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(54) Title: IN VITRO TEST FOR DERMAL CORROSIVE PROPERTIES (57) Abstract <p>An <i>in vitro</i> test kit and an <i>in vitro</i> test method which determine dermal corrosion properties of a test substance and/or mixture are disclosed. The test substance is applied directly to a biobarrier designed to give corrosion times comparable to those of the <i>in vivo</i> dermal corrosion test. A corrosive test substance destroys and passes through the biobarrier, after which the test substance enters and reacts with the adjacent chemical detection system, where the test substance is detected. The detection time, or the time from application to detection, can be correlated with UN Packing Groups and EEC classes.</p>		

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IN VITRO TEST FOR DERMAL CORROSIVE PROPERTIESTechnical Field

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This invention relates to the field of testing materials for their ability to be corrosive to human skin. More specifically, the invention relates to a device and a method for an *in vitro* test which can
15 predict the ability of a material to cause corrosion when placed in contact with the human skin.

Background Art

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As the potential for wider varieties of materials to cause cutaneous damage has become recognized, an increasing effort has been made by industry and environment protection groups to characterize this potential. Federal legislative
25 agencies and commercial manufacturers must consider these risks when developing registering, certifying and shipping materials which could produce toxic effects.

Acute primary irritation is defined as localized reversible inflammatory response of normal
30 living skin to direct injury and is caused by a single application of a chemical agent. Important manifestations are erythema (redness) and edema (swelling). Draize et al., J. Pharmacol. Exp. Ther.
35 82:337, 1944.

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Cumulative irritation is also reversible and consists of primary irritation resulting from repeated exposure of skin to materials that do not cause primary irritation, as reviewed by Guillot et al., Fd. Chem.

5 Toxic 20: 563, 1982.

Corrosion is defined as direct chemical action on normal living skin that results in its disintegration and irreversible alteration on site of chemical contact. According to Draize, et al. It is manifested by
10 ulceration or necrosis. (Draize, ibid.)

Animals have been used to test for toxic effects. However, the problems inherent in animal testing contribute to variability of all these methods.

1. Intra-laboratory variability in scoring
15 and handling
2. Individual responses vary considerably
3. Application and occlusive seal
4. Sex and age of animals

To evaluate dermal corrosivity, a procedure
20 based on the Department of Transportation Method of Testing Corrosion to skin has been widely used. Code of Federal Regulations, Transportation Title 49, Part 173, Appendix A. Method of Testing Corrosion to the Skin (1983). Six white New Zealand rabbits are shaved, the
25 test substance and a negative control is applied to the shaved skin of each rabbit. Each substances is held in place with a 1 x 1" square 12-ply surgical gauze pad. Corrosion is found if the test sample caused destruction or irreversible alteration of the tissue on at least two
30 of the six rabbits.

In 1977 the United Nations issued special recommendations for class 8 chemicals. (Transportation of Dangerous Goods. Orange Book. Special
35 Recommendations Relating to Class 8, p. 173.) The

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distinctions between chemicals in Packing Groups I, II and III were given as follows:

5 Group 1. Substances that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than three minutes.

10 Group 2. Substances, other than those in Packing Group 1, that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than 60 minutes.

15 Group 3. Substances, other than those meeting Packing Group 1 or 2 criteria, that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than four hours.

20 In recent years, different guidelines have evolved in Europe through legislative activity to classify and label potentially dangerous preparations. 1973 Off. J. Eur. Comm. 16 (L189); 1977 Off. J. Eur. Comm. 29 (L303); 1978 Off. J. Eur. Commission 21 (L296).
25 Different levels of skin corrosion define different classifications of the European Commission (EEC, 1983). Sect 4, No. 404 OECD Paris. A substance is corrosive if, when applied to intact animal skin, it produces full-thickness destruction of skin tissue in at least two
30 animals in four hours. If full-thickness destruction occurs within three minutes, the substance is in R35 class, which is comparable to Packing Group I. If full-thickness destruction occurs between three minutes and
35 four hours, the substance is classified as R34, which is comparable to Packing Groups II and III.

In Vitro Alternatives

The state of development of alternative models for dermal irritation and corrosion is improving rapidly. Attempts have been made to utilize other animals but these have not been well received. Attempts to develop true *in vitro* alternatives have been centered on three approaches. The first alternative uses patches of excised animal skin maintained in a glass diffusion cell (Parish, Fd. Chem. Toxic. 23:278, 1985; and Walker et al., Acta Pharm. Suec. 20:52, 1983). The second approach is to use cultured cells and to measure cytotoxicity. (Lamont et al. In Vitro Toxicology: New Directions, Vol. 7, Goldberg (ed.), Mary Ann Liebert, Inc., New York City; Naughton et al. In vitro Toxicology: New Directions, Vol. 7, Goldberg (ed.), Mary Ann Liebert, Inc., New York City).

The third approach uses mathematical SAR models (Free and Wilson, J. Med. Chem. 7:395, 1964; and Goldberg, L., Structure Activity Correlations as a Predictive Tool in Toxicology, Hemisphere Publishing Corp., N.Y., 1983) or physical parameters for prediction effects (Nago et al., Acta Derm. Venereal Stock 52:11, 1972; and Patrick et al., Tox. and Appl. Pharmacol. 81:476, 1985). One physical parameter frequently described as predictive of corrosivity is pH (Potokar et al., Fd. Chem. Toxicol. 23(6):615, 1985). The analysis of pH and acid/alkali reserve was proposed for classification of preparations as corrosive irritant or not classified as dangerous. High or low pH suggests a test sample will be irritant or corrosive but not how irritating. OECD (1981) recognized that test samples with pH <2 or pH >11.5 are so predictably corrosive that they need not be tested for dermal irritation. However, this basis has incorrectly classified and underestimated corrosive potential and appears to provide only broad

guidelines. It only is applicable to test materials which can be called acid or alkali.

All of the foregoing methods for predicting skin corrosion have limitations. The *in vitro* methods require living cells or isolated tissues or are very limited to specific chemicals such as in SAR. While these procedures do provide an alternative to animal testing, they do not achieve the simplicity and standardization one experiences with other standardized tests. The method of the present invention offers such a test. It provides a standard, quick, reproducible, objective measure of the capacity of materials to cause corrosion.

Scientists have utilized dermal biomacromolecules to study potential effects of chemical and formulations on the skin for the last forty years. As early as 1953, Scott and Lyon quantified an increase in exposed sulfhydryl groups of keratin after soaps and detergents were applied to the keratin. This exposure resulted from a separation of keratin chains (Van Scott and Lyon, J. Invest. Dermatol. 21:99, 1953). A relationship between the degree of denaturation or separation, the effects of different soaps and detergents on keratin, and the incidence of *in vivo* dermatitis due to these compounds was observed. Harrold (1959) expanded this work to include investigation of complete formulations on keratin denaturation and separation (J. Invest. Dermatol. 32:581). In 1971, Choman evaluated the swelling response of *in vitro* skin discs prepared from dermal calf collagen (J. Invest. Derm. 40:177). Sodium lauryl sulfate produced a swelling response, and similar responses for a series of anionic and nonionic surfactants were directly related to their skin irritation potential. Further research clearly established swelling of isolated epidermis and synthetic

dermal membranes as a parameter related to irritation. Such swelling is based on adsorption onto and disruption of the three-dimensional keratin protein matrix. Adsorption of the stratum corneum was thoroughly
5 investigated by Imokawa et al., who established a correlation between skin roughness *in vivo* and *in vitro* (J. Am. Oil Chem. Soc. 52:475). This and other studies established adsorption as a major step in the initiation of dermal irritation and as perhaps the most important
10 physicochemical parameter in the dermal toxicity of anionic surfactants.

A second major parameter in the initiation of irritation, integrity of the stratum corneum, has been investigated recently. Pemberton and Oliver used
15 monitoring of electrical resistance in skin slices as a measurement of barrier integrity and as an indicator of the corrosive potential of chemicals (Toxic. In Vitro 2:7, 1988). They showed that corrosive agents have a greater ability than noncorrosives to exert a direct
20 physicochemical lytic action on the stratum corneum. Many chemical toxicants produce changes in the keratin barrier matrix in direct proportion to their adsorption to and interaction with the barrier as studies by Van Scott, Harold, Choman and Imokawa (*supra*). These changes
25 correlate with their potential to produce dermal irritation and corrosion.

Disclosure of the Invention

One embodiment of the invention provides a
30 method for determining the degree of corrosive toxicity of a test substance to human skin or membrane. This method includes first applying the test substance at a first time to a first face of a biobarrier which also has
35 a second face. This biobarrier mimics human skin or membrane to be tested. The biobarrier has its a second

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face in contact with a chemical detection system which includes at least one indicator. The method secondly includes allowing the test substance to transit to the biobarrier's second face and contact the chemical
5 detection system. The method thirdly includes detecting the test substance in the chemical detection system at a second time. The method fourthly includes obtaining the difference between the first and second times, with the time difference being roughly inversely proportional to
10 the corrosive toxicity of the test substance. In another aspect of this invention, the chemical detection system is formulated to detect one of the breakdown products that the corrosive substance liberates in transit through the biobarrier. In another aspect of this invention,
15 after the test substance is applied to the biobarrier, sunlight, or ultra-violet light, is applied to the biobarrier; and the amount of protein liberated from the biobarrier is measured by the chemical, or protein, detection system, as well as any other physical, chemical
20 or optical detection system.

In a second embodiment, the invention provides a method to determine the effect of a test substance on the known detection time of a dermal corrosive substance. As a first step, this method provides combining the test
25 substance with a corrosive substance to obtain a mixture. Second, the method provides applying the mixture at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to be tested, the second face of the biobarrier
30 contacting a chemical detection system comprising at least one indicator. Third, the method provides allowing the mixture to transit to the biobarrier's second face and contact the chemical detection system. Fourth, the
35 method provides detecting the mixture in the chemical detection system at a second time. Fifth, the method

provides obtaining the difference between the first and second times to obtain a detection time for the mixture. And finally the method provides comparing the detection time of the mixture with the known detection time of the known corrosive substance. When the mixture has a longer detection time than the known corrosive substance, then the test substance has an anti-corrosive effect.

There is further provided a method of classifying a test substance as nontoxic or in toxic UN Packing Groups. First, this method provides for applying the substance at a first time to a first face of a biobarrier having a second face. This biobarrier mimics human skin in speed of response to known corrosive substances. The biobarrier also has a second face in contact with a chemical detection system, which includes at least one indicator. Second, the method provides for allowing the test substance to transit the biobarrier to the second face where it contacts the chemical detection system. Third, the method provides for detecting the substance in the chemical detection system at a second time. Fourth, the method provides for obtaining the difference between the first and second times. And fifth, the method provides for correlating this time difference with the times stated in the UN Packing Group designations, and thereby assigning the test substance a designation. In this method, if the time difference is less than 3 minutes, the test substance is assigned to UN Packing Group 1. If the time difference is more than 3 minutes but less than 60 minutes, the substance is assigned to UN Packing Group 2. If the time difference is greater than 60 minutes but not more than four hours, the test substance is assigned to UN Packing Group 3.

Another embodiment of this invention is a device for performing an *in vitro* test to determine the corrosiveness of a test substance to human skin or

membrane. This device has a biobarrier with a first face and a second face. The biobarrier mimics human skin or membrane and has characteristics so as to permit measurement of time for a test substance to traverse
5 therein. This device also has in contact with the second face of the biobarrier a chemical detection system, including at least one indicator, which detects when the test substance has passed through the biobarrier into the chemical detection system. This device further has a
10 means for recording a first time when the test substance is applied to the first face of the biobarrier and for recording a second time when the test substance has traversed the biobarrier and is detected by the chemical detection system. And finally, the device includes a
15 means for reporting the difference between the first and second times.

There is further provided a method of classifying a test substance as nontoxic or in toxic EEC R34 and R35 classes. First, this method provides for
20 applying the substance at a first time to a first face of a biobarrier having a second face. This biobarrier mimics human skin in speed of response to known corrosive substances. The biobarrier also has a second face in contact with a chemical detection system, which includes
25 at least one indicator. Second, the method provides for allowing the test substance to transit the biobarrier to the second face where it contacts the chemical detection system. Third, the method provides for detecting the substance in the chemical detection system at a second
30 time. Fourth, the method provides for obtaining the difference between the first and second times. And fifth, the method provides for correlating this time difference with the times stated in the EEC R34 and R35
35 classes, and thereby assigning the test substance a designation. In this method, if the time difference is

less than 3 minutes, the test substance is assigned to EEC class R35. If the time difference is more than 3 minutes but less than four hours, the test substance is assigned to EEC class R34.

5 There is further provided a method for determining the degree of corrosive toxicity of a substance to skin. This method involves applying the test substance at a first time to a first face of a biobarrier with a second face. The biobarrier has its second face
10 in contact with an electronic detection system, which comprises an indicator. In this method, the test substance transits the biobarrier to affect an indicator which is electronically detected at a second time. The difference in times is inversely proportional to the
15 corrosive toxicity of the test substance.

 In an alternative embodiment, the electronic detection system includes a sensor, or dosimeter. The dosimeter is affected by the test substance or material liberated from the biobarrier by the test substance. The
20 effect on the dosimeter is correlated with the degree of corrosivity.

 There is further provided a method for determining the degree of corrosive toxicity of a substance to skin. This method involves applying the test
25 substance at a first time to a first face of a biobarrier with a second face. The biobarrier has its second face in contact with an mechanical detection system. In one embodiment, the mechanical detection system includes at least one indicator. In this method, the test substance
30 transits the biobarrier to affect the indicator. The mechanical detection system detects the change in the indicator at a second time. The difference in times is inversely proportional to the corrosive toxicity of the
35 test substance.

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In an alternative embodiment, the mechanical detection system comprises a lever which is in contact with the biobarrier. The lever system shifts in response to contact with the test substance which has transited the biobarrier. The amount of shifting of the lever system is correlated to the degree of corrosivity of the test substance.

There is further provided a method for determining the degree of skin corrosive toxicity of a test substance with a test stick. The test substance is applied to the test stick, which includes a series of interspersed corrosive resistant materials and dyes. The stick's surface is a first layer composed of modestly corrosion-resistant material such that the second layer comprising a first dye immediately underlying the first layer is exposed by virtually all corrosive substances. The third layer underlies the second layer and is a more corrosion-resistant material than the first layer. The fourth layer is a second dye immediately under the third layer and is exposed by highly corrosive substances. The fifth layer underlies the fourth layer and is the most corrosion-resistant material of the three corrosion-resistant layers. Under the fifth layer is the sixth layer which is the third dye which is exposed only by extremely corrosive substances. The test substance contacts the test stick for more than 1 second. Then the color of the test stick is observed.

A further embodiment of a test for determining the degree of corrosive toxicity of a test substance utilizes microspheres. The microspheres comprise layers of increasingly corrosion-resistant materials and interspersed dyes, such that the most corrosive substances eat through more layers of the microspheres and expose dyes in or under these layers. The microspheres have a first, surface layer of modestly corrosion-resistant material,

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such that the second layer with a first dye immediately under the first layer is exposed by virtually all corrosive substances. The third layer underlies the second layer and is a more corrosion-resistant material than the first layer, such that the fourth layer (the second dye) immediately under the third layer is exposed by only highly corrosive substances. The fifth layer is under the fourth layer and is the most corrosion-resistant material of the corrosion-resistant layers, such that the sixth layer (the third dye) is exposed only by extremely corrosive substances. The test calls for applying the test substance to the microspheres, with which the test substance is in contact for more than 1 second, and observing the color of the test microspheres.

15 In yet another embodiment, there is provided a method for determining corrosivity by means of contact with microspheres. The microspheres are prepared by applying a layer of corrosion-resistant material of one color to an underlying particle of a second color. Corrosive substances will expose the second color. The test involves contact the microspheres with the test substance for more than one second. Then the color of the microspheres is observed to determine if the test substance exposed the second color. In a related 25 embodiment, the particles are coated with two or more different standard thicknesses of corrosion-resistant material and the underlying particles are two or more different colors; this permits gradation of the corrosivity by the number of colors which are exposed.

30 In another embodiment, the corrosivity of a test substance is measured by applying the test substance to a test stick covered with microspheres. The microspheres have been prepared applying a layer of corrosion-resistant material of one color to an 35 underlying particle of a second color. If the test

substance is corrosive, the second color is exposed. The test proceeds with the test substance in contact with the test stick for more than one second. The color of the test stick where the test substance was applied is
5 observed. In a related embodiment, the test stick is coated with microspheres containing particles coated with two or more different standard thicknesses of corrosion-resistant material and the underlying particles are two or more different colors; this permits gradation of the
10 corrosivity by the number of colors which are exposed.

In another embodiment, there is provided a method for determining the penetration of a test substance. In this method, a test substance is applied at a first time to a penetration barrier, which has a
15 first and a second face. This penetration barrier mimics human skin or other membrane to be tested. The second face of the penetration barrier contacts a detection system. The test substance is then allowed to transit the penetration barrier to the second face, where it
20 contacts the detection system. The detection system detects the test substance at a second time to obtain a detection time, which in turn can be correlated with in vivo penetration times.

In another embodiment, there is provided a
25 method for classifying test substance according to the UN Packing groups with a dry chemical indicator. This dry chemical indicator registers a change in response to exposure to a corrosive test substance which varies with the UN Packing Group. The test substance is exposed to
30 the dry chemical indicator, which is then observed for a change. The change is correlated with the UN Packing Group. The change can be in color or in curvature. In another embodiment, the change in the dry chemical
35 indicator can be correlated with the EEC classes of corrosivity.

Brief Description of the Drawings

Figure 1 illustrates in a cutaway view the biobarrier/chemical detection system of this invention.

Figure 2 illustrates a chemical detection
5 system modified to include electronic detection with a pair of electrodes and display of the test results.

Figure 3 illustrates modification of the chemical detection system to incorporate spectrophotometric observation of color change.

10 Figure 4 illustrates modification of the chemical detector to incorporate an electrode with a chemically reactive coating which is eroded by passage of corrosive substance through the biobarrier.

Figure 5 illustrates a network of conductive
15 traces in the biobarrier which is broken by a corrosive test substance, to reduce current and trigger a detection time.

Figures 6a and 6b illustrates a mechanical detection system in which corrosion triggers movement
20 which breaks an optical connection.

Figure 7 illustrates another mechanical embodiment.

Figure 8 illustrates test microspheres having three different thicknesses of a corrosion-resistant
25 material overlying three different colored particles.

Figure 9 illustrates a dip stick coated with test microspheres.

Figure 10 illustrates a comparison of corrosive substances from three UN Packing Groups and the two EC
30 classes with results of the method of the invention for the same substance.

Modes of Carrying Out the InventionA. Definitions

"Dermal corrosion" is the corrosive toxicity of a substance on the human skin and refers to the ability of the substance to cause alteration or destruction of human skin as measured by ulceration or necrosis. Corrosion is evidenced by cracking, fissures and blisters. Corrosion is a much more severe response than irritation, which is evidenced by erythema and edema.

A "chemical detection system" (CDS) refers to a chemical system that detects a test substance. The chemical detection system can be a liquid or a solid, usually a mixture, which may be clear or colored, and contains an indicator. When a substance is present in the CDS, a change in the CDS indicator occurs. Other chemical detection systems will detect breakdown products of the biobarrier, including protein. Other chemical detection systems contemplated in this invention include high pressure liquid chromatography, gas chromatography, mass spectroscopy, nuclear magnetic resonance and the like.

A "biobarrier" refers to the protein gel matrix to which test samples are applied. The biobarrier has a first face and a second face. The test sample is applied to the first face of the biobarrier, and the substance exits the biobarrier at the second face of the biobarrier. Generally, the biobarrier consists of biomacromolecules found in the skin or biomacromolecules which are structurally related to those found in the skin. The biobarrier includes a base membrane to which is optionally bound at least one protein. The base membrane is composed of non-proteinaceous, film-forming polymer, such as cellulose, nitrocellulose, or other similar material known to those skilled in the art. The protein which may be bound to the base membrane may be

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cross-linked keratin, cross-linked collagen or mixtures thereof. Alternately, the protein may be a synthetic protein or a plant protein capable of forming a gel network.

5 A test substance transits the biobarrier generally by breaking chemical bonds in the biobarrier.

Detection time is defined as the difference between a first time at which the test substance is applied to the biobarrier and a second time when the test
10 substance or other material is detected in the chemical detection system. The longer the detection time, the less corrosive is the test substance. The biobarrier can be designed to give corrosion times that approximate those of *in vivo* tests, or it can be designed to provide
15 a number which must then be corrected to arrive at a corrected detection time. Such an actual or corrected detection time is said to correlate with that of the *in vivo* test. Substances with short detection are more corrosive than those with longer detection times, while
20 those that correlate with *in vivo* corrosion times greater than four hours are considered noncorrosive.

An anti-corrosive substance is one which when combined with a known corrosive agent and exposed to the device of the current invention, has a detection time
25 greater than does the known corrosive agent alone.

B. General Description

B.1. General Parameters of the Testing Procedure

30 One aspect of the present invention provides a method and apparatus for performing an *in vitro* test for dermal corrosiveness of a test substance. The invention provides a biobarrier and chemical detection system
35 (CDS). The test substance is applied to the biobarrier at a first time. If it is corrosive, the test substance

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crosses the biobarrier and is detected by the CDS at a second time. The time it takes the test sample to move through and destroy the biobarrier depends in the degree of corrosivity of the test sample. The biobarrier and
5 CDS are designed to produce a response which is detectable at a time which is roughly inversely proportional to the corrosion of the sample to be tested.

The biobarrier has characteristics to permit transit times which correlate with the time of skin
10 response to known corrosive chemicals. The biobarrier is formed from a standardized mixture of materials which has the advantages and properties of standardized *in vitro* tests using chemical reagents. The biobarrier can be prepared in dishes, wells, discs, trays, etc.

15 In another aspect, the biobarrier is composed of a base membrane and any of a variety of macromolecular networks such as keratin, collagen, keratin/collagen mixtures and synthetic or plant proteins capable of forming gel networks.

20 When the test substance destroys the biobarrier, the test substance enters the CDS where it is detected, for example, by the formation of color and/or precipitate in the CDS. Color or precipitate formation can be assessed using a variety of techniques that are
25 described in detail below.

For example, in one embodiment, the invention provides a solution of chemical indicators which recognizes 14 classes of chemicals, such as oxidizing chemicals, reducing chemicals, alkali and acid. When the
30 test substance interacts with the CDS, a visible or measurable change can be observed or read spectrophotometrically.

35 In another aspect, the invention is directed to a method wherein the CDS provides a protein indicator to

detect the release of destroyed or degraded protein as an endpoint of corrosivity.

In another aspect, the invention is directed to a screening method to predict corrosivity of a test
5 sample where the test sample is applied to the biobarrier and its release can be detected by high pressure liquid chromatography (HPLC), gas chromatography (GC), ultraviolet (UV), and visible light spectrum (VIS) as well as any other physical, chemical, optical, mechanical
10 or electronic detection system. If the chemical is released quickly, it is corrosive. If the chemical takes longer and correlates with *in vivo* corrosion times greater than four hours, it is noncorrosive.

In another variation, a more sophisticated
15 chemical detection system could use HPLC, GC, mass spectroscopy or NMR to detect the test substance or a biobarrier breakdown product which is released at a particular time. The automated electronic or mechanical equipment can be set up to scan the CDS at intervals and
20 record the change, for example, on a strip chart. Then the detection time could be determined.

In another embodiment, the biobarrier/CDS can identify whether a complex mixture has at least one component which is corrosive. Then, identification of
25 the corrosive component can be made chemically. If substances from a complex mixture transit the biobarrier at different times, they can be collected and analyzed. A biobarrier/CDS testing the corrosivity of 1-20 components is contemplated. Standard absorbance readings
30 are made to detect the substance entering the CDS.

In another aspect of the invention, the degree of corrosivity, determined by the test substance's detection time, can be correlated with the UN Packing
35 Group Assignments. If the actual or corrected detection time is not more than three minutes, the test substance

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is classified as Group 1. If the detection time exceeds three minutes but is less than 60 minutes, the test substance is classified as Group 2. If the detection time exceeds 60 minutes but is not more than four hours, the test substance is classified as Group 3. The test substance is classified as noncorrosive if the CDS detects no test substance in four hours. The inventive method can also be used as a noncorrosive/ corrosive screen.

10 In another embodiment, the method quantifies the protein released from the biobarrier. The first detection of protein release from the biobarrier using optics, protein reagents, or fluorescence as an indication of the degree of corrosivity of the test substance.

15 In still another embodiment, the method of the invention can also be used to identify and quantitate the anticorrosivity of a test substance. Anticorrosives protect a tissue, organ, or molecule from the corrosive effects of a known corrosive substance. First, the biobarrier of the system is pretreated with an unknown potentially anticorrosive substance. Then a known corrosive chemical can be applied to the biobarrier, and the detection time is quantified. Then, the detection time for the corrosive chemical alone is compared to the detection time for the test substance and the corrosive chemical. If the detection time for the test substance is greater than that for the corrosive chemical alone, then the test substance is anticorrosive. The difference in detection times indicates the degree of anticorrosivity.

25 In another aspect, the anticorrosivity of a substance can also be determined by premixing a sample containing a potential anticorrosive and a known corrosive and then applying the resulting mixture to the

system. An increase in the detection time produced by the corrosive and anticorrosive with respect to the detection time produced by the corrosive alone is again indicative of the anticorrosivity potential of the anticorrosive. The optimal combination of the anticorrosive and the corrosive can be determined by varying the ratios of the substances.

B.2. The Biobarrier

As shown in Figure 1, the biobarrier 1 is formed from a colored or uncolored pliable network of macromolecules. The biobarrier is chosen to mimic the particular skin or membrane that would be corroded. In assembly, a base membrane 5, which is a semipermeable membrane, is placed between two concentric rings in the bottom of an individual well 15. The biobarrier 1 is placed on or poured onto the base membrane 5. In vial 20, under well 15, the chemical detection system (CDS) 25 is placed so that the CDS 25 is in contact with base membrane 5. In use, the test substance 30 is applied onto the top or first face of biobarrier 1, and the time is noted. If test substance 30 is corrosive, it destroys biobarrier 1 and passes through the biobarrier 1 to the second face of the biobarrier and base membrane 5 into the CDS 25. This change in CDS 25 is either visually observed or recorded by a machine, and a second time is noted. The difference in the two times is roughly inversely proportional to the corrosiveness of the test substance 30.

The biobarrier 1 itself is a combination of a semipermeable base membrane 5 and cross-linked protein. The semipermeable base membrane 5 is composed of non-proteinaceous, film-forming polymer, such as cellulose or nitrocellulose. The protein bound to the membrane is preferably keratin and/or collagen or a combination

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thereof. The protein is preferably crosslinked. The biobarrier can be made from keratin or collagen by using salt solutions as a diluent as well as glycols. Salt solutions include water solutions with 0% up to about 5 1.5% salts. Preferably, about 0.9% saline (sodium chloride) solution is used. Salts can be varied and can include but are not limited to magnesium chloride, potassium chloride, sodium bicarbonate and combinations thereof. Optionally, ethanol and other polar diluents 10 can be used with, or instead of, water. Examples of glycols include but are not limited to ethylene, propylene, hexylene and polypropylene glycol and mixtures thereof. Optionally, a bifunctional crosslinking reagent such as dimethylsuberimide may be used to crosslink the 15 proteins to the cellulose or nitrocellulose membrane. The resulting biobarrier mimics the stratum corneum barrier of the skin. Optionally, enzymes can be added to represent different tissues.

According to the present invention, the 20 biobarrier 1 can also be constructed so as to mimic mucous membrane such as the buccal, vaginal, or penile membrane. In such a form, no keratin or collagen is added to the biobarrier 1. Other skin types that can be mimicked with this system include, but are not limited 25 to, chapped hands, callouses, bunions, plantar's warts, eczema, sun burn and skin rashes of various types.

According to the present invention, the diluent components can be varied to produce biobarriers representing the skin of different animal species, 30 different locations on the animal's body and different ages of the animals. For example, when a higher percentage of diluent is used, there is less protein on the matrix, mimicking the body's more sensitive (less 35 exposed to the elements) skin and younger skin. For example, in older skin, there is less water and lipid,

and that can also be mimicked by adjusting biobarrier ingredients. Also, the ingredients used to make the biobarrier can be adjusted so that the biobarrier mimics facial skin, or other areas of the body, as indicated for
5 cosmetic, emollient or medication applications. With a higher level of glycol(s), the biobarrier is harder, mimicking older skin.

In addition to the above-described synthetic biobarriers 1, animal membranes and/or skin such as snake
10 or pig skin can be used for the biobarrier 1. In this case, it is not necessary to crosslink the keratin protein present because it is already extensively crosslinked naturally.

A lipid component can also be added to a
15 keratin/collagen crosslinked synthetic biobarrier 1 by covalently attaching, coupling, and/or binding fatty acid or cholesterol or acylceramide to the crosslinked biobarrier 1. The amount and kind of lipid in human skin varies widely depending on body location, diet,
20 ethnicity, and other parameters. A lipid component changes the permeability characteristics of the biobarrier 1. Therefore, any lipid should only be added to the basic synthetic membrane when its importance in the skin has been evaluated.

25 Other components such as preservatives (for example, formaldehyde) or stabilizers (for example crosslinking agents) may be added to the biobarrier. These components may affect the time and other biobarrier characteristics may need to be adjusted to accommodate
30 these chemicals.

The biobarrier height can also be adjusted for various conditions and embodiments. For example, the
35 biobarrier height can be calibrated so that UN Packing Group 3 corrosive substances corrode the biobarrier in the same time period as rabbit skin is eroded (60 minutes

to 4 hours). Alternately, the biobarrier height can be varied so that the test can run in different time periods, either more quickly for faster assessment or more slowly for convenient assessment after overnight
5 incubation. Then the detection time is corrected by multiplying by a correction factor, and the corrected time is compared with known times.

When a test substance 30 is evaluated for dermal corrosion by the method of the invention, the
10 substance is applied to the first face of biobarrier 1, and the time is noted. Then, corrosive substance 30 destroys the biobarrier 1 and causes release of portions of the substance 30 and/or protein and/or other
15 components from the biobarrier 1 into the CDS 25. When substance 30 or another material is detected in the CDS 25, a second time is noted. The difference between the first and second times is the detection time. The
greater the corrosion of the biobarrier 1, the faster the release of the test substance 30 into the CDS 25, and the
20 smaller is the detection time.

B.3. The Chemical Detection System (CDS)

In this embodiment, the CDS is provided in the form of a clear aqueous liquid with at least an indicator
25 which detects the presence of the test substance. The CDS is exposed to the test substance after the substance has been placed on the biobarrier and penetrates the permeable membrane.

The indicator is composed of active ingredients
30 which change color or precipitate. Color-changing indicators include, but are not limited to, cresol red and methyl orange. For precipitation, the active
ingredients of the CDS are solutes of colloidal particles
35 which remain in solution until the substance penetrates the biobarrier and enters the solution and produces

precipitation. The CDS can contain a number of different chemical indicators, which are well known to those skilled in the art.

To maintain a compatible pH range and ionic strength, the CDS is provided with ions and buffers which are used to adjust the buffering capacity and ionic strength of the precipitants and/or indicators used. Suitable compounds include but are not limited to phosphate salts, acetate salts, Tris-Cl, borate, and a variety of other compounds known in the art. Preferably, ionic strength can vary over a wide range of about 0.005 M to 0.05 M.

B.4. Electronic Detection System

The CDS detection system described above may be modified to include electronic detection and display of the test results. For example, a pair of electrodes 40 and 42 may be placed in the CDS 25 in vial 20, as shown schematically in Figure 2. A small voltage is applied across electrodes 40 and 42 by a voltage source 44 such as a battery or a transformer. The magnitude of the applied voltage is selected so that no current flows between the electrodes through the CDS in the initial composition of the CDS (i.e., prior to any reaction with the test substance).

The CDS is chosen so that when a quantity of test substance 30 and/or a portion of the biobarrier 1 enters the CDS, a chemical reaction occurs to increase the ion concentrations in the CDS. This increase in ion concentration increases the conductivity of the CDS, thereby permitting current to flow between electrodes 40 and 42, as measured by ammeter 45. A timer 46 determines and displays the detection time, that is, the time between when the test substance first contacts the biobarrier and the time when a current is first detected

by ammeter 45. A separate display 48 such as a CRT or a strip chart may also be provided to show plots of current versus time and/or digital representations of current and time information along with current change rate
5 information. An alternative embodiment includes a dosimeter, or sensor capable of sensing, for example, sodium hydroxide or potassium hydroxide, which are released from the biobarrier. When signals from the dosimeter increase, the biobarrier has been breached by
10 corrosive substance. The timing of the signals or rate of signals can be correlated with the corrosivity of the test substance.

Another modification of the CDS is shown schematically in Figure 3. In this embodiment, a color
15 change in the CDS (as described above) is used to detect the penetration of biobarrier 1 by the test substance. Instead of visual detection of the color change, however, an electronic color detection system is used. Specifically, a light source 50 and photodetector 52 are
20 disposed on opposite sides of transparent portions of vial 20. The amount of light transmitted through the CDS in the vial diminishes as the CDS color changes from transparent to the color of the selected pH indicator dye (e.g., blue or violet). As the amount of light received
25 by photodetector 52 diminishes, the voltage generated by photodetector 52 decreases, as measured by voltmeter 54. Once again, this embodiment has a timer 46 and a display 48 to provide information on detection time, or elapsed time between initial contact of the test substance with
30 the biobarrier and the later contact of the test substance with the CDS. This embodiment also can be adjusted to provide the rate of change of the CDS color.

Figure 4 shows yet another alternative to the
35 CDS detector described above. In this embodiment, vial 20 contains an electrically conductive solution such as

saline. Electrodes 60 and 62 are disposed in the CDS solution, as before. In place of the chemically reactive portions of the CDS, however, is a chemically reactive coating 61 on one of the electrodes, such as a substance
5 that will enzymatically produce ions for charge transfer from the saline to the electrode when contacted by the test substance. A timer 46 and display 48 are provided, as in the other embodiments.

Figure 5 shows an embodiment that does not rely
10 on the chemical interaction of the test substance with a CDS. The biobarrier 1 of this embodiment has a network of conductive traces 70 formed on the back surface of the biobarrier 1 and electrically interconnected to junctions 72 formed on opposite sides of the membrane. During the
15 test, a current source 71 applies current from junction 72 and to junction 74. A voltmeter 76 measures the voltage drop between the junctions. As the test substance corrodes the biobarrier, one or more conductive traces will be broken, thereby raising the electrical
20 resistance measured between junctions 72 and 74. A timer 46 and display 48 may be used to measure and display the elapsed time for the corrosive activity and the relationship between time and voltage rise.

25 B.5. Mechanical Detection System

Figures 6(a) and 6(b) show a mechanical detection embodiment of this invention. In this embodiment, biobarrier 1 is suspended between two gripping arms 80 and 81. Arms 80 and 81 are connected at
30 a pivot point 82, and a pair of springs 83 and 84 are disposed to move the arms apart in order to provide tension across biobarrier 1. A motion detector such as light sources 85 and photodetectors 86 are placed to
35 detect movement of either arm. In use, corrosion of the biobarrier by the test substance causes the biobarrier to

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weaken and stretch, thereby permitting arms 80 and 81 to move apart. Movement of either arm breaks the optical connection between a light source 85 and its respective photodetector 86, thereby sending a signal to system
5 timer 46 via a logic unit 88. Detection time may be read from display 48.

Figure 7 shows another mechanical embodiment of this invention. In this embodiment, a single arm 90 provides downward tension on biobarrier 1. A light
10 source/photodetector arrangement detects motion by arm 90 (only the photodetector 92 is shown in the drawing). In use, corrosion of the biobarrier by the test substance will cause the biobarrier to weaken and stretch, thereby permitting arm 90 to move downward toward the biobarrier.
15 This motion breaks the optical connection between the light source and the photodetector (as detected by logic unit 94) to stop the elapsed time clock 46. Detection time, or elapsed time, may be displayed on display 48.

In another embodiment of the mechanical
20 detection system, one arm of a lever system is in contact with the lower surface of the biobarrier. This arm of the lever shifts in response to contact with the test substance which has transited the biobarrier. The amount of shifting of the lever system can be correlated with
25 the degree of corrosivity.

B.6. Test Microspheres

In another embodiment, shown in Figure 8, there are provided test microspheres. The test microspheres
30 are composed of an outer, corrosion-resistant biobarrier of one color which coats an underlying particle of another color. The biobarrier has a standard thickness for reproducibility and correlation with other corrosion
35 test methods. When the microspheres are in contact with a corrosive test substance for at least one second, the

underlying color is exposed and is detectable. This color change is not dependent on the corrosive substance reacting to produce a color change. Therefore, this method is considered more universally applicable.

5 In another embodiment, the microspheres are prepared with multiple standard thicknesses of the biobarrier. The underlying particle has different colors depending on the overlying biobarrier thickness. The microspheres are exposed to the test substance for a
10 standard period of time, at least one second. Non-corrosive substances will not erode the biobarrier, so only the biobarrier color will be visible. Somewhat corrosive substances will only erode the thinnest layer of the biobarrier, revealing only one underlying color.
15 Increasingly corrosive substances erode the thicker biobarrier layers, revealing two or more underlying colors. Thus, the degree of corrosivity can be determined and correlated with other corrosivity tests. Optionally, the test substance is removed (for example,
20 rinsed off) after a standard time period and before observing for color. In lieu of visible colors, fluorescent dyes can be incorporated into the outer or underlying layers of the microspheres. Increases or decreases in fluorescence can be correlated with
25 corrosivity. Alternately, the microspheres can be designed so that a gas is liberated as the surface of the microspheres is eroded by corrosive substances, and the rate of gas formation can be correlated with corrosivity. In yet another embodiment, the outer layer of the
30 microspheres comprises magnetically attractive materials. As the outer layer is corroded, fewer of the microspheres are magnetically attracted; the proportion of microspheres magnetically attracted after a standard
35 interval can be inversely correlated with corrosivity.

B.7. Test Dipstick

In another embodiment, shown in Figure 9, a paper or wooden dipstick can be coated with microspheres having at least one standard thickness of biobarrier of a particular color. When the dipstick is exposed to a corrosive test substance, the biobarrier erodes off the microspheres revealing an underlying particle of other color(s), fluorescence, etc.

In an alternate method, the dipstick can be coated with alternating layers of corrosion-resistant materials and dyes. Thus, highly corrosive substances corrode off more layers and expose an underlying color denoting highly corrosive substances. The corrosion-resistant layers also can incorporate the various dyes.

B.8. The Method

A major virtue of the method of this invention is its simplicity. The crux of the procedure is the application of the substance to be tested to a synthetic or natural biobarrier and exposure of the biobarrier to a chemical detection system which is sensitive to many chemicals. The time to produce a change in the CDS can be read quantitatively by direct measurement or qualitatively by eye.

B.9. Evaluation of the Results

As set forth above, it is desirable to have a direct correlation between the results of the test as performed by the method of the invention and the capacity of a substance to corrode the human skin. However, results of human experience for a large number of corrosives for which testing is desired is not available. Therefore, a threshold criterion for predictive validity of the testing procedures of the invention is correlation

of its results with those of *in vivo* dermal corrosivity testing of the same substances.

Accordingly, the time curves shown in Figure 10 have been prepared to show the relationship between the detection time obtained using the biobarrier/CDS of the present invention. The tables state the concordance between the inventive method and prior test methods. Concordance of "1" is reported when there is perfect agreement in the classification of corrosivity. When the inventive method and the prior test method disagree by one classification level or more, concordance of "0" is reported.

C. Examples

The invention has been disclosed by direct description. The following are examples showing the efficacy of the method on many chemicals. The examples are only examples and should not be taken in any way as limiting to the scope of the method or device.

C.1. Protocol for Preparing the Biobarrier

To make a Biobarrier:

First, mix the following:

10.0 g Porcine Dermagelatin 300 bloom
(Sigma Chemical Co., St. Louis, MO)
0.9 g Sodium Chloride (NaCl)
50.0 ml Deionized H₂O

Second, heat to 66° or until the gelatin is dissolved. Use a thermometer to be certain that the temperature does not exceed approximately 70° C.

After the biobarrier protein solution reaches the appropriate temperature and becomes clear, add 50 ml of ethylene glycol. When the ethylene glycol is thoroughly mixed, remove the mixture from the heat.

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Immediately begin skimming the air bubbles and foam off the top of the gelatin with a spoon.

The gelatin is now ready to be pipetted onto cellulose base membrane discs in the bottom of wells that
5 measure 3 cm in height and 1 cm in diameter. Add 200 μ l per disc base membrane. Working time is approximately 30 minutes.

The biobarrier wells are packaged airtight and stored at 4°C until ready to use.

10 Note: The procedure must be carefully followed to ensure consistent assay results. In particular, do not boil the gelatin, or it will "skin-up" upon cooling. If it skins up, do not use it. As much as 80 ml of ethylene glycol may be used; however, the biobarrier will
15 need to be retested for whether it still reflects the corrosivity of human skin. Watch for air bubbles when injecting the gel into the discs.

C.2. Protocol for Chemical Detection System:

20 Preparation of indicator:

Indicator 1. Methyl Orange:

1) Add 50.0 mg of methyl orange solid reagent to 50.0 ml deionized water in a tapered cell culture centrifuge tube.

25 2) Shake well. This is a 0.1% solution.

Indicator 2. Cresol Red:

1) Add 50.0 mg of cresol red to 50.0 ml of deionized water.

2) Shake solution well. Some greenish
30 particles usually remain. To remove the particles, pour the solution onto analytical grade filter paper in a funnel, and transfer the filtered solution to another test tube.

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Chemical Detection System Preparation:

1) To 100.0 ml deionized water, add 1 ml of 0.1% methyl orange and 2.0 ml of 0.1% cresol red solutions. The final concentrations should be 0.001% methyl orange and 0.002% cresol red. Alternately, the same volumes of each indicator solution may be used.

2) Shake well. Label bottle with date, contents (CDS solution) and initials.

10 C.2. Results

Into each vial, 22 ml of the CDS solution was placed so that it was in contact with the lower surface of the biobarrier/base membrane. Then 500 μ l or 500 mg of the test substance was applied to the upper surface of the biobarrier. The time at which the test substance was applied was recorded. The test system was visually observed for a change in color, upon which a second time was recorded. The two times were subtracted, and the detection time was recorded. Over 300 chemicals and formulations have been analyzed to date. Corrosive classifications were assigned based on Table I. *In vivo* classifications were obtained from industrial laboratories or the Federal Hazardous Material Table (Imokawa, Cont. Derm. 5: 357, 1978).

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Table I. *In Vitro* Results

<u>Time (min)</u>	<u>Abbreviation</u>	<u>Classification</u>
0-3	I	Corrosive Packing Group I
3-60	II	Corrosive Packing Group II
60-240	III	Corrosive Packing Group III
> 240	NC	Noncorrosive

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Tables II-V list the results of the inventive method for the various chemicals, their *in vivo* classification as listed in Imokawa (Cont. Derm. 5: 357, 1978) or supplied by chemical manufacturers. As indicated, some of the test results were repeated and are shown either on the line below the earlier test results or in parenthesis after the earlier test results. When *in vivo* tests were repeated, the tests were performed according to the D.O.T. protocol (49 C.F.R. § 173.136).

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Table II. Test Packing Group I

CORROSITEX Results						
<u>Chemical</u>	<u>Conc.</u>	pH of <u>10%</u>	Time (h:m:s)	Class	<u>In Vivo</u>	Concordance
					DOT Results	
Nitric Acid	90%	0	0:00:34	I	I	1
Selenic Acid	95%	0	0:01:41	I	I	1
Fluorosulfonic Acid	Pure	0	0:00:13	I	I	1
Trifluoroacetic Acid	99%	0.75	0:05:25	II	I	0

Table III. Test Packing Group II
CORROSITEX Results

Chemical	Conc.	pH of 10%	Time (h:m:s)	Class	In Vivo	
					DOT Results	Concordance
Dimethylcyclohexyl-amine (2d test)	99+	11.79	0:35:00	II	II	1
Dichloroacetic acid	99+	0.64	1:05:00	III	II	0
Dichloroacetyl chloride	99+	0.46	0:08:00	II	II	1
			0:05:00	II	II	1
Chloroacetic acid	99+	1.44	0:08:30	II	II	1
Bromoacetic acid	99+	1.41	0:09:30	II	II	1
Cyclohexylamine	99%	12.34	0:47:00	II	II	1
NaOH, solid	pellets	13.81	0:12:00	II	II	1
Ethylenediamine	neat	12.13	0:13:00	II	II	1
Lithium hydroxide, monohydrate	98%	11.8	0:20:00	II	II	1
Potassium hydrogen sulfate	35-37%	0.85	0:21:00	II	II	1
Ammonium hydrogen sulfate	neat	0.78	0:13:00	II	II	1
Glacial acetic acid	99+	2.3	0:35:00	II	II	1
Aluminum chloride	pure	2.92	0:16:30	II	II	1
Acetic anhydride	pure	1.99	0:47:00	II	II	1
Trichloroacetic acid	99+	0.74	0:11:00	II	II	1

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Table III (continued)

	35	30	25	20	15	10	5	
Antimony tribromide			99%	0.35	0:22:00	II	II	1
Hydrobenzene			65w+%	0.55	0:13:00	II	II	1
sulfonic acid								
Sulfuric acid			99.99%	0	0:01:30	I	II (I)	0
Sulfurous acid			neat	1.78	0:18:00	II	II	1
Dimethylcarbaryl			98%	2.31	0:17:00	II	II	1
chloride								
Boron trifluoride-			98%	0.95	0:03:00	I	II	0
acetic acid complex (in vitro repeat)					0:03:34	II		1
Ortho-anisoyl			97%	0.72	0:10:20	II	II	1
chloride								
Diethylene triamine			99%	12.01	0:34:00	II	II	1
Dodecyl trichloro-			98%	0.5	0:11:35	II	II	1
silane								
Fluoboric acid			48w+%	1.3	0:02:45	I	II (III)	0
Valeryl chloride			98%	0.45	0:10:40	II	II	1
Tetramethylammonium			99%	13.61	0:11:30	II	II	1
hydroxide pentahydrate								
Formic acid			96%	1.55	0:06:30	II	II	1
Hydrogen bromide			48%	0.3	0:02:47	I	II	0
Thiophosphoryl			98%	5.81	0:10:08	II	II	1
chloride								

Table III (continued)				
	98%	0	0:07:00	II
Phenyl trichlorosi-lane	98%	0	0:07:00	II
Antimony trichloride	99.99%	0.3	0:04:15	II
Mercaptoacetic acid	97%	0.3	0:11:37	II
Acrylic acid	99%		0:29:00	II
Dichlorophenyl-phosphine	97%		0:02:00	I
Phosphoric acid	85%	0.85	0:15:00	II
Propionic acid	99+%	2.68	0:41:00	II
Ferric chloride (repeat)	98%	3.00	0:23:00	III
2,2 amino ethoxyl ethanol (repeat)	98%	11.30	0:21:18	II
			0:31:00	II

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Table IV. Test Packing Group III

CORROSITEX Results					
<u>Chemical</u>	<u>Conc.</u>	<u>pH of 10%</u>	<u>Time (h:m:s)</u>	<u>Class</u>	<u>In Vivo DOT Results</u>
Dicyclohexylamine	99%	9.57	3:30:00	III	III 1
Tetraethylene-	neat	11.85	0:56:20	II	III 0
pentamine (repeat)			1:01:00	III	1
Copper(II) chloride	97%	2.99	0:42:00	II	III 0
(repeat)			0:42:41		
Ethylhexylamine	98%	11.98	2:45:00	III	III 1
Benzene sulfonyl	neat	1.80	3:30:00	III	III 1
chloride					
Hydroxylamine	97+%	3.58	3:30:00	III	III 1
sulfate					
Crotonic acid	99+%	2.30	1:22:25	III	III 1
Butyric anhydride	99.2%	3.08	2:30:00	III	III 1
Hexanoic acid	99.5%	3.00	2:29:00	III	III 1
Pyridine	100	9.2	2:09:40	III	Not grouped 1

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Table V. CORROSITEX Noncorrosives

	Chemical	Conc.	pH of 10%	CORROSITEX Results			Concordance
				Time (hours)	Class	In Vivo DOT Results	
25	Acid dithiodipropionic	100		>4	NC	NC	1
30	Acrylate methoxy ethyl	100		>4	NC	NC	1
	Benzyl toluene	100		>4	NC	NC	1
	1813	100	10.2	>4	NC	NC	1
	SK 602	100	9.8	>4	NC	NC	1
	1807	100	10.3	>4	NC	NC	1
	2615	100	10.0	>4	NC	NC	1
	Butoxyethylacetate	100	3.9	>4	NC	NC	1
	2619	100	10.0	>4	NC	NC	1
	1816	100	9.9	>4	NC	NC	1

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In Table VI the DOT *in vivo* classifications are compared to the classifications obtained by the inventive method and another *in vitro* classification based on pH and acid alkali reserve (Oliver et al., Toxic. in Vitro. 2:7, 1988). The inventive method seems to more accurately assign substances with primarily acid/alkali effects than does a physical chemistry system based on pH determination and analysis of acid alkali reserve. Three substances were underestimated based on assignments using pH and acid/alkali reserve.

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Table VI
Comparison of CORROSITEX, In Vitro Results,
and Predictions Based on pH and Acid/Alkali Reserve

Chemical	CORROSITEX Grouping	pH	DOT Group	Time (h:m:s)	Assignment Based on pH and Acid/Alkali (24)
HoAc 25%	II	1.9	II	00:31:28	C
HoAc 10%	II	2.3	II	00:40:10	NC
NaOH 5%	II	13.7	II	00:21:20	C
NaOH 1%	II	13.3	II	00:33:04	NC
KOH 5%	II	13.9	II	00:18:51	C
KOH 1%	II	13.1	II	00:41:21	NC
HCl 1N	II	0.4	II	00:12:30	C

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C.3. Results of Anti-Corrosive Study

In one protocol, 500 μ l or 500 mg of a test sample was applied to the biobarrier described above. The time to produce a change in the CDS was recorded.

5 The results were categorized according to Table VII.

A second experiment was conducted as discussed above to determine the amount of anti-corrosive necessary to reduce the corrosivity of a test sample. The results are reported in Table VII.

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TABLE VII. Anti-Corrosive Test Results

	<u>Time</u>	<u>Class</u>
Corrosive	00:02:37	I
Anti-Corrosive	> 4 h	NC
15 50/50 Corrosive /	00:15:41	II
Anti-Corrosive		

20 In summary, the invention provides a convenient, inexpensive screening procedure to obtain preliminary data with respect to dermal corrosion potential of a substance. Results obtained were comparable to those obtained from the procedures involving whole animals.

25 This invention has been detailed both by example and by direct description. It should be apparent that one having ordinary skill in this art would be able to surmise equivalents to the invention as described in the claims which follow but which would be within the spirit of the description above. Those equivalents are
30 to be included within the scope of this invention.

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Claims

1. A method for determining the degree of corrosive toxicity of a test substance to human skin or
5 membrane which method comprises:

(a) applying the test substance at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to be tested, which biobarrier has its second
10 face in contact with a chemical detection system, the chemical detection system comprising at least one indicator;

(b) allowing the test substance to transit the biobarrier to the second face to contact the
15 chemical detection system;

(c) detecting the test substance in the chemical detection system at a second time; and

(d) obtaining the difference in the first and second times, whereby the time difference is roughly
20 inversely proportional to the corrosive toxicity of the test substance.

2. The method of claim 1 wherein the biobarrier comprises a base membrane to which is bound at
25 least one protein.

3. The method of claim 2 wherein the base membrane is cellulose or nitrocellulose.

30 4. The method of claim 2 wherein the protein is selected from cross-linked keratin, cross-linked collagen, mixtures of cross-linked keratin and collagen, or synthetic or plant proteins capable of forming gel
35 networks.

5. The method of claim 2 wherein the protein is mixed with a diluent before adding the protein to the base membrane.

5 6. The method of claim 5 wherein the diluent comprises a more polar solvent.

7. The method of claim 6 wherein the polar solvent consists of water, a salt solution or an alcohol.
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8. The method of claim 5 wherein the diluent comprises a less polar solvent.

9. The method of claim 8 wherein the less
15 polar solvent is selected from the group consisting of ethylene glycol, propylene glycol, hexylene glycol, polypropylene glycol and mixtures thereof.

10. The method of claim 1 wherein the chemical
20 detection system comprises at least one indicator and an aqueous medium of pH and ionic strength compatible with the biobarrier.

11. The method of claim 10 wherein the
25 indicator comprises a composition which interacts with the test substance to form a precipitant.

12. The method of claim 10 wherein the
indicator comprises a composition which interacts with
30 the test substance to form a colored product.

13. The method of claim 1 wherein the chemical
detection system is high pressure liquid chromatography,
35 gas chromatography, mass spectroscopy or nuclear magnetic resonance.

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14. A method to determine the effect of a test substance on the known detection time of a dermal corrosive material, which method comprises:

5 (a) combining the test substance with the corrosive material to obtain a mixture;

(b) applying the mixture at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to be tested, the second face of the biobarrier contacting a
10 chemical detection system comprising at least one indicator;

(c) allowing the mixture to transit the biobarrier to the second face to contact the chemical detection system;

15 (d) detecting the mixture in the chemical detection system at a second time;

(e) obtaining the difference in the first and second times to obtain a detection time for the mixture; and

20 (f) comparing the detection time of the mixture with the known detection time of the known corrosive material, wherein a longer detection time for the mixture indicates that the test substance has an anti-corrosive effect.

25

15. A method for determining the corrosive toxicity of a test substance to human skin or membrane, which method comprises:

(a) applying the test substance at a
30 first time to a first face of a biobarrier having a second face, the biobarrier mimicking human skin or membrane to be tested, the biobarrier comprising at least one component which is broken down by a skin corrosive
35 chemical to form a breakdown product, the biobarrier

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being in contact at its second face with a breakdown product detection system;

(b) allowing the test substance to react in transit with the biobarrier component to produce at least one breakdown product;

(c) allowing the breakdown product to transit to the second face of the biobarrier and to contact the breakdown product detection system;

(d) detecting the breakdown product in the breakdown product detection system at a second time;

(e) obtaining the difference in the first and second times, whereby the time difference is roughly inversely proportional to the corrosive toxicity of the test substance.

15

16. A method for determining the dermal corrosivity of a test substance to human skin or membrane in sunlight, which method comprises:

(a) applying the test substance at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to be tested and comprises at least one protein, the second face of the biobarrier being in contact with a protein detection system, the protein detection system comprising at least one indicator;

(b) exposing the treated biobarrier to ultra-violet light;

(c) allowing the test substance to react in transit with the biobarrier protein to release the protein;

(d) allowing the released protein to transit to the second face of the biobarrier and to contact the protein detection system;

(e) detecting the released protein in the protein detection system at a second time; and

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(f) obtaining the difference in the first and second times, whereby the time difference is roughly inversely proportional to the corrosive toxicity of the test substance exposed to sunlight.

5

17. A method of classifying a test substance as nontoxic or in toxic UN Packing Groups, the method comprising

(a) applying the substance at a first
10 time to a first face of a biobarrier having a second face, which biobarrier mimics human skin in speed of response to known corrosive substances, the biobarrier having a second face in contact with a chemical detection system, the chemical detection system comprising at least
15 one indicator;

(b) allowing the test substance to transit the biobarrier to the second face to contact the chemical detection system;

(c) detecting the substance in the
20 chemical detection system at a second time;

(d) obtaining the difference in the first and second times; and

(e) correlating the time difference with the times stated in the UN Packing Group designations,
25 and thereby assigning the designations.

18. The method of claim 17 wherein if the difference of (d) is less than 3 minutes, the UN Packing Group is group 1.

30

19. The method of claim 17 wherein if the difference of (d) is more than 3 minutes but less than 60 minutes, the UN Packing Group is group 2.

35

20. The method of claim 17 wherein if the difference of (d) is greater than 60 minutes but not more than four hours, the UN Packing Group is group 3.

5 21. A device for performing an *in vitro* test to determine the corrosiveness of a test substance to human skin or membrane, comprising

 (a) a biobarrier having a first face and a second face, the biobarrier mimicking human skin or
10 membrane, the biobarrier having characteristics so as to permit measurement of time for a test substance to traverse therein;

 (b) a chemical detection system comprising at least one indicator, the chemical detection system
15 contacting the second face of the biobarrier; and

 (c) a means for recording a first time when the test substance is applied to the first face of the biobarrier and for recording a second time when the test substance has traversed the biobarrier and is detected by
20 the chemical detection system; and

 (d) a means for reporting the difference between the first and the second times.

22. The device of claim 21 wherein the
25 characteristics of the biobarrier comprise thickness.

23. A method of classifying a test substance as nontoxic or in toxic EEC R34 and R35 classes, the method comprising

30 (a) applying the substance at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin in speed of response to known corrosive substances, the biobarrier having the
35 second face in contact with a chemical detection system,

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the chemical detection system comprising at least one indicator;

(b) allowing the test substance to transit the biobarrier to the second face to contact the chemical
5 detection system;

(c) detecting the substance in the chemical detection system at a second time;

(d) obtaining the difference in the first and second times;

10 (e) correlating the time difference with the times stated for the EEC R34 and R35 classes; and

(f) assigning the EEC class.

24. The method of claim 23 wherein if the time
15 difference of (d) is less than three minutes, the EEC designation is class R35.

25. The method of claim 23 wherein if the time difference of (d) between three minutes and four hours,
20 the EEC designation is class R34.

26. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane which method comprises:

25 (a) applying the test substance at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to be tested, which biobarrier has its second face in contact with an electronic detection system, the
30 electronic detection system comprising at least one indicator;

(b) allowing the test substance to transit the biobarrier to the second face to contact the electronic
35 detection system and affect an indicator;

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(c) detecting the change in the indicator with the electronic detection system at a second time; and

(d) obtaining the difference in the first and second times, whereby the time difference is roughly
5 inversely proportional to the corrosive toxicity of the test substance.

27. The method of claim 26 wherein the electronic detection system comprises a dosimeter, which
10 dosimeter is affected by the test substance or material liberated from the biobarrier by the test substance, the effect on the dosimeter being capable of correlation with the degree of toxicity.

15 28. The method of claim 26 wherein the electronic detection system comprises a liquid with two electrodes, the potential between which changes when sufficient test substance and/or biobarrier enters the liquid, at which the second time is recorded.

20 29. The method of claim 26 wherein the electronic detection system comprises a screen on which a constant electrical potential is maintained, until sufficient test substance and/or biobarrier contacts the
25 screen to change the electrical potential, at which the second time is recorded.

30 30. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane which method comprises:

(a) applying the test substance at a first time to a first face of a biobarrier having a second face, which biobarrier mimics human skin or membrane to
35 be tested, which biobarrier has its second face in contact with a mechanical detection system, the

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mechanical detection system comprising at least one indicator;

(b) allowing the test substance to transit the biobarrier to the second face to contact the mechanical
5 detection system;

(c) detecting the test substance in the mechanical detection system at a second time; and

(d) obtaining the difference in the first and second times, whereby the time difference is inversely
10 proportional to the corrosive toxicity of the test substance.

31. The method of claim 30 wherein the mechanical detection system comprises a surface which is
15 immediately under the biobarrier wherein the surface is part of a sensitive weight balance, which registers a weight change when sufficient test substance and/or biobarrier contacts the surface.

20 32. The method of claim 30 wherein the mechanical detection system indicator comprises a light beam which is immediately under the biobarrier, wherein the light beam is broken when sufficient test substance and/or biobarrier transits the biobarrier.

25 33. The method of claim 30 wherein the mechanical detection system comprises a lever system, the lever system in contact with the second face of the biobarrier, the lever system shifting in response to
30 contact with the test substance transiting the biobarrier, the shifting of the lever system being correlated with the degree of corrosive toxicity of the test substance.

35

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34. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane, which method comprises:

- (a) applying the test substance to test
5 microspheres, the microspheres having been prepared by applying a layer of corrosion-resistant material of one color to an underlying particle of a second color, such that if the microspheres are exposed to a sufficiently corrosive substance, the second color is exposed;
- 10 (b) leaving the test substance in contact with the microspheres for a time greater than 1 second; and
- (c) observing the color of the contacted
microspheres;

whereby the test substance is determined to be
15 corrosive if it exposes the second color.

35. The method of claim 34, wherein step (c)
is preceded by the step of removing the test substance
from the microspheres.

20

36. The method of claim 34, wherein the
microspheres have been prepared with two or more
different standard thicknesses of corrosion-resistant
material and the underlying particles are two or more
25 different colors, whereby the corrosivity of the test
substance is correlated with the number of colors
exposed.

37. The method of claim 36, wherein the test
30 substance exposes only one color under the corrosion-
resistant material, whereby the test substance is
determined to be less corrosive.

35

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38. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane which method comprises:

- 5 (a) applying the test substance to a test stick which comprises one color, the stick having been prepared by applying the test microspheres to the stick, the microspheres having been prepared by applying a layer of corrosion-resistant material of one color to an underlying particle of a second color, such that if the
10 microspheres are exposed to a sufficiently corrosive substance, the second color is exposed;
- (b) leaving the test substance in contact with the test stick for more than 1 seconds; and
- 15 (c) observing the color of the microspheres on the test stick where the test substance had been applied.

39. The method of claim 38 wherein step (c) is preceded by the additional step of removing the test substance from the test stick.

20

40. The method of claim 38 wherein the test microspheres of step (a) have at least two standard thickness of corrosion-resistant material overlying particles having at least two different colors, such that
25 the degree of corrosivity correlates with the number of colors which are exposed.

41. A method for determining the penetration of a test substance, the method comprising the steps of

- 30 (a) applying the test substance at a first time to a penetration barrier, the penetration barrier having a first and a second face, which penetration barrier mimics human skin or membrane to be tested, which
35 penetration barrier has its second face in contact with a detection system;

(b) allowing the test substance to transit the penetration barrier to the second face to contact the detection system;

(c) detecting the test substance with the
5 detection system at a second time, to obtain a detection time; and

(d) correlating the detection time with *in vivo* penetration times.

10 42. The method of claim 41 wherein the test substance which transits the penetration barrier to the second face is removed at timed intervals for detection and quantitation, and wherein the quantities of test substance per time are correlated with *in vivo*
15 penetration.

43. A method of classifying the corrosiveness of a test substance according to UN Packing Groups, the method comprising

20 (a) providing a dry chemical indicator which registers a change in response to exposure to a corrosive test substance, the change varying with the UN Packing Group;

(b) exposing the test substance to the dry
25 chemical indicator;

(c) observing the change in the chemical indicator; and

(d) correlating the change with the UN Packing Group.

30

44. The method of claim 43 wherein the dry chemical indicator registers changes in color which correlate with different UN Packing Groups.

35

45. The method of claim 43 wherein the dry chemical indicator registers a change in curvature which is correlated with the UN Packing Group.

5 46. The method of claim 43 wherein the dry chemical indicator registers a change in curvature correlated with the EEC class.

10 47. A method of classifying the corrosiveness of a test substance according to EEC R34 and R35 classes, the method comprising

- (a) providing a dry chemical indicator which registers a change in response to a corrosive test substance, the change varying with the EEC class;
- 15 (b) exposing the test substance to the dry chemical indicator;
- (c) observing the change in the dry chemical indicator; and
- (d) correlating the change with EEC R34 or R35
- 20 class.

25 48. The method of claim 47 wherein the dry chemical indicator registers changes in color corresponding to different EEC classes.

 49. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane which method comprises the steps of

- (a) applying the test substance to a test
- 30 stick, the stick having been prepared by applying a series of layers to an underlying material,
- the layers comprising both corrosive-resistant materials and dyes interspersed, the surface of
- 35 the test stick comprising a first layer, the first layer comprising a modestly corrosive-resistant material such

that a second layer comprising a first dye immediately under the first layer is exposed by virtually all corrosive substances, a third layer underlying the second layer and comprising a more corrosive-resistant material than the first layer, such that a fourth layer, comprising a second dye immediately under the third layer is exposed by only more corrosive substances, a fifth layer underlying the fourth layer and comprising the most corrosive resistant substance of the three corrosive-resistant layers, such that a sixth layer, comprising a third dye immediately under the fifth layer is exposed by highly corrosive substances;

(b) leaving the test substance in contact with the test stick for more than 1 second;

(c) observing the color of the test stick where the test substance was applied; and

(d) correlating the color of the test stick with the degree of corrosivity of the test substance.

50. The method of claim 49, wherein the test substance is removed from the test stick before the color of the test stick is observed.

51. The method of claim 49, wherein the dye layers and corrosive-resistant layers are combined, such that the first dye is incorporated into the third layer, the second dye is incorporated into the fifth layer and the third dye is incorporated into the underlying material of the test stick.

52. A method for determining the degree of corrosive toxicity of a test substance to human skin or membrane, which method comprises the steps of

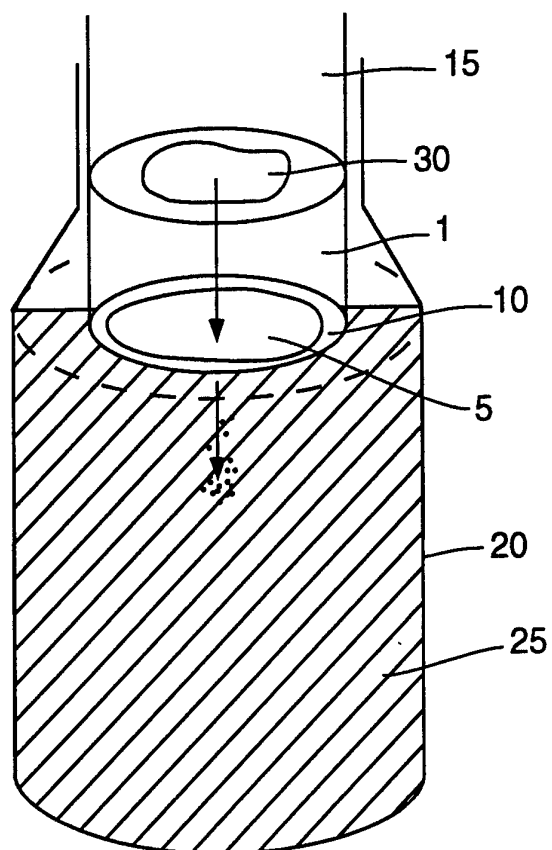
-57-

- (a) applying the test substance to test microspheres, the microspheres having been prepared by applying a series of layers to underlying particles, the layers comprising both corrosive-resistant materials and dyes interspersed such that a first layer, or surface layer comprises a modestly corrosive-resistant material such that the second layer comprising a first dye immediately underlying the first layer is exposed by virtually all corrosive substances, the third layer underlying the second layer and comprising a more corrosive-resistant material than the first layer, such that the fourth layer, comprising a second dye immediately under the third layer is exposed by only more corrosive substances, the fifth layer underlying the fourth layer and comprising the most corrosive-resistant material of the three corrosive-resistant layers, such that the sixth layer, comprising a third dye immediately under the fifth layer, is exposed only by highly corrosive substances;
- (b) letting the test substance contact the microspheres for more than 1 second; and
- (c) observing the color of the microspheres.

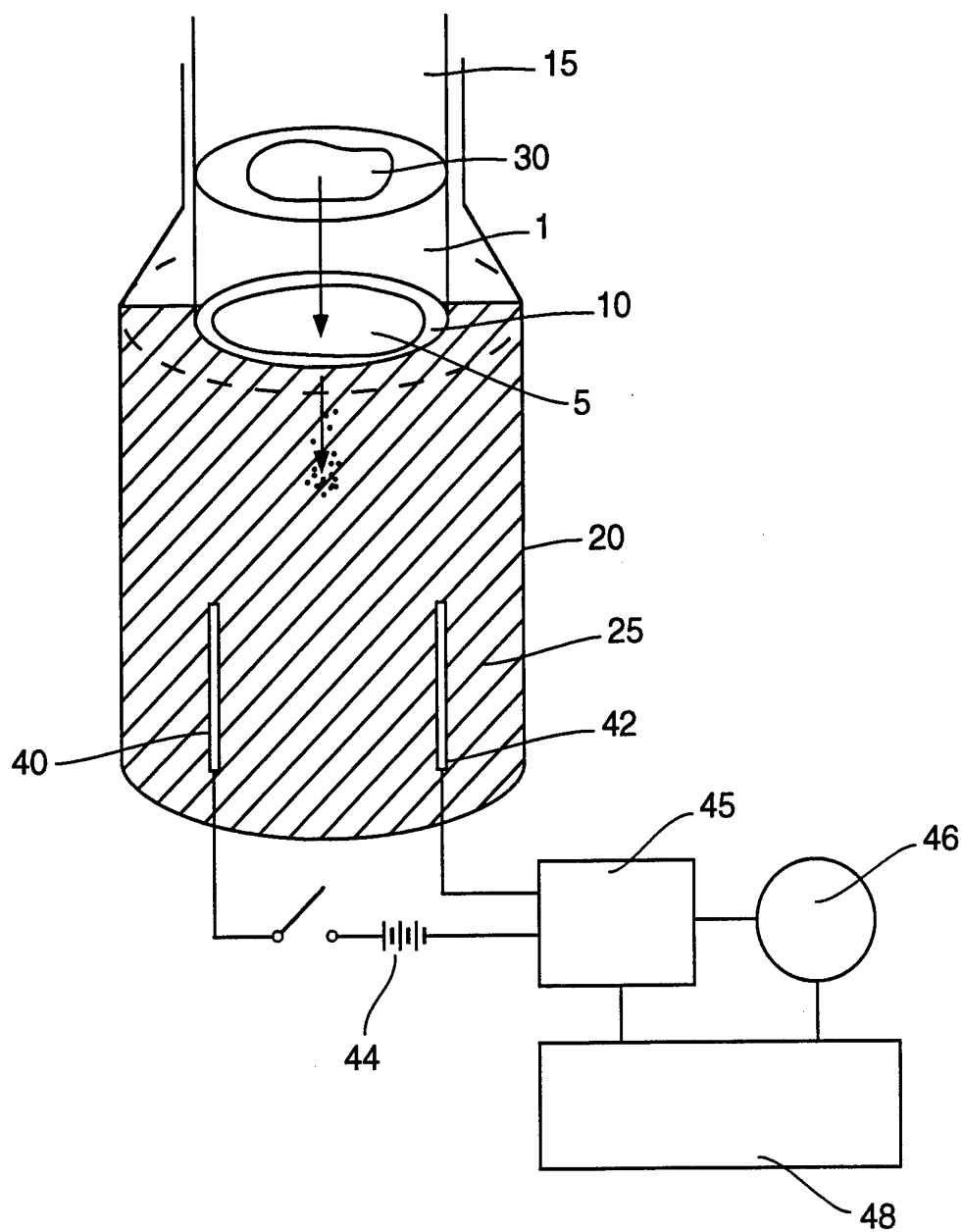
53. The method of claim 52, wherein the test substance is removed from the microspheres before the color of the microspheres is observed.

54. The method of claim 52, wherein the dye layers and corrosive-resistant layers are combined, such that the first dye is incorporated into the third layer, the second dye is incorporated into the fifth layer and the third dye is incorporated into the underlying material of the microspheres.

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**FIG. 1**

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**FIG. 2**

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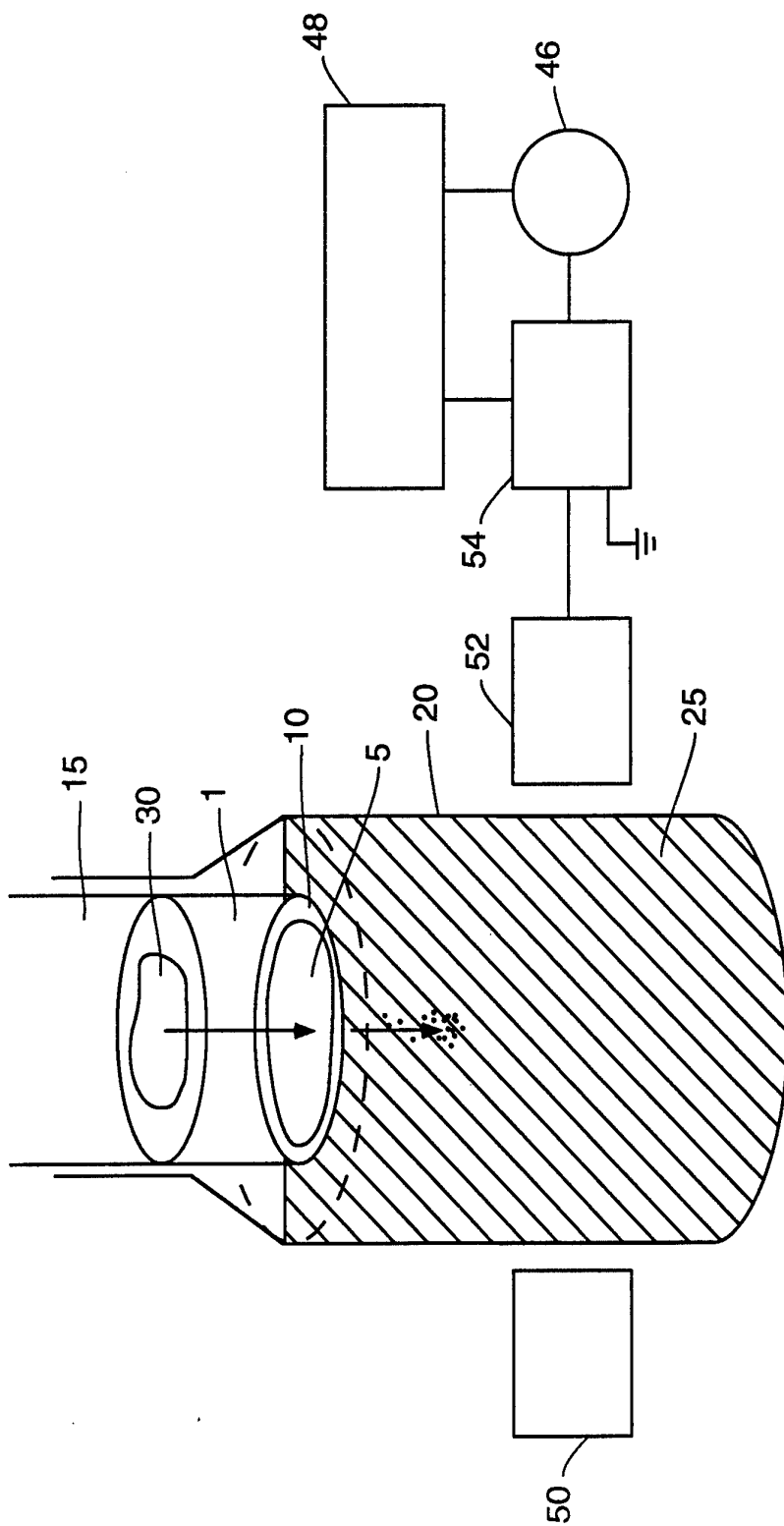
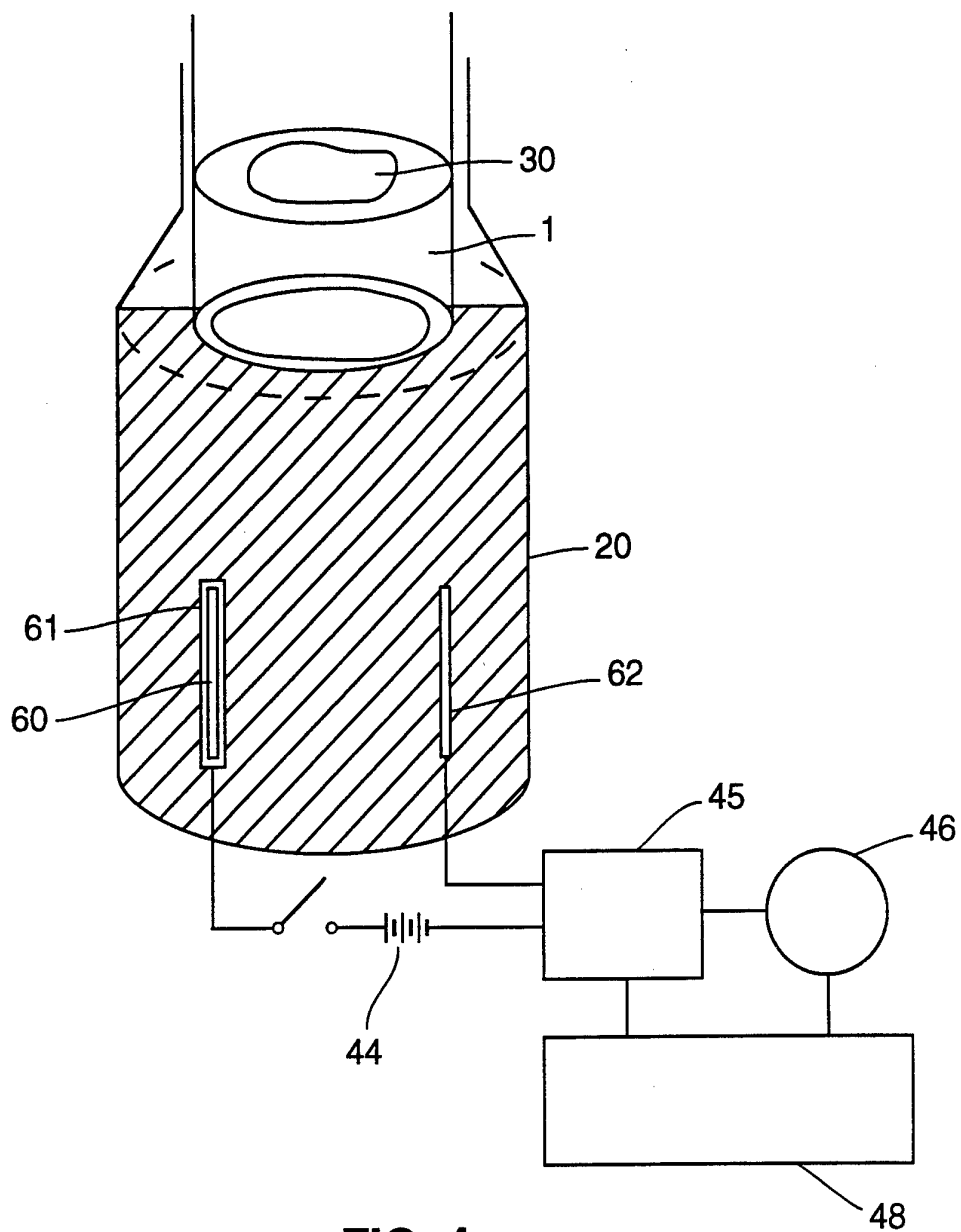


FIG. 3

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**FIG. 4**

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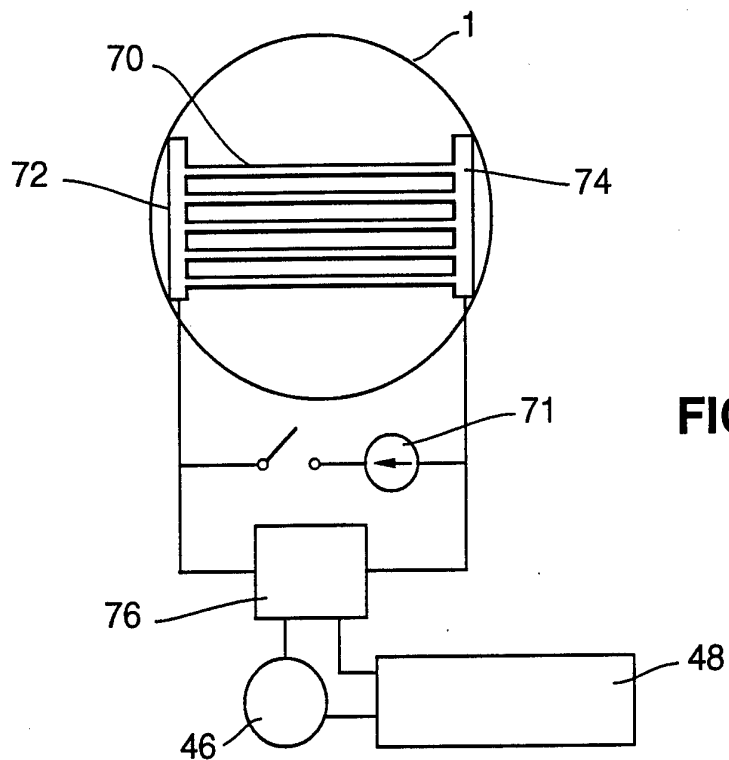


FIG. 5

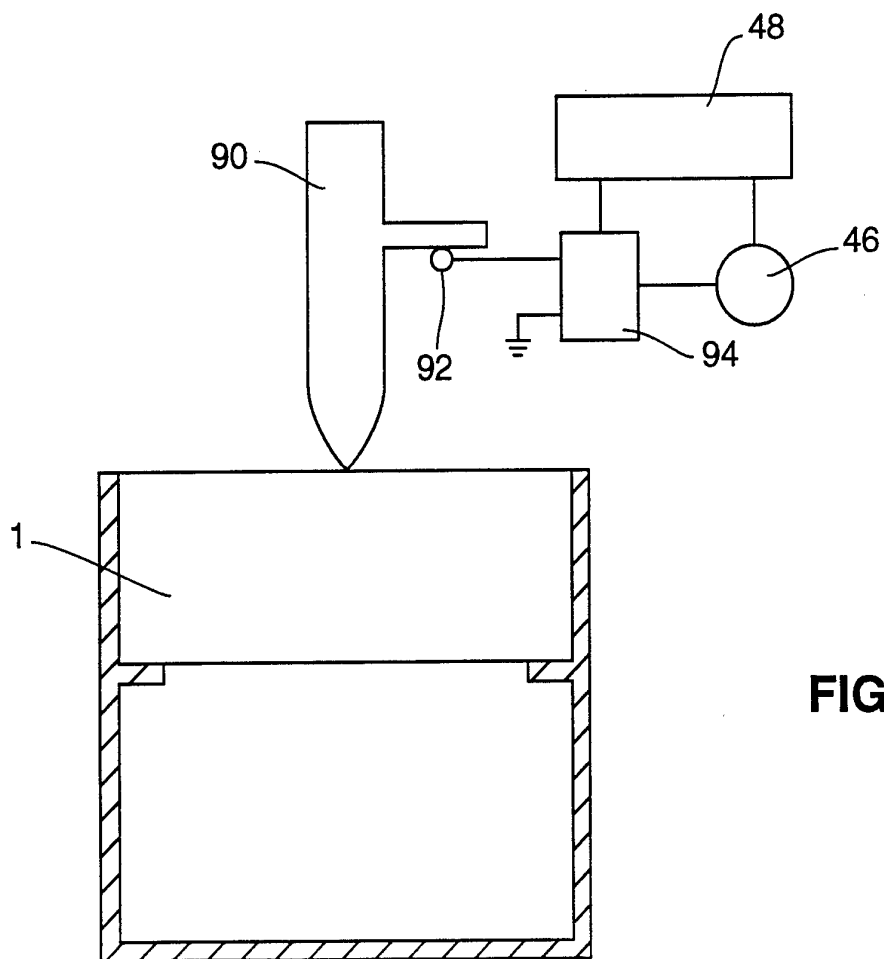


FIG. 7

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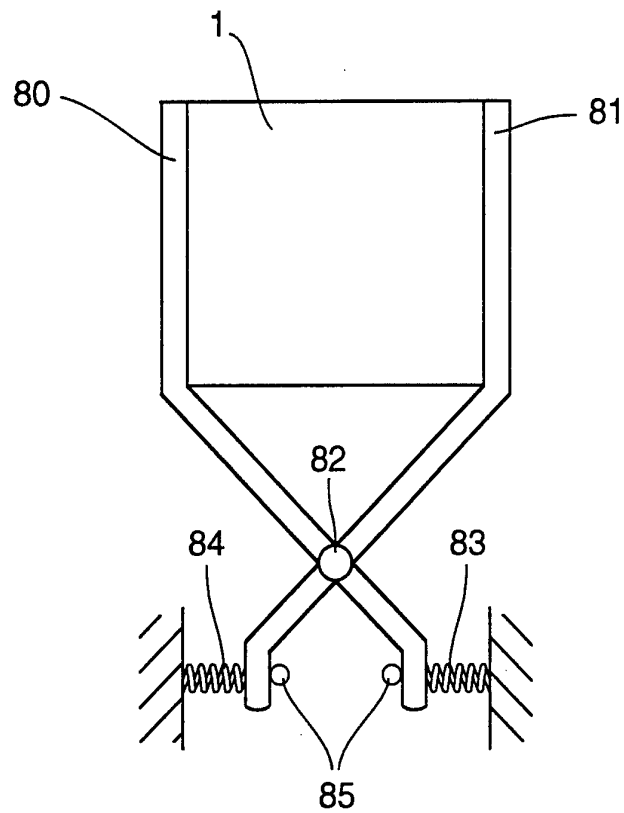


FIG. 6a

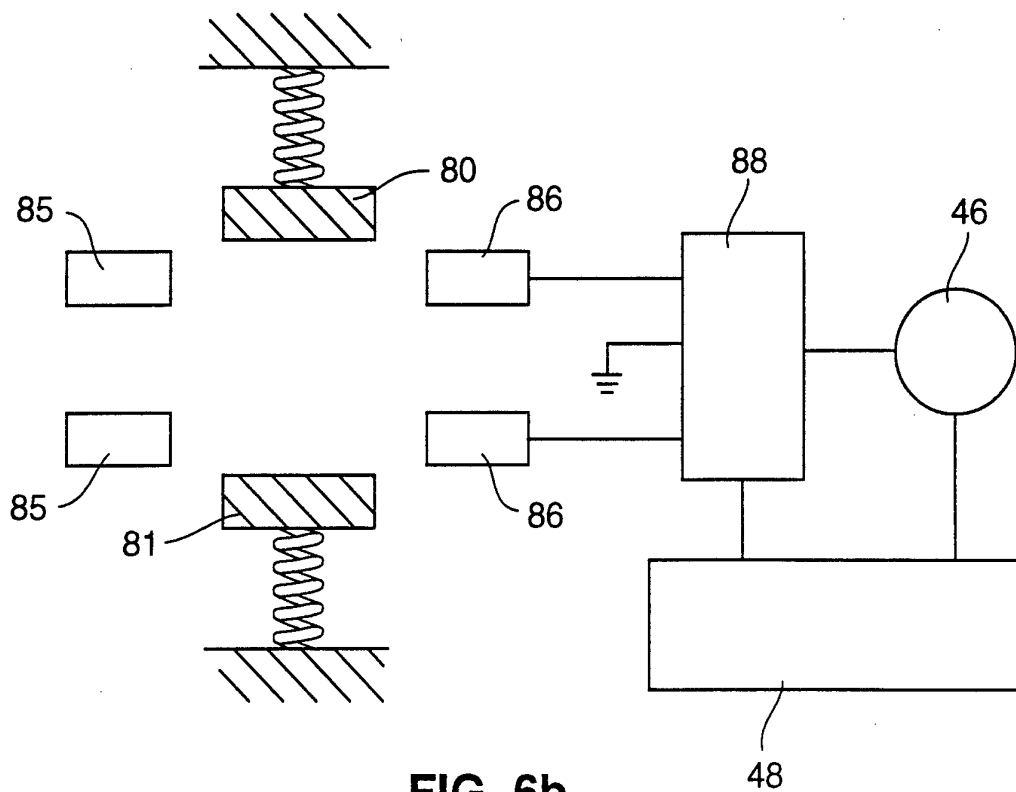
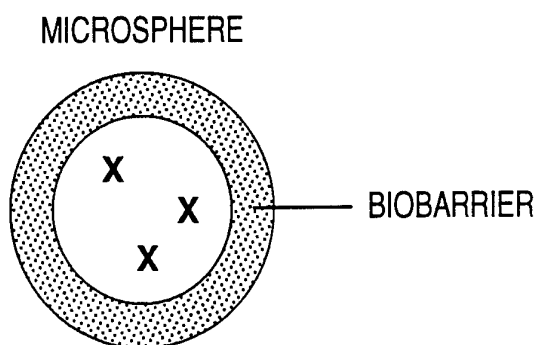


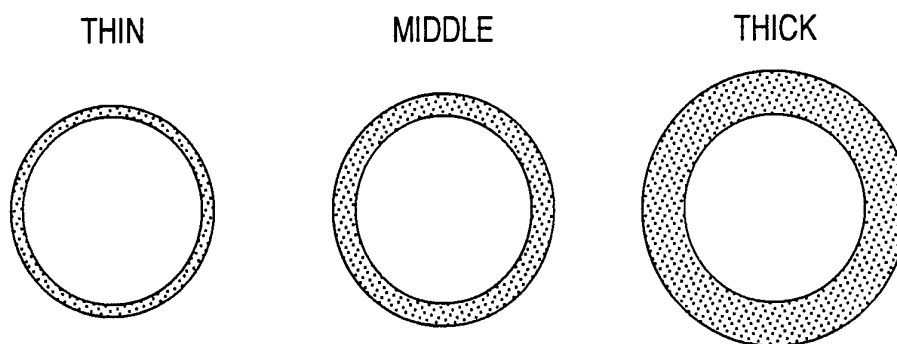
FIG. 6b

SUBSTITUTE SHEET

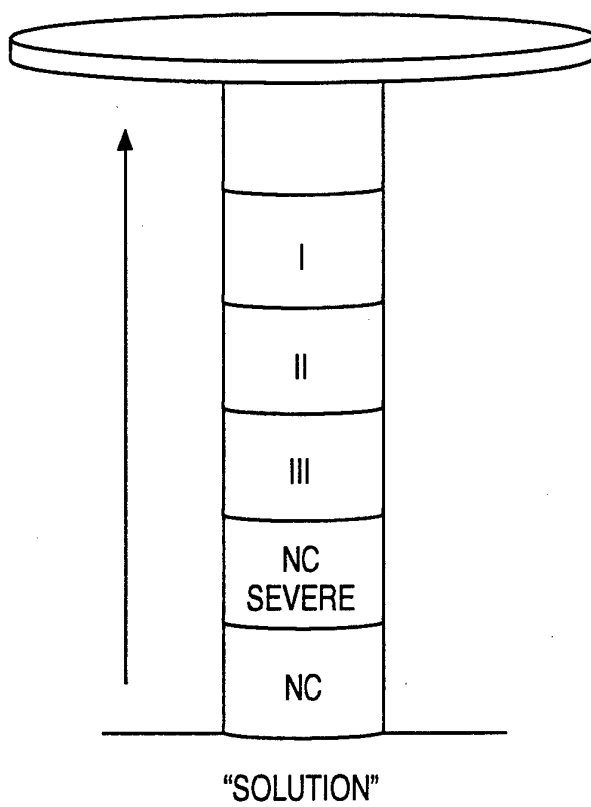
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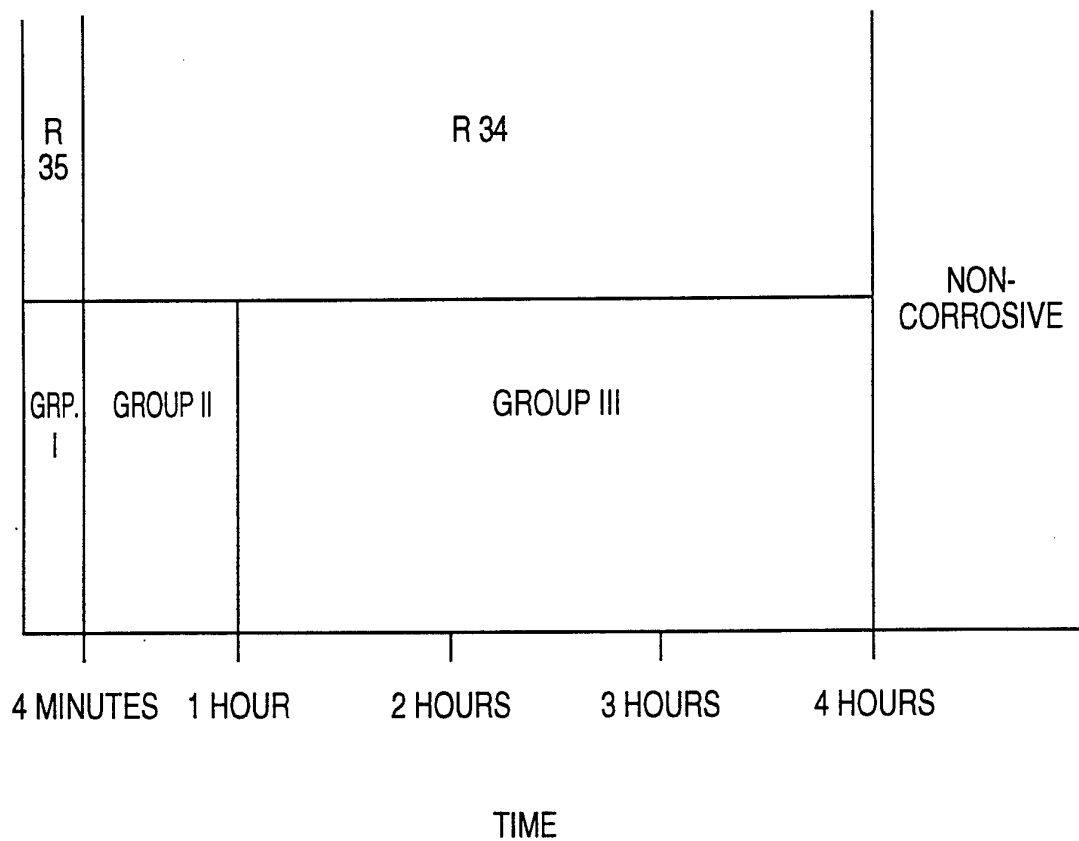
X COULD BE COLORED DYE: SOLID DYE, FLUORESCENT DYE, ETC.

**FIG. 8**

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**FIG. 9**

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**FIG. 10**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07071

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : G01N 33/48

US CL : 436/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/6, 8, 63, 161, 171, 173; 422/ 61, 70, 89; 73/64.47

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,053,340 (Bergman et al.) 01 October 1991, See Entire Document.	1-54
Y	Fd. Chem. Toxic, Volume 23, No.2, issued 1985, Parish, "Relevance of in vitro tests to in vivo acute skin inflammation: Potential in vitro applications of skin keratome slices, neutrophils, fibroblasts, mast cells, and macrophages", pages 275-285, See Entire Document.	1-54
A	US, A, 4,005,191 (Clark) 25 January 1977, See Entire Document.	1-54
A	US, A, 4,129,647 (Klein) 12 December 1978, See Entire Document.	1-54



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 October 1993

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,163,800 (Wickett et al.) 07 August 1979, See Entire Document.	1-54
A	US, A, 4,863,696 (Saydek et al.) 05 September 1989, See Entire Document.	1-54
A	US, A, 4,164,568 (Bywater) 14 August 1979, See Entire Document.	1-54
A	US, A, 4,593,046 (Gruber) 03 June 1986, See Entire Document.	1-54
A	US, A, 4,594,884 (Bondi et al.) 17 June 1986, See Entire Document.	1-54
A	US, A, 4,771,004 (Higuchi) 13 September 1988, See Entire Document.	1-54
A	US, A, 4,879,116 (Fox et al.) 07 November 1989, See Entire Document.	1-54
A	Acta Dermatovener (Stockholm), Volume 52, issued 1972, Nagao et al., "The effect of sodium hydroxide and hydrochloric acid on human epidermis", pages 11-23.	1-54
A	Toxicology and Applied Pharmacology, Volume 81, issued 1985, Patrick et al., "Mechanisms of chemically induced skin irritation", pages 476-490.	1-54
A	Fd. Chem. Toxicol., Volume 23, No. 6, issued 1985, Potokar et al., "Studies on the design of animal tests for the corrosiveness of industrial chemicals", pages 615-617.	1-54
A	Acta Pharm. Suec., Volume 20, issued 1983, Walker et al., "Absorption through human and laboratory animal skins: In vitro comparisons", pages 52-53.	1-54
A	In Vitro Toxicology: New Directions, Volume 7, issued 1989, G.S. Lamont et al., "Developing an Alternative to the Draize Skin Test: Comparison of Human Skin Cell Responses to Irritants In Vitro", See pages 175-181.	1-54
A	J. Med. Chem., Volume 7, issued 06 July 1964, Free et al., "A mathematical contribution to structure-activity studies", pages 395-399.	1-54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Goldberg, L., Ed., "Structure-Activity Correlations as a Predictive Tool in Toxicology", published 1983 by Hemisphere Publishing Corp. (N.Y.), see entire document.	1-54
A	J. Invest. Dermatol., Volume 32, issued 1959, Harrold et al., "Denaturation of epidermal keratin by surface active agents", pages 581-588.	1-54
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A	J. Am. Oil Chem. Soc., Volume 512, issued December 1975, Imokawa et al., "Study on skin roughness caused by surfactants: I. A new method in vitro for evaluation of skin roughness", pages 479-483.	1-54
A	Toxic. In Vitro, Volume 2, No. 1, issued 1988, Oliver et al., "An in vitro model for identifying skin-corrosive chemicals: I. Initial validation", pages 7-17.	1-54
A	J. Amer. Oil Chem Soc., Volume 52, issued 1975, Imokawa et al., "Study on skin roughness caused by surfactants: II. Correlation between protein denaturation and skin roughness", pages 484-489.	1-54
A	Contact Dermatitis, Volume 5, issued 1979, Imokawa et al., "Cumulative effect of surfactants on cutaneous horny layers: adsorption onto human keratin layers in vivo", pages 357-366.	1-54