric acid/ citric acid	acid mixture		+	+	II	II		concentration wt. - 5.0/5.0/5.0%
hydrogenated tallow amine	organic base/amine		-	-	NC	NC	Submission; Barratt et al. (1998);	Borderline C/NC chemical, as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
hydrogen bromide, aqueous sol.; hydrobromic acid	inorganic acid		+	+	II	I		pH tested on 10% solution
hydroxylamine sulfate	amine/inorganic salt		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
iodine monochloride	acid derivative/halogen derivative/halide salt		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
isopropanol	neutral organic/alcohol	industrial chemical	-	NQ	NC		Barratt et al. (1998), Fentem et al. (1998)	
isostearic acid	organic acid		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
Lanc Super		cleaner	-	-	NC	NC	Submission	
lauric acid	organic acid		-	NQ			Botham et al. (1995)	
liquid bleach		bleach	-	-	NC	NC	Gordon et al. (1994)	
liquid bleach		bleach	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
lithium hydroxide monohydrate	inorganic base		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution.
LMCRK		industrial chemical	+	+	I, II	II	Submission	
maleic acid	organic acid		-	+	NC	II	Submission	pH tested on 10% solution. Human reference data stated that the chemical caused skin burns.
maleic anhydride	acid derivative/anhydride		+	+	III	II	Submission	pH tested on 10% solution. Human reference data stated that the chemical caused skin burns.
mercaptoacetic acid	organic acid		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution
2-mercaptoethanol, sodium salt (45% aq.)	inorganic base		+	+	R34/II & III	R34	[illegible]	
mercaptopropanol	thioalkanol	organic solvent/ industrial chemical	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
methacrolein	electrophile/unsaturated aldehyde		+	-	R34/II & III	NC	Barratt et al. (1998); Fentem et al. (1998)	NQ Corrositex® data also obtained
o- methoxyphenol guaiacol	organic acid/phenol		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	Borderline C/NC chemical as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
3-methoxypropylamine	organic base/amine		+	+	R34/II & III	R34	Barratt et al. (1998); Fentem et al. (1998)	
2-methylbutyric acid	organic acid		+	+	R34	R34		
4,4-methylenebis(2,6-di-*tert*-butylphenol)	organic acid/phenol		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
methyl laurate	neutral organic/ester	surfactant	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
methyl palmitate	neutral organic/ester	surfactant	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
4-(methylthio)benzaldehyde	electrophile/aldehyde		-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	Reducing agent (may affect MTT status)
menthyl trimethylacetate; methyl pivalate	neutral organic/ester	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
nitric acid	inorganic acid		+	+	I	I	Gordon et al. (1994)	pH tested on 10% solution. Human reference data stated that the chemical caused skin burns.
n -nonanol	neutral organic/alcohol	industrial chemical	-	NQ			Botham et al. (1995)	
nonyl acrylate	acid ester	industrial chemical	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
octadecyltrichlorosilane	acid derivative/silane		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
octanoic acid; caprylic acid	organic acid		+	-	R34/II & III	NC	Submission;	Borderline C/NC chemical, as judged subjectively from the proximity of the chemical to the chemical to the classification boundary (SAR analysis).
octanoic/decanoic acids (65:35)	organic acid		+	-	R34/II & III	NC	Submission; Barratt et al. (1998); Fentem et al. (1998)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
octanoic/decanoic acids (55:45)	organic acid		+	-	R34/II & III	NC	Barratt et al. (1998); Fentem et al. (1998)	
octanoic/decanoic acids (60:40)	organic acid		+	-	R34/II & III	NC	Barratt et al. (1998); Fentem et al. (1998)	
octyltrichlorosilane	acid derivative/silane		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
oleic/caprylic acid	organic acid		-	-			Botham et al. (1995)	concentration wt.- 70/30%
oleine/octanoic acid (70/30)	organic acid		-	-	NC	NC	Submission; [illegible]	
phenethyl bromide	electrophile/alkyl halide	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
phenylacetyl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
2-phenylethanol	neutral organic/alcohol	industrial chemical	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
phenyltrichlorosilane	acid derivative/silane		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
phosphoric acid	inorganic acid		+	+	II, R34	II, R34	Submission; Barratt et al. (1998); Fentem et al. (1998)	Fentem et al. (1998) list the Corrositex® packing group as R34, while the submission lists it as II. Human reference data stated that the chemical causes skin burns and deep ulceration with ultimate scarring.
phosphorus pentachloride	acid derivative/halogen derivative/inorganic acid/Lewis acid		+	+	I, R35	I, R35	Submission; Barratt et al. (1998); Fentem et al. (1998)	Produces fumes on contact with water-decomposes. Fentem et al. (1998) lists the packing group designation at R35, while the submission lists it as I.

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
phosphorus tribromide	acid derivative/halogen derivative/inorganic acid/Lewis acid		+	+	I, R35	I, R35	Submission; Barratt et al. (1998); Fentem et al. (1998)	Highly volatile; decomposes. Fentem et al. (1998) lists the packing group designation as R35, while the submission lists it as I.
potassium bisulfate; potassium hydrogen sulfate	inorganic acid derivative/inorganic salt/metal salt		+	+	I, II	II	Submission; Gordon et al. (1994)	pH tested on 10% solution
potassium hydroxide, 10% aq.	inorganic base		+	+	II	II, R34	Barratt et al. (1998); Fentem et al. (1998); Gordon et al. (1994)	Colored test material. Fentem et al. (1998) lists the Corrositex® packing group as R34, while the submission lists it as II. Fentem et al. (1998) does not provide an *in vivo* packing group designation.
potassium hydroxide, 5% aq.	inorganic base		discordant	+		R34	Submission;	Submission lists *in vivo* response as C, while Fentem et al. (1998) lists it as NC.
propionic acid	organic acid		+	+	II	II	Submission	pH tested on 10% solution
Proxel AB	neutral organic	industrial chemical	-	-			Botham et al. (1995)	
Proxel BD	neutral organic	industrial chemical	+	NQ			Botham et al. (1995)	
pyrrolidine	amine/organic base		+	+			Botham et al. (1995)	
RP-26		surfactant	-	-	NC	NC	Submission	
RP-29		surfactant	-	-	NC	NC	Submission	
RP-33		surfactant	-	-	NC	NC	Submission	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
RP-34		surfactant	-	-	NC	NC	Submission	
RP-39		surfactant	-	-	NC	NC	Submission	
RP-40		surfactant		-	NC	NC	Submission	
RP-46		surfactant	-	-	NC	NC	Submission	
RP-49		surfactant	-	-	NC	NC	Submission	
RP-52		surfactant	-	-	NC	NC	Submission	
RP-53		surfactant	-	-	NC	NC	Submission	
RP-57		surfactant	-	-	NC	NC	Submission	
RP-61		surfactant	-	-	NC	NC	Submission	
RP-80B		surfactant	-	-	NC	NC	Submission	
RP-81B		surfactant	-	-	NC	NC	Submission	
selenic acid	inorganic acid		+	+	I	I	Gordon et al. (1994)	pH tested on 10% solution
sodium bicarbonate; sodium hydrogen carbonate	inorganic salt/acid derivative		-	-	NC	NC	Submission	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
sodium bisulfite; sodium sulfite carbonate	inorganic salt/acid derivative		-	-	NC	NC		
sodium carbonate, 50% aq.	inorganic base		-	+	NC	R34		
sodium hydrogen fluoride	inorganic salt/acid derivative		-	-	NC	NC	Submission; Gordon et al. (1994)	pH tested on 10% solution
sodium hydroxide	inorganic base		+	+	II	II	Botham et al. (1995); Gordon et al. (1994)	pH tested on 10% solution; solid. Human reference data indicated that various lyes caused chemical burns.
sodium hydroxide/BAC (1:10)	base mixture		+	+	NP	III	Submission	
sodium hydroxide/sodium dodecyl sulfate (SDS) (1:10)	base mixture		+	+	NP	III	Submission	
sodium hydroxide/SMS (1:10)	base mixture		+	+	II	II	Submission	
sodium hydroxide/SMS (5:3)	base mixture		+	+	NP	II	Submission	
sodium hydroxide/TX 100	base mixture		+	+	I, II	II	Submission	
sodium hypochlorite+5% chlorine	acid derivative/inorganic salt		-	-	NC	NC	Submission	pH tested on 10% solution
sodium lauryl sulfate, 20% aq.; SDS; SLS; sodium dodecyl sulfate; irium		soaps/surfactants	-	NQ	NC		Barratt et al. (1998); Fentem et al. (1998)	
sodium metasilicate	inorganic salt	cleaner	+	+			Botham et al. (1995)	concentration wt. - 20.0%
sodium perborate	inorganic salt	cleaner	-	+			Botham et al. (1995)	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
sodium percarbonate	inorganic salt	cleaner	-	+			Botham et al. (1995)	
sodium silicate A140	inorganic salt	cleaner	+	+			Botham et al (1995)	
sodium silicate H100	inorganic salt	cleaner	-	+			Botham et al. (1995)	
sodium undecylenate		soaps/surfactants	-	-	NC	NC		
Solcenic 2BW		industrial chemical	-	-	NC	NC	Submission	
Solcenic 3A		industrial chemical	-	-	NC	NC	Submission	
Solcenic 3B		industrial chemical	-	-	NC	NC	Submission	
sulfamic acid; midosulfonic acid	inorganic acid		-	+	NC	II, R34		Fentem et al. (1998) list the Corrositex® packing group as R34, while the submission lists it as II. Human reference data reported that exposure frequently causes burns and deep ulceration with chimate scarring.
sulfuric acid, 10% wt.	inorganic acid		+	+	I	R34, I	Submission; Barratt et al. (1998);	Fentem et al. (1998) list the designation as C, but further state that supporting data do not enable unequivocal classification as either R34 (II/III) or R35 (I); more probable to be R34 (II/III). The submission lists the classification as R34. Gordon et al. (1994) lists both the Corrositex® packing group and DOT packing group as I. Fentem et al. (1998) does not list an *in vivo* packing group.
sulfuric acid/Cellusolve	acid mixture		+	+	I, II	II		
sulfuric acid/ferric chloride	acid mixture		+	+	I, II	II	Submission	
sulfuric acid/SDS	acid mixture		+	+	I, II	II	Submission	concentration wt. - 5.0/10.0%

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
sulfuric acid /Triton X-100	acid mixture		+	+	I, II	II	Submission	concentration wt. - 5.0/10.0%
sulfur monochloride	acid derivative/halogen derivative		+	+	I	II	Gordon et al. (1994)	pH tested on 10% solution
sulfurous acid	inorganic acid		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
surfactant		amphoteric surfactant	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
surfactant		anionic surfactant	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
surfactant		anionic surfactant	-	-	NC	NC	Gordon et al. (1994)	pH tested on 10% solution
Synprolam	amine/organic base		+	-			Botham et al. (1995)	
tallow amine	organic base/amine		+	-	R35, II	NC	Submission: Barratt et al. (1998); Fentem et al. (1998)	The *in vivo* exposure time may have been greater than 3 min. Necrosis was observed in two of the three rabbits only from day 7.
TBQ		industrial chemical	-	+	NC	III		
2-*tert*- butylphenol	organic acid/phenol		+	NQ	R34/II & III		Barratt et al. (1998); Fentem et al. (1998)	Borderline CNC chemical as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
tetrachloroethylene; perchloroethylene; pcm	neutral organic/halogen derivative	cleaner	-	-	NC	NC	Barratt et al. (1998); Fentem et al. (1998)	NQ Corrositex® data also obtained
tetraethylenepentamine	amine/organic base		+	+	III	III	Gordon et al. (1994)	pH tested on 10% solution
tetramethylammonium hydroxide	base/quaternary ammonium salt	surfactant	+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
thiophosphoryl chloride	acid derivative/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
Tide		laundry detergent	-	+	NC	III	Submission	
Toilet Duck Green		cleaner	-	-	NC	NC	Submission	
tributylamine	organic amine		-	-	NC	NC	Submission	pH tested on 10% solution
trichloroacetic acid	organic acid/halogen derivative		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
trichlorotoluene	halogenated aromatic/ aryl halide		-		NC	NC	Submission; Gordon et al. (1994)	pH tested on 10% solution
triethanolamine	organic base/amine		-	+			Botham et al. (1995)	concentration wt % 95.0
triethylenetetramine	amine/organic base		+	+	II	II	Gordon et al. (1994)	pH tested on 10% solution
trifluoroacetic acid	organic acid/halogen derivative		+	+	I	II	Gordon et al. (1994)	pH tested on 10% solution
n -undecanol	neutral organic/alcohol	industrial chemical	-	NQ			Botham et al. (1995)	
10-Undecenoic acid	organic acid		-	-	NC	NC	Submission; Barratt et al. (1998); Fentem et al. (1998)	Borderline NC/C chemical, as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
valeryl chloride; pentanoyl chloride	acid derivative/halogen derivative		+	+	II	II		pH tested on 10% solution
Windex Blue		glass cleaner	-	+	NC	III	Submission	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Chemical Name	Chemical Class	Product Class	*In Vivo*	Corrositex®	*In Vivo* Packing Group	Corrositex® Packing Group	References	Comment
2,4-xylidine	organic base/amine		-	discordant	NC	NC	Submission; Barratt et al. (1998); Fentem et al. (1998)	The submission listed Corrositex® response as NC. Fentem et al. (1998) lists the Corrositex® packing group as R34 in 2/6 tests and NC in 4/6 tests.
ZFA		cleaner	-	-	NC	NC	Submission	
ZWS 9352		cleaner	+	+	NP	III	Submission	

Abbreviations: aq=aqueous; C=corrosive; NC=noncorrosive; NP=not provided; NQ=nonqualifying chemical; SAR=structure activity relationship; wt.=weight

Characterization of the Data Sources (Appendix A) Used for Performance Analyses of Corrositex® [1]

Table 1. Number of qualifying chemicals overlapping between sources (based on the total data set)

	Total	Submission (1998)	Fentem et al. (1998)	Botham et al. (1995)	Gordon et al. (1994)
IVI submission (1998)	118		32[a]	2[b]	9
Fentem et al. (1998)	40	32[a]		1	2
Botham et al. (1995)	37	2[b]	1		8
Gordon et al. (1994)	75	9	2	8	
Total Data Set	221[c]				

[a] Data submitted by InVitro International (IVI) in the Submission for twenty-three chemicals were obtained from the Fentem et al. (1998) Validation Study. Discordant Corrositex® results between IVI and Fentem et al. (1998) were found for two chemicals (2,4-xylidine and hexanoic acid); both were classified as positive by IVI. These chemicals were considered as positive in the total data set performance calculations. In the individual source data set calculations, neither of these chemicals was included in the performance calculations of the Fentem et al. (1998) data set since they were discordant between laboratories within the validation study. Discordant *in vivo* results between source papers were found for one chemical (potassium hydroxide, 5% aq.). When this chemical was included in the total data set calculations, it was considered to be positive. In the data sets for the individual papers, the *in vivo* finding reported in the respective paper was used.

[b] Discordant Corrositex® results between source papers were found for one chemical (hexanoic acid) (see footnote "a"). Discordant Corrositex® results between laboratories in the Botham et al. (1995) paper were found for one compound (Empicol LZPV/C); this compound was not included in the performance calculations for this paper.

[c] This total is less than the sum of the chemicals reported in the individual papers because of the overlap in chemicals reported in multiple papers. This total was derived from the following:

- number of chemicals reported in a single paper = 174 (includes one compound [2,4-xylidine] actually reported in two papers but only included in calculations for one paper due to discordance [see footnote "a"]);

[1] See tables 5-14

- number of chemicals reported in two papers = 45 (includes one compound [hexanoic acid] actually reported in three papers but only included in calculations for two papers due to discordance [see footnotes “a” and “b”]); and

- number of chemicals reported in three papers = 2.

Corrositex® Test Method Submission (May, 1998)

EXECUTIVE SUMMARY

InVitro International's Corrositex® test is a laboratory alternative to the *in vivo* methods that are commonly employed to assess dermal corrosivity. Previous investigations have demonstrated the utility of this *in vitro* method and it is now accepted by the U.S. Department of Transportation (DOT E-10904) as a satisfactory means of defining the corrosivity of Class 8 hazardous materials.
In addition, EPA has approved Corrositex as Method 1120 for the characterization of solid waste.

In early 1996, at the request of the chairman of the Consumer Product Safety Commission, the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) performed an initial technical review of much of the available Corrositex data. This review resulted in a request for additional information and clarification. InVitro International responded in June 1996 and provided the requested information with a data base consisting of 419 individual chemicals and compounds. However, a lot of the materials included in that data base can not be identified. Therefore, notebook copies of these data points can not be located. A new table with 118 chemicals and compounds is constructed and evaluated. This new table included the data points that can be backed up with notebook copies and those from the ECVAM validation study. Analysis of this revised information provided similar observations to the June 1996 submission:

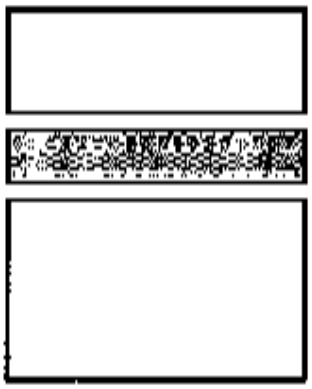
- The Corrositex test is comparable to the *in vivo* test with regard to its ability to assess the corrosivity of well-defined inorganic and organic acids and bases.
- The Corrositex test is comparable to the *in vivo* test with regard to its ability to assess the corrosivity of complex mixtures of inorganic and organic acids and bases.
- As judged by sensitivity, specificity, false positive rates, and false negative rates, the Corrositex test tends to "err on the side of safety."
- The Corrositex test appears to exhibit an increased false positive rate for industrial cleaner formulations, i.e., mixtures composed of dilute alkali, dilute sodium metasilicate, and a surfactant. This over-estimation of corrosivity tends to favor public safety.

Taken together, these observations suggest that the Corrositex test, as defined by the parameters embodied in DOT E-10904, is a very suitable and safe *in vitro* alternative to the *in vivo* methods that are employed to assess the dermal corrosivity of hazardous materials. We ask for regulatory support where Corrositex has been proven to work well; and that it not be held back because there may be some areas where Corrositex does not work as well.

A. ADDITIONAL INFORMATION REQUESTED

1. *Describe the proposed use of Corrositex® in the context of current regulatory requirements for corrosivity testing for each of the respective regulatory agencies. Describe if such will require changes in current testing requirements.*

As a small business, we do not have adequate internal or external resources to answer this question by conducting a complete review of federal regulations. However, based on our understanding of the mechanism of action of the Corrositex test (Figures 1 and 2) and an evaluation of our experience to date, it is most likely that this *in vitro* test method would be best suited for the determination of the corrosivity of acidic and alkaline compounds. Consequently, agencies whose corrosivity regulations are predicated on assessment of acidity and alkalinity might be expected to consider the use of this *in vitro* alternative.

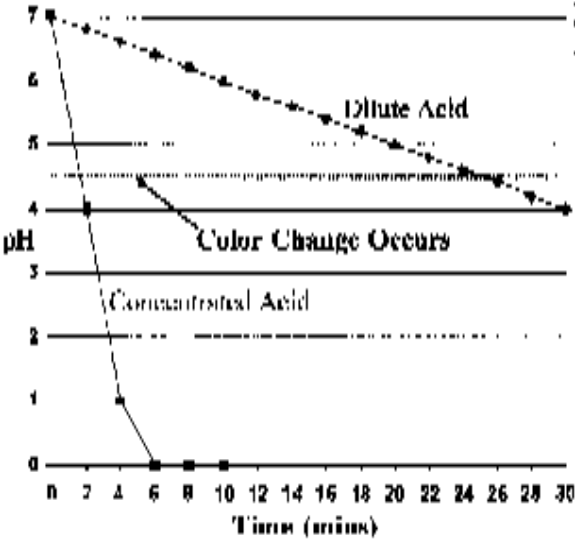


Collagen gel is predominantly composed of water, with small amounts of dissolved protein. Most test materials actually **diffuse through** the aqueous phase of this gel. Only the most corrosive materials (Packing Group I) will destroy this portion of the biobarrier.

Porous cellulose membrane permits free **diffusion** of chemicals whose molecular weight is <12,000. Only the most corrosive substances actually burn a hole in the membrane.

Chemical Detection System is composed of water and two pH indicator dyes. The pH of the CDS is 7. The acid indicator dye changes color when the pH of the solution drops below 4.5. The basic indicator dye changes color when the pH rises above 8.5. Therefore, acids and bases that enter the CDS are detected because they promote a visible change in the color of these indicator dyes. **NOTE: Chemicals that** do not cause the pH to change appreciably will **not qualify** for the assay because they fail to provoke a color change.

Figure 1. Detailed Description of the Components of the Corrositex® Test



When a test material diffuses through the biobarrier, it mixes with and alters the pH of the CDS. A color change occurs when the pH of the CDS falls below 4.5 or rises above 8.5. The time that is required to cause this change in pH is governed by three factors:

1. **the strength** of the acid or base,
2. **the rate of diffusion** of the test material and,
3. for very corrosive substances, **the rate of destruction** of the biobarrier.

These mechanisms are depicted in the graph shown at the left. In this **example**, concentrated and dilute **sulfuric acid** (H_2SO_4) were **analyzed** with the Corrositex® test. Because the rate of diffusion is proportional to concentration, the concentrated acid diffused through the biobarrier more rapidly than the dilute acid solution. Additionally, the concentrated H_2SO_4 reacted chemically with (hydrolyzed) the collagen and cellulose to cause destruction of the biobarrier. As a result, the concentrated acid entered the CDS and caused a color change in less than 2 minutes. By contrast, the dilute acid required 25 minutes to diffuse through the biobarrier and induce a visible color change. Accordingly, the concentrated acid was considered to be a Packing Group I material and the dilute acid was a Packing Group II substance.

Figure 2. Diffusion Times and Acid/Base Strength Govern "Breakthrough" Times

2. *Describe how use of Corrositex will produce results that are at least equivalent for risk assessment purposes as the method that it is proposed to replace. In particular, the predicted frequency and implications of false negatives and false positives should be addressed.*

The new database, which contained a description of 118 distinct samples (Appendix IV), was employed as the primary source of information for this response. The 2X2 contingency table is shown below.

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	59	16	75
	Noncorrosive	5	38	43
	Total	64	54	118

Table 1. Comparison of Corrositex Results with *In Vivo* Results.

These observations were analyzed further by performing the calculations shown in Table 2 shown below.

Parameter	Formula	Results
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 97/118 = 82%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 59/64 = 92%**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 38/54 = 70%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 59/75 = 79%**
Predictive Value = (Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 38/43 = 88%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 16/54 = 30%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 5/64 = 8%**

Table 2. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings

These findings demonstrate that the Corrositex test correlates well with the accepted *in vivo* method of assessing dermal corrosivity. The sensitivity and specificity of the laboratory test favors identification of corrosive substances. This observation is also substantiated by noting that the false positive rate is greater than the false negative rate. All of these observations are consistent with the stated developmental objective of providing an *in vitro* test that tended to "err on the side of safety."

3. *Provide in vivo reference data including a description of the quality of the data, protocol used to generate the data, the data from the tests, and a summary of the actual results. The nature, quality, and source of any other in vivo reference data should be provided. Describe the definition of corrosivity used in the in vivo tests, such as ulceration, necrosis, or other specified irreversible change. Indicate if the in vivo data are available for examination.*

In vivo data are included in Appendix V for data audit purpose. The entire content of Appendix V is confidential. The *in vivo* data are summarized together with Corrositex data in Appendix IV. The protocol and definition of corrosivity are described in Appendix V.

4. *Indicate if the Corrositex and in vivo data were generated in accordance with GLPs. Describe those aspects that were not performed in accordance with GLPs, and the potential impact of such deviations from non-adherence to GLPs.*

Notebook copies for data points summarized in Appendix IV are included in Appendix VI for data audit purpose. The entire content of Appendix VI is confidential. The scientific notebooks of InVitro International's scientists and technicians are maintained on file at the company's Irvine, CA facility. These documents were reviewed during the preparation of this response and both the quality and the quantity of the data contained in these records was found to be sufficient to support the current and prior applications. However, it was noted that strict adherence to GLP was not maintained. For example, not all of the notebooks have been signed by the technician who performed the work or the technicians' supervisor and not all partially completed notebook pages have been lined out. In spite of this, the underlying data is sound and it is unlikely that these technical failures to comply with GLP have a significant negative impact on the outcomes of the studies reported here.

5. *Indicate if laboratory records have been maintained for the testing that has been conducted, and if these records would be available for examination if requested.*

Laboratory records have been maintained for the testing that has been conducted. All of these records are available for on-site examination if requested.

6. *Indicate whether there are Corrositex testing data for the range of chemicals and products regulated by agencies that have or will be asked to accept this method, and indicate the specific classes of chemicals and products for which this method is being proposed.*

As noted in response to Question 1, the Corrositex test was specifically designed to assess the corrosivity of acids and bases. Consequently, the types of chemicals that have been assessed to date have predominantly been those that are known to be consistent with the DOT description of Class 8 corrosives. The current DOT exemption (Appendix VII) indicates that the Corrositex test is suited for assessing the corrosivity of the following classes of chemicals:

- Acids, inorganic and organic
- Acid derivatives (anhydride, haloacids, salts, etc.), inorganic and organic
- Acyl halides
- Alkylamines and polyalkylamines
- Bases, inorganic and organic
- Chlorosilanes
- Metal halides and oxyhalides.

7. *Develop 2X2 tables for each study showing the degree of agreement between the in vitro and in vivo tests. This should include calculations for concordance, sensitivity, specificity, false positive rate, false negative rate, and positive and negative predictivity. Also, it is important to know, as a function of chemical class and product category, the proportion of discordant calls that were "under" and "over."*

The revised data base consisting of 118 samples (Appendix IV) was sorted to identify compounds and chemicals that could be assigned to defined classes. This resulted in a data base consisting of **63** materials (Appendix VIII). The remaining **55** compounds consisted of mixtures and proprietary products that the composition is unknown. These undefined compounds are compiled on a separate list found in Appendix IX. Data found only in Appendix VIII was then utilized to develop the 2X2 contingency tables and performance analysis for each chemical class as shown below.

When **inorganic and organic acids** as well as **acid mixtures** were delineated within the data base found in Appendix VIII, the following results were obtained:

Total number of compounds: 22

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	15	2	17
	Noncorrosive	2	3	5
	Total	17	5	22

Table 3. Comparison of Corrositex Results with *In Vivo* Results for **Inorganic and Organic Acids** and **Acid Mixtures**.

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	$\frac{\text{Number of Compounds Correctly Identified}}{\text{Total Number of Compounds Tested}}$	**= 18/22 = 82%**
Sensitivity =	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosives}}$	**= 15/17 = 88%**
Specificity =	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosives}}$	**= 3/5 = 60%**
Predictive Value = (Corrosives)	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosive Results}}$	**= 15/17 = 88%**
Predictive Value = Noncorrosives)	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosive Results}}$	**= 3/5 = 60%**
False Positive Rate =	$\frac{\text{Number of Noncorrosives Classified as Corrosive}}{\text{Total Number of Noncorrosives Tested}}$	**= 2/5 = 40%**
False Negative Rate =	$\frac{\text{Number of Corrosives Classified as Noncorrosive}}{\text{Total Number of Corrosives Tested}}$	**= 2/17 = 12%**

Table 4. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Inorganic and Organic Acids** and **Acid Mixtures**

When **inorganic and organic acid derivatives** were delineated within the data base found in Appendix VIII, the following results were obtained:

Total number of compounds: 14

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	7	1	8
	Noncorrosive	0	6	6
	Total	7	7	14

Table 5. Comparison of Corrositex Results with *In Vivo* Results for **Acid Derivatives**.

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 13/14 = 93%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 7/7 = 100%**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 6/7 = 86%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 7/8 = 88%**
Predictive Value = Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 6/6 = 100%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 1/7 = 14%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 0/7 = 0%**

Table 6. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Acid Derivatives.**

When **amines** were delineated within the data base found in Appendix VIII, the following results were obtained:

Total number of compounds: 14

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	10	0	10
	Noncorrosive	1	3	4
	Total	11	3	14

Table 7. Comparison of Corrositex Results with *In Vivo* Results for **Amines**.

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	$\frac{\text{Number of Compounds Correctly Identified}}{\text{Total Number of Compounds Tested}}$	**= 13/14 = 93%**
Sensitivity =	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosives}}$	**= 10/11 = 91%**
Specificity =	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosives}}$	**= 3/3 = 100%**
Predictive Value = (Corrosives)	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosive Results}}$	**= 10/10 = 100%**
Predictive Value = Noncorrosives)	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosive Results}}$	**= 3/4 = 75%**
False Positive Rate =	$\frac{\text{Number of Noncorrosives Classified as Corrosive}}{\text{Total Number of Noncorrosives Tested}}$	**= 0/3 = 0%**
False Negative Rate =	$\frac{\text{Number of Corrosives Classified as Noncorrosive}}{\text{Total Number of Corrosives Tested}}$	**= 1/11 = 9%**

Table 8. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Amines.**

When **inorganic bases and base mixtures** were delineated within the data base found in Appendix VIII, the following results were obtained:

Total number of compounds: 8

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	7	1	8
	Noncorrosive	0	0	0
	Total	7	1	8

Table 9. Comparison of Corrositex Results with *In Vivo* Results for **Inorganic Bases and Base Mixtures**.

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	$\frac{\text{Number of Compounds Correctly Identified}}{\text{Total Number of Compounds Tested}}$	**= 7/8 = 88%**
Sensitivity =	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosives}}$	**= 7/7 = 100%**
Specificity =	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosives}}$	**= 0/1 = 0%**
Predictive Value = (Corrosives)	$\frac{\text{Number of Corrosives Correctly Identified}}{\text{Total Number of Corrosive Results}}$	**= 7/8 = 88%**
Predictive Value = Noncorrosives)	$\frac{\text{Number of Noncorrosives Correctly Identified}}{\text{Total Number of Noncorrosive Results}}$	**= 0/0 = N/A**
False Positive Rate =	$\frac{\text{Number of Noncorrosives Classified as Corrosive}}{\text{Total Number of Noncorrosives Tested}}$	**= 1/1 = 100%**
False Negative Rate =	$\frac{\text{Number of Corrosives Classified as Noncorrosive}}{\text{Total Number of Corrosives Tested}}$	**= 0/7 = 0%**

Table 10. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Inorganic Bases and Base Mixtures.**

The remaining of Appendix VIII are Cinnamaldehye, Trichlorotoluene, 20/80 Coconut/Palm Soap, Sodium Undecylenate, and Cellusolve + TX-100. The following results were obtained:

Total number of compounds: 5

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	0	0	0
	Noncorrosive	0	5	5
	Total	0	5	5

Table 11. Comparison of Corrositex Results with *In Vivo* Results for the remaining of Appendix VIII.

A performance summary was not prepared.

An interesting way of summarizing this data is suggested by the knowledge that the Corrositex Chemical Detection Solution is composed of an aqueous solution of an acidic indicator dye (methyl orange) and a basic indicator dye (phenyl red). As a result, the Corrositex test would be expected to perform most reliably as a means of characterizing the dermal corrosivity of acids and bases. Therefore, the acid and base data from Tables 3, 5, 7, and 9 presented above have been compiled and are presented below in Table 12.

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	39	4	43
	Noncorrosive	3	12	15
	Total	42	16	58

Table 12. Comparison of Corrositex Results with *In Vivo* Results for **Organic and Inorganic Acids and Bases.**

The performance of the Corrositex test for organic and inorganic acids and bases may then be calculated as follows:

Parameter	Formula	Results
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 51/58 = 88%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 39/42 = 93%**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 12/16 = 75%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 39/43 = 91%**
Predictive Value = (Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 12/15 = 80%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 4/16 = 25%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 3/42= 7%**

Table 13. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Inorganic and Organic Acids** and **Bases**.

The results reported here suggest that, for acids and bases, the Corrositex test is very comparable to the *in vivo* test of dermal corrosivity. In addition, with regard to corrosive materials, the Corrositex test displays excellent sensitivity and predictivity. The false positive rate of the Corrositex test is much greater than the false negative rate. In these regards, the results obtained with acids and bases display similar trends with those reported for all of the compounds in our current data base (see Table 2, page 3).

8. *Indicate any limitations for the use of this method. Specify any chemical classes or product categories where the method should or probably should not be used.*

As detailed in response to Question 13 in Section A, current evidence suggests that "industrial cleaners" tend to give an elevated false positive response rate in the Corrositex test. Initial investigations indicate that most of these formulations appear to be composed of a mixture of dilute (0.5 - 2.0%) alkali, dilute (0.5 - 5.0%) sodium metasilicate, and a surfactant. However, this over-estimation of corrosivity tends to favor public safety.

As noted in June 1996 submission, response to Question 2d in Section C, samples that have a pH in the range of 5.0 to 8.5 display a significant rate of non-qualification in the Corrositex test. This observation is consistent with the knowledge that the CDS indicator solution does not exhibit a color change in this pH range.

9. *Provide the complete detailed technical protocol for the proposed method.*

A copy of the current Corrositex Instruction Manual (revision 12/95) is found in Appendix X.

10. *Provide the complete protocols used to generate the different sets of data included in this submission. The technical protocol used, including any modifications and their impact, should be clearly stated for each data set.*

The original version of the Corrositex Instruction Manual (revision 1/95) provides a detailed description of the experimental protocol that was employed for all of the studies reported here. This version of the protocol is found in Appendix XI.

11. *Submit quality assurance procedures used to ensure lot-to-lot consistency of the test materials, and summarize data for such monitoring.*

The current versions of the Quality Control Assessment SOPs for Corrositex formulations and fully-assembled kits are found in Appendix XII. The results of initial lot-to-lot quality assurance studies can be found on pages 20 to 24 of the Application for Exemption submitted on September 28, 1992 (Appendix I). To date, 18 lots have been manufactured and all have passed the quality assurance specifications listed in the Standard Operating Procedures (SOP) found in the Appendix XII.

12. *Data submission:*

a. *Indicate which and if any of the studies were conducted with coded chemicals.*

The Application for Renewal of Exemption DOT E-10904 (Appendix II) contains several tables that detail the results of studies conducted with coded samples. Table 14 shown on the next page summarizes the appropriate references for these tables:

Table Number	Page Number	Number of Coded Samples
17	34	29
38 and 39	66 and 67	50
53	118	17

Table 14 . Summary of Tables Found in Appendix II Describing Results of Studies Conducted with Coded Samples.

Additionally, it should be noted that Tables 38 and 39 found in Appendix II contained a series of misaligned cells. Consequently, the data presented there is incorrect. These errors were corrected in the supplemental information provided in Appendix III (pages 13-21).

b. *Indicate the timeframe of the conduct of the various studies; i.e., the dates during which studies were conducted in each laboratory.*

The data included in Appendix VI were obtained from April 1993 through June 1994. The *in vivo* studies were conducted at various times as indicated in the reports (Appendix V).

c. *Indicate the lab in which each data set was generated, even if this is provided as a coded designation for each lab.*

The Corrositex results of the revised data base consisting of 118 samples were generated by InVitro International (Appendix VI). The *in vivo* results were generated by the contract lab in L. A. or were supplied by the manufactures as shown in Appendix V.

13. *Provide the chemical composition for chemical mixtures and products included in the data submission. Data for mixtures or products for which the chemical composition is not known or available should be evaluated separately from chemicals for which this information is available.*

The composition of **63** materials found in the revised data base (Appendix IV) is known. This information is summarized in Appendix VIII and has been evaluated and discussed in response to Question 7. The composition of the remaining **55** materials is proprietary and is unknown. The results of studies conducted on these materials are summarized in

Appendix IX and are utilized to develop the 2X2 contingency tables and performance analysis for each product class as shown below.

When **cleaners and detergents** were delineated within the data base found in Appendix IX, the following results were obtained:

Total number of compounds: 18

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	7	7	14
	Noncorrosive	1	3	4
	Total	8	10	18

Table 15. Comparison of Corrositex Results with *In Vivo* Results for **Cleaners and Detergents**.

Performance summary:

Parameter	Formula	Results
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 10/18 = 56%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 7/8 = 88%**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 3/10 = 30%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 7/14 = 50%**
Predictive Value = Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 3/4 = 75%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 7/10 = 70%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 1/8 = 12%**

Table 16. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Cleaners and Detergents.**

When **industrial chemical** were delineated within the data base found in Appendix IX, the following results were obtained:

Total number of compounds: 22

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	13	5	18
	Noncorrosive	1	3	4
	Total	14	8	22

Table 17. Comparison of Corrositex Results with *In Vivo* Results for **Industrial Chemical**

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 16/22 = 73%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 13/14 = 93%**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 3/8 = 38%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 13/18 = 72%**
Predictive Value = (Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 3/4 = 75%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 5/8 = 62%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 1/14 = 7%**

Table 18. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Industrial Chemical.**

When **surfactant** was delineated within the data base found in Appendix IX, the following results were obtained:

Total number of compounds: 14

Contingency table:

		***In Vivo* Results**		
		Corrosive	**Noncorrosive**	**Total**
Corrositex Results	**Corrosive**	0	0	0
	Noncorrosive	0	14	14
	Total	0	14	14

Table 19. Comparison of Corrositex Results with *In Vivo* Results for **Surfactant**.

Performance summary:

Parameter	**Formula**	**Results**
Equivalence =	Number of Compounds Correctly Identified / Total Number of Compounds Tested	**= 14/14 = 100%**
Sensitivity =	Number of Corrosives Correctly Identified / Total Number of Corrosives	**= 0/0 = NA**
Specificity =	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosives	**= 14/14 = 100%**
Predictive Value = (Corrosives)	Number of Corrosives Correctly Identified / Total Number of Corrosive Results	**= 0/0 = NA**
Predictive Value = (Noncorrosives)	Number of Noncorrosives Correctly Identified / Total Number of Noncorrosive Results	**= 14/14 = 100%**
False Positive Rate =	Number of Noncorrosives Classified as Corrosive / Total Number of Noncorrosives Tested	**= 0/14 = 0%**
False Negative Rate =	Number of Corrosives Classified as Noncorrosive / Total Number of Corrosives Tested	**= 0/0 = NA**

Table 20. Summary of the Performance of the Corrositex Test Compared to *In Vivo* Findings for **Surfactant.**

The degree of agreement between the Corrositex and *in vivo* tests for each chemical and product class is summarized in tables 21 and 22.

Chemical Class	N	Equivalence	Sensitivity	Specificity	Corrosive Predictivity	Noncorrosive Predictivity	False Positive	False Negative
Acid Derivative	14	93%	100%	86%	88%	100%	14%	0%
Acid[a]	22	82%	88%	60%	88%	60%	40%	12%
Amine	11	91%	91%	100%	100%	75%	0%	9%
Base[b]	8	88%	100%	0%	88%	NA	100%	0%

[a] Chemical class includes acid mixtures.

[b] Chemical class includes base mixtures.

Table 21. Summary of Performance of the Corrositex Test when Compared to *In Vivo* Test Results for Chemical Classes (Data from Appendix VIII)

Product Class	N	Equivalence	Sensitivity	Specificity	Corrosive Predictivity	Noncorrosive Predictivity	False Positive	False Negative
Cleaner & Detergent	18	56%	88%	30%	50%	75%	70%	13%
Industrial Chemical	29	79%	93%	38%	72%	75%	62%	7%
Surfactant	11	100%	NA	100%	NA	100%	0%	NA

Table 22. Summary of Performance of the Corrositex Test when Compared to *In Vivo* Test Results for Product Classes (Data from Appendix IX).

14. *Indicate and cite any scientific publications on the Corrositex test method.*

The Corrositex test has been described in the following three scientific papers:

V.C. Gordon, J.D. Harvell, and H.I. Maibach. Dermal Corrosion, The CORROSITEX System: A DOT Accepted Method to Predict Corrosivity Potential of Test Materials, *In Vitro* Skin Toxicology, ed. Andre Rougier, V10: 37-45.

V.C. Gordon, S. Mirhashemi, R. Wei and V. Harutunian, A New *In Vitro* Method to Determine the Corrosivity Potential of Surfactants and Surfactant-Based Formulations, Comunicaciones - Presentadas a la XXV Jornadas Del Comite Espanol De La Detergencia.

P.A., Botham, et al., A Prevalidation Study on *In Vitro* Skin Corrosivity Testing: The Report and Recommendations of ECVAM Workshop 6, ATLA 23, 219-255, 1995.

Copies of these manuscripts are included in Appendix XIII.

B. MISCELLANEOUS COMMENTS

1. *Corrositex results should be compared with the in vivo classification of corrosive or noncorrosive.*

This has been done in the current response.

2. *The chemicals that have been tested in a series of different concentrations need to be addressed separately from chemicals tested only once.*

Diluted samples of a chemical have not been included in the current data base (Appendix IV).

C. APPARENT INCONSISTENCIES AND DISCREPANCIES IN THE TABLES THAT SHOULD BE CORRECTED OR EXPLAINED

1. *Reproducibility within and between laboratories: Results for tests run on the same chemical or product in different labs to demonstrate interlaboratory reproducibility should be combined into one table. Similarly, multiple tests of chemicals to demonstrate intralaboratory repeatability or reproducibility should be combined into appropriate tables.*

The results of inter- and intra-laboratory reproducibility studies may be found on pages 23 and 36 of Appendix I and pages 73 through 81 of Appendix II.

2. *Performance of the test.*

a. Calculate performance when scored as C vs. NC.

See Question 2 in Section A.

b. Calculate performance when scored as I, II, III, or NC.

Data found in Appendix IV has been analyzed to define the performance of the Corrositex test when scored as Packing Group I, II, III or NC. The following contingency table demonstrates the performance of the Corrositex test when compared the *in vivo* data (Table 23). It should be noted that samples whose *in vivo* data are listed as "C" or "R34" are not included in the analysis

Reported in Table 23. Sample #44 and 59 of Appendix IV were not included either. Therefore the total number of samples described in this instance is 90.

		***In Vivo* Packing Group**				
		I	**II**	**III**	**NC**	**Total**
Corrositex Packing Group	**I**	**3**	1	1	0	5
	II	1	**28**	1	7	37
	III	0	0	**1**	8	9
	Noncorrosive	1	0	0	**38**	39
	Total	5	29	3	53	90

Table 23. Comparison of Packing Groups Defined by *In Vivo* Studies and the Corrositex Test.

c. *Performance of the test for different categories of substances.*

See Question 7 & 13 in Section A.

d. *Proportion of non-qualifying (NQ) substances among structure or use categories.*

The submission in June 1996 contained 83 non-qualified samples. Current data base does not contain non-qualified samples.

3. *Specific questions*

a. Explain why there are two conflicting in vivo classifications for 95% caprylic acid.

The results of *in vivo* studies demonstrated that 95% caprylic acid was corrosive. Briefly, the conflicting entries of corrosive and noncorrosive noted for this material resulted from the following series of errors. Initially, when Table 39 on page 67 of Appendix II was compiled, an incorrect designation of NC was entered in the *in vivo* results columns of line 35. This erroneous entry was transcribed to line 240 of Table 49 found on page 90 and lines 368 and 369 of Table 54 found on pages 131 and 132 of Appendix II. Please refer to pages 13-21 of the Supplement to Application for Revision and Renewal of DOT E-10904 found in Appendix III for a detailed explanation and correction of this error.

b. Explain why there are often 2-3 in vivo classifications listed for several chemicals.

These multiple classifications result from incomplete/insufficient *in vivo* studies reported by the contract laboratory. While this laboratory was expected to employ *in vivo* exposure periods of 4 hours, 1 hour, and 3 minutes respectively to determine Packing Groups III, II or I; in some cases only the 4 hour and 1 hour exposure periods were actually performed. In these instances when the 3 minute exposure period was omitted, it was not possible to distinguish if the tested material should be characterized as a packing group I or packing group II compound. Consequently, in these cases, the *in vivo* classification "I, II" has been included in the listing found in Table 49 of Appendix II.

c. Table 49, rows 952 and 953. The results for the two samples are virtually identical, yet 952 is classified B1/II and 953 is classified B2/III. Provide an explanation.

The samples are not duplicates with inconsistent results. The strippers should have been labeled Stripper A and Stripper B. These are individual formulations provided by an industrial company.

d. Eliminate duplicate entries from Table 49.

Current data base does not contain duplicate entries.

e. Table 49: Describe what the entries under the column titled "In lab" mean; e.g., if the space is blank or if there is an asterisk.

An asterisk entered under the column titled "In lab" indicates that the sample was evaluated at InVitro International. A blank entry indicates that the sample was not evaluated at InVitro International. All the corrositex results of the current data base were generated at InVitro International (Appendix VI).

f. Table 54, lines 11 and 30. The same patterns of response are assigned different concordances. This should be explained.

When Table 54 of Appendix II was originally formatted, lines 11 and 30 were as shown below:

#	Sample Name	Corrositex Group	DOT	*in vivo*	Concordance
11	Hydrobromic acid	I	II	II	over
30	Phosphorous pentachloride	I	II	I	1

Thus the Corrositex test was judged to over-estimate the packing group of hydrobromic acid (line 11) when compared to the *in vivo* test. By contrast, the results of the packing group determination of Phosphorous pentachloride obtained from the Corrositex test and the *in vivo* test were found to be concordant.

Prior to submission of the renewal application, Table 54 was re-formatted to clearly delineate the compounds that were identified to be corrosive by the *in vivo* test. This was achieved by replacing the *in vivo* packing group designations of I, II, or III with the letter "C." However, when this was done, the person responsible for compiling the new version of the table failed to edit and amend the results reported in the concordance column. Consequently the version of Table 54 found in Appendix II contained the following lines:

#	Sample Name	Corrositex Group	DOT	*in vivo*	Concordance
11	Hydrobromic acid	I	II	C	over
30	Phosphorous pentachloride	I	II	C	1

Thus the apparent discrepancies identified by the reviewer resulting from editing errors that occurred while the table was being compiled.

Every effort has been made to correct these types of errors in the data that is provided with the current response.

g. Table 54. If Corrositex groups I, II, and III are all equivalent to a classification of "C," why are some chemicals in Group I considered "over" (rows 10, 82, 83) and others considered "under" (rows 116, 117, 149, 150, 160, 161) when compared to the in vivo classification of "C?"

See Question 3f in Section C.

#	Sample Name	Product Category	Conc. wt%	Corrositex Results			*in vivo*	Corrositex data source	Category data source	*in vivo* data source
				Time	Category	Packing Group				
1	Boron Trifluoride-Dihydrate	Acid Derivative	96	1.53	1	I	I	p1	p87	1-3-70841, #1
2	Calcium Carbonate	Acid Derivative	Neat	>240	2	NC	NC	p31, #7	p75	1-3-78700, #23
3	Chromium (III) Fluoride	Acid Derivative	97	171.6	2	NC	NC	p13	p88	1-3-70841, #3
4	Copper (II) Chloride	Acid Derivative	99	35.71	1	II	NC	p58, 59	p99	1-3-70841, #12
5	Ferric Chloride	Acid Derivative	98	27.29	1	II	II	p16	p88	1-3-70841, #16
6	Glycol bromoacetate	Acid Derivative	Neat	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
7	Maleic Anhydride	Acid Derivative	99	35.65	1	II	III	p17	p87	1-3-70841, #18
8	Phosphorous Pentachloride	Acid Derivative	98	0.08	1	I	I	p18	p89	1-3-70841, #6
9	Phosphorous Tribromide	Acid Derivative	97	1.02	1	I	I	p2 & 18	p88	1-3-70841, #7
10	Potassium Bisulfate	Acid Derivative	Neat	25.11	1	II	I, II	p49, #2	p94	1-3-82069, #45
11	Sodium bicarbonate	Acid Derivative	Neat	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
12	Sodium bisulphite	Acid Derivative	Neat	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
13	Sodium Hydrogen Fluoride	Acid Derivative	99	71.57	2	NC	NC	p18	p89 & 91	1-3-70841, #8
14	Sodium hypochlorite w/ 5% Chlorine	Acid Derivative	5% Cl	>240	2	NC	NC	p48, #4	p75	1-3-82588-3, #20
15	Boron trifluoride-acetic acid complex	Acid Mixture	98	3.47	1	II	II	p56, #2	p86	1-3-70841, #2
16	HCl + Sulfuric acid + Citric acid	Acid Mixture	5,5,5	9.09	1	II	II	p72	p92	1-3-82069, #28
17	Sulfuric acid + Cellusolve	Acid Mixture	5,20	15.00	2	II	I, II	p71	p93	1-3-82069, #55
18	Sulfuric acid + Ferric Chloride	Acid Mixture	5, 2	14.98	2	II	I, II	p71	p93	1-3-82069, #56
19	Sulfuric acid + SDS	Acid Mixture	5, 10	16.15	2	II	I, II	p71	p93	1-3-82069, #57
20	Sulfuric acid + Triton X-100	Acid Mixture	5, 10	16.86	2	II	I, II	p71	p93	1-3-82069, #58
21	Fluoboric Acid	Acid, Inorganic	48	2.49	1	I	III	p1 & 16	p88	1-3-70841, #4
22	Hydrobromic Acid	Acid, Inorganic	48	2.28	1	I	II	p2	p85	1-3-70841, #5
23	Hydrochloric acid	Acid, Inorganic	14.4	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
24	Phosphoric Acid	Acid, Inorganic	85	10.96	1	II	II	p17	p88	1-3-70841, #19
25	Sulfamic Acid	Acid, Inorganic	99+	22.63	1	II	NC	p19	p89	1-3-70841, #22
26	Sulfuric acid	Acid, Inorganic	10	NA	NA	R34	C	ECVAM	ECVAM	ECVAM
27	10-Undecenoic acid	Acid, Organic	NA	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
28	2-Methylbutyric acid	Acid, Organic	NA	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
29	3-3'-Dithiodipropionic acid	Acid, Organic	NA	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
30	65/35 Octanoic/decanoic acids	Acid, Organic	65/35	NA	NA	NC	R34	ECVAM	ECVAM	ECVAM
31	70/30 Oleine/octanoic acid	Acid, Organic	70/30	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
32	Butyric Acid	Acid, Organic	99	40.57	1	II	II	p2 &14	p88	1-3-70841, #11

#	Sample Name	Product Category	Conc. wt%	Corrositex Results			*in vivo*	Corrositex data source	Category data source	*in vivo* data source
				Time	Category	Packing Group				
33	Hexanoic Acid	Acid, Organic	Neat	133.4	1	III	C	p13 & 20	p92	1-3-82069, #31
34	Maleic Acid	Acid, Organic	99	9.56	1	II	NC	p17	p88	1-3-70841, #17
35	Octanoic acid	Acid, Organic	NA	NA	NA	NC	R34	ECVAM	ECVAM	ECVAM
36	Propionic Acid	Acid, Organic	99+	19.19	1	II	II	p18, 19	p89	1-3-70841, #20
37	Cinnamaldehyde	Aldehyde	Neat	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
38	1-(2-Aminoethyl) piperazine	Amine	99	38.78	1	II	II	p18	p11, #3	1-3-70841, #9
39	2,4-Xylidine (2,4-Dimethylaniline)	Amine	NA	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
40	Butylamine (in EtOH/EG 1:1)	Amine	81.8	26.33	1	III	C	p61, #3	p80	1-3-80626-3, #60
41	Cyclohexylamine	Amine	Neat	42.79	1	II	C	p84	p57, last	1-3-82069, #19
42	Diaminopropane, 1,2-	Amine	99+	21.67	1	II	I	p1	p11, #6	1-3-70841, #13
43	Dicyclohexylamine	Amine	97	190.87	1	III	C	p52	p11, #10	1-3-82069, #20
44	Dimethyldipropylenetriamine	Amine	NA	NA	NA	R34	R35	ECVAM	ECVAM	ECVAM
45	Dimethylisopropylamine	Amine	NA	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
46	Dimthylbenzylamine, N,N-	Amine	99+	86.17	1	III	III	p1	p82	1-3-82069, #21
47	Ethanolamine	Amine	99+	23.88	1	II	II	p35	p11, #14	1-3-70841, #15
48	Hydrogenated tallow amine	Amine	NA	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
49	n-Heptylamine	Amine	NA	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
50	Tallow amine	Amine	NA	NA	NA	NC	R35	ECVAM	ECVAM	ECVAM
51	Tributylamine	Amine	99+	>240	2	NC	NC	p13	p75	1-3-82588-3, #21
52	NaOH/BAC (1:10)	Base Mixture	1,10	34.33	2	III	C	p22, #2	p74	1-3-82069, #34
53	NaOH/SDS (1/10)	Base Mixture	1,10	32.1	2	III	C	p23, #1	p74	1-3-82069, #35
54	NaOH/SMS (1/10)	Base Mixture	1,10	17.66	2	II	II	p28, #10	p74	1-3-82069, #42
55	NaOH/SMS (5/3)	Base Mixture	5,3	15.59	1	II	C	p32	p83	1-3-78830, #34
56	NaOH/TX100	Base Mixture	5,5	15.23	2	II	I, II	p32	p83	1-3-82069, #43
57	2-Mercaptoethanol, Na salt	Base, Inorganic	45	NA	NA	R34	R34	ECVAM	ECVAM	ECVAM
58	Potassium hydroxide	Base, Inorganic	10	NA	NA	R34	C	ECVAM	ECVAM	ECVAM
59	Sodium carbonate	Base, Inorganic	50	NA	NA	R34	NC	ECVAM	ECVAM	ECVAM
60	APL 9372	Cleaner	Neat	16.61	2	II	NC	p28, #2	p78	1-3-80626-2, #49
61	Boat Bottom Cleaner	Cleaner	Neat	23.78	2	II	I, II	p49, last	p93	1-3-82069, #32
62	COR 11	Cleaner	Neat	31.75	2	III	C	p4	p7	1-3-82069, #51
63	COR 18	Cleaner	Neat	30.78	2	III	NC	p60, last	p8	1-3-82069, #46
64	COR 19	Cleaner	Neat	23.92	NF	II	I, II	p4	NF	1-3-78540, #10

#	Sample Name	Product Category	Conc. wt%	Corrositex Results			*in vivo*	Corrositex data source	Category data source	*in vivo* data source
				Time	Category	Packing Group				
65	COR 20	Cleaner	Neat	30.17	2	III	C	p5	p8	1-3-82069, #47
66	COR 4	Cleaner	Neat	39.00	2	III	NC	p3	p10	1-3-80626-2, #32
67	COR 6	Cleaner	Neat	17.91	2	II	NC	p4	p8	1-3-78700, #19
68	COR 9	Cleaner	Neat	35.3	2	III	C	p4	p8	1-3-82069, #50
69	COR3	Cleaner	Neat	15.17	1	II	I, II	p67	p8	1-3-78540, #7
70	Coverage Plus	Cleaner	Neat	88.9	2	NC	C	p33	p77	1-3-78700, #16
71	Larc Super	Cleaner	Neat	>240	NF	NC	NC	p69, 70	NF	1-3-80626-3, #64
72	Toilet Duck Green	Cleaner	Neat	153.5	2	NC	NC	p53, #5	p75	1-3-78540, #11
73	ZFA	Cleaner	Neat	72.91	2	NC	NC	p34, #8	p79	1-3-80626-2, #45
74	ZWS 9352	Cleaner	Neat	31.48	2	III	C	p28, #11	p78	1-3-86156-1, #1
75	Windex Blue	Cleaner, glass	Neat	34.78	2	III	NC	p54, #3	p75	1-3-78540, #12
76	Cascade	Detergent, dishwashing	Neat	60.95	1	III	NC	p33, 73	p81	1-3-78700, #14
77	Tide	Detergent, Laundry	Neat	69.96	1	III	NC	p35	p76	1-3-78700, #13
78	Trichlorotoluene	Halogenated Aromatic Hy	99	>240	2	NC	NC	p14	p90	1-3-82588-3, #19
79	315	Industrial Chemical	Neat	28.61	NF	II	I, II	p46 #3	NF	1-3-84605-2, #9
80	485	Industrial Chemical	Neat	28.25	NF	II	I, II	p39, #1	NF	1-3-84605-2, #12
81	880	Industrial Chemical	Neat	15.86	NF	II	I, II	p46, #2	NF	1-3-84605-2, #5
82	3000	Industrial Chemical	Neat	24.17	NF	II	I, II	p41, #1	NF	1-3-84605-2, #10
83	4000	Industrial Chemical	Neat	20.34	NF	II	I, II	p40, #2	NF	1-3-84605-2, #11
84	122 B	Industrial Chemical	Neat	12.44	NF	II	I, II	p47, #4	NF	1-3-84605-2, #4
85	1702 BR	Industrial Chemical	Neat	26.13	NF	II	I, II	P41, #4	NF	1-3-84605-2, #6
86	1703 CR	Industrial Chemical	Neat	28.43	NF	II	I, II	p41, #2	NF	1-3-84605-2, #8
87	1709 B	Industrial Chemical	Neat	27.11	NF	II	I, II	p41, #3	NF	1-3-84605-2, #7
88	3-11-23x Undyed	Industrial Chemical	Neat	45.03	2	III	NC	p27, #9	p79	1-3-80626-3, #62
89	3-12-02P	Industrial Chemical	Neat	47.79	2	III	NC	p34, #4	p79	1-3-80626-3, #61
90	3-12-03K Undyed	Industrial Chemical	Neat	25.5	NF	II	NC	p30, #1	NF	1-3-80626-3, #63
91	BSS 9487	Industrial Chemical	Neat	13.98	1	II	C	p29, #12	p78	1-3-80626-2, #46
92	Formula # 100-016	Industrial Chemical	Neat	41.67	2	III	C	p98, #3	p9	1-3-86156-4, #4
93	Formula # 100-057B	Industrial Chemical	Neat	>240	2	NC	C	p102	p103	1-3-86156-5, #7
94	Formula # 100-088	Industrial Chemical	Neat	40.29	2	III	C	p 24,	p10, # 16	1-3-86156-4, #5
95	GIN	Industrial Chemical	Neat	11.19	2	II	NC	p34, #3	p92	1-3-80626-2, #44
96	LMCRK	Industrial Chemical	Neat	22.1	2	II	I, II	p26, #1	p77	1-3-82069, #13
97	Solcenic 2BW	Industrial Chemical	Neat	69.62	2	NC	NC	p105	p9	1-3-80749, #4

#	Sample Name	Product Category	Conc. wt%	Corrositex Results			*in vivo*	Corrositex data source	Category data source	*in vivo* data source
				Time	Category	Packing Group				
98	Solcenic 3A	Industrial Chemical	Neat	138.79	2	NC	NC	p73	p9	1-3-80749, #6
99	Solcenic 3B	Industrial Chemical	Neat	70.13	2	NC	NC	p104	p9	1-3-80749, #5
100	TBQ	Industrial Chemical	Neat	56.25	2	III	NC	p22, #8	p77	1-3-78700, #15
101	91440	Petrochemical	Neat	>240	2	NC	NC	p65	p100	1-3-73960, #17
102	20/80 Coconut/palm soap	Surfactant	Neat	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
103	RP-26	Surfactant	Neat	>240	2	NC	NC	p62	p95	1-3-76818, #14
104	RP-29	Surfactant	Neat	>240	2	NC	NC	p62	p95	RP data
105	RP-33	Surfactant	Neat	>240	2	NC	NC	p64	p95	RP data
106	RP-34	Surfactant	Neat	>240	2	NC	NC	p62	p97	RP data
107	RP-39	Surfactant	Neat	>240	2	NC	NC	p62	p97	RP data
108	RP-40	Surfactant	Neat	>240	2	NC	NC	p62	p95	1-3-76818, #16
109	RP-46	Surfactant	Neat	>240	2	NC	NC	p63	p97	RP data
110	RP-49	Surfactant	Neat	99.08	2	NC	NC	p55, #1	p95	RP data
111	RP-52	Surfactant	Neat	>240	2	NC	NC	p63	p95	RP data
112	RP-53	Surfactant	Neat	>240	2	NC	NC	p63	p95	RP data
113	RP-57	Surfactant	Neat	>240	2	NC	NC	p63	p95	RP data
114	RP-61	Surfactant	Neat	>240	2	NC	NC	p63	p95	RP data
115	RP-80B	Surfactant	Neat	>240	2	NC	NC	p66	p96	1-3-76818, #17
116	RP-81B	Surfactant	Neat	>240	2	NC	NC	p66	p97	1-3-76818, #18
117	Sodium undecylenate	Surfactant	33	NA	NA	NC	NC	ECVAM	ECVAM	ECVAM
118	Cellusolve + TX-100	Surfactant Blend	20/10	>240	2	NC	NC	p50, #14	p93	1-3-82069, #7

NA: Not available

NF: Not found

CORROSITEX®

INSTRUCTION MANUAL

DOT-E 10904

(December, 1995)

TABLE OF CONTENTS

CORROSITEX® INSTRUCTIONS

I. MATERIALS AND EQUIPMENT

A. **2-Sample Kit**

1) One vial containing one gram of biobarrier matrix and a micro stirbar.
2) One vial containing 10 ml of biobarrier diluent.
3) Two racks of seven vials filled with Chemical Detection System (CDS).
4) One tray of 12 membrane discs plus one additional membrane disc. **These discs must be stored in the refrigerator at 2-8°C.**
5) Two Data Sheets.
6) Three Qualify test tubes.
7) Two Categorize tests (two A test tubes, two B test tubes, and one bottle of Confirm Reagent).

B. **4-Sample Kit**

1) One vial containing one gram of biobarrier matrix and a micro stirbar.
2) One vial containing 10 ml of biobarrier diluent.
3) Four racks of seven vials filled with Chemical Detection System (CDS).
4) One tray of 24 membrane discs plus one additional membrane disc. **These discs must be stored in the refrigerator at 2-8°C.**
5) Four Data Sheets
6) Five Qualify test tubes.
7) Four Categorize tests (four A test tubes, four B test tubes, and one bottle of Confirm Reagent).

C. CORROSITEX Lab (Contains all equipment necessary to perform the CORROSITEX test method - must be purchased separately)

1) Nuova II stirring hot plate - 110 V (1)
2) Thermometer (1)
3) Digital timers (2)
4) Eppendorf repeat pipettor (1)
5) Eppendorf combitips 2.5 ml (1 case/100)
6) Lab Industries positive displacement pipettor (1)
7) Lab Industries positive displacement pipettor tips (1 case/250)
8) Spatula (1)
9) Forceps (1)
10) Uvex safety goggles (1 pair)
11) Permanent lab marking pens (2)
12) Water bath container (1)
13) Plastic wrap (1 roll)

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I. BIOBARRIER PREPARATION[1]

NOTE: Preparation must be completed at least 2 hours prior to running tests.

1) Place the water bath container on the hot plate and insert a thermometer.

2) Fill the water bath container with approximately one inch of water and heat to 68 - 70°C (turn the heat knob to 7-8). **Do not allow the temperature to exceed 70°C.**

3) While the water bath is warming, remove the membrane discs from the refrigerator. Remove the tray lid to prevent condensation.

4) Add the entire contents of the biobarrier diluent to the vial of biobarrier matrix powder. Place the vial inside the water bath on the hot plate.

5) Turn the stir knob to medium speed (4-5). Make sure the micro stirbar is rotating smoothly, but not too fast (avoid foaming). Adjust the stir knob if necessary.

NOTE: DO NOT VORTEX OR SHAKE THE VIAL VIGOROUSLY TO SOLUBILIZE.

6) Allow the biobarrier matrix powder to dissolve completely. This should take approximately 20 minutes.

7) Turn off the stir knob and the heat. Allow the solution to sit for 5 minutes in the water bath to allow any air bubbles to rise to the surface.

NOTE: To prevent the biobarrier solution from solidifying in the vial, do not remove the vial from the water bath. Immediately proceed to Step 8.

8) Assemble the repeat pipettor with a 7.5 ml combitip and set the pipettor to position four (4), to dispense a total of 200 ul.

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[1] The instructions detailed below describe the simultaneous preparation of all of the biobarriers. Occasionally, it may be more convenient to analyze only a single sample at one time. If this is the case, prepare only 3 biobarrier membranes with the dissolved biobarrier matrix powder prepared in Step 6. The unused matrix solution may then be stored at 2-8°C in its tightly sealed original vial for up to 1 month. To prepare the remaining biobarriers at a later date, solubilize the solidified matrix gel by warming it for 3 to 4 minutes without stirring in a 60°C water bath prior to pipetting it as described in Steps 8 - 12.

Pre-made biobarriers are also available. They may be ordered from In Vitro International by calling (800)-2-INVITRO.

Rev 12/91

9) Fill the pipettor tip with biobarrier solution, avoiding air bubbles. Dispense one aliquot to waste to ensure proper subsequent volume delivery. Wipe the pipettor tip to remove excess solution.

10) Pipet 200 µl into each membrane disc, ensuring the entire membrane is covered and no air bubbles have formed. Any air bubbles on the gel will alter the result of the test. If this occurs, the disc should not be used and should be replaced with the spare membrane disc supplied in the kit.

11) Note the preparation and expiration dates of the biobarriers on the tray label. Tightly wrap the filled tray with plastic wrap and immediately store the tray at 2-8°C.

12) **The tray must be stored at 2-8°C for 2 hours prior to beginning any testing.** The biobarriers are stable for 7 days if wrapped with an airtight seal and stored at 2-8°C. This procedure must be followed as described, as the biobarriers are sensitive to dehydration.

III. CORROSITEX TESTING PROTOCOL

STEP 1 - QUALIFY: This step ensures that your sample is compatible with the CORROSITEX system prior to running the test and may be performed prior to the biobarrier preparation if desired.

1) Fill in the sample information on the CORROSITEX Data Sheet.

2) Add the sample (150 µl if liquid, 100 mg if solid) to the Qualify test tube. Shake to dissolve solids. For immiscible liquids and insoluble solids, shake the vial and let stand for one minute. Observe the color change at the sample/testing fluid interface.

3) If the amber liquid changes color or consistency, check yes on the Sample Qualified section of the Data Sheet and proceed to Step 2.

4) If a physical change is not observed, your material is not suitable for the CORROSITEX system. Check no on the Sample Qualified section of the Data Sheet and contact your technical representative at (800)-2-INVITRO.

STEP 2 - CATEGORIZE: This step establishes the category of cut-off times for your sample.

1) Add the sample (150 µl if liquid, 100 mg if solid) to the tubes labeled Tube A (yellow solution) and Tube B (clear solution). Cap and shake until mixed.

2) If a color change is observed in either tube, match the color to the corresponding color charts on the CORROSITEX Testing Protocol Poster. Assign and record the appropriate category on the Data Sheet and proceed to Step 3.

3) If a color change is not observed in either tube, add two drops of the CONFIRM reagent to Tube B. Cap and shake until mixed

4) Match the resulting color to the color chart on the CORROSITEX Testing Protocol Poster (Step 2B) Assign and record the appropriate category on the Data Sheet and proceed to Step 3.

NOTE: For immiscible samples, shake the vial and let stand for one minute. Observe the color change at the sample/testing fluid interface.[2]

STEP 3 - CLASSIFY: This step determines the appropriate Packing Group for your sample.

1) Make certain all proper safety procedures are followed for the chemicals being tested. Consult your company's safety procedures prior to proceeding. USE PROPER SAFETY EQUIPMENT Fume hood, gloves, eye protection, etc.

2) Remove one tray of seven pre-filled CDS vials from the kit box.

3) The CDS vials must be at room temperature (17-25°C) before using.

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[2] Occasionally, either intensely colored samples or samples that promote subtle color changes in the Categorize tubes may be encountered. These types of samples may be accurately categorized by performing the following protocol:

1) Measure and record the pH of a 10% (v/v or w/v) aqueous solution of the sample.

2) If the pH of the 10% solution is < 7.0, utilize Tube A (yellow solution) to perform the categorization test. If the pH of the 10% solution is > 7.0, utilize Tube B (clear solution) to perform the categorization test.

3) Add the sample (150 ul if liquid, 100 mg if solid) to the tube that has been selected. Cap and shake until mixed.

4) Measure and record the final pH of the mixture in the tube.

5) For measurements performed with Tube A, if the pH is ≤ 5.0, the sample is Category 1. If the pH > 5.0, the sample is Category 2. For measurements performed with Tube B, if the pH ≥ 9.0, the sample is Category 1. If the pH < 9.0, the sample is Category 2.

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4) Vials 1 - 4 are to be utilized for sample replicate testing. The vial labeled (+) is to be utilized for a positive control sample, the vial labeled (-) is for a negative control sample and the vial labeled C serves as a CDS color control.

NOTE: Label each vial cap with the corresponding test material for appropriate disposal purposes.

The following table lists several chemicals which may be used as positive and negative controls, or you may establish your own controls. If the control falls outside the designated range, please contact your technical representative at **800-2-INVITRO.**

CORROSITEX POSITIVE AND NEGATIVE CONTROLS

	Chemical	Conc. (wt. %)	CORROSITEX Time (min.)
Positive Control	Nitric Acid	68-73	0.5 - 2.0
	Sulfuric Acid	95-98	0.5 - 2.0.
Negative Control	Citric Acid	10	> 60
	Propionic Acid	6	> 60

5) One timer may be used for the sample replicates and one timer may be used for the controls. Place the timers in front of the rack. Make certain they are set to zero and are ready to time the test.

6) Add a biobarrier disc to the top of the first vial. Do not allow the discs to be in the vial for longer than two minutes before adding the test sample.

NOTE: The tray of biobarrier discs should be kept on crushed ice when not in the refrigerator. Immediately re-wrap and place the tray of unused discs back into a 2-8°C refrigerator after completion of the test.

7) Evenly apply 500 ul or 500 mg of the test sample onto the top of the biobarrier disc and start the timer the instant the sample is added.

Repeat Steps 6 and 7 for the remaining vials, staggering each start time by one minute. The start time difference for each vial will be subtracted from the final time to determine the net response time. Staggering allows more accurate time recording if exact reaction times are desired.

DO NOT CAP THE VIALS WHILE THE TEST IS IN PROGRESS DUE TO POSSIBLE PRESSURE BUILD UP WITH SOME REACTIVE CHEMICALS.

8) The first indication of the presence of a chemical in the CDS will be a narrow stream of color change produced beneath the center of each biobarrier disc. As soon as this reaction is observed, record the detection time on the Data Sheet.

If exact reaction times are not required, observe the CDS vials for the first 5 minutes after each start time and for 5 minutes before and after each Packing Group cut-off time. For example, if you have a Category 1 sample the CDS vials would be observed for the first 5 minutes, and then again at the 55 to 65 minute interval and the 235 to 245 minute interval for each vial.

NOTE: Changes in the CDS may include various color changes (red, orange, or lightening) and flaking or precipitation.

9) When the test has been completed, remove the biobarrier discs and cap each vial. Use caution when handling vials. Follow your lab protocol for proper chemical disposal.

10) Repeat Steps III 1, 2 and 3 for each sample to be tested.

11) Calculate the CORROSITEX Time and the mean of the four sample replicates (CORROSITEX Time = Detection Time - Start Time.)

12) Using the table below, assign the appropriate Packing Group by sample category and CORROSITEX time.

PACKING GROUP DESIGNATION

	CORROSITEX Time (minutes)			
Category 1	0 to 3 min.	>3 to 60 min.	>60 to 240 min.	>240 min.
Category 2	0 to 3 min.	>3 to 30 min.	>30 to 60 min.	>60 min.
	Packing Group I	Packing Group II	Packing Group III	Non-corrosive

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INVITRO INTERNATIONAL

19038 Miliken Avenue, Irvine, California 92606

800-240-8487, 949-851-8386, FAX 949-851-8588

CORROSITEX® DATA SHEET

12426

Sample Name: _____ Company Name: _____

Sample Lot #: _____ Phone Number: ( ) -

Sample Description: (Please circle appropriate categories)

Solid / Liquid Product / Waste pH _____

Test Performed By: _____ Print Name

_____ Signature

Active Ingredients:

Chemical Names	Class	Concentration

Test Reviewed By: _____ Print Name

_____ Signature

Date Test Performed: _____

CORROSITEX Lot #: _____ Kit Expiration: _____ Preparation Date of Biobarriers: _____

	1	2	3	4
Detection Time in min:sec				
Start Time				
CORROSITEX Time				
Convert to Minutes				
Mean of 4 Replicates				

PACKING GROUP ASSIGNMENT TABLE

	CORROSITEX Time (in Minutes)			
Category 1	0 to 3 min.	>3 to 60 min.	>60 to 240 min.	>240 min.
Category 2	0 to 3 min.	>3 to 30 min.	>30 to 60 min.	>60 min.
	Packing Group I	Packing Group II	Packing Group III	Non Corrosive

Comments/Observations: _____

The following information is provided as an example of how the Corrositex® assay is performed. Color copies of the "How-To Guide" may be obtained by contacting InVitro International, Inc. at 1-800-2-INVITRO.

CORROSITEX®

A How-To Guide to the

Convenient and Cost-Effective

Identification of Corrosive

and Non-Corrosive Materials.

CORROSITEX®

CORROSITEX is an *in vitro* testing system that mimics the effect of corrosives on living skin and classifies the level of corrosivity in chemicals, formulations and waste.

CORROSITEX can save your business time and money by enabling you to accurately and efficiently package and ship hazardous materials.

Ensures Compliance - New regulations stipulate that all corrosives in commerce be classified prior to shipment into United Nations (U.N.) Packing Groups according to DOT and international requirements. CORROSITEX accurately assigns U.N. Packing Groups I, II, III, or verifies non-corrosivity.

Lower Costs - CORROSITEX costs up to 80% less than *in vivo* testing.

Quicker Results - CORROSITEX delivers results in as little as 3 minutes to 4 hours.

Greater Accuracy - Because CORROSITEX is more accurate than pH testing and is packing group specific, it prevents costly over-packing and eliminates any potential risk of under-packing corrosive materials.

Reduces Risk - CORROSITEX provides important information on potential dangers in the workplace and the type of emergency response required in case of an accident.

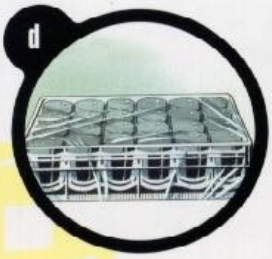
Government Approved - Government approved and internationally accepted, CORROSITEX is recommended by and/or meets the requirements of:

- *U.S. Department of Transportation (DOT): DOT-E 10904*
- *Environmental Protection Agency (EPA): SW-846 Method 1120*
- *International Air Transport Association (IATA)*
- *Occupational Safety and Health Administration (OSHA)*
- *Transport Canada*
- *United Nations Packing Groups*
- *European Community*

Faster Product Development -
CORROSITEX accelerates product development by allowing you to:

- Inexpensively pre-screen and modify new formulations
- Determine the toxic impact of formulations
- Rapidly bring to market better, safer brands

CORROSITEX® BIOBARRIER PREPARATION



a Add entire contents of Diluent to the vial of Biobarrier Matrix powder.

b Stir and warm to 68 - 70°C in a water bath until the Biobarrier Matrix powder has completely dissolved (approximately 20 min.).

c Pipet 200 µl into each membrane disc, ensuring the entire membrane is covered and no air bubbles have formed.

d Note the preparation/expiration dates, seal the tray with plastic wrap and refrigerate (2 - 8°C) immediately. Do not freeze.

Caution: Wear and use safety equipment and conduct test under approved fume hood for corrosive materials.

Pre-made biobarriers available from InVitro.
Ask for details when ordering.

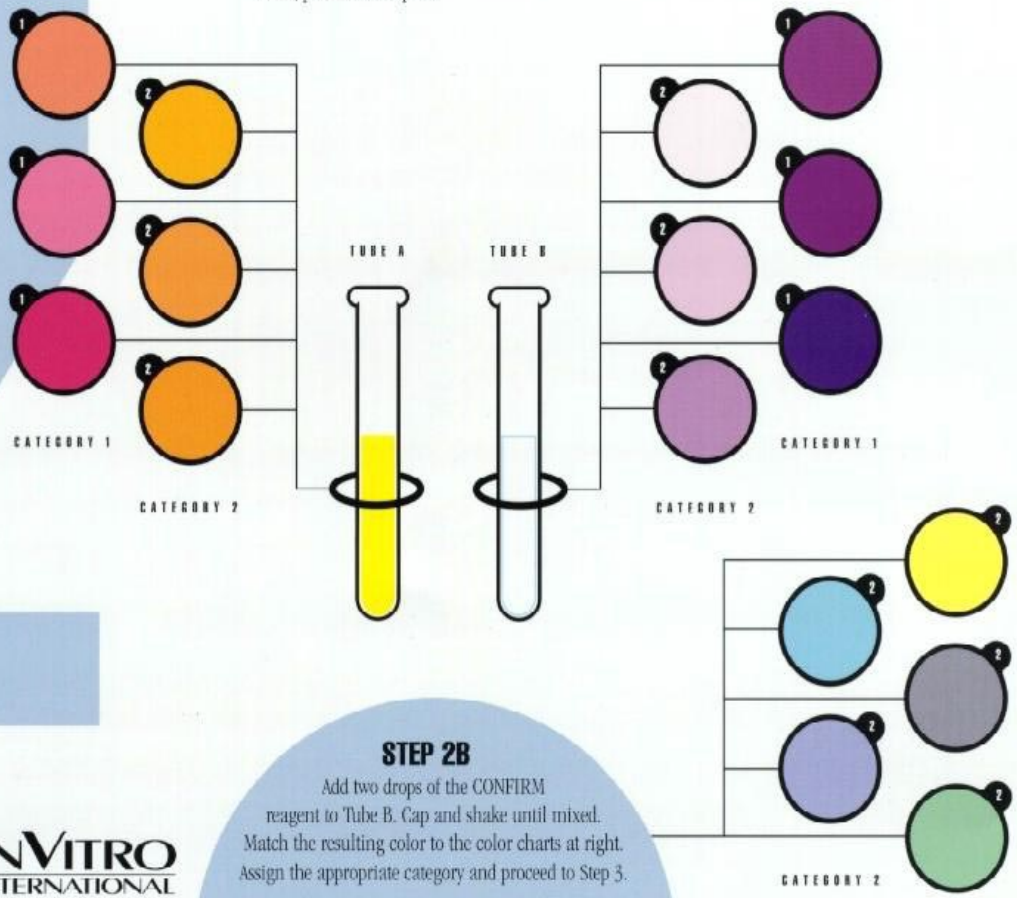
CORROSITEX® TESTING PROTOCOL

STEP 1- QUALIFY

Add your sample (150 µl if liquid, 100 mg if solid) to the Qualify test tube. If the amber liquid changes consistency or color, proceed to Step 2. If a physical change is not observed, your material is not suitable for the Corrositex system. Please call your technical representative at 1-800-2-INVITRO.

STEP 2- CATEGORIZE

Add your sample* (150 µl if liquid, 100 mg if solid) to Tube A and Tube B. Cap and shake until mixed. If a color change is observed in either tube, match the color to the corresponding color chart. Assign the appropriate category and proceed to Step 3. If a color change is *not* observed in either tube, proceed to Step 2B.



STEP 2B

Add two drops of the CONFIRM reagent to Tube B. Cap and shake until mixed. Match the resulting color to the color charts at right. Assign the appropriate category and proceed to Step 3.

*For intensely colored samples, please refer to the instruction manual.

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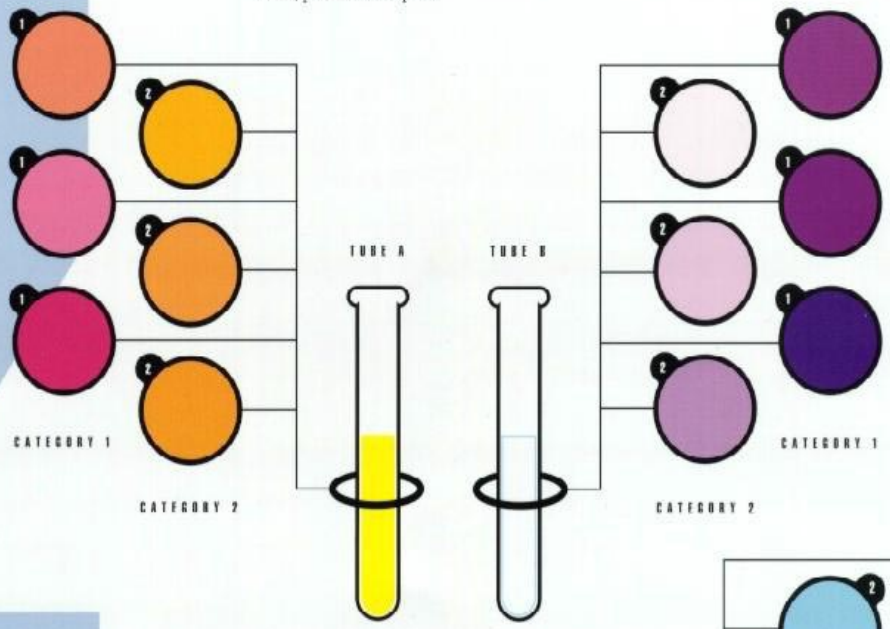
CORROSITEX® TESTING PROTOCOL

STEP 1- QUALIFY

Add your sample (150 µl if liquid, 100 mg if solid) to the Qualify test tube. If the amber liquid changes consistency or color, proceed to Step 2. If a physical change is not observed, your material is not suitable for the Corrositex system. Please call your technical representative at 1-800-2-INVITRO.

STEP 2- CATEGORIZE

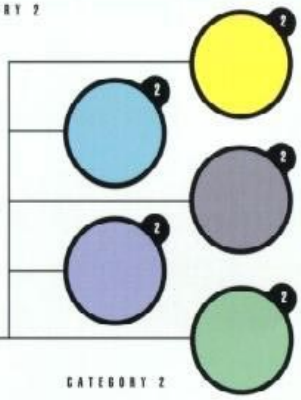
Add your sample* (150 µl if liquid, 100 mg if solid) to Tube A and Tube B. Cap and shake until mixed. If a color change is observed in either tube, match the color to the corresponding color chart. Assign the appropriate category and proceed to Step 3. If a color change is *not* observed in either tube, proceed to Step 2B.



STEP 2B

Add two drops of the CONFIRM reagent to Tube B. Cap and shake until mixed. Match the resulting color to the color charts at right. Assign the appropriate category and proceed to Step 3.

*For intensely colored samples, please refer to the instruction manual.



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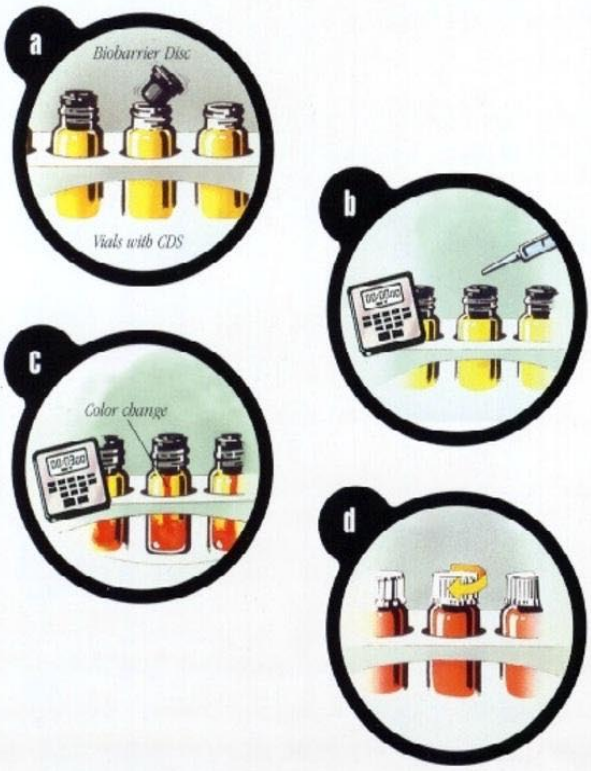
STEP 3- CLASSIFY



a Place one (prepared and refrigerated) Biobarrier Disc in the top of each vial. Begin test immediately (no later than 2 minutes).

b Add 500 µl (liquid) or 500 mg (solid) of your test sample and control chemicals into Biobarrier Discs in each of the 6 vials and start timers. *Caution: Do not cap the vials during the test due to possible pressure build up.*

c As soon as a reaction is observed in the Chemical Detection System, record the detection time.

d Remove each Biobarrier Disc, cap and dispose of vials using your lab protocol for proper chemical disposal.

Use the category determined in Step 2 and the reaction time determined in Step 3 to assign the proper Packing Group as shown in the table to the right.

U.N. PACKING GROUP ASSIGNMENT TABLE

Category	Time Required for CDS change (minutes)			
Category 1	0 to 3 min.	>3 to 60 min.	>60 to 240 min.	>240 min.
Category 2	0 to 3 min.	>3 to 30 min.	>30 to 60 min.	>60 min.
	Packing Group I	**Packing Group II**	**Packing Group III**	**Non-corrosive**

Note: Full compliance with DOT regulations requires metal corrosivity data before packaging as a non-corrosive.

CORROSITEX can be purchased and performed on-site, or you may send your samples off-site to a private laboratory for testing. For more information on how CORROSITEX can benefit your company, contact InVitro International at **1-800-2-INVITRO**.

InVitro International's CUSTOMIZED TECHNOLOGY SERVICES is dedicated to ensuring that our products and services meet your specific requirements.

WE FULLY GUARANTEE YOUR SATISFACTION.

16632 Millikan Ave. • Irvine, CA 92606 • 1-800-2-INVITRO • Fax: 714-851-0563
http://www.invitrointl.com

INVITRO INTERNATIONAL

NICEATM[1] Comparison of the Corrositex® Instruction Manuals

All data submitted in the 1998 Corrositex® Submission was performed according to the Corrositex® Instruction Manual (revised 1/95). The current Corrositex® Instruction Manual (Appendix D) was revised on 12/95. The procedures listed in the two manuals are similar, except for slight modifications including:

1. Biomembrane preparations are to be prepared in the same manner; however, the 12/95 revised manual states that biobarriers are stable for 7 days as compared to 10 days in the 1/95 manual.

2. "Prequalification Test" and "Screening" procedures in the 1/95 manual are similar to the "Qualify" and "Categorize" procedures listed in the 12/95 manual. The stepwise assay procedures are similar between the two manuals, as are the calculations for the Corrositex® breakthrough time. Positive and negative controls and their associated breakthrough times are listed only in the 12/95 revised manual. For determination of Packing Group, US Department of Transportation (US DOT) Approved Category A/B in the 1/95 manual is labeled "Category I", in the revised 12/95 Instruction Manual; the same breakthrough Corrositex® times (minutes) are used for both manuals. US DOT Pending Category A/B in the 1/95 manual is labeled "Category 2" in the revised 12/95 submission. At the request of the US DOT, the Corrositex® times (minutes) for Category 2 agents for Packing Group III designation has changed from ">30 to 45 minutes" in the 1/95 manual to ">30 to 60 minutes" in the 12/95 manual. Similarly, the Corrositex® times (minutes) for Category 2 agents for noncorrosive designation has changed from ">45 minutes" in the 1/95 manual to ">60 minutes" in the 12/95 manual.

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

NICEATM[1] List of Nonqualifying Chemicals for Corrositex®

The following table lists chemicals that were nonqualifying for Corrositex®. The chemicals were obtained from the following sources as indicated in the reference column of the table:

- Barratt, M. D., P. G. Brantom, J. H. Fentem, I. Gerner, A. P. Walker, and A. P. Worth. 1998. The ECVAM international validation study for *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicol. In Vitro 12:471-482.

- Botham, P. A., M. Chamberlain, M. D. Barratt, R. D. Curren, D. J. Esdaile, J. R. Gardner, V. C. Gordon, B. Hildebrand, R. W. Lewis, M. Liebsch, P. Logemann, R. Osborne, M. Ponec, J.-F. Régnier, W. Steiling, A. P. Walker, and M. Balls. 1995. A prevalidation study on *in vitro* skin corrosivity testing: The report and recommendations of ECVAM Workshop 6. ATLA 23:219-255.

- Fentem, J. H., G. E. B. Archer, M. Balls, P. A. Botham, R. D. Curren, L. K. Earl, D. J. Esdaile, H.-G. Holzhütter, and M. Liebsch. 1998. The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicol. In Vitro 12:483-524.

- Gordon, V. C., J. D. Harvell, and H. I. Maibach. 1994. Dermal corrosion, the Corrositex system: A DOT accepted method to predict corrosivity potential of test materials. Alternative Methods Toxicol. 10:37-45.

- InVitro International, Inc. Corrositex® ICCVAM Submission, May, 1998

- Appendix XIII of the October 11, 1996 submission. Please note that data sheets were not provided for this submission. However, to expand the data base of nonqualifiers, these data were provided for consideration the Peer Review Panel (PRP).

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Chemical Name	In Vivo	pH	References	Comment
agrochemical C	NP	6.6	1996 Submission	
agrochemical I	NP	4.6	1996 Submission	
agrochemical N	NP	5.7	1996 Submission	
alkyl benzene sulfonate	-	NP	1996 Submission	
alkyl (4EO) phosphoric acid	-	NP	1996 Submission	
alkylphosphoric acid 4M	-	NP	1996 Submission	
4-amino-1,2,4-triazole	-	5.5	Barratt et al. (1998); Fentem et al. (1998)	
ammonium lauryl sulfate	-	6.65	1996 Submission	
amphoteric/ anionic / nonionic 2 surfactant blend	-	8.16	1996 Submission	
anionic/ amphoteric blend 1	-	7.18	1996 Submission	
anionic blend 4	-	NP	1996 Submission	
Arquad 16-50	+	NP	1996 Submission; Botham et al. (1995)	
arquad 16-50	+	NP	1996 Submission	
Arquad DMMCB-50	+	NP	Botham et al. (1995)	
benzophenone	NP	7.82	1996 Submission	
benzyl alcohol	NP	7.82	1996 Submission	
betaine	-	6.38	1996 Submission	
2-bromobutane; butyl bromide	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; SAR=structure activity relationship

Chemical Name	In Vivo	pH	References	Comment
butylbenzene	-	7.59	1996 Submission	
n -butyl propionate	-	3.6	Barratt et al. (1998); Fentem et al. (1998)	
caprylic acid	+	2.97	1996 Submission	
carvacrol	+	3.9	Barratt et al. (1998); Fentem et al. (1998)	Borderline C/NC chemical, as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
catearyl alcohol	-	NP	1996 Submission	
chloroform	-	6.82	1996 Submission	
clyceryl stearate glyceryl	-	NP	1996 Submission	
cocoalkyl benzyl ammonium chloride	+	NP	1996 Submission	
dicocoalkylbenzyl-ammoniumchloride	+	NP	1996 Submission	
disodium sulfosuccinate	-	5.57	1996 Submission	
disodium cocoamphodiacetate	-	NP	1996 Submission	
disodium cocoamphodipropionate	-	NP	1996 Submission	
dodecanoic acid (lauric acid)	-	NP	Barratt et al. (1998); Fentem et al. (1998)	
Dowanol PNB	-	NP	Botham et al. (1995)	
Duoquad	+	NP	1996 Submission; Botham et al. (1995)	
Duoquad T-50	+	NP	1996 Submission	
Elfan OS46	-	NP	Botham et al. (1995)	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; SAR=structure activity relationship

Chemical Name	In Vivo	pH	References	Comment
2-ethoxyethyl methacrylate	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	
EXP 3830 D	-	NP	1996 Submission	
foralkyl AC8N	-	NP	1996 Submission	
glycine	NP	6.74	1996 Submission	
n -hexanol	-	NP	1996 Submission; Botham et al. (1995)	
Hostaphat KLD	-	NP	Botham et al. (1995)	
hydrogen peroxide	-	NP	1996 Submission	
isopropanol	-	3.6	Barratt et al. (1998); Fentem et al. (1998)	
isostearic acid	-	3.6	Barratt et al. (1998); Fentem et al. (1998)	
lauric acid	-	NP	1996 Submission; Botham et al. (1995)	
methacrylate d'allye	-	NP	1996 Submission	
o-m-e thoxyphenol Guaiacol	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	Borderline C/NC chemical as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
p -methylthiobenzaldehyde	-	NP	1996 Submission	
4,4-methylenebis(2,6-di-*tert*- butylphenol)	-	NP	Barratt et al. (1998); Fentem et al. (1998)	
methyl laurate	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	
methyl palmitate	-	NP	Barratt et al. (1998); Fentem et al. (1998)	
4-(methylthio)benzaldehyde	-	6.8	Barratt et al. (1998); Fentem et al. (1998)	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; SAR=structure activity relationship

Chemical Name	In Vivo	pH	References	Comment
menthyl trimethylacetate; methyl pivalate	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	
mixed C8 amphocarboxylic acid	-	NP	1996 Submission	
myristyl myristate	-	0.43	1996 Submission	
n -nonanol	-	8.97	1996 Submission; Botham et al. (1995)	
Noramium M2SH3	-	NP	1996 Submission	
petrochemical 12	NP	3.69	1996 Submission	
petrochemical 1A	NP	8.57	1996 Submission	
petrochemical 22	NP	6.2	1996 Submission	
petrochemical 26A	NP	7.54	1996 Submission	
petrochemical 40A	NP	7.54	1996 Submission	
petrochemical 48A	NP	8.54	1996 Submission	
petrochemical 49A	NP	9.05	1996 Submission	
petrochemical 5	NP	7.86	1996 Submission	
petrochemical 58A	NP	7.2	1996 Submission	
petrochemical 59A	NP	7.4	1996 Submission	
petrochemical 69A	NP	8.59	1996 Submission	
phenethyl bromide	-	3.6	Barratt et al. (1998); Fentem et al. (1998)	
2-phenylethanol	-	3.6	Barratt et al. (1998); Fentem et al. (1998)	
Proxel BD	+	NP	Botham et al. (1995)	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; SAR=structure activity relationship

Chemical Name	In Vivo	pH	References	Comment
sodium laureth carboxylate-13	-	NP	1996 Submission	
sodium benzyl sulfonate	-	8.99	1996 Submission	
sodium butoxyethoxy acetate	-	5.12	1996 Submission	
sodium caproamphoacetate	-	7.14	1996 Submission	
sodium cocoamphohydroxypropylsulfonate	-	NP	1996 Submission	
sodium cocoamphoproprionate	-	NP	1996 Submission	
sodium lauroamphoacetate/trideceth sulfate	-	NP	1996 Submission	
sodium lauryl sulfate (20% aq.); SDS; SLS; sodium dodecyl sulfate; Irium	-	3.9	Barratt et al. (1998); Fentem et al. (1998)	
sodium methyl cocoyl taurate	-	NP	1996 Submission	
sodium stearoamphoacetate	-	NP	1996 Submission	
sodium trideth sulfate	-	7.89	1996 Submission	
stearyl alcohol, cetrimonium bromide	-	NP	1996 Submission	
stearyl stearate	-	NP	1996 Submission	
sulfoccinate	-	5.69	1996 Submission	
2-*tert*- butylphenol	+	3.9	Barratt et al. (1998); Fentem et al. (1998)	Borderline C/NC chemical as judged subjectively from the proximity of the chemical to the classification boundary (SAR analysis).
thioglycolate d'isooctyle	-	NP	1996 Submission	
n -undecanol	-	6.8	1996 Submission; Botham et al. (1995)	
1-undecanol	-	6.8	1996 Submission	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; SAR=structure activity relationship

NICEATM[1] Evaluation of Nonqualifying Chemicals

Data Set Including the 1996 InVitro International, Inc. (IVI) Submission

The list of nonqualifying chemicals provided in Appendix F was expanded to include data from the 1996 IVI submission. A breakdown of the data sources for nonqualifying chemicals is provided in the table below. Of the 75 chemicals for which *in vivo* data was provided, 64 chemicals (85%) were classified as noncorrosive in the *in vivo* test. The remaining 11 chemicals (15%) were classified as corrosive according to the *in vivo* test.

pH data were provided for 50 chemicals, and the overall distribution is shown in the attached Figure 1. All but one of the nonqualifying chemicals were in the pH range of 3 to 10; for this analysis, all pH values were rounded to the nearest whole number.

Both *in vivo* and pH data were provided for 33 chemicals, and the pH distribution for these materials is shown in Figure 2. Of these 33 chemicals, one would be classified as corrosive using the pH test, although *in vivo* data indicates that this chemical is noncorrosive; 3 chemicals in the pH range between 3 and 3.9 would be classified as corrosive according to *in vivo* results.

Data Set Excluding the 1996 IVI Submission

Data on 30 nonqualifying chemicals were provided in the Fentem et al. (1998) and Botham et al. (1995) papers. Of these 30 materials, 24 (80%) were classified as noncorrosive in the *in vivo* test. The remaining 6 (20%) were classified as corrosive according to the *in vivo* test. Both pH and *in vivo* data were available for 18 chemicals in this data set; the pH distribution for these chemicals was nearly identical to that found for the 33 chemicals in the total data set.

Source of Data on Nonqualifying Test Materials

Source	NQ (Total)	With pH data	Without pH data	With *in vivo* data	Without *in vivo* data	With pH and *in vivo* data
Fentem et al. (1998)	18	15	3	18	--	15
Botham et al. (1995)	12	3	9	12	--	3
Gordon et al. (1994)	0	--	--	--	--	--
1998 IVI submission	0[a]	--	--	--	--	--
1996 IVI submission[b]	62	32	30	45	17	15
Total	92	50	42	75	17	33

[a]IVI was advised by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) not to submit data for nonqualifying chemicals.

[b]Note that data sheets were not submitted for these results; therefore, no quality assurance audit was conducted for these data.

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

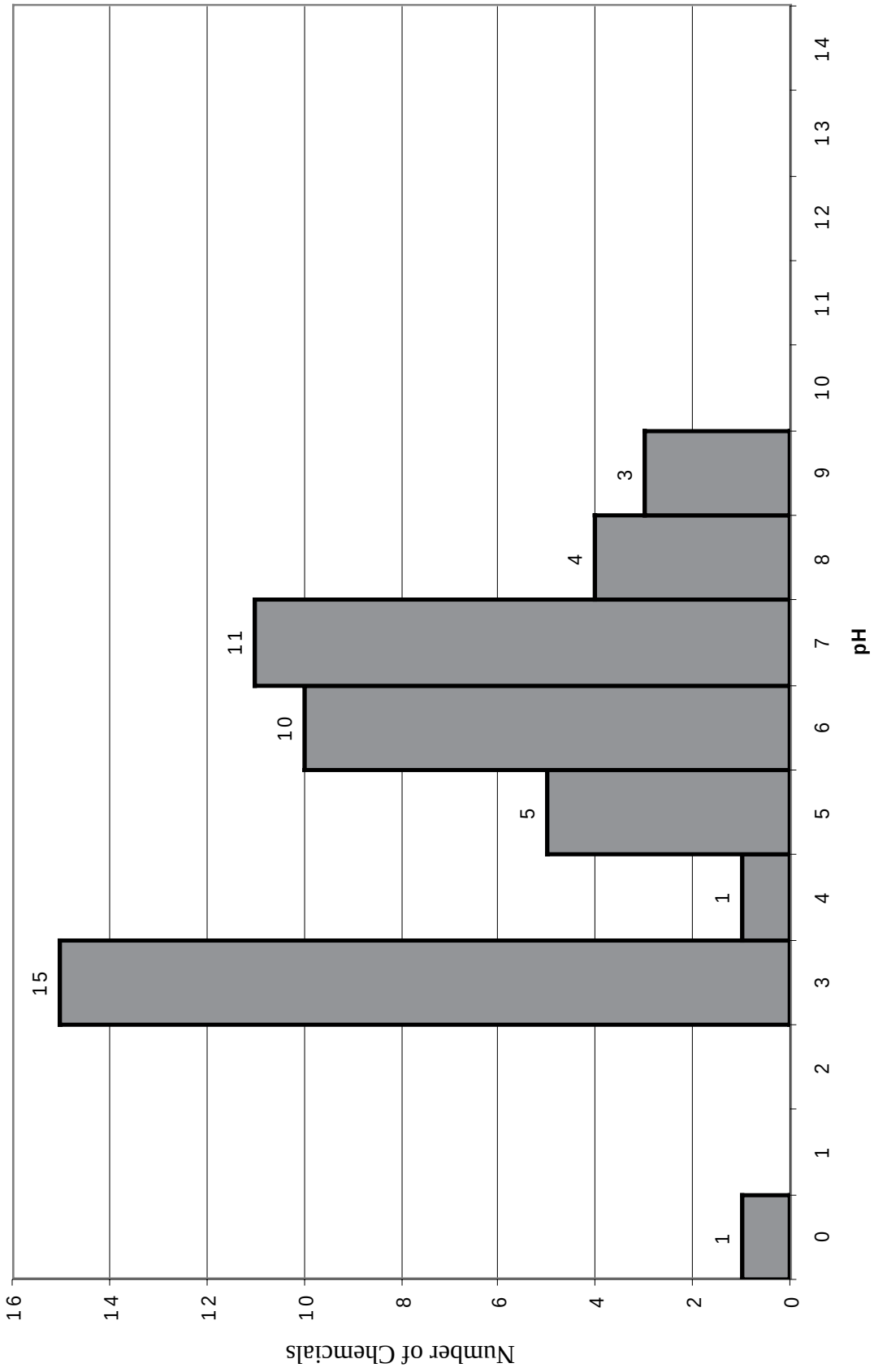
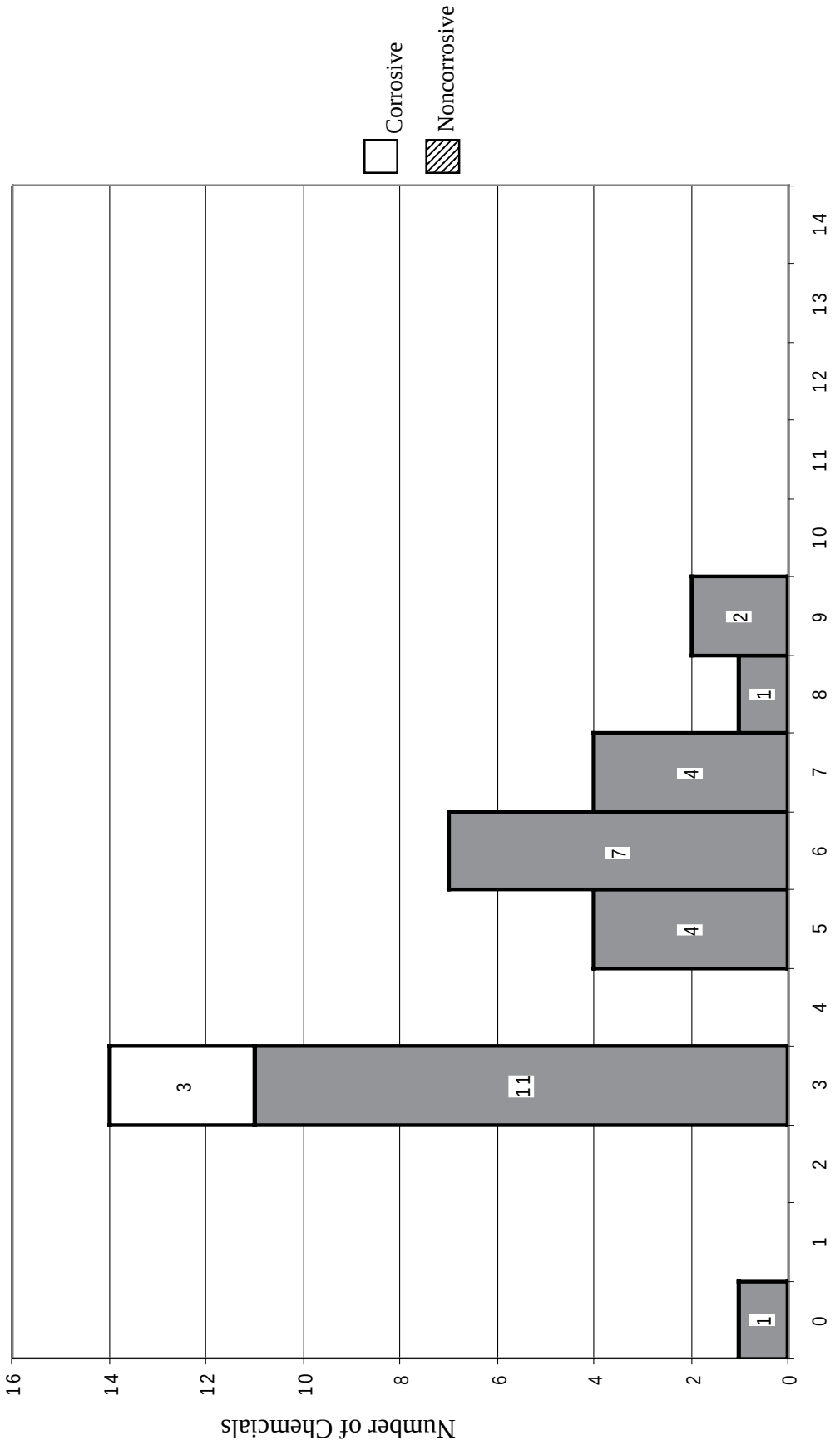


Figure 1. pH Distribution for Nonqualifying Chemicals (Total) (N=50)

Figure 2. pH Distribution for Nonqualifying Chemicals for which *In Vivo* Data was Available (N=33)



NICEATM Table of Breakthrough Times for Chemicals Provided by InVitro International, Inc.

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
315		28.88	28.75	28.63	28.57	28.47	28.37	**28.61**	**0.19**	
485		28.67	28.50	28.33	28.17	28.00	27.83	**28.25**	**0.31**	
880		16.33	16.37	15.65	16.17	15.37	15.25	**15.86**	**0.50**	
3000		24.17	24.08	24.48	24.80	23.90	23.58	**24.17**	**0.43**	
4000		20.25	20.50	20.53	20.03	20.37	20.33	**20.34**	**0.18**	
91440										Not found in provided data sheets.
1,2-diaminopropane	78-90-0	21.67	21.67					**21.67**	**0.00**	
1-(2-aminoethyl)piperazine	140-31-8	38.83	38.73					**38.78**	**0.07**	
10-Undecenoic acid	112-38-9									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
122B		12.60	12.25	12.22	12.28	12.53	12.73	**12.44**	**0.21**	
1702BR		25.70	25.20	28.12	25.13	24.93	27.92	**26.13**	**1.46**	
1703CR		28.67	28.63	28.58	28.33	27.92	28.42	**28.43**	**0.28**	
1709B		27.08	27.53	27.17	26.93	27.23	26.73	**27.11**	**0.27**	
2,4-xylidine	95-68-1									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
2-mercapoethanol, sodium salt (45% aq.)	37482-11-4									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
2-methylbutyric acid	600-07-7									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
20/80-coconut/palm soap										Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
3,3'-Dithiodipropionic acid	1119-62-6									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
boron trifluoride-acetic acid complex		3.57	3.40	3.43				**3.47**	**0.09**	
BSS 9487		13.98	13.98					**13.98**	**0.00**	
butylamine (in ethanol [EtOH]/ethylene glycol [EG] 1:1)	109-73-9 64-17-5 107-21-1	25.58	27.07					**26.33**	**1.05**	
butyric acid	107-92-6	40.58	40.55					**40.57**	**0.02**	
calcium carbonate		> 4 h								
Cascade		62.52	61.37					**60.95**	**0.81**	
Cellosolve + TX100		> 4 h								
chromium(III) fluoride		171.67	171.52					**171.60**	**0.11**	
cinnamaldehyde	14371-10-9									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
copper(II) chloride		35.68	35.85	35.60				**35.71**	**0.13**	
COR 11		31.83	31.67					**31.75**	**0.11**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
boron trifluoride-acetic acid complex		3.57	3.40	3.43				**3.47**	**0.09**	
BSS 9487		13.98	13.98					**13.98**	**0.00**	
butylamine (in EtOH/EG 1:1)	109-73-9 64-17-5 107-21-1	25.58	27.07					**26.33**	**1.05**	
butyric acid	107-92-6	40.58	40.55					**40.57**	**0.02**	
calcium carbonate		> 4 h								
Cascade		62.52	61.37					**60.95**	**0.81**	
Cellosolve + TX100		> 4 h								
chromium(III) fluoride		171.67	171.52					**171.60**	**0.11**	
cinnamaldehyde	14371-10-9									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
copper(II) chloride		35.68	35.85	35.60				**35.71**	**0.13**	
COR 11		31.83	31.67					**31.75**	**0.11**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
COR 18		29.58	31.98					**30.78**	**1.70**	
COR 19		23.83	24.00					**23.92**	**0.12**	
COR 20		30.17	30.17					**30.17**	**0.00**	
COR 3		15.17	15.17					**15.17**	**0.00**	
COR 4		39.08	38.92					**39.00**	**0.11**	
COR 6		17.92	17.90					**17.91**	**0.01**	
COR 9		35.42	35.33					**35.30**	**0.06**	
Coverage Plus		88.17	88.00					**88.90**	**0.12**	
cyclohexylamine	108-91-8	42.82	42.75					**42.79**	**0.05**	
dicyclohexylamine	101-83-7	182.48	196.30	187.88	196.82			**190.87**	**6.93**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
dimethyldipropylenetriamine	10563-29-8									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
dimethylisopropylamine	996-35-0									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
ethanolamine	141-43-5	23.92	23.83					**23.88**	**0.06**	
ethanolamine 80%		22.03	22.15					**22.09**	**0.08**	
ferric chloride; iron (III) chloride	7705-08-0	27.33	27.25					**27.29**	**0.06**	
fluoboric acid; hydrogen tetrafluoroborate	16872-11-0	2.43	2.55					**2.49**	**0.08**	
Formula # 100-016		41.83	41.67	41.50				**41.67**	**0.17**	
Formula # 100-057B		> 4 h								
Formula # 100-088		40.50	40.37	40.3	40.25	40.13	40.17	**40.29**	**0.14**	
GIN		11.20	11.18					**11.19**	**0.01**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
glycol bromoacetate (85%)	3785-34-0									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
hexanoic acid	142-62-1	133.43	133.37					**133.40**	**0.04**	
hydrochloric acid (14.4% wt)	7647-01-1									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
hydrochloric acid + sulfuric acid + citric acid		9.07	9.10					**9.09**	**0.02**	
hydrogen bromide, aqueous sol.; hydrobromic acid	10035-10-6	2.53	2.03					**2.28**	**0.35**	
hydrogenated tallow amine	61788-45-2									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
Larc Super		> 4 h								
LMCRK		21.97	22.23					**22.10**	**0.18**	
maleic acid		9.58	9.53					**9.56**	**0.04**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
Master Chemical 019A		81.47	81.42	81.7				**81.53**	**0.15**	
N,N -dimethylbenzylamine	103-83-3	86.17	86.17					**86.17**	**0.00**	
n- Heptylamine	111-68-2									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
octanoic acid; caprylic acid	124-07-02									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
phosphoric acid	7664-38-2	10.95	10.97					**10.96**	**0.01**	
phosphorus pentachloride	10026-13-8	0.08	0.08					**0.08**	**0.00**	
phosphorus tribromide	7789-60-8	1.00	1.03					**1.02**	**0.02**	
potassium bisulfate; potassium hydrogen sulfate	7646-93-7	21.78	28.13					**24.96**	**4.49**	Mean is listed as 25.11 in the Submission.
potassium hydroxide (5% aq.)	1310-58-3									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
potassium hydroxide 3%	1310-58-3	21.08	21.20					**21.14**	**0.08**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
propionic acid		19.17	19.20					**19.19**	**0.02**	
propionic acid		38.35	36.22	38.98	37.57			**37.78**	**1.19**	
RP-26		> 4 h								
RP-29		> 4 h								
RP-33		> 4 h								
RP-34		> 4 h								
RP-39		> 4 h								
RP-40		> 4 h								
RP-46		> 4 h								
RP-49		> 4 h								
RP-52		> 4 h								

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
RP-53		> 4 h								
RP-57		> 4 h								
RP-61		> 4 h								
RP-62		> 4 h								
RP-80B		> 4 h								
RP-81B		> 4 h								
sodium bicarbonate; sodium hydrogen carbonate	144-55-8									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
sodium bisulfite; sodium sulfite carbonate	7631-90-5									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
sodium carbonate (50% aq.)	497-19-8									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
sodium hydrogen fluoride		71.57	71.17					**71.37**	**0.28**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
sodium hydroxide/N,N'-bis(acrylyl)cystamine (BAC) (1:10)		34.35	34.30					**34.32**	**0.04**	
sodium hydroxide/sodium dodecyl sulfate (SDS) (1:10)		32.13	32.07					**32.10**	**0.04**	
sodium hydroxide/SMS (0.5:1)		24.87	24.80					**24.84**	**0.05**	
sodium hydroxide/SMS (1:10)		17.65	17.67					**17.66**	**0.01**	
sodium hydroxide/SMS (5:3)		15.80	15.38					**15.59**	**0.30**	
sodium hydroxide/tx 100		15.13	15.32					**15.23**	**0.13**	
sodium hypochlorite w/ 5% chlorine		> 4 h								
sodium undecylenate	3398-33-2									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
Solcenic 2BW		69.65	69.60	69.53	69.67	69.56	69.68	**69.62**	**0.06**	
Solcenic 3A		138.80	138.75					**138.78**	**0.04**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
Solcenic 3B		70.23	70.18	70.12	70.10	70.08	70.06	**70.13**	**0.06**	
sulfamic acid; midosulfonic acid	5329-14-6									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
sulfuric acid (10% wt.)	7664-93-9	22.63	22.63					**22.63**	**0.00**	
sulfuric acid + Cellusolve		15.08	14.92					**15.00**	**0.12**	
sulfuric acid + ferric chloride		15.05	14.92					**14.98**	**0.09**	
sulfuric acid + SDS		16.22	16.08					**16.15**	**0.09**	
sulfuric acid + Triton x-100	9002-93-1	16.93	16.78					**16.86**	**0.11**	
tallow amine	61790-33-8									Source: ECVAM Validation Study (Fentem et al., 1998). No original data submitted.
TBQ		56.27	56.22					**56.25**	**0.04**	
Tide		77.58	62.35					**69.96**	**10.77**	

Chemical Name	CASRN	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Mean	Standard Deviation	Comments
Toilet Duck Green		177.87	177.75					**177.81**	**0.08**	
tributylamine		> 4 h								
trichloro-toluene		> 4 h								
Windex Blue		34.45	35.18	34.70				**34.78**	**0.37**	
ZFA		75.33	70.48					**72.91**	**3.43**	
ZWS 9352		31.42	31.53					**31.48**	**0.08**	

NICEATM[1] Evaluation of the Performance of Corrositex® and the pH Test

The tabulated data as shown in the List of Chemicals (Appendix A) were used to compare the performance of Corrositex® and the pH Test; both of the tests were compared against *in vivo* data. Only those chemicals for which pH was available were used in the analysis. Additionally, any chemicals that produced discordant results between papers/submissions or had discordant results in multiple labs within the same study were not included in this analysis.

Also included in this section are graphs of the distribution of chemicals over the entire pH range. The first graph depicts the distribution of pH for the entire data set (Submission, Prevalidation Study [Botham et al., 1995], ECVAM Validation Study [Fentem et al., 1998], and Gordon et al., 1994). The next two graphs depict the distribution of pH for the chemicals provided in the Submission and the ECVAM Validation Study, respectively.

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

NICEATM Evaluation of the Performance of the Corrositex® and the pH Test[1] in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings

Comparison	Number of Chemicals	Accuracy %	Accuracy Number	Sensitivity %	Sensitivity Number	Specificity %	Specificity Number	Positive Predictivity %	Positive Predictivity Number	Negative Predictivity %	Negative Predictivity Number	False Positive Rate %	False Positive Rate Number	False Negative Rate %	False Negative Rate Number
For Chemicals with a pH <2															
Corrositex® vs. *In Vivo*	51	94	(48/51)	100	(48/48)	0	(0/3)	94	(48/51)		NA	100	(3/3)	0	(0/48)
pH Test vs. *In Vivo*	51	94	(48/51)	100	(48/48)	0	(0/3)	94	(48/51)		NA	100	(3/3)	0	(0/48)
For Chemicals with a pH between 2 and 4															
Corrositex® vs. *In Vivo* [2]	30	67	(20/30)	55	(11/20)	90	(9/10)	92	(11/12)	50	(9/18)	10	(1/10)	45	(9/20)
pH Test vs. *In Vivo*	44	48	(21/44)	0	(0/23)	100	(21/21)		NA	48	(21/44)	0	(0/21)	100	(23/23)
For Chemicals with a pH between 4 and 8															
Corrositex® vs. *In Vivo* [3]	17	100	(17/17)	100	(4/4)	100	(13/13)	100	(4/4)	100	(13/13)	0	(0/13)	0	(0/4)
pH Test vs. *In Vivo*	22	82	(18/22)	0	(0/4)	100	(18/18)		NA	82	(18/22)	0	(0/18)	100	(4/4)
For Chemicals with a pH between 8 and 11.5															
Corrositex® vs. *In Vivo* [4]	15	93	(14/15)	100	(7/7)	88	(7/8)	88	(7/8)	100	(7/7)	13	(1/8)	0	(0/7)
pH Test vs. *In Vivo*	16	56	(9/16)	0	(0/7)	100	(9/9)		NA	56	(9/16)	0	(0/9)	100	(7/7)
For Chemicals with a pH > 11.5 [5]															
Corrositex® vs. *In Vivo*	27	96	(26/27)	100	(23/23)	75	(3/4)	96	(23/24)	100	(3/3)	25	(1/4)	0	(0/23)
pH Test vs. *In Vivo*	27	85	(23/27)	100	(23/23)	0	(0/4)	85	(23/27)		NA	100	(4/4)	0	(0/23)

[1] For the purposes of this analysis, a chemical/compound was deemed corrosive in the pH Test if that chemical/compound had a pH less than or equal to 2 or greater than or equal to 11.5 (40 CFR 158.690).

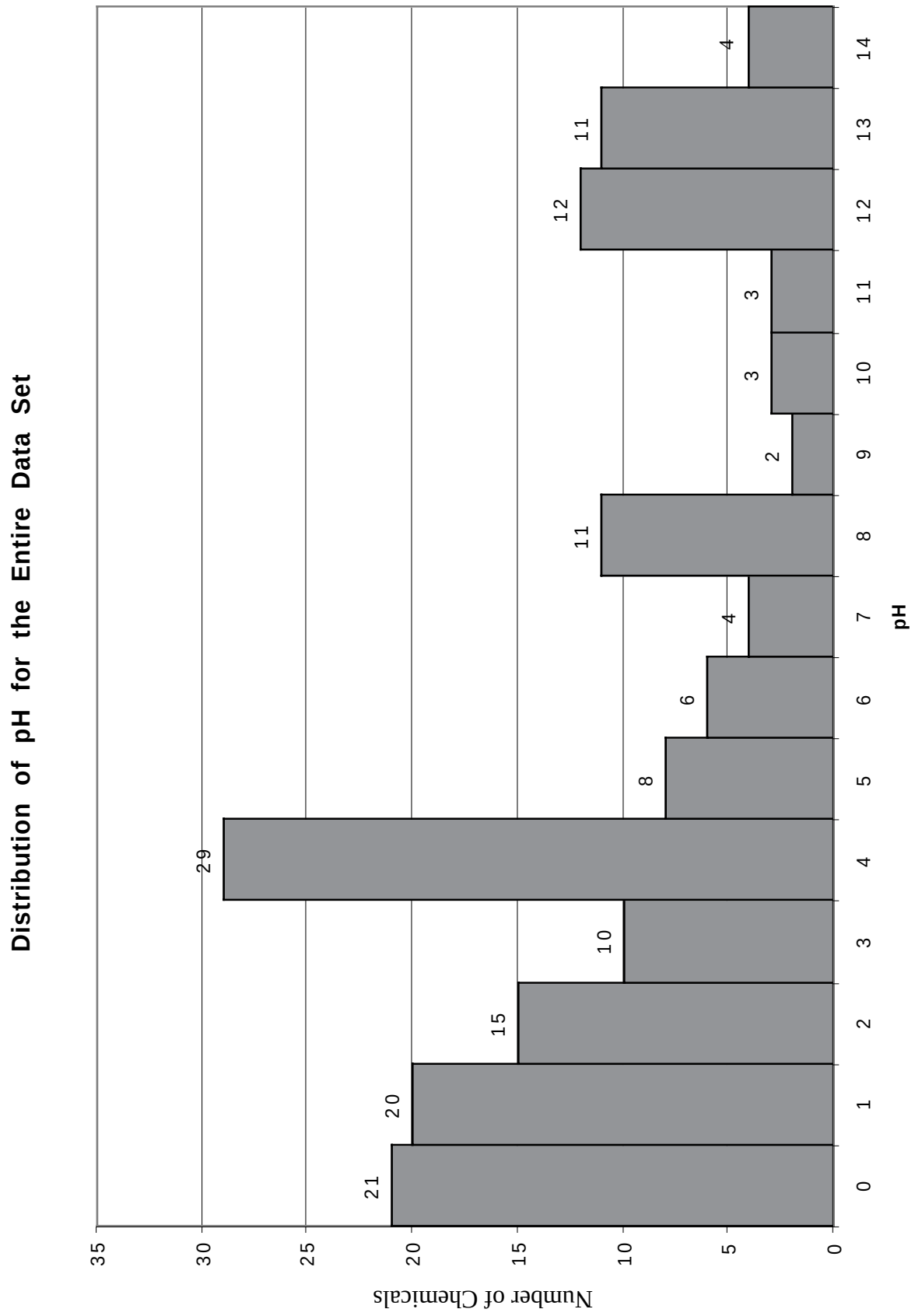
[2] One chemical (hexanoic acid) was not included in this analysis because discordant Corrositex® results were obtained. Thirteen chemicals were nonqualifying in Corrositex®, and were therefore, not included (isostearic acid; *n* -butyl propionate; isopropanol; phenethyl bromide; 2-phenylethanol; *o* -methoxyphenol guiacol; 2-bromobutane; 2-ethoxyethyl methacrylate; methyl trimethylacetate; methyl laurate; sodium lauryl sulfate; carvacrol; and 2-*tert* -butylphenol).

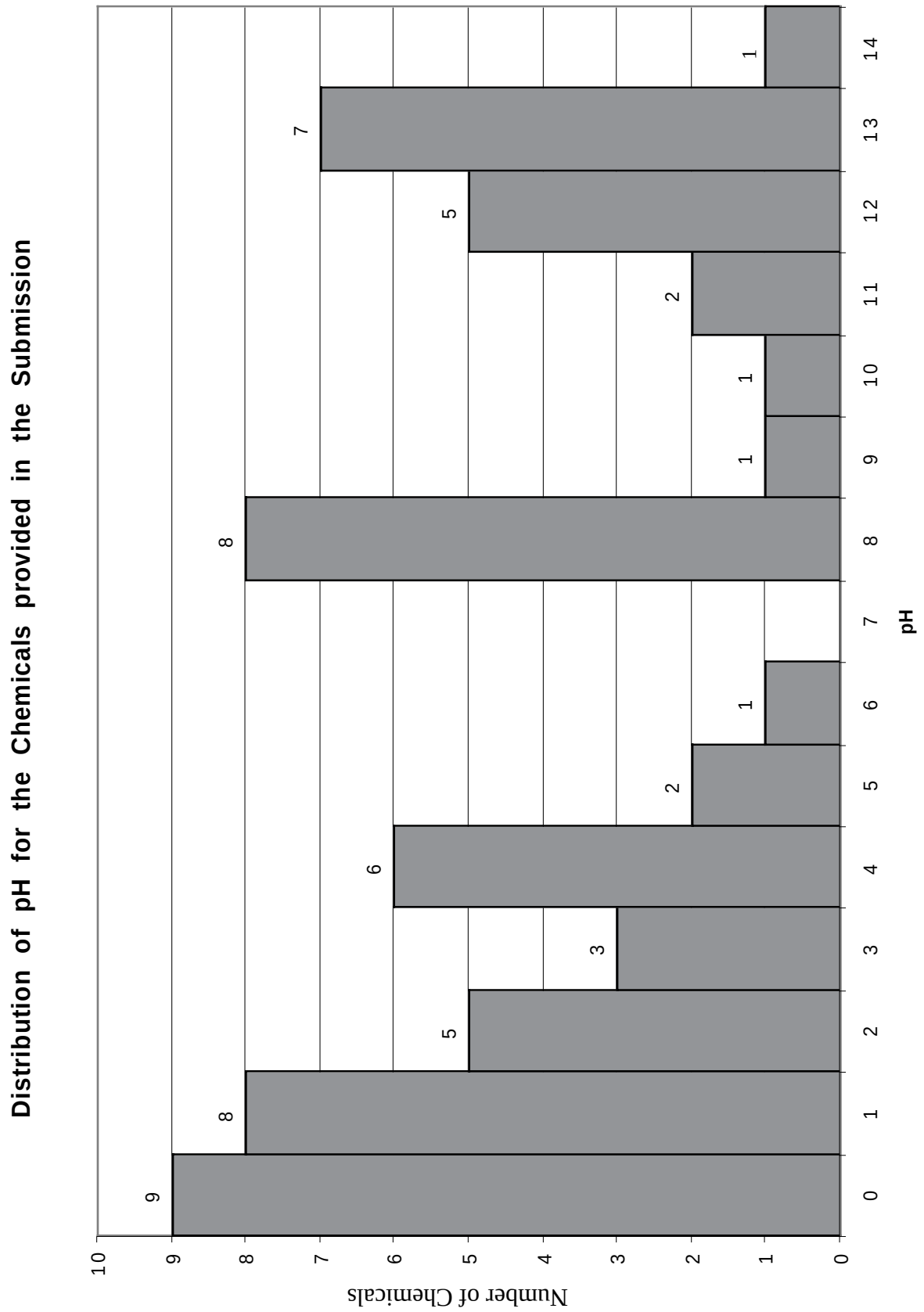
[3] One chemical (2,4-xylidine) was not included in this analysis because discordant Corrositex® results were obtained. Four chemicals were nonqualifying in Corrositex®, and were therefore, not included (4-amino-1,2,4-triazole; butylbenzene; 4-(methylthio)benzaldehyde; *n* -undecanol).

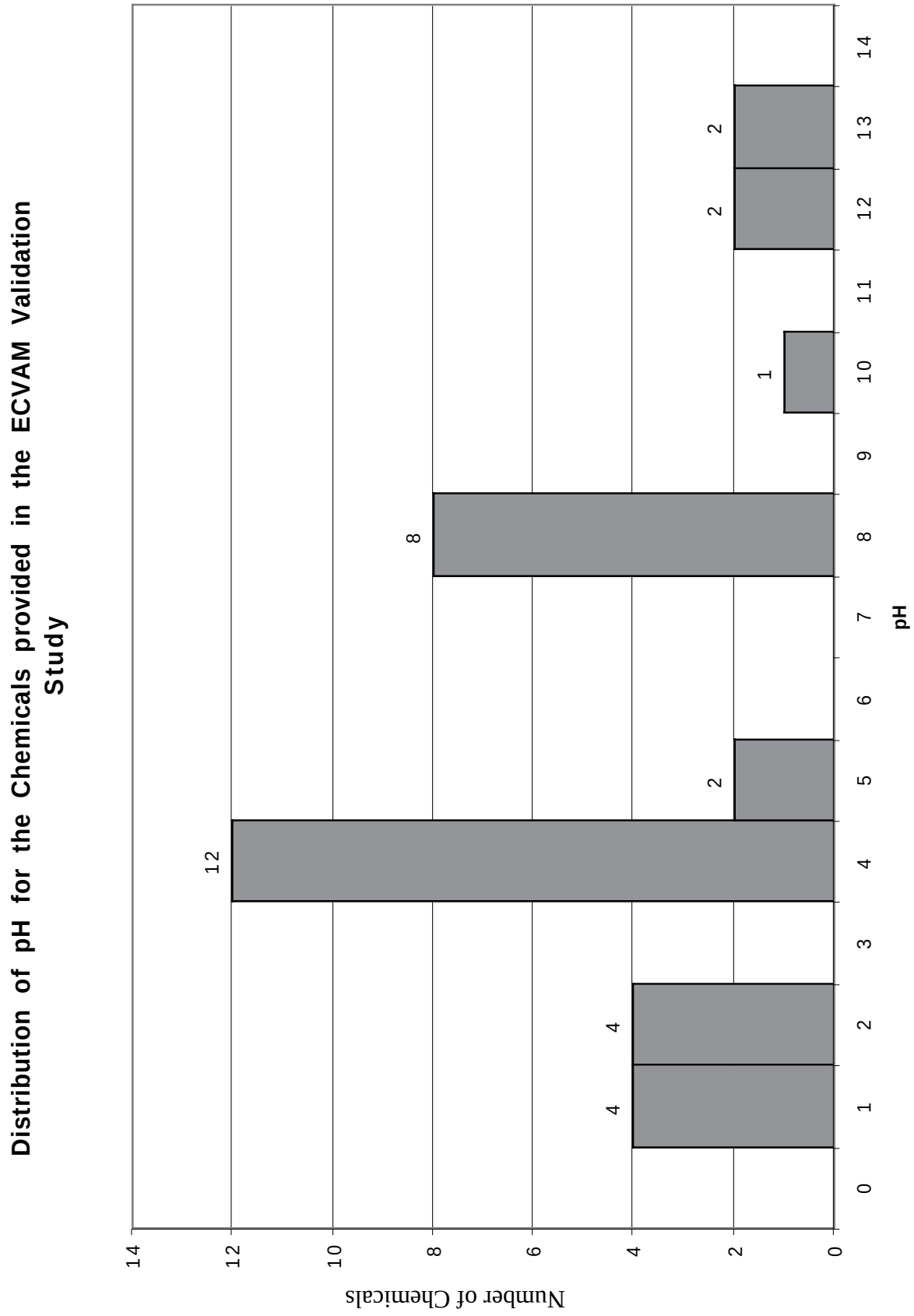
[4] One chemical (*n* -nonanol) was nonqualifying in Corrositex®, and was therefore, not included.

[5] One chemical (potassium hydroxide) was not included in the either of the following comparisons because discordant *in vivo* results were obtained.

Abbreviations: NA = not applicable


Distribution of pH for the Entire Data Set
Number of Chemicals
0
5
10
15
20
25
30
35
pH
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
21
20
15
10
29
8
6
4
11
2
3
3
12
11
4


Distribution of pH for the Chemicals provided in the Submission
Number of Chemicals
pH
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
9
8
5
3
6
2
1
8
1
1
2
5
7
1


Distribution of pH for the Chemicals provided in the ECVAM Validation Study
Number of Chemicals
0
2
4
6
8
10
12
14
pH
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
4
4
12
2
8
1
2
2

NICEATM[1] Description of the Data Set used to Evaluate the Performance of Corrositex® and the pH Test at Varying pH Levels

The following information describes the data set that was used to evaluate the performance of the Corrositex® Test and the pH Test for corrosivity versus *in vivo* results. This is a subset of the data provided in Appendix A.

pH less than or equal to 2

Total number of chemicals with pH data = 51

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 3
Number of chemicals that are discordant between *in vivo* and pH results = 3

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
gin	Industrial Chemical	-	+	+
maleic acid	organic acid	-	+	+
sulfamic acid	inorganic acid	-	+	+

Abbreviations: - = noncorrosive, + = corrosive

pH between 2 and 4

Total number of chemicals with pH data = 44

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 10
Number of chemicals that are discordant between *in vivo* and pH results = 22

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
acrylic acid	organic acid	+	+	-
ferrous chloride tetrahydrate	acid derivative	+	+	-
butyric acid	organic acid	+	+	-
acetic acid	organic acid	+	+	-
crotonic acid	organic acid	+	+	-
dimethylcarbamyl chloride	acid derivative	+	+	-
aluminum chloride	acid derivative	+	+	-
butyric anhydride	acid derivative	+	+	-
hydroxylamine sulfate	amine	+	+	-
2-methylbutyric acid	organic acid	+	+	-
benzyl chloroformate	acid derivative	+	-	-

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
caprylic acid	organic acid	+	-	-
capric/caprylic acid	organic acid	+	-	-
methacrolein	aldehyde	+	-	-
octanoic acid	organic acid	+	-	-
65/35 octanoic/decanoic acids	organic acid	+	-	-
55/45 octanoic/decanoic acids	organic acid	+	-	-
60/40 octanoic/decanoic acids	organic acid	+	-	-
allyl bromide	industrial chemical	+	-	-
Copper (II) chloride	acid derivative	-	+	-
isostearic acid	organic acid	-	nq	-
n-butyl propionate	neutral organic	-	nq	-
isopropanol	neutral organic	-	nq	-
phenethyl bromide	alkyl halide	-	nq	-
2-phenylethanol	neutral organic	-	nq	-
o-methoxyophenol guaiacol	organic acid	-	nq	-
2-bromobutane	industrial chemical	-	nq	-
2-ethoxyethyl methacrylate	industrial chemical	-	nq	-
methyl trimethylacetate	industrial chemical	-	nq	-
methyl laurate	surfactant	-	nq	-
sodium lauryl sulfate	soap/surfactant	-	nq	-
carvacrol	organic acid	+	nq	-
2-*tert*-butylphenol	organic acid	+	nq	-
hexanoic acid	organic acid	+	discordant	-

Abbreviations: - = noncorrosive, + = corrosive, nq = nonqualifying

pH between 4 and 8

Total number of chemicals with pH data = 22

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 0
Number of chemicals that are discordant between *in vivo* and pH results = 3

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
ammonium hydrogen difluoride	acid derivative	+	+	-
sulfur monochloride	acid derivative	+	+	-
1-(2-aminoethyl)piperazine	amine	+	+	-
4-amino-1,2,4-triazole	amine	-	nq	-
butylbenzene	industrial chemical	-	nq	-
4-(methylthio)benzaldehyde	aldehyde	-	nq	-
n-undecanol	industrial chemical	-	nq	-
2,4-xylidine	amine	-	discordant	-

Abbreviations: - = noncorrosive, + = corrosive, nq = nonqualifying

pH between 8 and 11.5

Total number of chemicals with pH data = 16

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 1
Number of chemicals that are discordant between *in vivo* and pH results = 7

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
1,2-diaminopropane	amine	+	+	-
dimethyldipropylenetriamine	amine	+	+	-
dimethylisopropylamine	amine	+	+	-
n-heptylamine	amine	+	+	-
dicyclohexylamine	amine	+	+	-
3-methoxypropylamine	amine	+	+	-
N,*N*-dimethylbenzylamine	amine	+	+	-
n-nonanol	industrial chemical	-	nq	-
triethanolamine	amine	-	+	-

Abbreviations: - = noncorrosive, + = corrosive, nq = nonqualifying

pH greater than 11.5

Total number of chemicals with pH data = 28

If pH 11.5 is used as the cutoff for corrosivity:

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 1
Number of chemicals that are discordant between *in vivo* and pH results = 4

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
sodium hypochlorite w/ chlorine	acid derivative	-	-	+
calcium carbonate	acid derivative	-	-	+
degreaser	degreaser	-	-	+
sodium carbonate	inorganic base	-	+	+
potassium hydroxide	inorganic base	discordant	+	+

If pH 12.5 is used as the cutoff for corrosivity:

Number of chemicals that are discordant between *in vivo* and Corrositex® results = 1
Number of chemicals that are discordant between *in vivo* and pH results = 12

Breakdown of discordant results:

Chemical	**Chemical/Product Class**	***In Vivo***	**Corrositex**	**pH**
calcium carbonate	acid derivative	-	-	+
degreaser	degreaser	-	-	+
sodium carbonate	inorganic base	-	+	-
lithium hydroxide monohydrate	inorganic base	+	+	-
ethanolamine	amine	+	+	-
tetraethylenepentamine	amine	+	+	-
triethylenetetramine	amine	+	+	-
2-ethylhexylamine	amine	+	+	-
2-mercaptoethanol, sodium salt	inorganic base	+	+	-
diethylamine	amine	+	+	-
diethylenetriamine	amine	+	+	-
ethylenediamine	amine	+	+	-
cyclohexylamine	amine	+	+	-
potassium hydroxide	inorganic base	discordant	+	+

Abbreviations: - = noncorrosive, + = corrosive, nq = nonqualifying

NICEATM[1] Assessment of the Performance of Corrositex® Compared to the Performance of the pH Test for Test Materials in the Extreme pH Ranges

In an effort to evaluate the usefulness of Corrositex® compared to the pH Test for test materials with pH values in the extreme ranges (i.e., pH ≤ 2.0 and pH ≥ 11.5), the database of test materials used in the analysis provided in Appendices I and J was expanded to include information from the following sources:

- Fentem, J. H., G. E. B. Archer, M. Balls, P. A. Botham, R. D. Curren, L. K. Earl, D. J. Esdaile, H.-G. Holzhütter, and M. Liebsch. 1998. The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicol. In Vitro 12:483-524.

- Gordon, V. C., J. D. Harvell, and H. I. Maibach. 1994. Dermal corrosion, the Corrositex system: A DOT accepted method to predict corrosivity potential of test materials. Alternative Methods Toxicol. 10:37-45.

- InVitro International, Inc. Corrositex® ICCVAM Submission, May, 1998

- October, 1996 Corrositex® ICCVAM Submission, provided by InVitro International, Inc. [Please note: Supporting data sheets for Corrositex® and *in vivo* data were not provided for the test materials in this submission; quality assurance analysis was not conducted on these results.]

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Table 1. Summary of Results for Test Materials with a pH ≤ 2.

In Vivo	pH	Corrositex	Number of Test Materials
+	+	+	102
-	+	+	13
-	+	-	6[a]
-	+	discordant [1]	1[b]
-	+	nonqualifying	1[c]

[a] The six test materials were as follows, with test concentrations in parentheses: chemical #9, citric acid (5%), citric acid (10%), citric acid (14.6%), ferric chloride (2%), and oxalic acid (10%).
[b] The discordant test material was identified as chemical #83151 (a petrochemical).
[c] The nonqualifying chemical was methyl myristate.

Table 2. Summary of Results for Test Materials with a pH ≥ 11.5.

In Vivo	pH	Corrositex	Number of Test Materials
+	+	+	100
+	+	-	2[a]
discordant	+	+	2[b]
-	+	+	29
-	+	-	10[c]
-	+	discordant	1[d]

[a] The two test materials were cellusolve/SMS (20/1%) and Cleaner #13.
[b] The test materials that were discordant based on *in vivo* tests were potassium hydroxide (5%) and sodium hydroxide (5%).
[c] The 11 test materials were as follows, with test concentrations in parentheses: bathroom cleaner, bathroom cleaner #2, calcium carbonate (neat), three different cleaners, degreaser, sodium hydroxide (0.1%), sodium hypochlorite (5%), and sodium metasilicate (2%).
[d] The test material that was discordant in Corrositex tests was cleaner #14.

Table 3. Summary of Results for Test Materials with a pH ≤ 2 or ≥ 11.5.

In Vivo	pH	Corrositex	Number of Test Materials[a]
+	+	+	202
+	+	-	2
-	+	+	42
-	+	-	16
-	+	discordant	2
-	+	nonqualifying	1
discordant	+	+	2

[a]Please see the footnotes to Tables 1 and 2 for specific test material information.

[1] Different labs within a study and/or source papers reported different results.

Table 4. Performance of Corrositex® and the pH Test as Compared to *In Vivo* Findings for Test Materials in the Extreme pH Ranges (i.e., pH ≤ 2 and pH ≥ 11.5)

Comparison	Number of Test Materials	Accuracy [1]		Sensitivity [2]		Specificity [3]		Positive Predictivity [4]		Negative Predictivity [5]		False Positive Rate [6]		False Negative Rate [7]	
		%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number
Total Data Set															
Corrositex® vs. *In Vivo*	262	83%	(218/262)	99%	(202/204)	28%	(16/58)	83%	(202/244)	89%	(16/18)	72%	(42/58)	1%	(2/204)
pH vs. *In Vivo*	265	77%	(204/265)	100%	(204/204)	0%	(0/61)	77%	(204/265)	NA	(0/0)	100%	(61/61)	0%	(0/204)
pH ≤ 2															
Corrositex® vs. *In Vivo*	121	89%	(108/121)	100%	(102/102)	32%	(6/19)	90%	(102/113)	100%	(6/6)	68%	(13/19)	0%	(0/102)
pH vs. *In Vivo*	123	83%	(102/1230	100%	(102/102)	0%	(0/21)	83%	(102/123)	NA	(0/0)	100%	(21/21)	0%	(0/102)
pH ≥ 11.5															
Corrositex® vs. *In Vivo*	141	78%	(110/141)	98%	(100/102)	26%	(10/39)	78%	(100/129)	83%	(10/12)	74%	(29/39)	2%	(2/102)
pH vs. *In Vivo*	142	72%	(102/142)	100%	(102/102)	0%	(0/40)	72%	(102/142)	NA	(0/0)	100%	(40/40)	0%	(0/102)

[1] Accuracy: (a) The closeness of agreement between a test result and an accepted reference value. (b) The proportion of the correct outcomes of the method. Often used interchangeably with concordance.

[2] Sensitivity: The proportion of all positive test materials that are correctly classified as positive in a test.

[3] Specificity: The proportion of all negative test materials that are correctly classified as negative in a test.

[4] Positive Predicitivity: The proportion of correct positive responses among materials testing positive.

[5] Negative Predictivity: The proportion of correct negative responses among materials testing negative.

[6] False Positive Rate: The proportion of all negative (noncorrosive) test materials that are falsely identified as positive.

[7] False Negative Rate: The proportion of all positive (corrosive) test materials that are falsely identified as negative.

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
9	NP	NC	NC	1.7	October, 1996 Submission		
83151	NP	NC	discordant	1.47	October, 1996 Submission		Table XIX lists the Corrositex® packing group as II, while Table 23 lists it as NC.
91437	NP	C	III	1.73	October, 1996 Submission		
1130811	NP	C	III	1.76	October, 1996 Submission		
1860811	NP	C	III	1.8	October, 1996 Submission		
A	NP	C	II	13.54	October, 1996 Submission		
acetic acid	99	C	II	0	October, 1996 Submission		
acetic acid	46	C	II	1.49	October, 1996 Submission		
acetic acid	37	C	II	1.53	October, 1996 Submission		
acetic acid	25	C	II	1.92	October, 1996 Submission		
acetic anhydride	pure	II	II	1.99	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
acetyl bromide	99	II	I	~2.0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
agrochemical U	neat	C	II	12.1	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
aluminum bromide, anhydrous	98	II	II	1.22	Gordon et al. (1994)	October, 1996 Submission	pH tested on 10% solution. US DOT packing group used in place of *in vivo* packing group.
ammonium hydrogen sulfate	neat	II	II	0.78	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
ammonium hydroxide	29	NC	II	13.55	October, 1996 Submission		
o -anisoyl chloride	97	II	II	0.72	Gordon et al. (1994)	October, 1996 Submission	pH tested on 10% solution. US DOT packing group used in place of *in vivo* packing group.
antimony tribromide	99	II	II	0.35	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
antimony trichloride	100	II	II	0.3	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
B	NP	C	II	13.33	October, 1996 Submission		
bathroom cleaner	neat	NC	NC	12.5	October, 1996 Submission		
bathroom cleaner2	NP	NC	NC	12.26	October, 1996 Submission		
benzene sulfonyl chloride	neat	III	III	1.8	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
boat bottom cleaner	neat	II	II	0.52	October, 1996 Submission		
boron fluoride-dihydrate	96	I	I	0.41	May, 1998 Submission	October, 1996 Submission	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
boron trifluoride-acetic acid complex	98	II	II	0.41, 0.95	May, 1998 Submission	October, 1996 Submission	
boron trifluoride-dihydrate	96	I, R35	I, R35	0.41, 0.95, 1.5	May, 1998 Submission; Barratt et al. (1998); Fentem et al. (1998)	October, 1996 Submission	Decomposes. Fentem et al. (1998) list R35 as the Corrositex® packing group designation, while the Submission lists it as I.
bromoacetic acid	99	II	II	0, 1.41	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
bromoacetyl bromide	98	II	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
butylamine (in ethanol [EtOH]/ethylene glycol [EG] 1:1)	40	NC	III	12.59	October, 1996 Submission		
butylamine	NP	C	C	12.96	Botham et al. (1995)	October, 1996 Submission	
butylamine (in EtOH/EG 1:1)	82	C	II, III	13.43	May, 1998 Submission	October, 1996 Submission	
butyric acid	99	II,III	II	0	May, 1998 Submission	October, 1996 Submission	
C	NP	C	II	13.61	October, 1996 Submission		
calcium carbonate	neat	NC	NC	12.56	May, 1998 Submission	October, 1996 Submission	
Calgon LpHse	neat	III	II	0.51	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
cellusolve + SMS	20.0/10.0%	II	NC	12.96	October, 1996 Submission		
chloroacetic acid	99	II	II	1.44	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
citric acid	50	II	II	0.5	October, 1996 Submission		
citric acid	25	NC	II	1.15	October, 1996 Submission		
citric acid	20	NC	II	1.28	October, 1996 Submission		
citric acid	15	NC	NC	1.37	October, 1996 Submission		
citric acid	10	NC	NC	1.58	October, 1996 Submission		
citric acid	5	NC	NC	1.78	October, 1996 Submission		
citric acid + sodium dodecyl sulfate (SDS)	20/10	II	II	1.38	October, 1996 Submission		
cleaner	NP	C	III	0.51	October, 1996 Submission		
cleaner	NP	NC	NC	12.18	October, 1996 Submission		
cleaner	NP	C	II	12.2	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
cleaner	NP	NC	NC	12.74	October, 1996 Submission		
cleaner	NP	NC	NC	12.86	October, 1996 Submission		
cleaner 12	neat	C	III	13.02	October, 1996 Submission		
cleaner 13	neat	C	NC	12.83	October, 1996 Submission		
cleaner 14	neat	NC	discordant	12.7	October, 1996 Submission		Table 30 lists the Corrositex® response as NC, while Appendix IV lists it as packing group III.
cleaner 15	neat	NC	III	12.64	October, 1996 Submission		
cleaner 16	neat	NC	III	12.86	October, 1996 Submission		
cleaner 17	neat	NC	III	12.74	October, 1996 Submission		
cleaner 20	neat	NC	III	12.18	October, 1996 Submission		
cleaner 21	neat	NC	III	12.67	October, 1996 Submission		
cleaner 27	neat	NC	II	12.97	October, 1996 Submission		
cleaner 3	neat	C	II	13.57	October, 1996 Submission		
cleaner 5	neat	C	II	13.56	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
cleaner 6	neat	C	II	13.34	October, 1996 Submission		
cleaner A	neat	C	II	13.54	October, 1996 Submission		
cleaner B	neat	C	II	13.33	October, 1996 Submission		
cleaner C	neat	C	II	13.61	October, 1996 Submission		
cleaner cor14	neat	II	II	0.72	October, 1996 Submission		
cleaner D	neat	NC	III	13.01	October, 1996 Submission		
cleaner E	neat	C	II	13.35	October, 1996 Submission		
cleaner F	neat	NC	II	12.23	October, 1996 Submission		
cleaner G	neat	C	II	13.19	October, 1996 Submission		
cleaner K	neat	C	II	12.85	October, 1996 Submission		
cleaner L	neat	C	II	13.34	October, 1996 Submission		
cleaner M	neat	C	II	0.72	October, 1996 Submission		
cleaner N	neat	C	II	13.64	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
cleaner O	neat	C	II	13.29	October, 1996 Submission		
cleaner P	neat	C	II	13.85	October, 1996 Submission		
cleaner R	neat	C	II	13.44	October, 1996 Submission		
cleaner S	neat	C	II	13.17	October, 1996 Submission		
COR1	NP	C	II	13.54	October, 1996 Submission		
COR11	NP	C	II	12.85	October, 1996 Submission		
COR13	NP	C	II	13.34	October, 1996 Submission		
COR14	NP	C	II	0.72	October, 1996 Submission		
COR15	NP	C	II	13.64	October, 1996 Submission		
COR16	NP	C	II	13.29	October, 1996 Submission		
COR17	NP	C	II	13.85	October, 1996 Submission		
COR19	NP	C	II	13.44	October, 1996 Submission		
COR2	NP	C	II	13.33	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
COR20	NP	C	II	13.17	October, 1996 Submission		
COR3	NP	C	II	13.61	October, 1996 Submission		
COR4	NP	NC	II	13.01	October, 1996 Submission		
COR5	NP	C	II	13.35	October, 1996 Submission		
COR6	NP	NC	II	12.23	October, 1996 Submission		
COR7	NP	C	II	13.19	October, 1996 Submission		
COR9	NP	C	II	12.99	October, 1996 Submission		
cyanuric chloride	99	III	III	1.72	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
cyclohexylamine	99	II	II	12.34	May, 1998 Submission; Gordon et al. (1994); Botham et al. (1995)	October, 1996 Submission	US DOT packing group used in place of *in vivo* packing group.
D	NP	C	II	12	October, 1996 Submission		
D	NP	NC	III	13.01	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
degreaser	NP	NC	NC	13.64	Gordon et al. (1994)		* - #870 in table; other degreasers in table as well
degreaser/cleaner	neat	C	III	12.99	October, 1996 Submission		
degreaser/cleaner	neat	C	II	13.34	October, 1996 Submission		
degreaser/cleaner	neat	C	II	13.64	October, 1996 Submission		
1,4-diaminobutane	34	C	III	13.6	Botham et al. (1995)	October, 1996 Submission	
1,2-diaminopropane	99	I	II	12.06	October, 1996 Submission		
dichloroacetic acid	99	II	II	0.64	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
dichloroacetic acid	3	NC	III	0.98	October, 1996 Submission		
dichloroacetyl chloride	99	II	II	0.46	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
dichlorophenyl-phosphine	97	II	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
3-diethylaminopropyl-amine	99	III	II	12.17	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
diethylene triamine	99	II	II	12.01	Gordon et al. (1994)	October, 1996 Submission	US DOT packing group used in place of *in vivo* packing group.

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
2,3-dimethylcyclohexyl-amine	99	II	III	11.79	October, 1996 Submission		
dodecyltrichloro-silane	98	II	II	0.5	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
E	NP	C	II	13.35	October, 1996 Submission		
ethanolamine	2	NC	III	11.5	October, 1996 Submission		
ethanolamine	3	NC	III	11.65	October, 1996 Submission		
ethanolamine	4	NC	III	11.76	October, 1996 Submission		
ethanolamine	5	NC	III	11.8	October, 1996 Submission		
ethanolamine	25	NC	II	12.24	October, 1996 Submission		
ethanolamine	50	II	II	12.92	October, 1996 Submission		
ethanolamine	60	C	II	12.94	October, 1996 Submission		
ethanolamine	80	C	II	13.57	October, 1996 Submission		
ethylenediamine	99	II	I, II	12.13	May, 1998 Submission; Gordon et al. (1994); October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group. Appendix IV lists the Corrositex™ packing group as I, while the 1998 Submission and the 1996 Submission list it as II.

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
ethylhexylamine	98	III	III	11.98	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
F	NP	NC	II	12.23	October, 1996 Submission		
ferric chloride; iron (III) chloride	NP	II, R34	II, R34/R35	1	May, 1998 Submission; Barratt et al. (1998); Fentem et al. (1998)		In the Fentem et al. (1998) study, 4/6 calls designated the packing group as R34, while 2/6 designated the packing group as R35. The Submission lists the packing group as II.
ferric chloride; iron (III) chloride	2	NC	NC	1.62	October, 1996 Submission		
flouboric acid; hydrogen tetrafluoroborate	48	II, III	I	1.3	May, 1998 Submission; Gordon et al. (1994); October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group; pH tested on 10% solution; Gordon paper references the US DOT designated packing group as II, while the Submission lists the *in vivo* packing group designation as III.
fluorosulfonic acid	neat	I	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
formic acid	50	C	II	0.05	October, 1996 Submission		
formic acid	40	C	II	0.38	October, 1996 Submission		
formic acid	34	C	II	0.62	October, 1996 Submission		
formic acid	30	C	II	0.72	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
formic acid	96	II	II	1.55	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
formic acid	7	NC	II	1.61	October, 1996 Submission		
formic acid	25	C	II	1.10, 1.48	October, 1996 Submission		
fumaryl chloride	95	II	II	0.05	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
G	NP	C	II	13.19	October, 1996 Submission		
GIN	NP	NC	II	0.18	May, 1998 Submission	October, 1996 Submission	* - GIN 8672
gin8672	neat	II	II	0.18	October, 1996 Submission		
glass cleaner	NP	NC	II	12.23	October, 1996 Submission		
glass cleaner 1	neat	NC	II	12.23	October, 1996 Submission		
glycol bromoacetate	85	R34, II & III	R34	2	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission		
heavy duty stripper	neat	C	II	13.54	October, 1996 Submission		
hydrobenzene sulfonic acid	65	II	II	0.55	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
hydrochloric acid	10	NC	II	0.17	October, 1996 Submission		
hydrochloric acid + oxalic acid + sulfuric acid	2.0/1.6/2.0	II	II	0.12	October, 1996 Submission		
hydrochloric acid + sulfuric acid + citric acid	5/5/5	II	II	0	October, 1996 Submission		
hydrochloric acid + sulfuric acid + citric acid	1/1/1	II	II	0.44	October, 1996 Submission		
hydrochloric acid	25	C	II	0	October, 1996 Submission		
hydrochloric acid	35	C	II	0	October, 1996 Submission		
hydrochloric acid	14.4-25	C	II	0	October, 1996 Submission		
hydrochloric acid	14	R34/II & III	R34	1.5	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission		
hydrogen bromide, aqueous sol.; hydrobromic acid	48	II	I	0, 0.3	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
I	NP	C	II	12.99	October, 1996 Submission		
ibc 7970	NP	II	II	0.12	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
ibc 7970	NP	II	II	0.12	October, 1996 Submission		
industrial cleaner	NP	C	II	12.4	October, 1996 Submission		
industrial cleaner	neat	C	II	12.9	October, 1996 Submission		
industrial cleaner	NP	C	II	13.35	October, 1996 Submission		
iodine monochloride	98	II	II	0.75	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
J	NP	C	II	12	October, 1996 Submission		
K	NP	C	II	12.85	October, 1996 Submission		
L	NP	C	II	13.34	October, 1996 Submission		
lithium hydroxide, monohydrate	98	II	II	11.8	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
LpHse	neat	III	II	0.51	October, 1996 Submission		
M	NP	C	II	0.72	October, 1996 Submission		
mag 8320	neat	II	II	0.69	October, 1996 Submission		
maleic acid	99	NC	II	1.3	May, 1998 Submission	October, 1996 Submission	pH tested on 10% solution

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
maleic anhydride	99	III	II	1.05	May, 1998 Submission	October, 1996 Submission	pH tested on 10% solution; US DOT packing group used in place of *in vivo* packing group.
mercaptoacetic acid	97	II	II	0.3	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
2-mercapoethanol, sodium salt	45	R34/II & III	R34	12	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission		
myristyl myristate	NP	NC	NQ	0.43	October, 1996 Submission		
N	NP	C	II	13.64	October, 1996 Submission		
nitric acid	20	C	II	0	October, 1996 Submission		
nitric acid	90	I	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
nitric acid	5	C	II	0.18	October, 1996 Submission		
O	NP	C	II	13.29	October, 1996 Submission		
octadecyltrichloro-silane	95	II	II	0.3	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
octyltrichlorosilane	97	II	II	0.1	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
oxalic acid (in 50% ethanol)	10	NC	NC	0.81	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex* Packing Group	pH	References	pH reference (if different)	Comment
P	NP	C	II	13.85	October, 1996 Submission		
petrochemical 1130811	neat	C	III	1.76	October, 1996 Submission		
petrochemical 1140811	neat	NC	II	1.18	October, 1996 Submission		
petrochemical 1860811	neat	C	III	1.8	October, 1996 Submission		
petrochemical 83151	neat	NC	III	1.47	October, 1996 Submission		
petrochemical 91437	neat	C	III	1.73	October, 1996 Submission		
phenyl acetyl chloride	98	II	II	0.92	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
phenyl trichlorosilane	98	II	II	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
phosphoric acid	10	NC	II	0.35	October, 1996 Submission		
phosphoric acid	25	C	II	0, 0.44	October, 1996 Submission		
phosphoric acid	85	II	II	0, 0.85	October, 1996 Submission		
phosphorous pentachloride	98	I	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
phosphorus tribromide	97	I, R35	I, R35	0, 1	May, 1998 Submission; Barratt et al. (1998); Fentem et al. (1998)	October, 1996 Submission	US DOT packing group used in place of *in vivo* packing group. Highly volatile-decomposes. Fentem et al. (1998) lists the packing group designation as R35, while the 1998 and 1996 Submissions list it as I.
1(2-aminoethyl)piper-azine	99	II	II	11.78	October, 1996 Submission		
potassium bisulfate; potassium hydrogen sulfate	neat	I,II	II	0.75, 0.85	May, 1998 Submission; Gordon et al. (1994)	October, 1996 Submission	pH tested on 10% solution
potassium bisulfate; potassium hydrogen sulfate	35-37	II	II	0.85	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
potassium hydroxide	1	NC	II, III	13.05	October, 1996 Submission		Tables XI and 26 listed the Corrositex® packing group as II, while Table 35 lists it as III.
potassium hydroxide	5	discordant	R34	13.1, 13.83	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission	October, 1996 Submission	The 1996 and 1996 Submissions list the *in vivo* response as C, while Fentem et al. (1998) lists it as NC. Fentem et al. (1998) and the 1998 Submission list the Corrositex® packing group as R34, while Tables XI and 26 of the 1996 Submission list it as II.

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
potassium hydroxide	10	II	II, R34	13.2, 14	Barratt et al. (1998); Fentem et al. (1998); Gordon et al. (1994)	October, 1996 Submission	Colored test material. Fentem et al. (1998) lists the Corrositex® packing group as R34, while the 1998 Submission and Table 35 of the 1996 Submission list it as II. Table 35 of the 1996 Submission lists the *in vivo* packing group as II., while Fentem et al. (1998) does not provide an *in vivo* packing group designation.
proponic acid	99	II	II	0.35	October, 1996 Submission		
R	NP	C	II	13.44	October, 1996 Submission		
S	NP	C	II	13.17	October, 1996 Submission		
scj cor 014	neat	II	II	0.72	October, 1996 Submission		
selenic acid	95	I	1	0	October, 1996 Submission		pH tested on 10% solution
sho 9047	neat	II	II	0.29	October, 1996 Submission		
shower room cleaner	neat	C	II	0.72	October, 1996 Submission		
sodium metasilicate	3	NC	III	12.71	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
sodium carbonate	neat	II	II	0	October, 1996 Submission		
sodium hydrogen sulfate	neat	III	II	0.75	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
sodium hydrogen sulfate	neat	NC	II	0.75	October, 1996 Submission		
sodium hydroxide	0.5	NC	III	12.82	October, 1996 Submission		
sodium hydroxide	1	NC	III	13.11	October, 1996 Submission		
sodium hydroxide	2	NC	II	13.37	October, 1996 Submission		
sodium hydroxide	3	NC	II	13.41	October, 1996 Submission		
sodium hydroxide	10	I,II	II	13.6	October, 1996 Submission		
sodium hydroxide	pellets	II	II	13.81	Botham et al. (1995); Gordon et al. (1994)	October, 1996 Submission	pH tested on 10% solution. US DOT packing group used in place of *in vivo* packing group.
sodium hydroxide	5	discordant	II	13.49, 13.67	October, 1996 Submission		Table 35 lists the *in vivo* response as NC, while Tables XI and 26 list it at C.
sodium hydroxide	0.1	NC	NC	12.25	October, 1996 Submission		
sodium hydroxide	100	C	II	13.79	October, 1996 Submission		
sodium hydroxide/*N*-*N*'-bis(acrylyl)cyst-amine (BAC)	1.0/10.0	III	III	12.96	May, 1998 Submission	October, 1996 Submission	

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
sodium hydroxide/SDS	0.5/10.0	III	III	12.99	October, 1996 Submission		
sodium hydroxide/SDS	1.0/5.0	NC	III	13.02	October, 1996 Submission		
sodium hydroxide/SDS	1.0/10.0	III	III	13.07	May, 1998 Submission	October, 1996 Submission	
sodium hydroxide/SDS	2.0/5.0	C	II	13.29	October, 1996 Submission		
sodium hydroxide/SDS	3.0/5.0	I,II	II	13.29	October, 1996 Submission		
sodium hydroxide/SDS	5.0/5.0	II	II	13.43	October, 1996 Submission		
sodium hydroxide/SMS	1.0/3.0	NC	II	13	October, 1996 Submission		
sodium hydroxide/SMS	2.0/3.0	I,II	II	13.12	October, 1996 Submission		
sodium hydroxide/SMS	1.0/10.0	II	II	13.21	May, 1998 Submission	October, 1996 Submission	
sodium hydroxide/SMS	0.5/10.0	II	II	13.33	October, 1996 Submission		
sodium hydroxide/SMS	3.0/3.0	I,II	II	13.33	October, 1996 Submission		
sodium hydroxide/SMS	5.0/3.0	C	II	13.37	May, 1998 Submission	October, 1996 Submission	
sodium hydroxide/SMS	5.0/3.0	I,II,III	II	13.37	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
sodium hydroxide/SMS/SDS	5.0/3.0/5.0	I, II,III	II	13.28	October, 1996 Submission		Table 35 lists the *in vivo* response as II, III, while Table 35 lists it as I, II.
sodium hydroxide/TX100	5.0/5.0	I,II,III	II	13.5	May, 1998 Submission	October, 1996 Submission	
sodium hypochlorite	5	NC	NC	11.65	October, 1996 Submission		5% concentration represents the amount of available chlorine in the solution.
sodium metasilicate	2	NC	NC	12.7	October, 1996 Submission		
sodium metasilicate	15	C	II	13.23	October, 1996 Submission		
stripper 1	neat	C	II	13.85	October, 1996 Submission		
stripper 2	neat	C	II	13.29	October, 1996 Submission		
stripper 3	neat	C	II	12.6	October, 1996 Submission		
stripper 4	neat	C	II	12.5	October, 1996 Submission		
stripper 5	neat	C	II	13.19	October, 1996 Submission		
stripper 7	neat	C	II	12.8	October, 1996 Submission		
stripper 8	neat	C	III	12.85	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
sulfamic acid; midosulfonic acid	99	NC	II	0.65	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission	October, 1996 Submission	Fentem et al. (1998) list the Corrositex® packing group as R34, while the Submission lists it as II.
sulfuric acid	5	NC	II	0.03, 0.31	October, 1996 Submission		
sulfuric acid	15	C	II	0	October, 1996 Submission		
sulfuric acid	99	I	I	0	October, 1996 Submission		
sulfuric acid	100	I	I	0	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
sulfuric acid	15-25	C	II	0	October, 1996 Submission		
sulfuric acid	10	I, II	R34, I, II	0, 1.2	Barratt et al. (1998); Fentem et al. (1998); May, 1998 Submission; Gordon et al. (1994)	October, 1996 Submission	Fentem et al. (1998) lists the designation as C, but further state that supporting data do not enable unequivocal classification as either R34 (II/III) or R35 (I); more probable to be R34 (II/III). The 1998 Submission lists the classification as R34. Gordon et al. (1994) lists both the Corrositex® packing group and US DOT packing group as I. Fentem et al. (1998) does not list an *in vivo* packing group. The 1996 Submission lists the *in vivo* and Corrositex® packing group as II.
sulfuric acid + cellusolve	5/20	I,II	II	0.09	October, 1996 Submission		
sulfuric acid + ferric chloride	5/2	I,II	II	0.15	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

Chemical Name	Concentration (%)	*In Vivo* Packing Group	Corrositex® Packing Group	pH	References	pH reference (if different)	Comment
sulfuric acid + sds	5/10	I,II	II	0.19	October, 1996 Submission		
sulfuric acid + triton x-100	5/10	I,II	II	0.1	October, 1996 Submission		
sulfurous acid	neat	II	II	1.78	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
tetraethylenepentamine	neat	III	III	11.85	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
tetramethylammonium hydroxide pentadydrate	99	II	II	13.61	Gordon et al. (1994)	October, 1996 Submission	US DOT packing group used in place of *in vivo* packing group.
thioglycolic acid	50	II	II	0.99	October, 1996 Submission		
trichloroacetic acid	99	II	II	0.74	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
triethylene tetramine	1	II	II	11.91	October, 1996 Submission		
trifluoroacetic acid	60	I	II	0.75	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
U	NP	C	II	12.1	October, 1996 Submission		
valeryl chloride; pentanoyl chloride	98	II	II	0.45	October, 1996 Submission		US DOT packing group used in place of *in vivo* packing group.
wpa 8215	neat	II	II	0	October, 1996 Submission		
X	NP	C	II	11.5	October, 1996 Submission		

Abbreviations: C=corrosive; NC=noncorrosive; NP=not provided; nq=nonqualifying chemical

NICEATM[1] Summary of Results for Chemicals that Were Tested in Both the Submission and the ECVAM[2] Validation Study (Fentem et al., 1998)

Chemical Name	Chemical Class	Submission		ECVAM Validation Study						
		In Vivo	Corrositex	*In Vivo*	Lab 4 – Run 1	Lab 4 – Run II	Lab 5 – Run 1	Lab 5 – Run II	Lab 6 – Run 1	Lab 6 – Run II
Boron trifluoride dihydrate	Inorganic acid	I	I	R35/I	R35	R35	R35	R35	R35	R35
Phosphoric acid	Inorganic acid	II	II	R34/II	R34	R34	R34	R34	R34	R34
Sulfamic acid; midosulfonic acid	Inorganic acid	NC	II	NC	R34	R34	R34	R34	R34	R34
Ferric chloride; iron (III) chloride	Acid derivative	II	II	R34/II	R35	R35	R34	R34	R34	R34
Phosphorus pentachloride	Acid derivative	I	I	R35/I	R35	R35	R35	R35	R35	R35
Phosphorus tribromide	Acid derivative	I	I	R35/I	R35	R35	R35	R35	R35	R35
1- (2-aminoethyl) piperazine	Amine/organic base	II	II	R34/II	R34	R34	R34	R34	R34	R34
1,2 – diaminopropane	Amine/organic base	I	II	R35/I	R34	R34	R34	R34	R34	R34
Hexanoic acid[3]	Organic acid	C	III	R34/II & III	NC	NC	NC	NC	R34	R34

[1] NICEATM = The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

[2] ECVAM = The European Centre for the Validation of Alternative Methods

[3] Hexanoic acid was also tested by two labs as part of the ECVAM Prevalidation Study. (Botham et al., 1995) Both labs found the chemical to be corrosive, but no packing group was assigned.

Abbreviations: NC = Noncorrosive; I = US Department of Transportation (DOT) Packing Group I; II = US DOT Packing Group II; III = US DOT Packing Group III; R34 = United Nations (UN) Packing Groups II & III; R35 = UN Packing Group I.

NICEATM Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings

The following provides an overview of the performance of Corrositex® using the following sources: 1998 IVI Submission, Fentem et al. (1998), and Gordon et al. (1994). Chemicals mentioned only in the Botham et al. (1995) were specifically excluded at the request of the Peer Review Panel (PRP). This data set is an addition to Table 5 (page 21 of the Peer Review Panel [PRP] report).

As compared to the performance characteristics for the entire data set Table 5 (page 21 of the PRP report) the accuracy of the data set excluding the Botham et al. (1995) paper was slightly higher (87% as compared to 84%). Similarly sensitivity and specificity were also slightly higher for this data set (sensitivity = 92% as compared to 89%; specificity = 78% as compared to 75%).

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Evaluation of the Performance of Corrositex® in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Overall)

Data Source	Number of Chemicals	Accuracy[1] %	Accuracy[1] Number	Sensitivity[2] %	Sensitivity[2] Number	Specificity[3] %	Specificity[3] Number	Positive Predictivity[4] %	Positive Predictivity[4] Number	Negative Predictivity[5] %	Negative Predictivity[5] Number	False Positive Rate[6] %	False Positive Rate[6] Number	False Negative Rate[7] %	False Negative Rate[7] Number
Revised Data Set[8]	193	87%	(167/193)	92%	(110/120)	78%	(57/73)	87%	(110/126)	85%	(57/67)	22%	(16/73)	8%	(10/120)

[1] Accuracy: (a) The closeness of agreement between a test result and an accepted reference value. (b) The proportion of the correct outcomes of the method. Often used interchangeably with concordance.

[2] Sensitivity: The proportion of all positive test materials that are correctly classified as positive in a test.

[3] Specificity: The proportion of all negative test materials that are correctly classified as negative in a test.

[4] Positive Predicitivity: The proportion of correct positive responses among materials testing positive.

[5] Negative Predictivity: The proportion of correct negative responses among materials testing negative.

[6] False Positive Rate: The proportion of all negative (noncorrosive) test materials that are falsely identified as positive.

[7] False Negative Rate: The proportion of all positive (corrosive) test materials that are falsely identified as negative.

[8] Chemicals from the following sources were included in this analysis: 1998 Submission, Fentem et al. (1998), and Gordon et al. (1994). See Appendix A of this report for complete citations of these sources.

Quality Assurance Audit Summaries

NICEATM[1] Verification of Data in Appendix IV of the May 1998 Submission Based on Confidential Laboratory Notebooks Provided by the Sponsor and the ECVAM[2] Validation Study (Fentem et al., 1998)

In Vivo Data (Appendix V)

Handwritten data in the original laboratory notebooks substantiated the *in vivo* packing group label except for compounds numbered 1, 8, and 9 in Appendix IV of the Submission. The notebooks did contain typewritten summaries with the appropriate data (tissue destruction/irreversible changes within 3 minutes).

The laboratory notebook sample numbers designated for the compounds in Appendix IV of the Submission are sometimes incorrect. For compound 40 in Appendix IV, the laboratory notebook sample number is 60; for compound 51, #21; for compound 60, #49; for compound 66, #32; for compound 71, #64; for compound 73, #45; for compound 78, #19; for compound 80, #12; for compound 82, #10; for compound 83, #11. For compounds 88, 89, 90, 91, and 95, the notebook sample numbers are 62, 61, 63, 46, and 44, respectively.

ECVAM Validation Study Results

The European Union (EU) risk phrase listed for the Corrositex® results in Appendix IV of the Submission for compound 45 is R34. However, in the table of data on page 46 of the ECVAM report by Fentem et al. (1998), 2 of the 3 laboratories listed R35.

Corrositex® Laboratory Notebook Data (Appendix VI) of the Submission

For compound 13, the mean breakthrough time given in Appendix IV is 71.57 minutes.
The notebook gives 71.37 minutes.

For compound 80, named "485," the notebook gives a mean breakthrough time of 55.22 minutes. The value in Appendix IV of the Submission (28.25 minutes) was given for "485B" in the laboratory notebook.

For compound 94, Appendix IV of the Submission gives a mean breakthrough time of 40.29 minutes whereas the laboratory notebook page cited gives a value of 35.62 minutes.

None of the differences noted above would change the packing group designations.

All of the packing group designations are appropriately assigned for the Corrositex® results (mean breakthrough times).

All other mean breakthrough times were accurately reproduced from the laboratory notebook data.

All designations in the Category column under Corrositex® Results (except NF and NA) were accurately reproduced from the laboratory notebooks.

Data audit conducted by Bonnie Carson, ILS, Inc./NICEATM on July 28, 1998.

[1] NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

[2] ECVAM = European Centre for the Validation of Alternative Methods

NICEATM Summary Comments on NIEHS[3] Audit of the May, 1998 Corrositex® ICCVAM Submission

At the request of NICEATM, the NIEHS Quality Assurance Unit (QAU) conducted an audit of Sponsor-submitted information to determine the accuracy, consistency, and completeness of the transcribed summary tables as compared to the original study records. Confidential *in vivo* and *in vitro* data associated with the validation studies of the Corrositex® test system were compared against the summary tables in the May, 1998 Corrositex® Submission. The audit was conducted from September 4, 1998 through October 2, 1998. The following comments provide a summary of the information determined during the course of that audit.

A. Audit procedures and scope

Several points must be considered regarding the audit. As stated in both the Submission and the audit, neither the *in vivo* or *in vitro* segments of the report were conducted under full Good Laboratory Practice (GLP) compliance. Records concerning a number of areas were not available and not considered during the audit. These include animal receipt, quarantine, randomization, and serology; animal husbandry and environmental controls; test chemical receipt, inventory and usage; test chemical identity and purity; dose preparation analysis; standard operating procedures; instrument calibrations; and chemical-specific protocols, amendments, and deviations. Since the objective of the audit was to assess the extent to which summarized *in vitro* and *in vivo* test information was supported by hand-written source data and other records, the following audit procedures on the Submission were performed.

Audited data included all pertinent data and factual information applicable to the *in vivo* screening studies (as provided in the test reports [TR] and Appendix IV) and *in vitro* studies (as reported in Appendix IV). Information pertaining to test dates, animal strain and supplier, sample numbers and/or chemical name, concentration, times of exposure, and dermal reactions were verified in the *in vivo* test reports. The information in the *in vivo* test reports was then compared with the tabulated *in vivo* summary results in Appendix IV. Information pertaining to reaction time in the Corrositex® system, Corrositex® category, and packing group designation for *in vitro* study results in Appendix IV were verified from corresponding notebooks in Appendix VI. For *in vitro* studies, the packing group classifications were verified from the packing group designation table on page 6 of Appendix X. A total of 118 chemical samples were tabulated in Appendix IV, of which original data were present for 95 chemical samples.

B. Assessment

In assessing the audit report, NICEATM staff focused on audit findings that would impact the integrity of the data and the ability of the Peer Review Panel (PRP) to properly evaluate the data in the Submission. The majority of audit findings related to transcriptional errors or the lack of full documentation on test records. Whereas these errors demonstrate lack of completeness and/or GLP compliance within the Submission package, they do not effect the integrity of the data or an ability to assess performance of the data presented. Of more importance are cases where audit findings would modify actual Corrositex® results, packing group designation, or category designations. In general, the audit findings showed that (a) mean breakthrough times are accurately produced

[3] NIEHS = National Institute of Environmental Health Sciences

from laboratory notebook data, (b) packing group designations are appropriately assigned for mean breakthrough times, and (c) designations in the category column under Corrositex® results are accurately reproduced from the laboratory notebooks. Most of the recording errors noted in the audit would not alter packing group designations or Corrositex® results.

There were only a few instances where audit findings did affect packing group designations or Corrositex® results. *In vivo* Packing Group designation for sodium hydroxide (NaOH)/SMS was listed as corrosive (C) in the study report whereas the original study records list "inconclusive" as the result. The *in vitro* packing group for butylamine was entered in the report as "III," but original study records reported it as "II". *In vitro* Corrositex® time (minutes) for sample name 485 (sample number 50) was shown to be 55.22 minutes in the original study records but was listed as 28.25 minutes in the report entry. This would modify the packing group designation in the report for compound 485 from Packing Group designation II to category III.

In summary, the audit noted that the studies were not conducted under GLP compliance. A number of cases of transcriptional errors were identified and the lack of documentation completeness in the Submission was noted. NICEATM's assessment of the audit report found that most of the findings are inconsequential to the overall Submission. There were a few cases where designation of Corrositex® data could be affected. However, correction of data entries in these few cases does not affect the overall assessment of the Submission because these changes would either slightly alter or have no effect on the evaluation of concordance, specificity, sensitivity, positive or negative predictivity, or false positive or false negative rates. Whereas the auditors' findings may result in a lowering of confidence in the data presented in the Submission, they do not appear to have significant impact on the assessment and evaluation of the test submission.

*Note that on October 27, 1998, NICEATM sent the auditors report to the Sponsors for them to clarify and respond to the audit findings. The Sponsors acknowledged the errors, but added that these would have minimal effect on the overall data assessment.

Evaluation Guidance to the Peer Review Panel

A. Instructions for Peer Review Panel (PRP) Members

The PRP was charged with developing a consensus on the usefulness of Corrositex® as an alternative method to the *in vivo* methods that are commonly used for assessing dermal corrosivity. In reaching this determination, the PRP was asked to evaluate all of the available information in the Submission in accordance with the published criteria for validation and acceptance of toxicological test methods (NIEHS, 1997). The PRP was charged with preparing a written report that summarized the extent to which each of these criteria have been addressed.

An outline of the major items that were addressed in the PRP report is provided below in "B. Points for Evaluation." Specific questions or considerations that were addressed by the reviewers in their assessment were added by the Corrosivity Working Group (CWG) to ensure that the assessment provided adequate information to facilitate agency decisions on the regulatory acceptability of the method.

One primary and at least two secondary reviewers were designated for each section by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in consultation with the PRP Chair. These individuals were requested to prepare written responses for their assigned sections. However, all reviewers were encouraged to familiarize themselves with the entire set of questions and to comment on any (or all) section(s). All reviewers were asked to complete the summary conclusions section (Section C of this Appendix).

In conducting this review, the primary focus of the PRP was to evaluate the proposed Corrositex® protocol and supporting submission materials. Based on the supporting information provided in the Submission, the PRP was asked the extent to which Corrositex® is an acceptable alternative to standard *in vivo* dermal corrosivity assays for identifying human corrosives. The review focused on the following:

> ***Has** Corrositex® **been evaluated sufficiently and is its performance satisfactory to support its proposed use for assessing the dermal corrosivity potential of substances?***
>
> ***Does** Corrositex® **offer advantages with respect to animal welfare considerations (refinement, reduction, and replacement)?***

The focus of the PRP evaluation was on the utility of Corrositex®, as described in the proposed Instruction Manual (Appendix D) for detecting possible human corrosives. Suggestions for future evaluations or workshops to review proposed test method revisions or other test methods were submitted to NICEATM for consideration by Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and were described in Section D, Related Issues.

B. Points for Evaluation

1. Test Method Description

a. Are the test method and protocol described in sufficient detail, including the scientific and mechanistic basis of the test, range of applications, endpoints, numbers of replicates, need for dose-response curves, and acceptable variations in the protocol?

1) Is the protocol used to generate the supporting submission data in agreement with the proposed protocol? If not, discuss the adequacy of the rationale provided for changes incorporated in the proposed protocol.

2) Evaluate the appropriateness of the dose and breakthrough time selection procedure. Discuss the need for determination of categorization or acute toxicity data prior to conducting the actual test.

b. Comment on the adequacy and completeness of the test method protocol, including:

1) Description of the material and equipment needed to conduct the test. Is the number of tests per test agent appropriate?

2) Description of what is measured and how it is used.

3) Description of data analysis, evaluation, and decision criteria used to identify substances as:

- qualified or not qualified
- category 1 or category 2
- corrosive or noncorrosive

c. Are there appropriate provisions for the use of positive, negative, and [other endpoints] control chemicals?

d. What are the strengths and/or limitations of Corrositex® and are they described adequately, including the usefulness for testing various chemical classes and/or mixtures.

e. Are there editorial/technical corrections necessary for the proposed protocol?

2. Test Method Data Quality

Is there evidence of sufficient quality assurance/quality control (i.e., were experiments conducted and data collected and maintained in accordance with Good Laboratory Practice

[GLP] standards and procedures or in the "spirit" of GLPs [e.g., GLP standards without audits])? If not, is there clear indication from the technical data that there was adequate record-keeping or data collection.

a. Is there an assurance provided that indicates there was adherence to the protocol during the validation studies? Are deviations from the standard protocol clearly described and justified?

b. Was a data audit conducted by a Quality Assurance Unit? If so, is the data quality satisfactory based on the audit results (e.g., adequate adherence to protocols, record-keeping following GLPs)?

3. Test Method Performance

a. Are the data provided in sufficient detail for you to evaluate the results and conclusions obtained with Corrositex®? Are sufficient data provided to adequately evaluate the performance of the method for its proposed use?

b. Does the method adequately predict the endpoint of interest (corrosivity) by demonstrating a linkage between the test and the current test method (rabbit skin test)?

c. Comment on the adequacy of the methods used to evaluate the suitability and performance of the test method. Are results of Corrositex® and the reference test(s) compared and evaluated appropriately?

d. Comment on the adequacy of the statistical/analytical methods used to evaluate the performance of the test method.

e. Comment on the adequacy of the chemicals/products (numbers/types) selected to evaluate the performance of the method for each chemical/product class. Is it appropriate to generalize the performance of the method for all chemicals/products in each class based on the performance of the selected test chemicals/products?

f. Comment on the sensitivity, specificity, concordance, false positive rate, and false negative rates for the chemical/product classes that the method is proposed to be used for.

    1) To what extent does the method classify corrosives correctly for those that qualify in the Chemical Detection System (CDS): for all chemicals/products or some classes?

    2) To what extent does the method classify non-corrosives correctly for those that qualify in the CDS: for all chemicals/products or some classes?

g. Are the sensitivity, specificity, concordance, and false positive and negative rates acceptable for the chemical/product classes tested?

h. Are the conclusions on the usefulness of this method scientifically sound?

1) Are results of Corrositex® clinically relevant and is the test predictive for human corrosivity?

2) Is the utility of the method clearly established for regulatory use in hazard assessment of chemicals as potential corrosives?

4. Determination of Test Method Reliability (Repeatability/Reproducibility)

Are intra- and inter-laboratory reproducibility adequately evaluated?

a. Comment on the adequacy of the evaluation of intralaboratory repeatability and reproducibility of the test method, and the data used to define and describe the level of intralaboratory variability.

b. Comment on the adequacy of the evaluation of interlaboratory reproducibility of the test method, and the data used to define and describe the level of interlaboratory variation.

1) Consider the range of vehicle control data within and across laboratories in the validation studies. Do these differences affect data quality (reproducibility, sensitivity, etc)?

c. Was the reproducibility of the test method evaluated on a series of appropriate reference chemicals or products, and do these adequately represent the types of substances for which the test method is proposed to be used?

d. Are the results obtained with Corrositex® sufficiently repeatable and reproducible?

e. Comment on the adequacy of the reproducibility and reliability of Corrositex®. How does this compare to currently used methods for determining corrosivity.

5. Other Scientific Reviews

Comment on and compare the conclusions published in independent peer-reviewed reports or other independent scientific reviews of the test method, compared to the conclusions reached in this submission, and comment on any other ongoing evaluations of this method.

6. Other Considerations

a. Can the test method be readily transferred among properly equipped and staffed laboratories; that is:

1) Is it relatively insensitive to minor changes in protocol (e.g., the acceptable pH/temperature range for reagents and for the location where the test will be conducted)?

2) Are the level of training and expertise required to conduct the test reasonable?

3) Are the necessary equipment and supplies relatively easy to obtain?

b. Is the method cost-effective, relative to the cost of conducting an *in vivo* test or pH extreme?

c. Is the time needed to conduct the test reasonable?

d. Is there any other information that should be added to the report, published or unpublished?

e. Has there been adequate consideration and appropriate incorporation of animal use refinement, reduction, and replacement alternatives? Will Corrositex® reduce the number of animals required or refine the procedure to reduce or eliminate pain or distress compared with the reference tests?

f. How does the performance of this method compare to the current regulatory use of pH extremes (pH<2.0 or >11.5) to classify and label a chemical or product as corrosive?

g. Does the test method appear to be effective for assessing corrosivity/noncorrosivity for CDS-qualified chemicals of any pH, those in the listed effective range of <5.0 or >8.5, or only for certain other pH ranges?

h. Should pH determination be required prior to use of this test method?

C. Summary Conclusions

Based on the information provided:

a. Does this method adequately identify the dermal corrosivity potential for some or all chemicals? Specify those for which it would be considered adequate, and those for which it is not adequate.

b. Is this method adequate for identifying the absence of dermal corrosivity potential for some or all chemicals? Specify those for which it would be considered adequate, and those for which it would not be considered adequate.

c. Could this method be used to provide equivalent or better prediction of corrosivity or noncorrosivity for some or all chemicals than current methods? If applicable, discuss how it should be used in conjunction with current methods.

d. Discuss conditions/limitations/restrictions that may affect the intended use of Corrositex®, and that are justified based upon the presence or lack of scientific evidence.

e. Discuss advantages of the proposed Corrositex®, as compared to the standard corrosivity test methods.

f. Has there been adequate consideration and appropriate incorporation of animal use refinement, reduction, and replacement alternatives? Will Corrositex® reduce the number of animals required or refine the procedure to eliminate pain or distress compared with the commonly used corrosivity tests.

D. Related Issues

1. This evaluation is for a specific assay proposed as an alternative for currently accepted *in vivo* dermal corrosivity tests. Are there other test methods for this endpoint, or other endpoints that you would you like to see evaluated by ICCVAM in the future?

2. Are there suggestions/recommendations for workshops or validation efforts that you think that ICCVAM or others should support in this area of corrosivity?

Reference:

NIEHS (National Institute of Environmental Health Sciences). 1997. Validation and regulatory acceptance of toxicological test methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

ICCVAM[1] Validation and Regulatory Acceptance Criteria

Validation Criteria[2]

For a new or revised test method to be considered validated for regulatory risk assessment purposes, it should generally meet the following criteria (the extent to which these criteria are met will vary with the method and its proposed use). However, there needs to be flexibility in assessing a method given its purpose and the supporting database. Because tests can be designed and used for different purposes by different organizations and for different categories of substances, the determination of whether a specific test method is considered by an agency to be useful for a specific purpose must be made on a case-by-case basis. Validation of a test method is a prerequisite for it to be considered for regulatory acceptance.

- The scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.

- The relationship of the test method's endpoint(s) to the biologic effect of interest must be described. Although the relationship may be mechanistic or correlative, tests with biologic relevance to the toxic process being evaluated are preferred.

- A detailed protocol for the test method must be available and should include a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the species for which the test results are applicable, and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess.

- The extent of within-test variability, and the reproducibility of the test within and among laboratories must have been demonstrated. Data must be provided describing the level of intra- and interlaboratory reproducibility and how it varies over time. The degree to which biological variability affects this test reproducibility should be addressed.

- The test method's performance must have been demonstrated using reference chemicals or test agents representative of the types of substances to which the test method will be applied, and should include both known positive and known negative agents. Unless it is hazardous to do so, chemicals or test agents should be tested under code to exclude bias.

- Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace. Performance should be evaluated in relation to existing relevant toxicity testing data, and relevant toxicity information from the species of concern. Reference data from the comparable traditional test method should be available and of acceptable quality.

- The limitations of the method must be described; for example, *in vitro* or other non-animal test methods may not replicate all of the metabolic processes relevant to chemical toxicity that occur *in vivo*.

[1] ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

[2] NIEHS (National Institute of Environmental Health Sciences). 1997. Validation and regulatory acceptance of toxicological test methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

- Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs). Aspects of data collection not performed according to GLPs must be fully described, along with their potential impact.

- All data supporting the assessment of the validity of the test method must be available for review.

- Detailed protocols should be readily available and in the public domain.

- The method(s) and results should be published or submitted for publication in an independent, peer-reviewed publication.

- The methodology and results should have been subjected to independent scientific review

Regulatory Acceptance Criteria[2]

Validated methods are not automatically accepted by regulatory agencies; they need to fit into the regulatory structure. Flexibility is essential in determining the acceptability of methods to ensure that appropriate scientific information is considered in regulatory risk assessment. A test method proposed for regulatory acceptance generally should be supported by the following attributes:

- The method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable in the method, and financially unencumbered by the outcome of the evaluation.

- There should be a detailed protocol with standard operating procedures (SOPs), a list of operating characteristics, and criteria for judging test performance and results.

- Data generated by the method should adequately measure or predict the endpoint of interest and demonstrate a linkage between either the new test and an existing test, or the new test and effects in the target species.

- There should be adequate test data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.

- The method should generate data useful for risk assessment purposes, (i.e., for hazard identification, dose-response assessment, and/or exposure assessment). Such methods may be useful alone or as part of a battery or tiered approach.

- The specific strengths and limitations of the test must be clearly identified and described.

- The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.

- The method should be time and cost effective.

- The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.

- The method should be suitable for international acceptance.

- The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

[2] NIEHS (National Institute of Environmental Health Sciences). 1997. Validation and regulatory acceptance of toxicological test methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

Guidelines and Regulations for Dermal Corrosivity

AGENCY OR ORGANIZATION	GUIDELINES AND REGULATIONS[1]	COMMENTS
Consumer Product Safety Commission (CPSC)	**16CFR1500** **§1500.3**-Definitions **§1500.4**-Human experience with hazardous substances **§1500.41**-Method of testing primary irritant substances	The method involves the application of the test substance on the hair-free intact and abraded skin of at least 6 albino rabbits.
Occupational Safety and Health Administration (OSHA)	**29CFR1917** **§1917.28** (2) Definition of Corrosive **29CFR1910** **§1910.1200** Hazard Communication (includes Appendix A)	OSHA accepts determination of dermal corrosivity by Corrositex® and other *in vitro* tests. States that OSHA does not require chemical manufacturers or importers to conduct animal tests for the evaluation of the hazard potential of chemical products.
US Department of Transportation (USDOT)	Exemption allowing use of Corrositex® as an alternative test method. **49CFR 173** **§173.136**(a)(1) Class 8 Definitions **§173.137**(a), (b), (c)(1) Class 8 Assignment of Packing Group **49CFR172** **§172.442** Corrosive Label **§172.558** Corrosive Placard	Original exemption granted 28 April 1993. Current exemption expires 30 November 2000. §173.137 requires determination of the packing group based on data from tests conducted in accordance with 1992 OECD Guideline for Testing of Chemicals, No. 404, Acute Dermal Irritation/Corrosion.
US Environmental Protection Agency (EPA), Office of Solid Waste and Emergency Response	**62FR32452** (13 June, 1997) (final rule) affecting 40 CFR Parts 260, 264, 265, and 266. Hazardous Waste Management System; Testing and Monitoring Activities. Incorporates by reference update III of "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," EPA Publication SW-846, 3rd ed. SW-846 Method 9040 (**40CFR261.22**) determines corrosivity by the pH extremes (≤ 2 or ≥ 11.5).	State-of-the-art analytical technologies for RCRA-related testing include Method 1120, Dermal Corrosion, which describes the use of the Corrositex® test kit. h*ttp://www.epa.gov:80/epaoswer/hazwaste/test/1120.pdf* *http://www.access.gpo.govlsu_docs/aces/aces140.html*

AGENCY OR ORGANIZATION	GUIDELINES AND REGULATIONS	COMMENTS
EPA, Office of Pollution Prevention and Toxic Substances (OPPTS)	**OPPTS 870.2500** Acute Dermal Irritation	EPA Health Effects Test Guidelines *http://www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Drafts/*
EPA, Office of Pesticides	**40CFR152** **§152.170** Criteria for restriction to use by certified applicators **40CFR156** **§156.10** Labeling requirements (skin corrosives are assigned toxicity category I) **40CFR157** §157 Subpart B - Child-Resistant Packaging (§157.22 states requirement for pesticides corrosive to the eyes or skin) **40CFR158** **§158.690** (acute dermal toxicity testing requirement for biochemical pesticides is waived if corrosive to skin or falls within the corrosive pH ranges)	
US Food and Drug Administration (US FDA)	**21CFR 70** **§70 Subpart C** - Safety Evaluation. §70.42(b) Criteria for evaluating the safety of color additives	Corrosivity not mentioned *per se*. Sensitization and primary irritation mentioned. No other formal regulations found with respect to corrosivity.
Organization for Economic Cooperation and Development (OECD)	Harmonized integrated hazard classification system for human health and environmental effects of chemical substances.	Provides a tiered testing strategy for the evaluation of dermal corrosivity. *http://www.oecd.org//ehs/class/hclfinaw.doc*

[1]Unless otherwise specified in the comments column, guidelines may be accessed via the US Government Printing Office (GPO) Code of Federal Regulations database *http://www.access.gpo.gov/nara/cfr/cfr-table-search.html.*

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences; National Toxicology Program: Request for Comments on Test Methods Undergoing Review by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods

Background

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), with participation by 14 Federal regulatory and research agencies and programs, was established in 1997 to facilitate cross-agency communication and coordination on issues relating to validation, acceptance, and national/international harmonization of toxicological test methods. The Committee seeks to promote the scientific validation and regulatory acceptance of toxicological test methods that will enhance agencies's ability to assess risks and make decisions, and that will refine, reduce, and replace animal use whenever possible. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (Center), in collaboration with ICCVAM, carries out related activities such as independent peer reviews and workshops for test methods of interest to Federal agencies. Peer review panels are convened to develop scientific consensus on the usefulness of test methods to generate information for specific human health and/or ecological risk assessment purposes. Expert workshops are convened as needed to evaluate the adequacy of current methods for assessing specific endpoints, to identify areas in need of improved or new methods, to evaluate proposed validation studies, and to evaluate the validation status of methods. Following the peer review of proposed test methods, the ICCVAM forwards recommendations regarding their usefulness to appropriate agencies for their consideration. Federal agencies then determine the regulatory acceptability of a method according to their mandates.

Additional information on the activities and functions of the ICCVAM can be found in the publication, Validation an Regulatory Acceptance of Toxicological Test Methods, a Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication 97–3981, March 1997). This report was prepared in response to the National Institutes of Health (NIH) Revitalization Act of 1993 (Pub. L. 103–43) which required the NIEHS to develop criteria and recommended processes for the validation and regulatory acceptance of alternative toxicological test methods. The report is available on the Internet at http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.html, or may be requested from the NTP Center address listed below.

Request for Comments

Interested parties are encouraged to submit information and data that would be helpful in evaluating the usefulness of two test methods for which upcoming independent scientific peer review meetings are being planned. The methods are: (1) Corrositex®, an in vitro method proposed for assessing the dermal corrosivity potential of chemicals and products, and (2) the Frog Embryo Teratogenesis Assay in Xenopus (FETAX), a method for demonstrating developmental toxicity. Potential regulatory applications of FETAX in human health developmental toxicity assessments include screening and prioritizing compounds for further testing, evaluating complex mixtures in environmental samples, and as supplemental information in a weight-of-evidence evaluations of human developmental toxicity hazards.

The Center would welcome receiving information and data from completed, ongoing, or planned studies using or evaluating these test methods. Data and information submitted should address one or more of the criteria for validation and regulatory acceptance as provided in NIH publication 97–3981, "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods." Where possible, data and information should adhere to the guidance provided in the document "Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM," which is available on request from the NTP Center at the address provided below. Relevant information submitted in response to this request will be used to prepare test method background review documents for use by peer review and expert panels. Peer review meetings and/or workshops for these methods will be announced in future notices as they are scheduled.

Information on these test methods should be sent by mail, fax, or e-mail to the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods within 45 days of the appearance of this notice. The NTP Center mailing address, phone, fax, and e-mail are as follows: MD EC–17, P.O. Box 12233, Research Triangle Park, NC 27709; 919–541–3398 (phone); 919–541–4984 (FAX); ICCVAM@niehs.nih.gov (e-mail). Additional information can be obtained from: Dr. William S. Stokes, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Environmental Toxicology Program, NIEHS/NTP, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541–3398, FAX (919) 541–0947, email:stokes@niehs.nih.gov

Dated: July 17, 1998

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 98–20040 Filed 7–27–98; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Toxicology Program; National Institute of Environmental Health Sciences (NIEHS); National Institutes of Health (NIH); Notice of Meeting to Review the Corrositex® Assay as an Alternative Test Method for Assessing the Skin Corrosivity Potential of Chemicals; Request for Comments

SUMMARY: Pursuant to Public Law 105–43, notice is hereby given of a public meeting sponsored by the NIEHS and the National Toxicology Program (NTP), and coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The agenda topic is the scientific peer review of the Corrositex® assay, which is proposed as an in vitro alternative toxicological test method for assessing the skin corrosivity potential of chemicals and products. The meeting will be held on January 21, 1999, at the Natcher Center, National Institutes of Health, 45 Center Drive, Bethesda, MD 20892. The meeting will take place from 8:30 a.m. to 5:00 p.m. and is open to the public.

Background

Public Law 105–43 directed the NIEHS to develop and validate alternative methods that can reduce or eliminate the use of animals in acute or chronic toxicity testing, establish criteria for the validation and regulatory acceptance of alternative testing methods, and recommend a process through which scientifically validated alternative methods can be accepted for regulatory use. Criteria and procedures for validation and regulatory acceptance were developed in conjunction with 13 other Federal agencies and programs with broad input from the public. These are described in the document "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods" NIH publication 97–3981, March 1997, which is available on the Internet at http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.htm. Additional information on ICCVAM and NICEATM can be found through the ICCVAM/NICEATM web site http://iccvam.niehs.nih.gov.

An Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was subsequently established as a collaborative effort by NIEHS and 15 other Federal regulatory and research agencies and programs. The Committee's functions include the coordination of interagency reviews of new and evolving test methods and communication with stakeholders throughout the process of test method development and validation. The following Federal regulatory and research agencies and organizations are participating in this effort:

Consumer Product Safety Commission
Department of Defense
Department of Energy
Department of Health and Human Services
- Agency for Toxic Substances and Disease Registry
- Food and Drug Administration
- National Institute for Occupational Safety and Health/CDC
- National Institutes of Health
- National Cancer Institute
- National Institute of Environmental Health Sciences
- National Library of Medicine

Department of the Interior
Department of Labor
- Occupational Safety and Health Administration

Department of Transportation
- Research and Special Programs Administration

Environmental Protection Agency

The Corrositex® assay was proposed to ICCVAM for consideration as a test to identify the potential of chemicals to cause skin corrosion. An ICCVAM Corrosivity Working Group composed of Federal employees determined that there was sufficient information available to merit an independent scientific peer review of the Corrositex® assay test method. Peer review has been determined to be an essential prerequisite for consideration of a method for regulatory acceptance. The peer review panel will be charged with developing a scientific consensus on the usefulness of the test method to generate information for human hazard identification purposes. Following evaluation of the peer review meeting, the proposed test method and results of the peer review will be forwarded by ICCVAM to Federal agencies for consideration. Federal agencies will determine the regulatory acceptability of a method according to their mandates.

Agenda

There will be a brief orientation on ICCVAM and the ICCVAM review process, followed by peer review of the proposed Corrositex® test method and supporting information. The peer review panel will discuss the usefulness of the Corrositex® assay as an alternative to test methods currently accepted by government regulatory authorities for the assessment of skin corrosivity potential of chemicals and products. Copies of the Corrositex® Test Method Protocol and supporting documentation may be obtained from NICEATM, MD EC–17, P.O. Box 12233, Research Triangle Park, NC 27709 (919–541–3398), FAX (919–541–0947), e-mail: ICCVAM@niehs.nih.gov. The Corrositex® test method documents and copies of written public comments can also be viewed at the Consumer Products Safety Commission, Reading Room, 4330 East West Highway, Bethesda, MD 20814 on Monday through Friday from 8 a.m. to 5 p.m.

Public Comment

NICEATM invites the submission of written comments on the proposed Corrositex® test method, and other available information regarding the usefulness of the Corrositex® assay, including information about completed, ongoing, or planned studies. Written comments and additional information should be sent by mail, fax, or e-mail to NICEATM at the address listed above by December 10, 1998. Written comments will be made available to the peer review panel members, ICCVAM agency representatives and experts, and will be made available for attendees at the meeting. Members of the public who wish to present oral statements at the meeting should also contact NICEATM as soon as possible, but no later than January 10, 1999. Speakers will be assigned on a first-come, first-serve basis and will be limited to a maximum of five minutes in presentation length. Written comments accompanying the oral statement should be submitted in advance so that copies can be made and distributed to the peer panel members.

NICEATM will furnish an agenda and a roster of peer review panel members just prior to the meeting. Summary minutes and a final report of the Corrositex® assay peer review meeting will be available subsequent to the meeting upon request to the Center. Persons needing special assistance, such as sign language interpretation or other special accommodations, should contact NICEATM as described above.

Dated: October 20, 1998.

Kenneth Olden,

Director, National Toxicology Program.

[FR Doc. 98–28713 Filed 10–26–98; 8:45 am]

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Corrositex® Peer Review Meeting Agenda

Interagency Coordinating Committee on the
Validation of Alternative Methods (ICCVAM)
and the
National Toxicology Program (NTP) Interagency Center for the
Evaluation of Alternative Toxicological Methods (NICEATM)

January 21, 1999, 8:30 a.m. to 5:30 p.m.

Rooms E1 & E2
William H. Natcher Conference Center
45 Center Drive
Bethesda, MD

8:30 a.m.	Introductions	Dr. Robert Scala
8:40 a.m.	Welcome from the NTP	Dr. George Lucier
8:45 a.m.	Introduction to NICEATM and ICCVAM Overview of the Corrositex® Peer Review Process	Dr. William Stokes
9:00 a.m.	Summary of Current Agency Requirements	Dr. Richard Hill
9:15 a.m.	Overview of the Proposed Corrositex® Assay	Dr. Rosalind Wei
9:35 a.m.	Questions Regarding the Test Method Protocol	Dr. Rosalind Wei
9:55 a.m.	<u>Peer Review Panel Discussion</u> Test Method Description	Dr. John Harbell, Coordinator Drs. Karen Kohrman and John Stegeman
10:30 a.m.	Break	
10:50 a.m.	<u>Peer Review Panel Discussion (continued)</u> Test Method Data Quality	Dr. Sidney Green, Coordinator Drs. Michael Derelanko, John Harbell and Hajime Kojima
	Test Method Performance	Dr. A. Wallace Hayes, Coordinator Drs. Karen Kohrman and James Chen
12:45 p.m.	Public Comment	

1:05 p.m.	Lunch Break	
2:05 p.m.	<u>Peer Review Panel Discussion (continued)</u>	
	Test Method Reliability	Dr. Julia Fentem, Coordinator Drs. James Chen and Daniel Sauder
	Other Literature and Scientific Reviews	Dr. Michael Derelanko, Coordinator Dr. Hajime Kojima
3:00 p.m.	Break	
3:20 p.m.	<u>Peer Review Panel Discussion (continued)</u>	
	Presentation of Corrositex® Performance Compared to the pH test	Dr. Thomas Goldsworthy
	Other Considerations and Related Issues	Dr. John Stegeman, Coordinator Dr. Daniel Sauder
4:10 p.m.	Final Public Comments	
4:30 p.m.	Peer Review Panel Conclusions	Drs. Robert Scala and Julia Fentem
5:30 p.m.	Adjourn	

Corrositex® Peer Review Meeting Summary Minutes

January 21, 1999
Bethesda, Maryland

Introduction

A public meeting of an independent peer review panel (PRP) was convened on January 21, 1999, in Bethesda, Maryland to review Corrositex®, which was proposed as an alternative toxicological test method for assessing the corrosivity potential of chemicals and products. The meeting was coordinated by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and was sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the NTP.

The following expert scientists served on the PRP:

- Robert Scala, Ph.D., retired from Exxon Biomedical Sciences, Rehoboth Beach, Delaware (Chair)
- Julia Fentem, Ph.D., Unilever Research Colworth, Bedfordshire, United Kingdom (Executive Secretary)
- James Chen, Ph.D., National Center for Toxicological Research (NCTR), Little Rock, Arkansas
- Michael J. Derelanko, Ph.D., Allied-Signal, Inc., Morristown, NJ
- Sidney Green, Ph.D., Howard University College of Medicine, Washington, D.C.
- John Harbell, Ph.D., Institute for In Vitro Sciences, Inc. (IIVS, Inc.), Gaithersburg, Maryland
- A. Wallace Hayes, Ph.D., the Gillette Company, Boston, Massachusetts
- Karen Kohrman, Ph.D., the Procter & Gamble Company, Cincinnati, Ohio
- Hajime Kojima, Ph.D., Nippon Menard Cosmetic Company, Ltd., Nagoya, Japan
- Daniel Sauder, M.D., University of Toronto, Toronto, Ontario
- John Stegeman, Ph.D., Woods Hole Oceanographic Institution, Woods Hole, Massachusets

List of Attendees

- Susan Aitken, Consumer Product Safety Commission (CPSC)
- Surender Ahir, Occupational Safety and Health Administration (OSHA)
- June Bradlaw, Food and Drug Administration (FDA)
- Loretta Brammell, NIEHS/NICEATM
- Robert Bronaugh, FDA
- Rodger Curren, IIVS, Inc.
- George Cushmac, US Department of Transportation (US DOT)
- Alan Goldberg, Johns Hopkins University
- Thomas Goldsworthy, Integrated Laboratory Systems, Inc. (ILS, Inc.)/NICEATM
- Ben Gregg, US Environmental Protection Agency (US EPA)
- George Lucier, NIEHS
- Karen Haneke, ILS, Inc./NICEATM
- Ann Hanger, US EPA
- David Hattan, FDA
- Patrick Herron, ILS, Inc./NICEATM
- Barbara Hill, FDA
- Erin Hill, IIVS, Inc.
- Richard Hill, US EPA
- Vera Hudson, National Library of Medicine (NLM)
- Leonard Keifer, US EPA
- Francis Kraszewski, Gillette Company
- Marianne Lewis, US EPA
- Jeanie McAndru, US EPA
- Barry Margolin, ILS, Inc./NICEATM
- Kathy Miner, ILS, Inc./NICEATM

- Hari Mukhoty, US EPA
- Thomas Re, Cosmair
- John Redden, US EPA
- Katherine Stitzel, Procter & Gamble
- William Stokes, NIEHS/NICEATM
- Raymond Tice, ILS, Inc./NICEATM
- Mark Torreson, National Institute for Occupational Safety and Health (NIOSH)
- Heather Vahdat, ILS, Inc./NICEATM
- Kay Valeda, National Heart, Lung and Blood Institute (NHLBI)
- Sherry Ward, the Gillette Company
- Rosalind Wei, InVitro International, Inc. (IVI)
- Neil Wilcox, FDA
- Yung Yang, US EPA
- Errol Zeiger, NIEHS

Meeting—Background Information

Introductions

Dr. Scala, Chair, called the meeting to order at 8:30 a.m. and asked each person in attendance to state their name and affiliation.

Welcome from the NTP

Dr. George Lucier, Director of the NTP, thanked the ICCVAM participating agencies and stakeholders, the Corrositex® Sponsor, and the PRP for their efforts. Dr. Lucier also presented an overview of the NTP and the ICCVAM process.

Introduction to NICEATM and ICCVAM/ Overview of the Corrositex® Peer Review Process

Dr. William Stokes, ICCVAM Co-Chair and Director of NICEATM, read the conflict of interest statement that had been signed by each member before agreeing to serve on the PRP. Dr. Stokes asked if any PRP members had a change in their conflict of interest status; none were raised.

Dr. Stokes explained the ICCVAM review process, and the steps that had been undertaken in the review of Corrositex®. He discussed the role of the ICCVAM committee, its expert subgroup (Corrosivity Working Group [CWG]), the PRP, and the process by which regulations are reviewed and forwarded to agencies for action.

Public Law 103-43 directed the NIEHS to develop and validate alternative methods that can reduce or eliminate the use of animals in acute or chronic toxicity testing, establish criteria for the validation and regulatory acceptance of alternative testing methods, and recommend a process through which scientifically validated alternative methods can be accepted for regulatory use. Criteria and processes for validation and regulatory acceptance were developed in conjunction with 13 other Federal agencies and programs with broad input from the public. These are described in the document "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods," NIH Publication 97-3981, March, 1997. This document is available in the internet at http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.htm. ICCVAM was subsequently established in a collaborative effort by NIEHS and 13 other Federal regulatory and research agencies and programs. The Committee's functions include the coordination of interagency reviews of toxicological test methods and communication with stakeholders throughout the process of test method development and validation. The following Federal regulatory and research agencies and organizations are participating in this effort:

- Consumer Product Safety Commission
- Department of Defense
- Department of Energy
- Department of Health and Human Services

- Agency for Toxic Substances and Disease Registry
- Food and Drug Administration
- National Institutes of Health
  - National Cancer Institute
  - National Institute of Environmental Health Sciences
  - National Library of Medicine
- National Institute for Occupational Safety and Health/CDC
- Department of the Interior
- Department of Labor
  - Occupational Safety and Health Administration
- Department of Transportation
  - Research and Special Programs Administration
- Environmental Protection Agency

Corrositex® was proposed to ICCVAM for consideration as an *in vitro* method for use in determining the dermal corrosivity potential of chemicals. The test method submission was prepared by IVI. Independent peer review is an essential prerequisite for consideration of a method for regulatory acceptance (NIEHS, 1997). The PRP was charged with developing a scientific consensus on the usefulness of the method to generate information for human health risk assessment purposes. The proposed test method and results of the peer review will be forwarded by ICCVAM to Federal agencies for consideration. Federal agencies will determine the regulatory acceptability of the method according to their mandates.

Summary of Current Agency Requirements

Dr. Richard Hill, ICCVAM and CWG Co-Chair, presented an overview of current agency regulations with regard to dermal corrosion testing. He stated that corrosion is not universally defined, but generally focuses on destruction of the skin or the irreversability of effects on the skin. Dr. Hill further stated that testing is usually done using the *in vivo* rabbit skin corrosivity test. The test results serve as a basis for determining appropriate materials labeling and hazard identification. An international harmonization effort has been in progress in order to develop internationally consistent labeling. Measurement of pH is used to define potential corrosives, where chemicals which have a pH in the extreme ranges are considered to be potential corrosives for labeling purposes. Currently, the US DOT has accepted Corrositex® as a method to determine the corrosive potential of seven chemical classes. Dr. Hill also mentioned that a tiered testing scheme has been proposed by OECD for determining dermal corrosivity potential of chemicals/products.

Overview of the Corrositex®

Dr. Rosalind Wei, Director of Research and Development at IVI, described the procedure used to test chemicals or compounds using Corrositex®. The presentation was followed by assay-related questions from the PRP.

Meeting—Review of the Corrositex® Submission

Test Method Description

Dr. Harbell, the section coordinator, presented the analysis and conclusions reached by the test method description section reviewers, which included Drs. Kohrman and Stegeman.

The PRP concluded that the basis for the test was adequately described, and the protocol was complete and consistent. They further concluded that the decision rules were adequately defined, and that the range of applications is known to some degree.

Test Method Data Quality

Dr. Green, the section coordinator, presented the analysis and conclusions reached by the test method data quality section reviewers, which included Drs. Derelanko, Harbell, and Kojima.

With regard to data quality, the PRP concluded that the studies presented in the Submission were not conducted under Good Laboratory Practice (GLP) standards, but that the data were credible, based on results from two data audits. Studies conducted as part of the European Centre for the Validation of Alternative Methods (ECVAM) prevalidation and validation studies were conducted under the "spirit" of GLP.

Test Method Performance

Dr. Hayes, the section coordinator, presented the analysis and conclusions reached by the test method performance section reviewers, which included Drs. Kohrman and Chen.

The PRP concluded that certain limitations were present in the data set (i.e., complex mixtures were not defined and thus could not be evaluated; category definitions were vague, so some could not be considered in the evaluation; and the number of chemicals in some chemical classes was limited such that performance analysis for these classes may not be representative). However, the PRP concluded that the accuracy (82%), sensitivity (85%), specificity (70%), and positive and negative predictivity (78% and 80%, respectively) were adequate for the data set including the Submission, Prevalidation Study (Botham et al., 1995), and ECVAM Validation Study (Fentem et al., 1998). The PRP felt that the assay was useful as a stand-alone method for predicting the corrosive potential of acids and bases. The test can also be used as part of a tier assessment approach for determining the dermal corrosion potential of substances in other chemical classes.

Test Method Reliability

Dr. Fentem, the section coordinator, presented the analysis and conclusions reached by the test method reliability section reviewers, which included Drs. Chen and Sauder.

The PRP concluded that the reproducibility of the test was adequate, although one peer reviewer felt that additional interlaboratory investigations would be helpful. The PRP suggested the inclusion of positive and negative controls and analysis of variance in future intra- and inter-laboratory evaluations.

Other Literature and Scientific Reviews

Dr. Derelanko, the section coordinator, presented the analysis and conclusions reached by the other literature and scientific reviews section reviewers, which included Dr. Kojima.

Key papers evaluated are listed below:

- Barratt, M. D., P. G. Brantom, J. H. Fentem, I. Gerner, A. P. Walker, and A. P. Worth. 1998. The ECVAM international validation study for *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicol. In Vitro 12:471-482.
- Botham, P. A., M. Chamberlain, M. D. Barratt, R. D. Curren, D. J. Esdaile, J. R. Gardner, V. C. Gordon, B. Hildebrand, R. W. Lewis, M. Liebsch, P. Logemann, R. Osborne, M. Ponec, J.-F. Régnier, W. Steiling, A. P. Walker, and M. Balls. 1995. A prevalidation study on *in vitro* skin corrosivity testing: The report and recommendations of ECVAM Workshop 6. ATLA 23:219-255.
- Fentem, J. H., G. E. B. Archer, M. Balls, P. A. Botham, R. D. Curren, L. K. Earl, D. J. Esdaile, H.-G. Holzhütter, and M. Liebsch. 1998. The ECVAM international validation study on *in vitro* tests for skin corrosivity.

2. Results and evaluation by the management team. Toxicol. In Vitro 12:483-524.

- Gordon, V. C., J. D. Harvell, and H. I. Maibach. 1994. Dermal corrosion, the Corrositex system: A DOT accepted method to predict corrosivity potential of test materials. Alternative Methods Toxicol. 10:37-45.

The PRP concluded that generally, the results reported in these papers were similar to those presented in the Submission. It was noted that the Gordon et al. (1994) publication was not peer reviewed.

Presentation of Corrositex® Performance Compared to the pH Test

Dr. Thomas Goldsworthy, NICEATM, presented the findings of an evaluation of the performance of pH compared to that of Corrositex®; both tests were compared against *in vivo* rabbit skin corrosivity data as the standard. The analysis found that both the pH test and Corrositex® are adequate for identifying the corrosive potential of chemicals with a pH value in the extreme ranges (i.e., pH less than or equal to 2 or greater than or equal to 11.5). However, Corrositex® was slightly but consistently more predictive than pH for chemicals with a pH value in the extreme ranges. Further, Corrositex® correctly identified several noncorrosive chemicals with pH values in the extreme ranges; these chemicals would be false positive calls if analyzed only by pH. Additionally, a number of chemicals with pH values in the non-extreme range (i.e., pH greater than 2 and less than 11.5) were identified as corrosive using the *in vivo* test; Corrositex® correctly identified the majority of these compounds. Given the ease and cost effectiveness of conducting a pH test, the PRP recommended that pH testing be conducted prior to the use of Corrositex®. Such information could be used in the future to re-evaluate the agreement between pH and Corrositex® in identifying corrosivity.

Summary of Nonqualifying Chemicals

Ms. Karen Haneke, NICEATM, presented an overview of available data on nonqualifying chemicals, focusing on nonqualifying test materials for which there was also pH and *in vivo* data. Of the 75 nonqualifying test materials identified in published sources and a 1996 Corrositex® submission, 85% of these materials were considered noncorrosive according to *in vivo* test results. pH data were found for 50 nonqualifying materials, of which all but one were in the pH range of 3 to 10. pH distribution was similar when the database of nonqualifiers was limited to only test materials for which both pH and *in vivo* data was available (N = 33); when this limited data set was evaluated, 91% of the chemicals were noncorrosive according to *in vivo* tests.

Other Considerations and Related Issues

Dr. Stegeman, the section coordinator, presented the analysis and conclusions reached by the other considerations and related issues section reviewers, which included Dr. Sauder.

The PRP noted several advantages of the Corrositex® test compared to the *in vivo* rabbit skin corrosivity test. Corrositex® is a non-animal test that is also relatively quick and easy to perform. The PRP stated that the large proportion of test materials that do not qualify for testing by the Corrositex® method is one limitation of the assay.

The PRP also agreed that the assay, whether used alone or as a component of a tiered assessment approach, provides for the reduction and replacement of animal use for certain defined chemical classes. Additionally, chemicals that test negative or do not qualify for Corrositex®

have a low likelihood of causing corrosive lesions if tested in animals. Any follow-up tests using *in vivo* methods could employ small numbers of animals and test agent dilution schemes to minimize numbers of animals and possible distress in any individual animal.

Public Comments

Dr. Rodger Curren, IIVS, Inc., stated that since this is only the second ICCVAM review, the review is a precedent-setting activity. The PRP must determine whether the use of Corrositex® would provide an equivalent level of protection compared to the currently accepted *in vivo* rabbit skin corrosivity test. Dr. Curren added that with regard to reproducibility, he felt that data from only a few labs was adequate because there are performance standards (i.e., positive and negative controls). To address a PRP discussion on the adequacy of evaluating interlaboratory data from only three labs, with one being naïve, Dr. Curren stated that none of the labs were naïve; they all had experience in conducting the test. In response to the PRP's comment that the number of chemicals for some classes was inadequate for performance assessment purposes, he mentioned the difficulty in obtaining adequate *in vivo* data for comparison.

Dr. Alan Goldberg, Johns Hopkins University, asked two questions to members of the PRP. First, he asked for clarification on the statement that auditors concluded that the discrepancies did not affect the conclusions reached from the data. Dr. Green responded that the data deficiencies and missing data were very few, and were thus determined to have minimal effect. Second, Dr. Goldberg noted that one of the data sources evaluated (submissions and published sources) was slightly different in performance compared to the others, and asked how that would affect the totality of the data. Dr. Kohrman stated that the variability probably deals with small sample size. Dr. Goldsworthy added that evaluations were done on a wide variety of sources and combinations thereof, and generally, the data sets were found to be similar to each other.

Dr. Katherine Stitzel, Procter & Gamble, felt that the PRP should give additional thought to the statement that 20 chemicals per class would be an adequate number for evaluation. She stated that this may be setting a precedent that may be difficult to meet, strictly based on the prevalence of some chemical classes. She further added that making such a statement may be setting a standard for the *in vitro* test that was not set for the *in vivo* test.

Dr. Errol Zeiger, NIEHS, made additional comment on the issue of prevalence and how many chemicals are needed for an adequate evaluated. He pointed out that when speaking of prevalence, the discussion is not the prevalence of chemicals classes in the universe, but rather the prevalence of chemical classes in specific industries. Dr. Zeiger noted that the prevalence of certain chemical classes thus changes based on the industry evaluated and the endpoint of interest. Dr. Zeiger also provided comment on the issue of the interlaboratory reliability study and how dependent and nonindependent labs play a role in these types of assessments. He stated that one method of assessment is to include only labs with experience in conducting the assay, while a second is to include only labs with limited experience with the assay. Dr. Zeiger felt that the equivalence of training among the three labs is an asset to the evaluation, and urged caution in evaluating how labs are determined to be dependent versus independent.

Dr. Francis Kraszewski, the Gillette Company, asked if the PRP was satisfied with the mechanistic basis of the assay. Dr. Hayes clarified that the test is not mechanistically based, but instead is significantly correlated.

Dr. David Hattan, FDA, asked, from a regulatory standpoint, whether the PRP felt that the results on neat materials could be translated to reflect the response of final formulations. Dr. Kohrman replied that the answer was dependent on what is known about the matrix. She stated that with proper information, it is possible to make an assessment of the entire mixture based on results found using neat materials. Dr. Sauder added that the question is very valid, and that information/studies pertinent to the topic would be helpful.

Dr. Ben Gregg, US EPA, stated that most materials reviewed by his agency are mixtures, and that US EPA may be interested in using Corrositex® as a replacement for *in vivo* testing of mixtures. He stated that more work should be directed toward how the test performs for mixtures.

Dr. Robert Bronaugh, FDA, asked for clarification from the PRP about the database, and whether it is considered adequate versus inadequate. Dr. Scala answered that the database is considered to be adequate, but that data for certain chemical classes may be inadequate due to the few numbers of chemicals in those classes.

PRP Conclusions

Based on their review, the PRP concluded that the Corrositex® method is equivalent to the *in vivo* rabbit skin corrosivity test for predicting corrosivity and noncorrosivity for specified chemical classes (i.e., primarily acids and bases). Therefore the test may be used either as a stand-alone assay for determining the dermal corrosion potential of acids or bases or as part of a tier assessment approach for determining the dermal corrosion potential of substances in other chemical classes.

The meeting was adjourned.

Summary of Public Comments on Corrositex®

Two Federal Register Notices were published on July 28, 1998 (Vol. 63, No. 144) and October 27, 1998 (Vol. 63, No. 207) respectively, requesting public comments on the Corrositex® test method. Information was also included regarding the availability of a public test method document located at the Consumer Product Safety Commission (CPSC) Reading Room. Responses were received from three individuals as listed below:

Karen E. Purves (September 11, 1998) Animal Protection Institute

Ms. Purves' comments focused on validation and regulatory acceptance criteria as listed in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) document "Validation and Regulatory Acceptance of Toxicological Methods" (NIH Pub No. 97-3981). The following points summarize Ms. Purves' comments:

- Corrositex® can be used to properly determine corrosivity for US Department of Transportation (US DOT) shipping classifications quickly and at a reduced cost
- Detailed protocol for test method is readily available from InVitro International (IVI).
- Reproducibility and reliability have been demonstrated both in tests performed by IVI and in pre-validation studies conducted under European Centre for the Validation of Alternative Methods (ECVAM).
- Adequate data is available from *in vivo* and pH studies to allow for comparisions with Corrositex®.
- Procedural information for the performance and determination of results is provided for Corrositex®.
- There is a 93% correlation between Corrositex® and *in vivo* responses.
- Corrositex® provides data that allows for classification, labeling, and packaging of hazardous materials making it useful for risk assessment.
- Corrositex® is less expensive as well as time effective when compared to animal testing and more accurate when compared to pH testing.
- Corrositex® provides a replacement alternative to animal use in corrosivity testing.

Linda Sternberg (October 30, 1998) BetzDearborn, Inc.

Ms. Sternberg stated that while she recognizes that Corrositex® may be expedient and cost-effective in some circumstances, the assay did not appear to perform satisfactorily for the BetzDearborn product lines. BetzDearborn provides "engineered chemical treatments for influent water, boiler, cooling systems, wastewater and process systems" and most of the products tested using Corroistex® were mixtures. Experiments were performed on 74 of the company's products using Corrositex® (11 of which did not qualify for use). A 65% (41/63) error rate was observed among the qualifying products with a lack of agreement between data from Corrositex® and animal data and/or supplier information. Ms. Sternberg concedes that theoretically, a 'correlation factor' could be gen-

erated to compensate for these differences but points out that the money and time required to do so would contradict BetzDearborn's purpose in using Corrositex®.

Tom Hearty (January 18, 1999) Mallinckrodt, Inc.

Mallinckrodt tested two of their products using both Corrositex® and the *in vivo* corrosivity test outlined in OECD Guideline 404. One product (pH 12.7) was identified by both *in vivo* and Corrositex® test results as a noncorrosive substance. Corrositex® test results identified this product as a Category II material with a mean breakthrough time of greater than 70 minutes. The second product (pH 13.2) was found to be noncorrosive using the *in vivo* test, but was identified as corrosive by Corrositex®. The product was assigned to Packing Group III by Corrositex® since the product was a Category II material and had a mean breakthrough time of 47 minutes. The *in vivo* test results indicated that this compound was a moderate to severe irritant. Based on this result, Mr. Hearty felt that Corrositex® is too sensitive in its responses.

Additional Public Comment

The following public comments were presented during the public comment period at the January 21, 1999 peer review panel (PRP) meeting:

Dr. Rodger Curren, Institute for In Vitro Sciences, stated that since this is only the second ICCVAM review, the review is a precedent-setting activity. The PRP must determine whether the use of Corrositex® would provide an equivalent level of protection compared to the currently accepted *in vivo* rabbit skin corrosivity test. Dr. Curren added that with regard to reproducibility, he felt that data from only a few labs was adequate because there are performance standards (i.e., positive and negative controls). To address a PRP discussion on the adequacy of evaluating interlaboratory data from only three labs, with one being naïve, Dr. Curren stated that none of the labs were naïve; they all had experience in conducting the test. In response to the PRP's comment that the number of chemicals for some classes was inadequate for performance assessment purposes, he mentioned the difficulty in obtaining adequate *in vivo* data for comparison.

Dr. Alan Goldberg, Johns Hopkins University, asked two questions to members of the PRP. First, he asked for clarification on the statement that auditors concluded that the discrepancies did not affect the conclusions reached from the data. Dr. Green responded that the data deficiencies and missing data were very few, and were thus determined to have minimal effect. Second, Dr. Goldberg noted that one of the data sources evaluated (submissions and published sources) was slightly different in performance compared to the others, and asked how that would affect the totality of the data. Dr. Kohrman stated that the variability probably deals with small sample size. Dr. Goldsworthy added that evaluations were done on a wide variety of sources and combinations thereof, and generally, the data sets were found to be similar to each other.

Dr. Katherine Stitzel, Procter & Gamble, felt that the PRP should give additional thought to the statement that 20 chemicals per class would be an adequate number for evaluation. She stated that this may be setting a precedent that may be difficult to meet, strictly based on the prevalence of some chemical classes. She further added that making such a statement may be setting a standard for the *in vitro* test that was not set for the *in vivo* test.

Dr. Errol Zeiger, National Institute of Environmental Health Sciences (NIEHS), made additional comment on the issue of prevalence and how many chemicals are needed for an adequate

evaluation. He pointed out that when speaking of prevalence, the discussion is not the prevalence of chemicals classes in the universe, but rather the prevalence of chemical classes in specific industries. Dr. Zeiger noted that the prevalence of certain chemical classes thus changes based on the industry evaluated and the endpoint of interest. Dr. Zeiger also provided comment on the issue of the interlaboratory reliability study and how dependent and nonindependent labs play a role in these types of assessments. He stated that one method of assessment is to include only labs with experience in conducting the assay, while a second is to include only labs with limited experience with the assay. Dr. Zeiger felt that the equivalence of training among the three labs is an asset to the evaluation, and urged caution in evaluating how labs are determined to be dependent versus independent.

Dr. Francis Kraszewski, the Gillette Company, asked if the PRP was satisfied with the mechanistic basis of the assay. Dr. Hayes clarified that the test is not mechanistically based, but instead is significantly correlated.

Dr. David Hattan, Food and Drug Administration (FDA), asked, from a regulatory standpoint, whether the PRP felt that the results on neat materials could be translated to reflect the response of final formulations. Dr. Kohrman replied that the answer was dependent on what is known about the matrix. She stated that with proper information, it is possible to make an assessment of the entire mixture based on results found using neat materials. Dr. Sauder added that the question is very valid, and that information/studies pertinent to the topic would be helpful.

Dr. Ben Gregg, US Environmental Protection Agency (US EPA), stated that most materials reviewed by his agency are mixtures, and that US EPA may be interested in using Corrositex® as a replacement for *in vivo* testing of mixtures. He stated that more work should be directed toward how the test performs for mixtures.

Dr. Robert Bronaugh, FDA, asked for clarification from the PRP about the database, and whether it is considered adequate versus inadequate. Dr. Scala answered that the database is considered to be adequate, but that data for certain chemical classes may be inadequate due to the few numbers of chemicals in those classes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Date: June 4, 1999

From: Co-Chairs, Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Subject: Peer review report on Corrositex®; Request to forward to agencies for regulatory and non-regulatory acceptance consideration

To: Director, National Toxicology Program (NTP)

Thru: Director, Environmental Toxicology Program,
National Institute of Environmental Health Sciences _____

We are pleased to provide you with the peer review report, *Corrositex®: A Test Method for Assessing the Dermal Corrosivity Potential of Chemicals* (Attachment 1) which was reviewed and approved by ICCVAM at its May 22, 1999 meeting. The review of Corrositex® was coordinated by ICCVAM and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Corrositex® is the first *in vitro* method and the second test method to undergo evaluation by the ICCVAM on behalf of its 14 participating Federal agencies and programs.

ICCVAM concurred with the conclusions and recommendations of the Peer Review Panel (PRP) and the ICCVAM Corrosivity Working Group (CWG) (Attachment 2) regarding the potential usefulness of Corrositex®. The PRP concluded that Corrositex® may be used as part of a tiered testing strategy for assessing the dermal corrosion potential of chemicals, such as the one endorsed by the Organization for Economic Cooperation and Development (OECD). The OECD tiered testing strategy for dermal irritation/corrosivity provides for the use of validated and accepted *in vitro* methods. In this approach, positive responses do not generally require further testing, while negative responses would usually be followed by *in vivo* dermal irritation/corrosion testing. The PRP recommended that with either positive or negative Corrositex® results, there should be the opportunity for confirmatory testing if false positive or negative results are suspected based on a weight-of-evidence evaluation of supplemental information, such as pH, structure-activity relationships (SAR), and other chemical and testing information. ICCVAM concurs with the PRP that Corrositex®, in specific testing circumstances, may be useful as an assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives (e.g., US Department of Transportation [US DOT]).

The PRP concluded that the use of Corrositex® offers several advantages with respect to animal welfare. Corrositex®, when used as a stand-alone assay for some testing applications such as

transportation purposes, can replace the use of animals for corrosivity testing of qualified chemicals in some chemical classes. When used as part of a tiered testing strategy for corrosivity, there is a reduction in the number of animals required because positive results usually eliminate the need for animal testing, and when further testing in animals is determined to be necessary, only one animal is required to confirm a corrosive chemical. Corrositex® also provides for refinement in that most of the chemicals that are identified as negative by Corrositex® or nonqualifying in the detection system are unlikely to be corrosive when tested in the *in vivo* test. If any skin lesions are produced in test animals, they are usually limited to some degree of irritation.

Independent scientific peer review, considered a prerequisite for regulatory acceptance, has now been completed for Corrositex®. The final stage in the regulatory process involves determination of the acceptability of Corrositex® by regulatory agencies, and informing the appropriate regulated community and others of the acceptability of the method for meeting regulatory requirements.

We therefore ask that you forward the attached report to the participating ICCVAM agencies requesting their consideration of Corrositex® for regulatory acceptance or other non-regulatory applications where appropriate.

Thank you for your continuing support of ICCVAM and NICEATM's efforts to achieve validation acceptance of new methods that will provide for improved protection of human health and the environment, and improved animal welfare.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

MEMORANDUM

Date: May 3, 1999

To: ICCVAM

From: Co-chairs, ICCVAM CWG

Subject: ICCVAM CWG Recommendations on Corrositex® as a Test Method for Assessing Dermal Corrosivity Potential of Chemicals

The purpose of this memorandum is to inform ICCVAM that the CWG unanimously endorses the conclusions and recommendations stated in the PRP Report entitled *Corrositex®: An in vitro test method for assessing the dermal corrosivity potential of chemicals—Results of an independent peer review evaluation coordinated by ICCVAM and NICEATM* (Attachment 1). The Report consists of the PRP's written deliberations and conclusions from the public peer review meeting convened in Bethesda, Maryland on January 21, 1999. The PRP concurred in a public meeting via teleconference on April 22, 1999 that the Report accurately reflects the conclusions and recommendations of the January 21 meeting. Appendices to the Report include data and information submitted to substantiate the validation status of the method, and supporting analyses and information prepared by NICEATM. The CWG recommends that ICCVAM forward the Corrositex® Report to applicable Federal and international organizations for regulatory acceptance consideration for the uses recommended by the PRP.

The CWG concurs with the PRP's conclusion that Corrositex® may be used as part of a tiered testing strategy for assessing dermal corrosion potential of chemicals, such as the one endorsed by the OECD (Attachment 2). The OECD tiered testing strategy for dermal irritation/corrosivity provides for the use of validated and accepted *in vitro* methods. In this approach, positive responses do not generally require further testing, while negative responses would usually be followed by *in vivo* dermal irritation/corrosion testing. The PRP recommended that with either positive or negative Corrositex® results, there should be the opportunity for confirmatory testing if false positive or negative results are suspected based on a weight-of-evidence evaluation of supplemental information, such as pH, SAR, and other chemical and testing information. The CWG concurs with the PRP that Corrositex®, in specific testing circumstances, may be useful as an assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives (e.g., US DOT).

The PRP concluded that Corrositex® demonstrated excellent intra- and inter-laboratory reproducibility. The ease of transferability among laboratories and the simplicity of the assay were considered by the PRP to be attractive features of Corrositex®. The manufacturer's protocol for Corrositex® was considered to be complete and to provide the necessary details for users to conduct the assay correctly. Methods for data analysis and decision criteria were considered to be straightforward, and the PRP noted that concurrent positive and negative controls are included to determine whether each test trial is performing correctly. The PRP concluded that the test is more rapid and less expensive than the *in vivo* rabbit corrosivity test. Since it is an *in vitro* alternative test, it also has the advantage that it does not require the use of animals and can be conducted in laboratories without animal facilities.

Animal Welfare Considerations

The PRP concluded that the use of Corrositex® offers several advantages with respect to animal welfare. When used as a stand-alone assay for some testing applications such as determining transportation packing groups, Corrositex® can replace the use of animals for corrosivity testing of qualified chemicals in some chemical classes. When used as part of a tiered testing strategy for corrosivity, there is a reduction in the number of animals required because positive results usually eliminate the need for animal testing, and when further testing in animals is determined to be necessary, only one animal is required to confirm a corrosive chemical. Corrositex® also provides for refinement in that most of the chemicals that are identified as negative by Corrositex® or nonqualifying in the detection system are unlikely to be corrosive when tested in the *in vivo* test. If any skin lesions are produced in test animals, they are usually limited to some degree of irritation.

Limitations

A limitation noted by the PRP was that some corrosive chemicals and mixtures do not qualify for testing with Corrositex®. That is, they do not cause a color change in the Chemical Detection System (CDS). A color change is needed to enable the detection of the chemical or mixture if it breaks through the biobarrier membrane, and requires that the chemical change the pH of the solution to less than 5 or greater than 8.5. Many noncorrosive chemicals do not cause the required change in pH, and thus do not qualify for evaluation by Corrositex®. Of the 75 nonqualifying chemicals and mixtures for which *in vivo* corrosivity data were available, 85% (64/75) were classified as noncorrosive in the *in vivo* test. This indicates that nonqualifying chemicals are often noncorrosive.

Comparative Corrositex® and *in vivo* rabbit data were provided for complex mixtures that generally consisted of industrial chemicals, cleaners, detergents, and surfactants. While the performance data for Corrositex® are promising for some of these mixtures, the PRP did not consider it appropriate to reach conclusions for these classes in the assessment of the validation of the method because the composition of these materials was not provided. Based on the performance of Corrositex® for mixtures, the CWG thinks that Corrositex® can yield useful information for those materials that qualify for the test.

The evaluation of Corrositex® for some chemical/product classes was limited by the relatively small number of chemicals and/or the unbalanced distribution of corrosive and noncorrosive

chemicals/mixtures evaluated. As a consequence, conclusions about the performance of Corrositex® for some chemical classes were not made by the PRP.

CWG recommendations

The CWG concurs with the PRP's recommendations, and agrees that the following specific changes to the protocol should be made (Note: The wording for these recommendations has been slightly revised from those provided in the Report, but it does not change their intent):

- Specific information should be added to the protocol:

(1) It should be explicitly stated that the biobarrier should be allowed to harden on a level surface and should be refrigerated at 2 to 8 °C overnight before use.

(2) Even though replicate variability has been shown to be very low, guidance should be provided on how to evaluate an aberrant value.

(3) The IVI Corrositex® Data Sheets provided with the test kit should include a provision for recording the performance of the positive and negative controls. This information should be used to determine the suitability of the test results.

(4) Description of the test protocol would benefit from the addition of a flow diagram illustrating the steps in the procedure.

- In future studies, compliance with Good Laboratory Practice (GLP) Guidelines and inclusion of quality control procedures, would improve data quality and credibility.

- Positive and negative control values should be reported concurrently with each assay to demonstrate that the test is working properly.

- Laboratories unfamiliar with conducting the test should obtain appropriate training and conduct tests with reference chemicals before undertaking any testing of unknown chemicals and chemical mixtures.

- pH testing should be conducted prior to use of Corrositex®. Such information could be used in the future to re-evaluate the agreement between pH and Corrositex® in identifying corrosivity.