METHOD 1120

DERMAL CORROSION

1.0 SCOPE AND APPLICATION

1.1 The dermal corrosion assay system is an *in vitro* test method which determines the corrosive potential of a substance toward human skin. The method is simple, rapid, accurate, and may be applied to both solids, liquids and emulsions. The liquids may be aqueous or non-aqueous. Solids can be water-soluble or non-soluble. The samples may be pure chemicals, dilutions, formulations, or waste. No prior treatment of the sample is required. This method may be used to meet certain regulatory applications, e.g., DOT corrosivity measurement for Packing Groups, but is not required for determining if a waste passes or fails the characteristic of corrosivity per the RCRA definition.

1.2 This method is applicable to a limited number of materials, specifically: Acids, inorganic and organic; acid derivatives (anhydride, halo acids, salts, etc.), inorganic and organic; acyl halides; alkylamines and polyalkylamines; bases, inorganic and organic; chlorosilanes; and metal halides and oxyhalides.

2.0 SUMMARY OF METHOD

2.1 The assay system is an *in vitro* test method which is composed of two components, a synthetic macromolecular biobarrier and a Chemical Detection System (CDS). Test samples are applied on top of the macromolecular biobarrier. Corrosive samples are able to disrupt the macromolecular structure of the biobarrier. A color change in the CDS, located beneath the biobarrier, is detected visually and indicates that the test sample has altered the biobarrier sufficiently to allow its passage through the full thickness of the biobarrier. The time it takes a sample to disrupt the biobarrier is inversely proportional to the degree of corrosivity of the sample - the longer it takes to observe a color change, the less corrosive the substance is. Noncorrosive samples do not disrupt the biobarrier, or disrupt the biobarrier after a predetermined time period (see Section 2.4).

Corrosive samples may be placed into three different classes of corrosivity, established by the time required for the sample to break through the biobarrier. These classes are called Packing Groups by the U.S. Department of Transportation (DOT). Packing Groups are assigned according to the degree of danger presented by the corrosive material; Packing Group I indicates great danger; Packing Group II, medium danger; Packing Group III, minor danger. For consistency, these same definitions are used for this test method and are referred to as Group I, Group II, and Group III.

2.2 Prior to performing the assay, samples are pre-qualified to establish their compatibility with the assay system. The sample is placed in a small amount of CDS fluid. If any detectable change occurs in the CDS, the sample is qualified and can be analyzed by the test. If a sample is non-qualified, it is incompatible with the CDS and must be tested by another method.

2.3 Test samples are classified into categories by the screening test which is supplied with the assay kit. The category that a sample is assigned to will determine how the Groups will be assigned. Test samples are classified by pH changes produced in two well-defined buffers - one designed to buffer acids and another that buffers bases. These buffers are supplied as part of the screening test. Four different categories are defined as follows:

2.3.1 Category A_1 substances produce a large change in pH when they are added to the acid buffer. This change in pH is indicated by a strong color change of the acid buffer solution.

2.3.2 Category B_1 substances produce a large change in pH when they are added to the base buffer. This change in pH is indicated by a strong color change of the base buffer solution.

2.3.3 Category A_2 substances produce little or no pH changes when added to the acid buffer, and therefore, little or no color change in the buffer solution is observed.

2.3.4 Category B_2 substances produce little or no pH changes when added to the base buffer, and therefore, little or no color change in the buffer solution is observed.

2.4 Groups are assigned in the assay system by taking into account the category that is assigned to a sample by the screening test, and the time it takes to detect a color change in the CDS in the assay. Category A_1 and B_1 samples are assigned to Group I if a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to one hour, and to Group III if a color change is observed after one hour and up to four hours. If no color change occurs in four hours, the chemical is classified as Noncorrosive.

Category A_2 and B_2 samples are assigned to Group I if a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to 30 minutes, and to Group III if a color change is observed after 30 minutes and up to 60 minutes. If no color change occurs in 60 minutes, the chemical is classified as Noncorrosive.

3.0 INTERFERENCES

3.1 The test is not subject to interference from color, turbidity, colloidal matter or high salinity.

3.2 The Pre-qualification Test, the Screening Test and the Assay must be performed at room temperature. The samples must also be at room temperature (17- 25°C).

4.0 APPARATUS AND MATERIALS

4.1 Corrositex Assay Kit (InVitro International, 16632 Millikan Avenue, Irvine, CA 92714). The following three items are supplied in the Corrositex Assay Kit:

4.1.1 Four racks holding seven vials with black caps.

4.1.2 One tray of 24 membrane discs.

4.1.3 Four data sheets (color charts).

4.2 Combination hot plate/stir plate or equivalent - able to heat to 75°C. Stirring speed should be adjustable.

4.3 Digital thermometer - able to read to 75°C.

4.4 Timers (6) - able to measure hours, minutes and seconds.

4.5 Repeat pipettor - this pipet is different than the pipet specified in Section 4.12. Delivers 200 µL repeatedly, without refilling between individual deliveries.

4.6 2.5 mL combitip for repeat pipettor.

4.7 Lab Industries or equivalent sample pipettor - a positive displacement pipettor useful when pipetting viscous samples.

4.8 Pipet tips for Lab Industries, or equivalent, pipettor.

4.9 Test tubes

4.10 Balance - capable of weighing 100 mg accurately.

4.11 Spatula - capable of transferring 0.1 - 0.5 g.

4.12 Pipets - microliter, with disposable tips. Should be able to measure 100 µL accurately.

4.13 Tweezers.

4.14 Permanent marker pens.

4.15 Plastic wrap.

5.0 REAGENTS

5.1 All reagents listed below are provided in the Corrositex Assay Kit except for the positive and negative controls mentioned in Section 5.7. The Corrositex Assay Kit is available from InVitro International, 16632 Millikan Avenue, Irvine, CA 92714.

5.2 Chemical Detection System (CDS).

5.3 Screening test buffer solutions.

5.4 Confirmation Test Solution.

5.5 One gram of the biobarrier matrix and a microstir bar.

5.6 10 mL of biobarrier diluent.

5.7 Positive and negative controls, if desired, for GLP purposes.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Appropriate precautions should be taken for handling potentially corrosive substances such as wearing gloves and having proper eye protection.

6.2 Samples should be analyzed as soon as possible after collection.

7.0 PROCEDURE

7.1 Follow the established laboratory procedures for working with hazardous test samples. Wear lab coat, gloves and safety glasses when working with any potentially corrosive material.

7.2 Pre-Qualification Test

7.2.1 Add 100 mg or 150 µL of sample to 1.0 mL of CDS in duplicate test tubes.

7.2.1.1 Sample qualifies if there is a color reaction within 5 minutes: proceed with assay.

7.2.1.2 If no reaction is observed, the sample is non-qualified. Seek other methods to determine corrosivity.

7.3 Screening Test

7.3.1 Liquid samples

7.3.1.1 Add 150 µL of sample to Test Tubes A and B. Cap the test tubes and shake vigorously for 10 seconds. Read the color change of the mixture within one minute. If the sample is immiscible in the solution, wait one minute and then read the color change at the interface.

7.3.1.2 Assign the category. If an intense color change (similar to the Category 1 color chart) is observed in Tube A or Tube B, assign the sample to Category 1. If a less intense color change (similar to the Category 2 color chart) is observed in either Tube A or Tube B, assign the sample to Category 2 . If no color change is observed in either Tube A or Tube B, proceed to the next step.

7.3.1.3 Confirm test. Add two drops of the Confirm reagent to Tube B. Cap the test tube and shake vigorously for 5 seconds. The color of the solution will match one of the colors shown in the accompanying color chart, confirming that the sample is Category 2 material.

7.3.2 Solid samples

7.3.2.1 Add 100 mg of sample to Test Tubes A and B. Cap the test tubes and shake vigorously for one minute. Wait another minute and read the color change of the mixture. If the sample is insoluble in the solution, allow the mixture to settle and read the color change at the interface of the solution and the solid.

7.3.2.2 Assign the category. If an intense color change (similar to the Category 1 color chart) is observed in either Tube A or Tube B, assign the sample to Category 1. If a less intense color change (similar to the Category 2 color chart) is observed in either Tube A or Tube B, assign the sample to Category 2. If no color change is observed in either Tube A or Tube B, proceed to the next step.

7.3.2.3 Confirm test. Add two drops of the Confirm reagent to Tube B. Cap the test tube and shake vigorously for 5 seconds. The color of the solution will match one of the colors shown in the accompanying color chart, confirming that the sample is Category 2 material.

7.4 Assay

7.4.1 Biobarrier preparation

7.4.1.1 Biobarrier matrix preparation - must be completed at least two hours prior to running assay.

7.4.1.2 Place scintillation vial containing biobarrier matrix powder on the hot plate pad. Begin spinning the stir bar before adding the diluent.

7.4.1.3 Add the entire contents of the biobarrier diluent vial slowly and constantly to the vial of biobarrier matrix powder. Make sure that the stir bar is turning while adding the diluent. The stir bar should be turning rapidly, but not so fast that the solution foams.

7.4.1.4 Turn the heat on low; monitor the temperature of the solution as it is warming. Gradually increase the heat as necessary to warm the solution to 68°C (±1°C) to solubilize the matrix. This may take approximately 20 minutes. **DO NOT** allow the temperature to exceed 70°C.

7.4.1.5 While the solution is warming, remove the tray of 24 membrane discs from the refrigerator. Remove the tray lid.

7.4.1.6 After the biobarrier matrix solution reaches 68°C and has been completely solubilized, turn off the heat and move the vial toward the edge of the heat pad to keep it warm while aliquotting into the membrane discs.

7.4.1.7 Dispense solubilized solution into membrane discs. Using the repeat pipettor, set to dispense 200 µL. Slowly fill the pipette tip with biobarrier solution, avoiding air bubbles. Dispense one aliquot back into the biobarrier vial to ensure proper subsequent volume delivery. Wipe the tip before dispensing each aliquot. Dispense 200 µL into each disc, ensuring that the entire membrane is covered and no air bubbles have formed. Any air bubbles in the gel will alter the results of the test, therefore the disc cannot be used.

7.4.1.8 Label the lid with the date, time, lot number, and initials of the technician preparing the biobarrier. Wrap the filled tray evenly with plastic wrap; do not bunch up the plastic wrap underneath the plate.

7.4.1.9 Store the tray at 2 - 8°C for at least two hours before beginning any testing. The biobarrier is stable for seven days if wrapped and stored at 2 - 8°C.

7.4.2 Running the assay.

7.4.2.1 On the data sheet, complete the lot number, date, time, name of technician, name of chemical, whether it is solid or liquid, and pH of a 10% solution diluted in water or appropriate solvent.

7.4.2.2 When ready to test, remove one tray of seven pre-filled black-capped scintillation vials from the kit box.

7.4.2.3 Remove the tray of 24 membrane discs from the refrigerator. Place on a tray of ice.

7.4.2.4 Place disc into first scintillation vial. Do not allow the discs to be in contact with the CDS for longer than two minutes before applying the test sample. Within two minutes, add 500 µL (using the Lab Industries pipettor, or equivalent) or 500 mg (using spatula or tweezers) of test sample to disc. Start timer the instant the sample is added.

Note: Do not cap the vials while test is in progress due to potential pressure build-up.

7.4.2.5 Watch the vial for three minutes, ensuring that the color reaction is not missed if it is a Group I sample. Changes in the CDS may include various color changes, flaking or precipitation.

7.4.2.6 Add three more discs and samples to vials, staggering each start time so that the most accurate reaction times are recorded.

7.4.2.7 Allow assay to run until color or physical reaction occurs. Category 1 samples should be checked for reactions at 3 minutes, 1 hour and 4 hours. Category 2 samples should be checked for reactions at 3, 30 and 60 minutes.

7.4.2.8 At the first indication of the presence of a chemical reaction in the CDS, there will be a color change produced beneath the bottom-center of each biobarrier disc. As soon as a reaction is observed, immediately record net time of each vial on the data sheet.

7.4.2.9 Run positive and negative controls in the other two vials, if desired, for GLP purposes.

7.4.3 Assignment of Groups.

7.4.3.1 Category 1 samples are assigned to Group I if a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to one hour, and to Group III if a color change is observed after one hour and up to four hours. If no color change occurs in four hours, the chemical is classified as Noncorrosive.

7.4.3.2 Category 2 samples are assigned to Group I if a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to 30 minutes, and to Group III if a color change is observed after 30 minutes and up to 60 minutes. If no color change occurs in 60 minutes, the chemical is classified as Noncorrosive.

8.0 QUALITY CONTROL

8.1 Samples should be analyzed in quadruplicate. The test may be analyzed in duplicate if a simple screening of corrosives and noncorrosives is all that is required. However, it is recommended that for greater accuracy, samples be analyzed in quadruplicate. It is suggested that positive and negative controls be analyzed to conform with GLP.

9.0 METHOD PERFORMANCE

9.1 Interlaboratory and intralaboratory studies were conducted with five different laboratories. Ten different chemicals were tested with six replicates. The data are presented in Table 1.

9.2 More than 200 data points have been collected at InVitro International for six reference samples. Statistical analysis of this data shows the standard deviation for ethylenediamine and ferric chloride is about 5% of their respective assay times, and about 10% for maleic anhydride, sodium hydroxide, and dicyclohexylamine. The standard deviation for sulfuric acid approaches 18% of its assay time, but when taken into account that the mean assay time is less than 1 minute, a standard deviation of 0.13 minutes is actually a reflection of the difficulty of measuring such brief time periods.

9.3 The Corrositex assay has been used by more than 300 laboratories to test approximately 4,000 test materials in its first phase of utilization in industry. Diverse chemicals and formulations which include liquids, solids, insolubles and immiscibles have been studied from many major industries including petrochemical, agrochemical, surfactant, textile, paper and pulp, electroplating and water treatment. Examples of dermal corrosion values compared to pH for selected compounds are shown in Table 2.

9.4 Data results from 1,050 samples that have been tested using the assay system were complied and compared with *in vivo* data. Ninety-two percent of the samples (965 samples) passed the Pre-qualification Test and were then analyzed in the screening test and the assay. Assay was found to be highly concordant with corrosive/noncorrosive *in vivo* results. Of 406 corrosive samples with *in vivo* data, 377 (93%) were correctly identified as corrosive by assay. Of 296 noncorrosive samples with *in vivo* data, 83% were identified as noncorrosives, demonstrating the ability of this *in vitro* method to correctly identify corrosives and noncorrosives. Assay was also shown to accurately predict Packing Groups. Six hundred out of 702 samples (85.5%) were placed in the same Packing Group as that indicated by *in vivo* testing. Only 38 test samples out of 702 samples that had *in vivo* data were found to underestimate (5.4%). Of these 38 samples, 28 were distinct samples and the remainder were samples that had been tested in more than one laboratory. When taking this information into account, the percent of underestimation decreased to about 4%.

10.0 REFERENCES

1. Code of Federal Regulations, Transportation Title 49, Hazardous Materials Table, Section 172.101 (1991).

2. Code of Federal Regulations, Transportation Title 49, Method of Testing Corrosion to the Skin, Part 173, Appendix A (1991).

3. Schlesselman, J.J. (1973) Planning a Longitudinal Study: I. Sample Size Determination. J. Chron. Dis. **26**, 553-560.

4. ASTM Standards on Precision and Bias for Various Applications, “Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method”; ASTM: Philadelphia, PA, 1992; E 691-92.

5. Gordon, V.C., Harvell, J., and Maibach, H. (1994). Dermal Corrosion, The Corrositex System, A DOT Accepted Method to Predict Corrosivity of Test Materials. *In Vitro* Toxicology. Ed. Mary Ann Liebert, 1994.

TABLE 1
LABORATORY DATA

Dichloroacetyl chloride

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	3.30	0.59	17.88%
2	2.99	0.30	10.03%
3	3.88	0.47	12.11%
4	2.50	0.26	10.40%
5	3.30	0.28	8.48%

Formic acid

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	6.32	0.51	8.07%
2	5.08	0.46	9.06%
3	5.18	0.77	14.86%
4	4.82	0.29	6.02%
5	4.02	0.29	7.21%

Dichloroacetic acid

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	6.92	0.32	4.62%
2	5.21	0.25	4.80%
3	6.32	0.98	15.51%
4	5.78	0.26	4.50%
5	5.65	0.46	8.14%

Chloroacetic acid

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	5.46	0.36	6.59%
2	7.83	0.00	0.00%
3	4.95	0.35	7.07%
4	6.91	0.94	13.60%
5	4.95	0.34	6.87%

TABLE 1 (continued)

Dodecyltrichlorosilane

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	10.78	0.05	0.46%
2	11.59	0.36	3.11%
3	11.22	0.82	7.31%
4	11.96	0.56	4.68%
5	10.98	0.29	2.64%

Ammonium hydrogen sulfate

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	10.47	0.56	5.35%
2	9.02	0.33	3.66%
3	13.82	1.0	7.24%
4	11.17	0.93	8.33%
5	7.88	0.26	3.30%

Ethylenediamine

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	17.24	0.38	2.20%
2	21.33	0.53	2.48%
3	26.12	1.30	4.98%
4	20.76	0.19	0.92%
5	22.48	1.40	6.23%

Aluminum chloride

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	11.91	0.41	3.44%
2	21.33	0.53	2.48%
3	26.12	1.30	4.98%
4	20.76	0.19	0.92%
5	22.48	1.40	6.23%

TABLE 1 (continued)

Acetic acid

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	28.52	0.87	3.05%
2	27.00	0.00	0.00%
3	34.98	0.22	0.63%
4	36.30	0.41	1.13%
5	29.67	0.62	2.09%

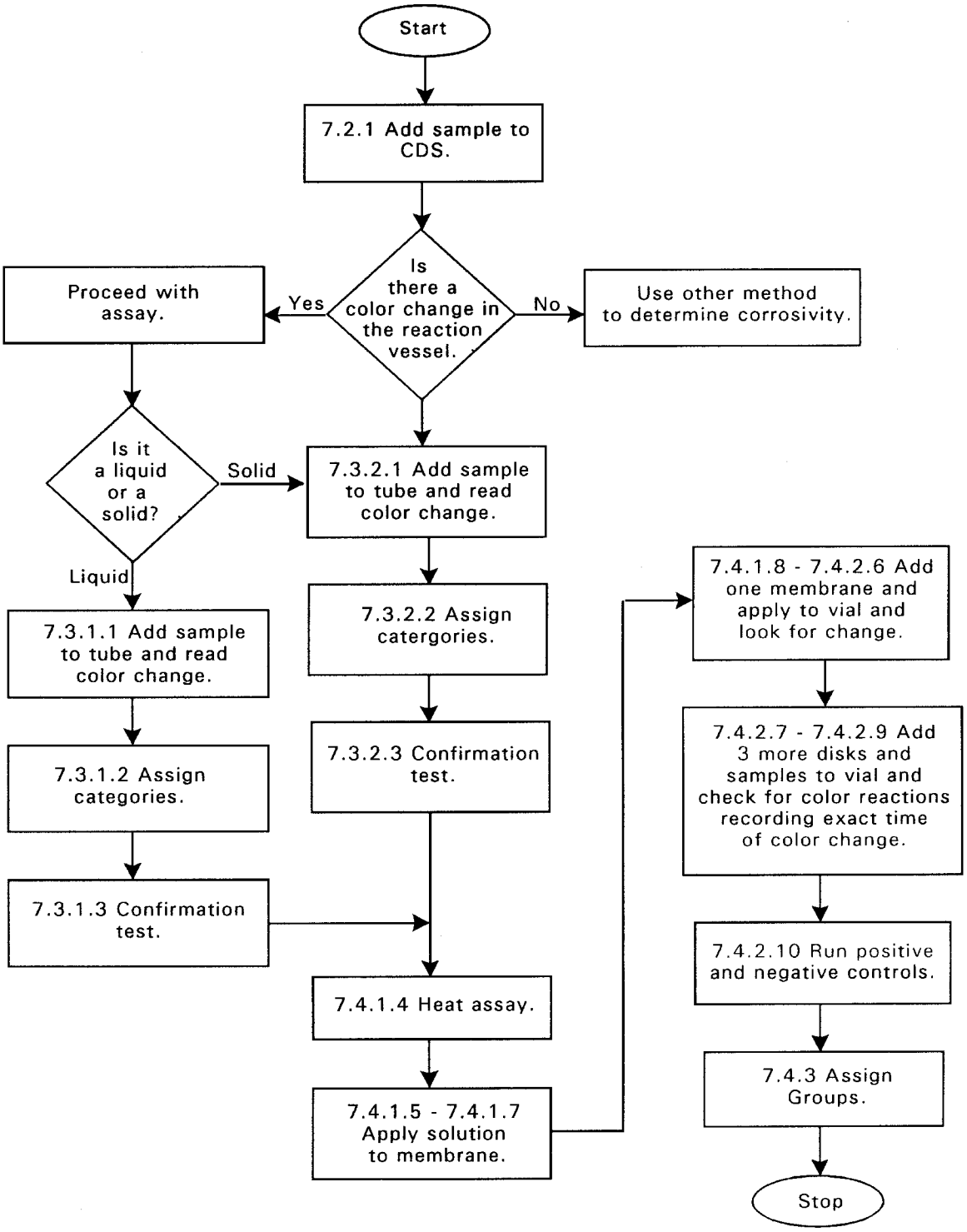
Dicyclohexylamine

Laboratory	Mean of 6 Test Results (minutes)	Standard Deviation	Relative Standard Deviation
1	181.73	0.47	0.26%
2	168.83	9.11	5.40%
3	210.70	7.68	3.64%
4	159.04	7.58	4.77%
5	126.75	0.62	0.49%

TABLE 2. EXAMPLES OF DERMAL CORROSION VALUES FOR SELECTED COMPOUNDS

Compound Name	Concentration (weight %)	pH[1]	Time (minutes)
Acetic acid	99+	0.00	29.31
Aluminum chloride	pure	2.92	16.50
Ammonium hydroxide	10.00	12.37	5.41
Bromoacetic acid	55.60	0.93	9.17
Butylamine	40.00	12.96	>240
Citric acid	20.00	1.28	47.65
1,2-Diaminopropane	99+	12.06	21.67
Dichloroacetic acid	3.10	0.98	37.63
Dicyclohexylamine	99.00	9.57	210.00
Diethylamine	98.00	13.86	5.89
Diethylene triamine	99.00	12.01	34.00
Ethanolamine	99+	11.82	21.68
Ferric chloride	98.00	3.00	21.30
Formic acid	33.90	0.62	>240
Hydrochloric acid	35.00	0.00	5.80
Hexanoic acid	99.00	3.00	149.00
Maleic acid	99.00	1.30	15.55
Mercaptoacetic acid	15.10	1.60	42.09
Nitric acid	90.00	0.00	0.57
Phosphoric acid	85.00	0.00	15.00
Potassium hydroxide	pellets	14.00	6.82
Propionic acid	99+	0.35	34.59
Sodium hydroxide	pellets	13.81	14.67
Sodium metasilicate	20.00	13.28	17.17
Sulfuric acid	15.00	0.00	11.48
Thiophosphoryl chloride	98.00	5.81	10.13
Tributylamine	99+	10.70	>240
Trichlorotoluene	99.00	3.32	>240
Triethanolamine	60.00	11.02	41.03
Triphosphoryl chloride	98.00	5.80	10.25

[1] pH of a 10% solution of the compound in water.


Start
7.2.1 Add sample to CDS.
Is there a color change in the reaction vessel.
Yes
No
Use other method to determine corrosivity.
Proceed with assay.
Is it a liquid or a solid?
Solid
Liquid
7.3.2.1 Add sample to tube and read color change.
7.3.2.2 Assign catergories.
7.3.2.3 Confirmation test.
7.3.1.1 Add sample to tube and read color change.
7.3.1.2 Assign categories.
7.3.1.3 Confirmation test.
7.4.1.4 Heat assay.
7.4.1.5 - 7.4.1.7 Apply solution to membrane.
7.4.1.8 - 7.4.2.6 Add one membrane and apply to vial and look for change.
7.4.2.7 - 7.4.2.9 Add 3 more disks and samples to vial and check for color reactions recording exact time of color change.
7.4.2.10 Run positive and negative controls.
7.4.3 Assign Groups.
Stop