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(54) Titre : INACTIVATION DE VIRUS ENVELOPPES ET DU SPERME
(54) Title: INACTIVATING ENVELOPED VIRUSES AND SPERM

(57) **Abrégé/Abstract:**

Disclosed is a composition for inhibiting the activity of an enveloped virus or sperm, the composition comprising a mixture of (a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof, (b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof, and (c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition. Also disclosed is a contraceptive device containing the composition as a spermicide.



ABSTRACT

Disclosed is a composition for inhibiting the activity of an enveloped virus or sperm, the composition comprising a mixture of (a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof, (b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof, and (c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition. Also disclosed is a contraceptive device containing the composition as a spermicide.

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INACTIVATING ENVELOPED VIRUSES AND SPERM

BACKGROUND OF THE INVENTION1. Field of the Invention

The present invention relates to a novel method and novel articles for inhibiting the activity of
5 enveloped viruses. The invention also relates to treatment of viral infections. More particularly, the invention relates to a method for inhibiting the development of diseases and infections caused by enveloped viruses. In one aspect the invention
10 particularly relates to a method of inhibiting the activity of viruses whose major mode of transmission is sexual. In other aspects the invention also relates to methods of inhibiting the activity of enveloped viruses whose mode of transmission is nonsexual. The
15 compositions of use in the invention are also effective in the inhibition of bacteria and fungi which coexist with viruses or viral infections. Additionally, the invention relates to a treatment for virus related diseases, particularly sexually transmitted diseases
20 related to AIDS, and to diseases related to this and other opportunistic infections of the immune-compromised host.

The method of the invention relates to using mixtures of betaines and amine oxides in the inhibition
25 of viruses and treatment of viral infections. The betaine is selected from the group consisting of (a) an alkyl-N-betaine, an alkyl-N-sulfobetaine, an acyl-N-betaine, an alkyl N-substituted aminopropionic acid and an alkylimidazolinium betaine and the amine oxide is

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selected from the group consisting of (b) an alkyl-N, N-dimethylamine oxide, an alkyl-N, N-dihydroxyethylamine oxide and an acylamide t-amide oxide. The term "betaine" when used herein means an N-dimethyl glycine and their lower alkyl homologs. The term includes N-dimethyl amino propionic acids and sulfo betaines which are the sulfonic acid analogs of such compounds. Unless otherwise specified a N-dimethyl compound is intended. More particularly, the invention relates to a method for employing mixtures of alkyl N-di(lower alkyl) betaines and alkyl N-di(lower alkyl) amine oxides in the inhibition of enveloped viruses and use of these mixtures in the treatment of infections caused by these viruses. The mixtures may also be used as spermicides, either alone or in combination with conventional spermicides. These mixtures may be used in combination with contraceptive devices or may be incorporated into the contraceptive device.

2. Description of the Prior Art

It is known that certain mixtures of amines are effective antimicrobial agents against gram negative and gram positive bacteria. For instance, it is known that certain mixtures of a) alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, alkyl N-substituted aminopropionic acid or an alkylimidazolinium betaine and (b) alkyl-N,N-dimethylamine oxide, alkyl-N, N-dihydroxyethylamine oxide or acylamide t-amide oxide can be used for skin degerming, cleansing and deodorizing (see for example U.S. Patent 4,183,952, issued to E.B. Michaels; U.S. Patent No. 4,075,350 issued to E.B. Michaels, and U.S. Patent No. 4,107,328; issued to E.B. Michaels). These compositions are also useful for long term inhibition of body odor. Mixtures of the same active ingredients are also known to enhance oral hygiene by reducing oral microflora and inhibiting formation of dental plaque. (U.S. Patent 4,839,158 issued to E.B. Michaels). Compositions of this type are

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also known to be effective fungicides. Corner, A.M., et al. Antimicrobial Agents and Chemotherapy. 32(3):350 - 353 (March 1988); Corner, A.M. The Journal of Clinical Dentistry. Vol. 2(2):34 - 38. (1990)). (See also U.S. Patents 4,075,350, 4,107,328, 4,183,952 and 4,839,158 issued to E.B. Michaels.)

It is also known that nonionic surface-active agents possessing ether or amide linkages between the hydrophilic and hydrophobic portions of the molecule rapidly inactivate the infectivity of herpes simplex virus I and II. (Asculai, S.S., et al. Antimicrobial Agents Chemotherapy. Vol. 13:686 - 690 (1978)). Asculai et al. also states that cytocidal activity has been used as an index of antiviral activity. The compositions utilized in this invention have an advantage over the compositions described in Asculai et al. in that these compositions inactivate viruses and bacteria while exhibiting low cytocidal activity.

The spermicide Nonoxynol-9 (N-9), has been found to have bactericidal and viricidal effects. For example, N-9 has been found to inactivate viruses related to AIDS (e.g. HIV, human immunodeficiency virus) and herpes simplex viruses (HSV I and II). In vitro, N-9 has been shown to be effective against the organisms that cause gonorrhea, chlamydia, syphilis, trichomoniasis, and other sexually transmitted diseases. (U.S. Pharmacopeial Convention USPDI Update 1990 pps. 755 - 756).

SUMMARY OF THE INVENTION

It has now unexpectedly been found that certain hereinbelow defined mixtures of betaines such as (a) alkyl-N-betaines, alkyl-N-sulfobetaines, acyl-N-betaines, alkyl-N-substituted aminopropionic acids and alkylimidazolinium betaines, and amine oxides such as (b) alkyl-N, N-dimethylamine oxides, alkyl-N, N-dihydroxyethylamine oxides or acylamide t-amide oxides in acceptable diluents, carriers and excipients are

effective in inhibiting the activity of enveloped viruses. The invention provides a method of inhibiting the activity of enveloped viruses for which a major mode of transmission is sexual. The invention relates to a method for treating
5 diseases and infections caused by enveloped viruses. These compositions are also effective in the inhibition of bacteria and fungi that co-exist with viruses or viral infections, and for treatment of bacterial and fungal infections which co-exist with viruses or viral infections.
10 The invention also provides a method for disinfecting air and surfaces contaminated with enveloped viruses.

More specifically, the present invention provides use of a composition for inhibiting the activity of an enveloped virus or as a spermicide, the composition comprising a
15 mixture of (a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof, (b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-
20 dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof, and (c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

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The present invention also provides a contraceptive device containing a spermicide composition, wherein the spermicide comprises a mixture of (a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof, (b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof, and (c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

The present invention also provides a composition for inhibiting the activity of an enveloped virus or sperm, the composition comprising a mixture of (a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof, (b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof, and (c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine

4b

and amine oxide together comprise 0.5 weight percent of the composition.

The invention also provides a method for preventing the transmission of enveloped viruses.

5 The compositions used in the method of the present invention can also be used as spermicides. These compositions can be used alone, with other known spermicides and with, or incorporated into, contraceptive devices such as condoms, sponges, vaginal inserts, contraceptive films,
10 diaphragms, suppositories, contraceptive patches or sustained release devices. These compositions can also be incorporated into douches or wipes. For use as viricides, these compositions can be applied alone, with other spermicides, and with, or incorporated into, the
15 contraceptive devices described above. The compositions can also be applied in the form of a foam, gel, cream, salve, jelly or lotion. The compositions can also be applied in the form of a liquid, aerosol, mist or spray.

DESCRIPTION OF THE DRAWINGS

20 Figure 1: is a graph showing the effect of C31G (a composition according to the present invention) and the effect of Nonoxynol-9 (N-9) on sperm motility.

Figure 2: is a graph showing the effect of C31G and the effect of Nonoxynol-9 (N-9) on total sperm count.

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Figure 3: is a graph showing the effect of C31G and the effect of Nonoxonyl(N-9) on the mean progressive velocity of sperm.

Figure 4: is a graph showing in vitro toxicity of C31G vs. N-9 on sup-TI cells.

Figure 5: is a graph showing a five day toxicity study of C31G vs. N-9 on CEM cells.

Figure 6: is a graph showing comparing the effects on C31G vs. N-9 on HSV.

Figure 7: is a graph showing the effect of C31G on HIV.

Figure 8: is a graph comparing inactivation of HIV by C31G and N-9.

Figure 9: is a chart comparing inactivation of HIV by C31G and N-9.

DETAILED DESCRIPTION OF THE INVENTION

The method of the invention relates to using mixtures of betaines and amine oxides in the inhibition of enveloped viruses and treatment of viral infections caused by enveloped viruses. The method of the invention also relates to using mixtures of betaines and amine oxides in the inactivation of enveloped viruses. The betaines that can be used in this invention are alkyl-N-betaines, alkyl-N-sulfobetaines, acyl-N-betaines, alkyl N-substituted aminopropionic acids or alkyimidazolinium betaines or mixtures thereof. The amine oxides that can be used in this invention are alkyl-N, N-dimethylamine oxides, alkyl-N, N-dihydroxyethylamine oxides or acylamide t-amide oxides or mixtures thereof. These compositions are also effective for the treatment of viral, fungal, and microbial infections and contaminations. These compositions are effective in inhibiting enveloped viruses that are transmitted sexually or nonsexually. These compositions are also effective in the inhibition of bacteria and fungi which coexist with viruses or viral infections. These compositions can also be used

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topically.

Typically, these compositions are applied to areas where viruses are or can be transmitted. This includes application in the vagina, to mucous membranes, and to the skin.

These compositions can also be used as spermicides, either alone or in combination with other spermicides. These compositions can also be used with or incorporated into contraceptive devices such as, for example, condoms, diaphragms, sponges, contraceptive films, suppositories, sustained release devices, and contraceptive patches. For example, the composition may be incorporated into a lubricant applied to a condom or as part of a reservoir at the tip of a condom, or incorporated into a contraceptive sponge, contraceptive film, suppository, sustained release device or contraceptive patch. A contraceptive film can comprise the composition described above, together with, Type A gelatin, cellulose gum and a polyhydric alcohol. The compositions can also be used in contraceptive foams, gels, jellys, creams. The compositions can also be used in douches or use in preventing the transmission of viruses, these compositions can be used alone, with other spermicides and with or incorporated into a contraceptive device as described above. This invention also comprises a method for inhibiting the transmission of viruses that cause sexually transmitted diseases which comprises applying the compositions to those parts of the anatomy that are exposed to body fluids emanating from another during sexual activity.

These compositions have also unexpectedly been found to be less toxic to mammalian cells than Nonoxonyl-9 (N-9) (See Figures 4 and 5). Table 1 shows that these compositions have equivalent spermicidal activity to N-9, which is one of the few surfactants approved by the FDA for use as a spermicide.

For use in topical applications, the

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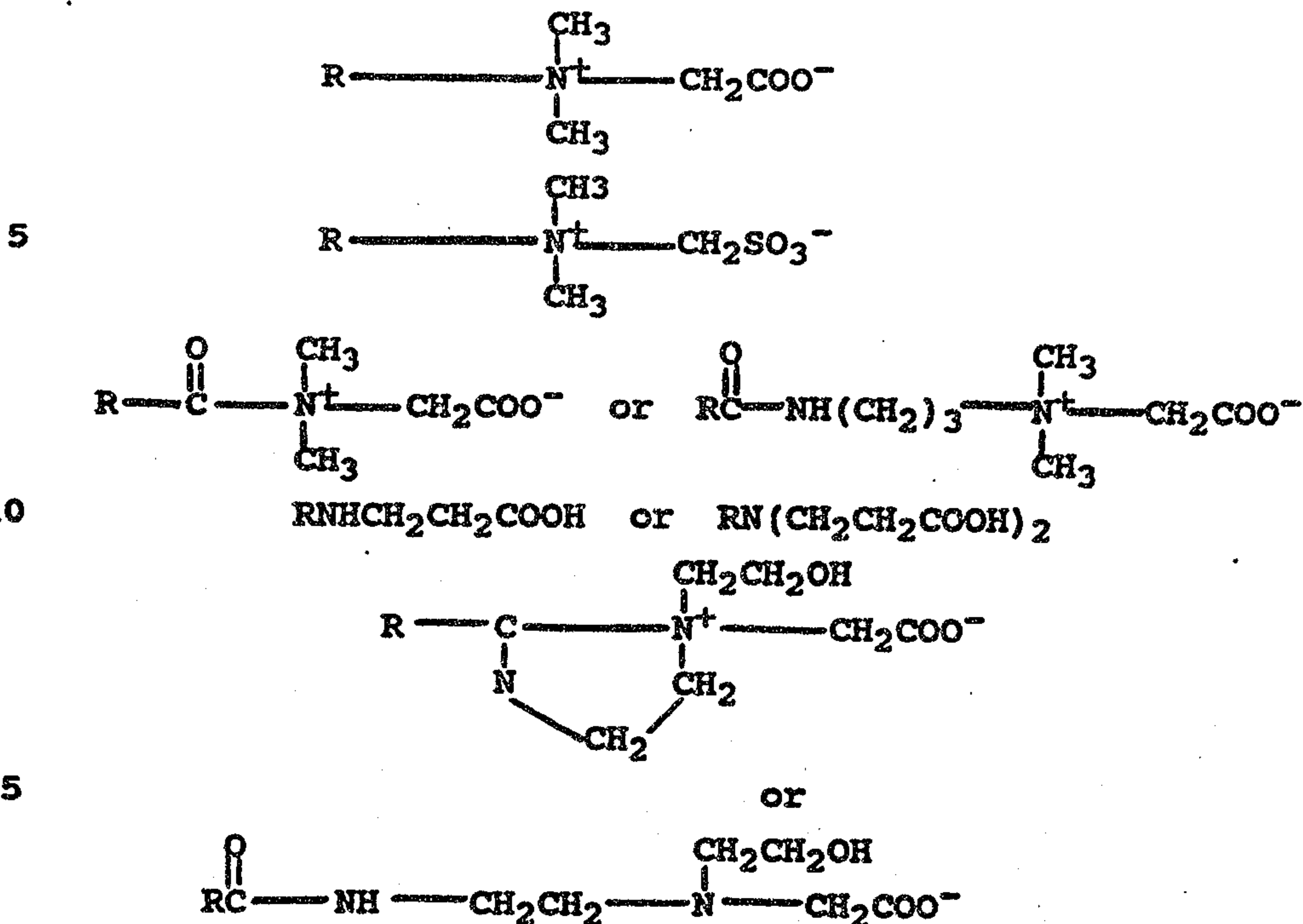
compositions can also be incorporated into liquids, creams, salves, lotions, foams and gels.

5 The invention also provides a method for disinfecting air and inanimate surfaces, for example, in an operating room or laboratory. More particularly, this invention provides a method for disinfecting areas which are contaminated with enveloped viruses. These compositions can also be incorporated into sprays, mists, wipes, aerosols or devices which produce sprays, mists or aerosols. These compositions can also be applied as liquids.

According to the method of this invention, these compositions may be used as viricides, fungicides and bactericides.

15 The compositions employed in the method of the invention comprise an admixture of betaines and amine oxides. The betaines used in this invention are selected from the group consisting of (a) alkyl-N-betaines, alkyl-N-sulfobetaines, acyl-N-betaines, alkyl N-substituted aminopropionic acid, alkyimidazolinium betaines and mixtures of two or more thereof. Typically the betaines have two lower alkyl groups bonded to the nitrogen atom. Most effectively they have two methyl substituents on the nitrogen atom. The amine oxides used in this invention are selected from the group consisting of b) a an alkyl-N,N-dimethylamine oxides, alkyl-N,N-dihydroxyethylamine oxide or acylamide t-amine oxides and mixtures of two or more thereof. Typically, the betaine and amine oxide components are present in a molar ratio of from 1:5 to 5:1, preferably in a molar ratio of 1:1. The alkyl-N-betaine, the alkyl-N-sulfobetaine, the acyl-N-betaine, the alkyl N-substituted 2-aminopropionic acid and alkyimidazolinium betaine (also referred to as cocoamphoacetates) employed as the components (a) of the composition of the invention have structures, respectively, as follows:

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where R is a higher alkyl group having from 10 to 18 carbon atoms, preferably from 12 - 16 carbon atoms.

20 When used herein the term lower alkyl means an alkyl group of from 1 to 3 carbon atoms.

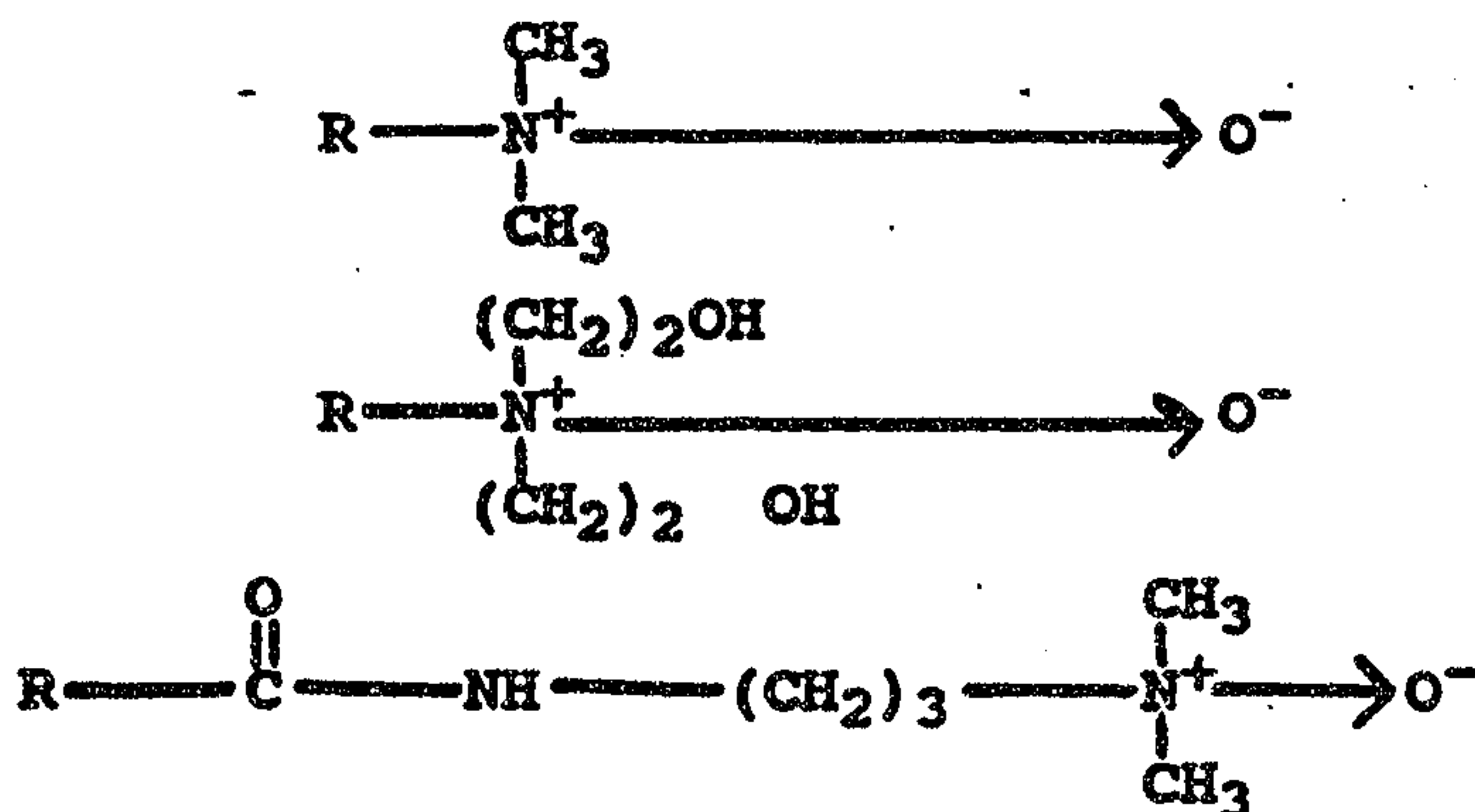
Illustrative of these aforementioned substances are: (1) coco-N-betaine, cetyl-N-betaine, stearyl-N-betaine, isostearyl-N-betaine, oleyl-N-betaine; (2) coco-N-sulphobetaine, cetyl-N-sulphobetaine, stearyl-N-sulphobetaine, isostearyl-N-sulphobetaine, oleyl-N-sulphobetaine; (3) cocoamido-N-betaine, cetylamido-N-betaine, stearyl-amido-N-betaine, isostearyl-amido-N-betaine, oleyl-amino-N-betaine; (4) N-coco-2 aminopropionic acid, N-cetyl-2-aminopropionic acid, N-stearyl-2-aminopropionic acid, N-isostearyl-2-aminopropionic acid, N-oleyl-2-aminopropionic acid, N-stearyl-bis(2-aminopropionic acid), N-oleyl-bis(2-aminopropionic acid), N-coco-bis(2-aminopropionic acid), N-cetyl-bis(2-aminopropionic acid),

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(5) N-lauryl-bis(2-aminopropionic acid) 1-hydroxyethyl-
 1-carboxymethyl-2-decylimidazolium betaine;
 1-hydroxyethyl-1-carboxymethyl-2-dodecylimidazolium
 betaine; 1-hydroxyethyl-1-carboxymethyl-2-
 5 cocoimidazolium betaine; 1-hydroxyethyl-1-carboxymethyl-
 2-stearylimidazolium betaine; 1-hydroxyethyl-1-
 carboxymethyl-2-oleylimidazolium betaine; or mixtures of
 the same.

When used here the term "coco" is that used in
 10 the CTFA (designations of Cosmetic and Toiletry and
 Fragrance Association, Wash., D.C.) and is used to
 indicate alkyl groups present in coconut oil, i.e. a
 mixture of alkyl groups of from 10 to 18 carbon atoms.
 The designations of the compounds listed herein are
 15 those of the CTFA.

The (1) alkyl-N,N-diethylamine oxide, (2)
 alkyl-N,N-dihydroxyethylamine oxide, or (3) acylamide
 t-amine oxide employed as component (b) of the
 aforementioned mixture, respectively, have the
 20 structure:



30 where R is a higher alkyl group of from 10 to 18 carbon
 atoms, for instance, radicals such as decyl, undecyl,
 lauryl, tridecyl, myristyl, cetyl, stearyl, isostearyl
 or oleyl. Exemplary of the amine oxides are: decyl-
 N,N-dimethylamine oxide, lauryl-N,N-dimethylamine oxide,
 35 stearyl-N,N-dimethylamine oxide, oleyl-N,N-dimethylamine
 oxide, coco-N,N dihydroxyethylamine oxide, cetyl-N,N-

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dihydroxyethylamine oxide, oleyl-N,N-dihydroxyethylamine oxide, N,N-dihydroxyethylamine oxide, oleyl-N,N-dihydroxyethylamine oxide and mixtures of the same.

5 The components (a) and (b) are usually admixed and acid is then added in an amount necessary to adjust the pH of a 0.5% solution to between 4 - 8, preferably to pH 4.5 - 5.5. The pH of an aqueous solution comprising the above enumerated components of the invention is determined by employing an aqueous solution
10 of 0.5%, by weight, total of active components typically at a glass electrode, to precisely define the acidity of the composition.

In general, the acid used to adjust the overall composition to the required pH is any organic or
15 inorganic acid that is compatible with the intended use of the composition, for example, hydrochloric acid, phosphoric acid, sulfuric acid, citric acid, acetic acid or nicotinic acid. The balance of the composition, after allowing for the acid is usually an acceptable
20 solvent, such as water or a lower (C₁-C₄) monohydric aliphatic alcohol, for a total of 100 parts or more. Where water is employed, small amounts of a lower alkyl alcohol, such as ethanol or propanol, may also be added to provide ease in formulation. Acceptable diluents,
25 carriers and excipients are, for example, ethyl or isopropyl alcohols, polyethylene glycol, povidone, polyhydric alcohols, glycerine, cellulose gums, gelatin, colorants and fragrances. If necessary, the pH of the total composition is then adjusted to the requisite pH
30 by adding a suitable inorganic or organic acid thereto.

The result is a substantially uniform, homogeneous, relatively nontoxic composition having enhanced activity against enveloped viruses, bacteria and fungi.

35 It has been unexpectedly found that these compositions exhibit (1) low mammalian cell toxicity, a property which is known to correlate with low irritation

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and low toxicity when used in contact with mucous membranes (Journal Clinical Dentistry 2(2):34 - 38, (1990)), and (2) highly efficacious inactivation of enveloped viruses although cytocidal activity is low.

5 In the past, research has used cytocidal activity as an index of activity. (Asculai, S.S., Antimicrob. Agents Chemother. 13:678 - 690 (1978)).

In practice, the total amount of the components (a) and (b) of the overall composition can
10 range from .01% - 40%, preferably from .03% - 30% depending on the intended means of use. For example, concentrations used are, approximately 20% - 30% in contraceptive films and 0.2% to 2% in gels.

Compositions for use in this invention
15 comprise alkyl N-di(lower alkyl) glycines and alkyl N-di(lower alkyl) amine oxides, wherein the lower alkyl is C₁₋₃. One class of betaines I have found to be particularly useful in this invention are the alkyl N-dimethyl betaines, such as cocobetaines and lauryl
20 betaines. Particularly useful amine oxides for use in this invention are the an alkyl N-dimethyl amine oxides, such as cocodimethyl amine oxides and lauryl dimethyl amine oxides.

One particular composition that can be used in
25 this invention comprises cocobetaine, cocamine oxide and citric acid monohydrate.

Another composition that can be used in this invention comprises lauryl betaine, lauramine oxide and citric acid monohydrate.

30 In such compositions, the molar ratio of betaines to amine oxides is normally from 5:1 to 1:5, preferably in a molar ratio of 2:1 to 1:2, more preferably about 1:1.

Other compositions that can be used in this
35 invention comprise mixtures of betaines, amine oxides, gelatin having a Bloom strength of 100 - 300 and a molecular weight from about 75,000 to about 300,000,

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polyhydric alcohols and cellulose gums. Such compositions are described in a copending U.S. application being filed simultaneously with the present application and having U.S. application Serial No. 07/673,631.

Compositions used in this invention can inhibit the activity of viruses that are related to AIDS. It is also expected that these compositions can inhibit the activity of HSV-1 and HSV-2. It is also expected that the compositions used in this invention can inhibit Hepatitis A, B and C. It is expected that the compositions can be used to inhibit viruses, bacteria and fungi which are associated with sexually transmitted diseases (STD's).

The compositions of this invention may be useful in relation to the following:

1) Transmission of HIV is often associated with the co-transmission of other viral and/or microbial pathogens. Indeed, some investigators have suggested that HIV may not be the sole agent responsible for AIDS (see Duesberg, P.H. (1991) Proc. Natl. Acad. Sci. 88:1575 - 1579; Lemaitre, M., Guetard, D., Henin, Y., Montagnier, L. and Zerial, A. (1990). Res. Virol. 141:5 - 16). For this reason, antimicrobial agents, such as those described in this application, with a broad spectrum of activities against viruses, bacteria, and yeasts such as Candida may be of particular value in the prevention and treatment of Acquired Immune Deficiency Syndrome (AIDS).

2) It is thought that certain bacteria known to cause STD's may aid in HIV transmission. In persons who have been exposed to HIV, certain bacteria which cause STD's often fail to respond to therapies that are otherwise highly effective. HIV infection may help the spread of a bacterial STD that in turn helps to spread HIV. STD's such as chlamydia, chancroid, syphilis, genital herpes and gonorrhea which cause ulcerations of

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the genital skin seem to increase the risk of acquiring or transmitting HIV infection sexually. Aral, S.O., et al. Scientific American, 264(2):62 - 69 (February 1991).

5 The compositions described above may also be of use to inactivate other enveloped viruses, including vaccinia, varicella, herpes zoster, cytomegalovirus, Epstein Barr virus, influenza, mumps, measles, rhinovirus, rabies and rubella. In order to facilitate a further understanding of the invention, the following
10 examples are presented primarily for the purposes of illustrating more specific details thereof. The invention is not to be deemed as limited thereby except as defined in the claims.

Example 1

15 The composition described below is a concentrate of C31G, an equimolar preparation of cocobetaine and cocodimethylamine oxides (designations of CTFA Cosmetic and Toiletory and Fragrance Association, Wash., D.C.) [CFTA] which can be used in a number of
20 different configurations.

Cocobetaine; 31.5% active ingredient (A1):
183.9 kg (405.5 lb),

Cocamine oxide, 31.5% (A1): 147.4 kg (325 lb),
Citric acid monohydrate, USP: 11.8 kg (26 lb),
25 Purified water, USP: 11.8 kg (26 lb),

To make about 354.9 kg (782.5 lb) C31G at
29.6% A1, at a dilution 1% A1; pH = 4.9.

Example 2

30 The spermicidal activity of C31G of example 1 was compared to the spermicidal activity of nonoxynol-9 (N-9).

The studies were conducted using the Hamilton-Thorn Sperm Motility Analyzer. Samples of pooled washed semen were incubated with dilutions of the two compounds
35 for fifteen seconds and were analyzed (in triplicate) for determination of the minimum concentration for inactivation by the following criteria:

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1. Inhibition of sperm motility (Figure 1)
2. Decrease in total sperm count (Figure 2)
3. Inhibition of the mean progressive sperm velocity: (Figure 3)

5 As shown in Table 1, C31G and N-9 gave identical results:

Table 1
Minimum Concentration for Spermicidal Activity

	C31G	N-9
10 Total Inhibition of Motility	150 ppm	150 ppm
90% Decrease in Sperm Count	150 ppm	150 ppm
50% Decrease in Progressive Velocity	125 ppm	125 ppm

Example 3

15 The cytotoxic effects of C31G and N-9 on mammalian cells were determined in several types of assays. Cell toxicity is an indication of the relative safety and comfort in use of surfactants in contraceptives or prophylactics.

20 As shown in Figure 4, a two week study monitoring drug effects on SUP-TI cells, (a human lymphocytic cell line), C31G demonstrated minimal toxicity at 0.001% [10 ppm] while N-9 at the same concentration was extremely toxic.

25 Example 4

Figure 5 shows MMT reduction in CEM cells after five days of continuous exposure. Under these conditions C31G showed no toxicity to mammalian cells at 3 ppm [.0003%] while N-9 showing toxicity, reducing mammalian cell viability by 50% at the same concentration. It is unexpected that a compound having the same spermicidal activity as N-9 would be less toxic to mammalian cells than N-9.

Example 5

35 Antiviral activity of C31G was tested using HSV-1 and HIV-1.

To test the effect of C31G and N-9 on herpes

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simplex virus type-1 (KOS strain), the virus stock was prepared in Vero (African green monkey kidney) cells. The virus was released from the cells by one freeze-thaw cycle followed by sonication. The virus titer was 4.5×10^8 plaque forming units (PFU)/ml, as determined on Vero cells. The drug formulations used were identical to the drugs in the spermicidal study.

The stock concentration of each drug was 5%. Two-fold serial dilutions were made, down to 0.04%, in PBS pH 7.4 at room temperature. 24 ul of each dilution was added to 220 ul of virus (10^8 PFU), giving final concentrations of 0.5 - 0.004%. These samples were incubated at room temperature for 10 min, and then 100ul of each was immediately diluted to 1 ml with ice-cold DMEM/5% FBS. 10-fold serial dilutions were then made, down to 10^{-7} for titration of remaining infectious virus on Vero cells in 24-well plates. Plaques were counted 36 hours post-infection.

Table 2

20	<u>Concentration (%)</u>	<u>Residual Virus (PFU/ml)</u>	
		<u>N-9</u>	<u>C31G</u>
	0.004	3.5×10^8	3.5×10^8
	0.008	2.7×10^8	3.3×10^8
	0.015	1.1×10^8	2.5×10^5
25	0.03	3200	<167
	0.06	<167	<167
	0.13	<167	<167
	0.25	<167	<167
	0.5	<167	<167

30 Note that these are titers (ie. PFU/ml), rather than absolute numbers of PFU. The titer determined for untreated virus was 4.7×10^8 PFU/ml in the experiment using N-9 and 4.5×10^8 PFU/ml in the experiment using C31G.

35 It is noted that complete inhibition of plaque formation by C31G occurs at more than one dilution below that of N-9. See Figure 6.

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Example 6

The inactivation of HIV-1 (AIDS) virus was studied in two experiments. The first study compared the effects of C31G of HIV-1 at two different exposure times, 2 minutes and 45 minutes. The next study compared the relative activity of C31G and N-9 on HIV using the same virus strain and measuring antiviral activity by reduction of virus titer as determined by reduction of P-24 HIV antigen.

The protocol and results of the first study follow:

EFFECTS OF C31G ON HUMAN IMMUNODEFICIENCY VIRUS

Materials:

Virus: HIV-1 strain 3B (5×10^5 ID50 units/ml)

Cells: SUP-T1 cell line (CD4+ lymphoid cells which produce characteristic cell fusion with giant cells and syncytia when infected with HIV)

Protocol:

1. Serial fourfold dilutions of C31G in PBS (4.0% - 0.004% and PBS alone as control) were prepared. pH was adjusted to 5.5.
2. Pooled aliquots of viral stock were prepared and C31G was added at each concentration, at 1:9 detergent:virus ratio (1:10 detergent dilution). These were incubated for either 2 or 45 minutes.
3. Serial fourfold dilutions of each virus/detergent mixture down to 1:4096 were prepared.
4. 16.6 ul of each virus/detergent dilution from each series were added to four replicate wells of SUP-T1 cells (10^4 cells in 150 ul volume; 1:10 detergent dilution followed by further fourfold dilutions).
5. The wells were examined twice weekly for two weeks and each well was scored positive or negative for the presence of characteristic viral syncytia or non-syncytial ghost formation due to detergent lysis.

Note:

Virus was exposed to C31G at a 1:10 dilution of

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initial concentration for either 2 or 45 minutes, followed by another 1:10 dilution and then serial four-fold dilutions for the entire period.

5 Cells were exposed to C31G at a 1:100 and then serial fourfold dilutions of initial concentration for entire period.

See Figure 7.

Example 7

The protocol and results of the second study follows:

10 Materials:

Cells: CEM cell line ATCC #119 T cell

Virus: HIV_{IIIb}

15 Test compounds: Compounds C31G and N-9, supplied as 5% solutions, were filtered through a low binding 0.22 filter prior to diluting.

Protocol:

1. Serial 2 fold dilutions of compounds N-9 and C31G in PBS adjusted to pH 5.5 were prepared. The highest concentration was 4% and the lowest was
20 0.03%. The initial concentrations were 4, 2, 1, 0.5, 0.25, 0.125, 0.06, and 0.03%, as well as a control without drug. The control titration was done twice.
2. 14 12 x 75mm sterile plastic tubes with 0.9 ml each
25 of concentrated virus (at least 1×10^6 TCID₅₀/ml) were set-up. 0.1 ml of each compound dilution were transferred to a tube (dilution of 1:10 of the initial drug concentrations).
3. Incubation was for 10 minutes at room temperature,
30 and was terminated (or slowed) by diluting 1:10 in complete growth medium (on ice).
4. Additional serial 5 fold dilutions (7 dilutions, with the final dilution equal to 1:781250) in complete medium on ice were prepared.
- 35 5. 50 ul of the virus dilutions were added to quadruplicate wells of 96 well trays (U bottom) containing 3×10^4 CEM cells in 50 ul.

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6. 60 minutes were allowed for virus absorption. The cells were washed twice by centrifuging the plates at 1500 rpm for 5 minutes and aspirating the supernatants. The final cell pellets were resuspended in 100 ul of medium and transferred to flat bottom 96 well trays. The results are shown in Figures 8 and 9.

Example 8Formulations for Contraceptive/AntiInfective Preparations

- 10 Concentrate (CCon) comprises:

Lauryl Betaine (30% ai)	1000 pts
Lauramine Oxide (30% ai)	870 pts
Citric Acid monohydrate	69 pts

- 15 The above are stirred to a uniform solution. At a dilution of one part to 30, the composition should have a pH of 4.85 at the glass electrode. Putative concentration equal to 28.5% active ingredients (ai).

Example 9Suppositories (inserts) for use with or without Condoms

20 CCon (of Example 8)	526 pts
PEG-32	4000 pts
PEG-20	1000 pts
Povidone	74 pts

- 25 The above are stirred to solution at 45°C. and injected into molds, cooled and ejected for packaging.

Example 10Contraceptive Sponge

- | | |
|------------------------|---------|
| 30 CCon (of Example 8) | 3.5 gms |
|------------------------|---------|
- Added to each mold for curing urethane. Charge in mold provided with polyester loop for post coital removal of the sponge.

Example 11

- 35 Spermicidal Gels, Creams or Jellies for use with Cervical Caps Condoms, Diaphragms or alone prior to coitus.

Gel-1	CCon (of Example 8)	7.50 pts
	Gelatin A200	1.5 pts

- Glycerine 10.0 pts
Hydroxyethyl Cellulose
(high viscosity Grade) 0.4 pts
Water 81.1 pts
- 5 Gel-2 As above with the substitution of
1.0 pt of Nonoxynol-9 for 3.5 pts of CCon (of
Example 8).

Procedure - The gelating and cellulose gum are
triturated in the glycerine and added to the water at
10 45°C. and stirred to solution. The surfactants are
added to the vessel and the warm solution removed for
packaging. A uniform fluid high viscosity gel forms on
cooling.

- Jelly 1 and 2
- 15 Clear fluid contraceptive jellies are prepared by
substitution of 1.5 pts of high viscosity grade
hydroxypropyl or hydroxypropylmethyl cellulose for the
gelatin and hydroxyethyl cellulose of the above
examples.

- 20 Creme 1 and 2
- The Gel formulations above are converted to Cream
formulations by incorporation of 0.5 pts of cetyl
alcohol in Gel 1 or 2 formulations by dissolving the
alcohol in the glycerine at 49°C. before trituration of
25 the gelating and cellulose gums.

Example 12

Contraceptive Film

- 6.2 lbs of gelatin Type A100 is trituated
with 0.5 lbs of hydroxyethylcellulose. 31 lbs of
30 glycerine are added. Solution is mixed thoroughly to
form a slurry. Add 33 lbs of CCon of Example 8 and 15
lbs of water. Warm to 40°C. Mix until gums and gelatin
are completely hydrated. Solution is poured on
polyethylene sheet to cast a film of about 3 mm thick to
35 be cut for films to be used as contraceptive films after
cooling.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Use of a composition for inhibiting the activity of an enveloped virus or as a spermicide, the composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

2. Use of a composition for inhibiting the activity of an enveloped virus, the composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition. -

3. A use according to claim 1 or 2, wherein the virus is selected from human immunodeficiency virus, a virus associated with AIDS, herpes simplex virus-type I, herpes simplex virus-type II, hepatitis A, hepatitis B, hepatitis C, vaccinia, varicella, herpes zoster, cytomegalovirus, Epstein Barr virus, influenza, mumps, measles, rhinovirus, rabies and rubella.

4. A use according to claim 3, wherein the virus is a virus associated with a sexually transmitted disease and is selected from human immunodeficiency virus, herpes simplex virus-type I, herpes simplex virus-type II, cytomegalovirus, and Epstein Barr virus.

5. Use of a composition as a spermicide, the composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

6. A use according to any one of claims 1 to 5, wherein the composition comprises an alkyl-N-di-(lower alkyl) betaine and an alkyl N-di-(lower alkyl) oxide, where the lower alkyl is C₁-C₃.

7. A use according to any one of claims 1 to 6, wherein the composition comprises:

- (a) an alkyl dimethyl betaine;
- (b) an alkyl dimethyl amine oxide; and
- (c) acid in an amount sufficient to adjust the pH of the medicine or composition from 4 - 8 when measured in an aqueous solution of components (a) and (b) in an acceptable carrier.

8. A use according to any one of claims 1 to 7, wherein the composition comprises at least one alkyl dimethyl betaine and at least one alkyl dimethyl amine oxide.

9. A use according to any one of claims 1 to 7, wherein the betaine is cocobetaine or lauryl-betaine, and the amine oxide is cocoamine oxide or lauramine oxide.

10. A use according to claim 9, wherein the betaine is a cocobetaine and the amine oxide is a cocamine oxide.

11. A use according to any one of claims 1 to 10, wherein the betaine and the amine oxide are present in a molar ratio of from 1:5 to 5:1.

12. A use according to any one of claims 1 to 11, wherein the composition has a molar ratio of betaine to amine oxide of 1:5 to 5:1, and the betaine and amine oxide together are present in the composition at a concentration of 0.01 weight percent to 40 weight percent.

13. A use according to claim 12, wherein the composition has a molar ratio of betaine to amine oxide of 1:2 to 2:1, and the betaine and amine oxide together are present in the

composition at a concentration of 0.03 weight percent to 30 weight percent.

14. A use according to claim 13, wherein the medicine or composition has a molar ratio of betaine to amine oxide of 1:1, and the betaine and amine oxide together are present in the medicine or composition at a concentration of 0.2 weight percent to 2 weight percent.

15. A contraceptive device containing a spermicide composition, wherein the spermicide comprises a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

16. A contraceptive device according claim 15, wherein the composition comprises an alkyl-N-di-(lower alkyl) betaine and an alkyl N-di-(lower alkyl) oxide, where the lower alkyl is C₁-C₃.

17. A contraceptive device according claim 15 or 16, wherein the composition comprises:

(a) an alkyl dimethyl betaine;

(b) an alkyl dimethyl amine oxide; and

(c) acid in an amount sufficient to adjust the pH of the composition from 4 - 8 when measured in an aqueous solution of components (a) and (b) in an acceptable carrier.

18. A contraceptive device according to claim 15, 16 or 17, wherein the composition comprises at least one alkyl dimethyl betaine and at least one alkyl dimethyl amine oxide.

19. A contraceptive device according to any one of claims 15 to 18, wherein the betaine is cocobetaine or lauryl-betaine, and the amine oxide is cocoamine oxide or lauramine oxide.

20. A contraceptive device according to claim 19, wherein the betaine is a cocobetaine and the amine oxide is a cocamine oxide.

21. A contraceptive device according to any one of claims 15 to 20, wherein the betaine and the amine oxide are present in a molar ratio of from 1:5 to 5:1.

22. A contraceptive device according to any one of claims 15 to 21, wherein the composition has a molar ratio of betaine to amine oxide of 1:5 to 5:1, and the betaine and amine oxide together are present in the medicine or composition at a concentration of 0.01 weight percent to 40 weight percent.

23. A contraceptive device according to claim 22, wherein the composition has a molar ratio of betaine to amine oxide of 1:2 to 2:1, and the betaine and amine oxide together are

present in the medicine or composition at a concentration of 0.03 weight percent to 30 weight percent.

24. A contraceptive device according to claim 23, wherein the composition has a molar ratio of betaine to amine oxide of 1:1, and the betaine and amine oxide together are present in the medicine or composition at a concentration of 0.2 weight percent to 2 weight percent.

25. A contraceptive device according to any one of claims 15 to 24, wherein the composition is in a form selected from a foam, gel, cream, salve, jelly, lotion, liquid, spray, mist, aerosol and wipe.

26. A contraceptive device according to any one of claims 15 to 25, wherein the device is a condom in which the composition is incorporated in the device in a manner selected from incorporation into a lubricant applied to the condom and incorporation in a reservoir at a tip of the condom.

27. A contraceptive device according to any one of claims 15 to 25, wherein the composition is incorporated into a device selected from a wipe, a douche, a contraceptive sponge, a contraceptive film, a sustained-release device and a suppository.

28. A composition for inhibiting the activity of an enveloped virus or sperm, the composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted

aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

29. A composition for inhibiting the activity of an enveloped virus, the composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkyimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

30. A composition according to claim 28 or 29, wherein the virus is selected from human immunodeficiency virus, a virus associated with AIDS, herpes simplex virus-type I, herpes simplex virus-type II, hepatitis A, hepatitis B, hepatitis C, vaccinia, varicella, herpes zoster, cytomegalovirus, Epstein Barr virus, influenza, mumps, measles, rhinovirus, rabies and rubella.

31. A composition according to claim 30, wherein the virus is a virus associated with a sexually transmitted disease and is selected from human immunodeficiency virus, herpes simplex virus-type I, herpes simplex virus-type II, cytomegalovirus, and Epstein Barr virus.

32. A spermicidal composition comprising a mixture of:

(a) a betaine selected from an alkyl-N-betaine, alkyl-N-sulfobetaine, acyl-N-betaine, an alkyl-N-substituted aminopropionic acid and an alkylimidazolinium betaine, or a mixture of two or more thereof;

(b) an amine oxide selected from an alkyl-N,N-dimethylamine oxide, alkyl-N,N-dihydroxyethylamine oxide, acylamide t-amine oxide, or a mixture of two or more thereof; and

(c) acid in an amount to provide the composition with a pH of 4 - 8 as measured when the betaine and amine oxide together comprise 0.5 weight percent of the composition.

33. A composition according to any one of claims 28 to 32, wherein the composition comprises an alkyl-N-di-(lower alkyl) betaine and an alkyl N-di-(lower alkyl) oxide, where the lower alkyl is C₁-C₃.

34. A composition according to any one of claims 28 to 33, wherein the composition comprises:

(a) an alkyl dimethyl betaine;

(b) an alkyl dimethyl amine oxide; and

(c) acid in an amount sufficient to adjust the pH of the medicine or composition from 4 - 8 when measured in an aqueous solution of components (a) and (b) in an acceptable carrier.

35. A composition according to any one of claims 28 to 34, wherein the composition comprises at least one alkyl dimethyl betaine and at least one alkyl dimethyl amine oxide.

36. A composition according to any one of claims 28 to 34, wherein the betaine is cocobetaine or lauryl-betaine, and the amine oxide is cocoamine oxide or lauramine oxide.

37. A composition according to any one of claim 36, wherein the betaine is a cocobetaine and the amine oxide is a cocamine oxide.

38. A composition according to any one of claims 28 to 37, wherein the betaine and the amine oxide are present in a molar ratio of from 1:5 to 5:1.

39. A composition according to any one of claims 28 to 38, wherein the composition has a molar ratio of betaine to amine oxide of 1:5 to 5:1, and the betaine and amine oxide together are present in the composition at a concentration of 0.01 weight percent to 40 weight percent.

40. A composition according to claim 39, wherein the composition has a molar ratio of betaine to amine oxide of 1:2 to 2:1, and the betaine and amine oxide together are present in the medicine or composition at a concentration of 0.03 weight percent to 30 weight percent.

41. A composition according to claim 40, wherein the medicine or composition has a molar ratio of betaine to amine oxide of 1:1, and the betaine and amine oxide together

are present in the medicine or composition at a concentration of 0.2 weight percent to 2 weight percent.

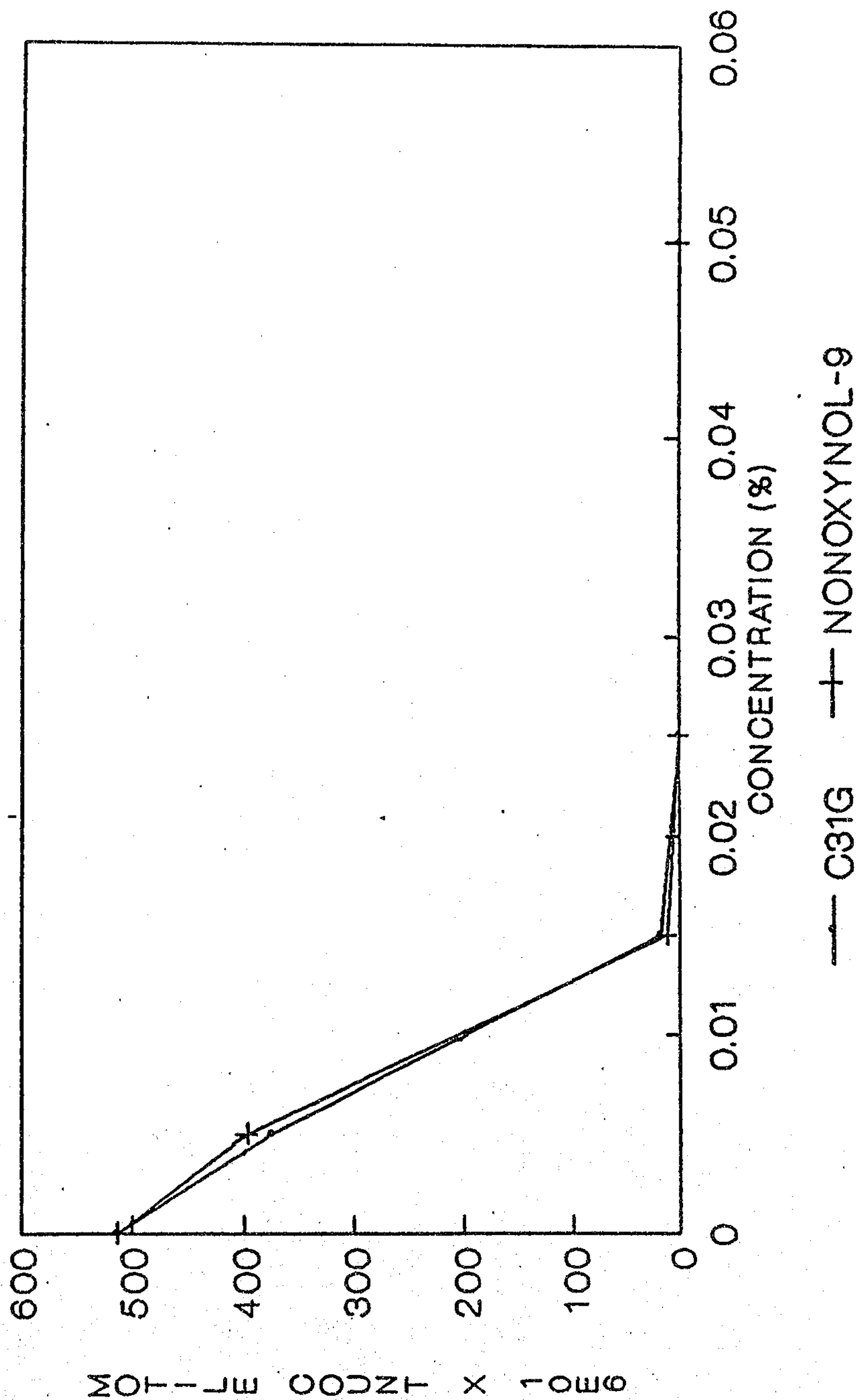
42. A composition according to any one of claims 15, wherein the composition is in a form selected from a foam, gel, cream, salve, jelly, lotion, liquid, spray, mist, aerosol and wipe.

43. A composition according to claim 42, wherein the composition is in a form selected from a foam, gel, cream and jelly.

44. A composition according to any one of claims 28 to 42, further comprising an acceptable diluent or carrier.

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FIG. 1
EFFECT ON SPERM MOTILITY

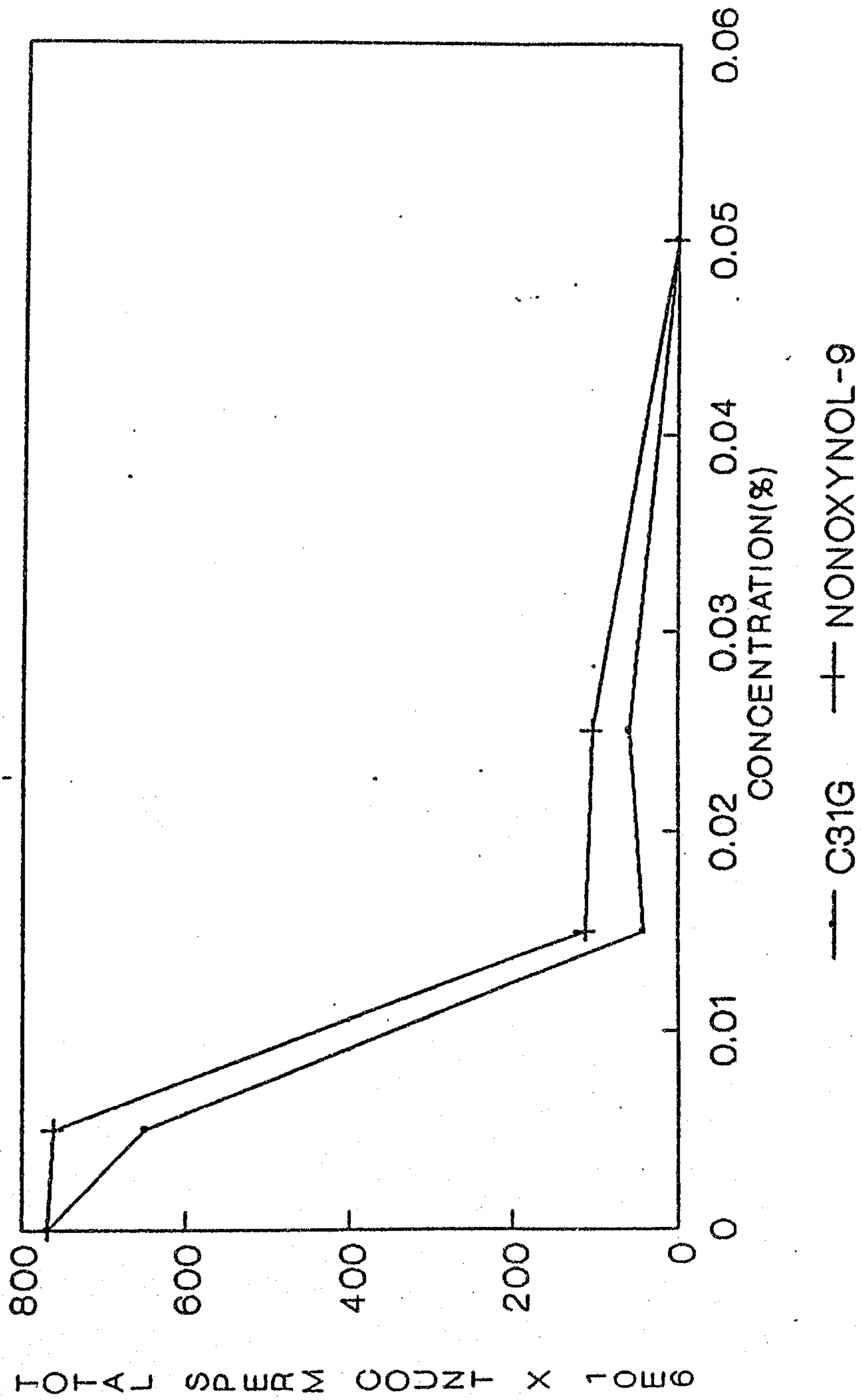


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FIG. 2
EFFECT ON TOTAL SPERM COUNT

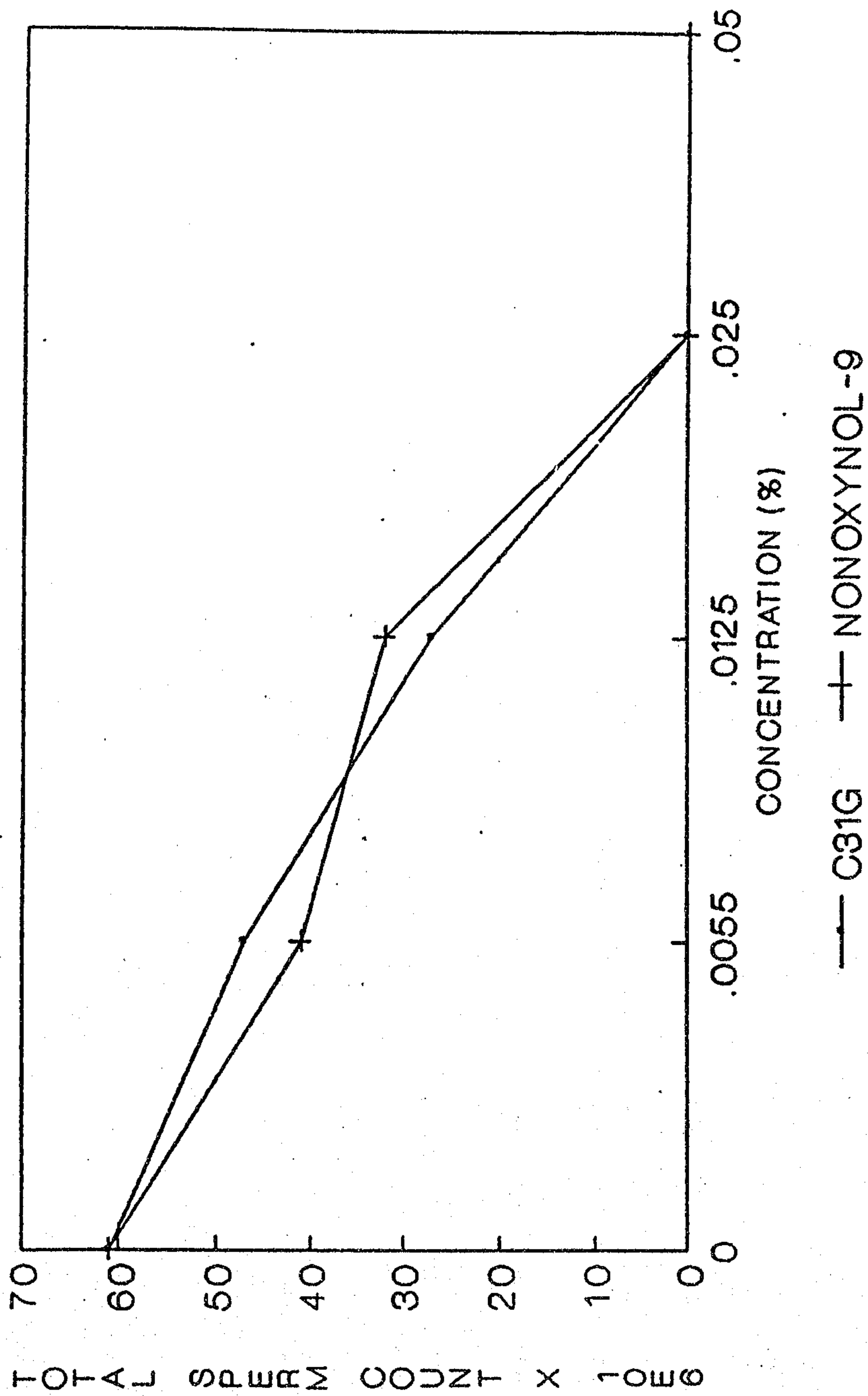


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FIG. 3

MEAN PROGRESSIVE VELOCITY MICRONS/SEC

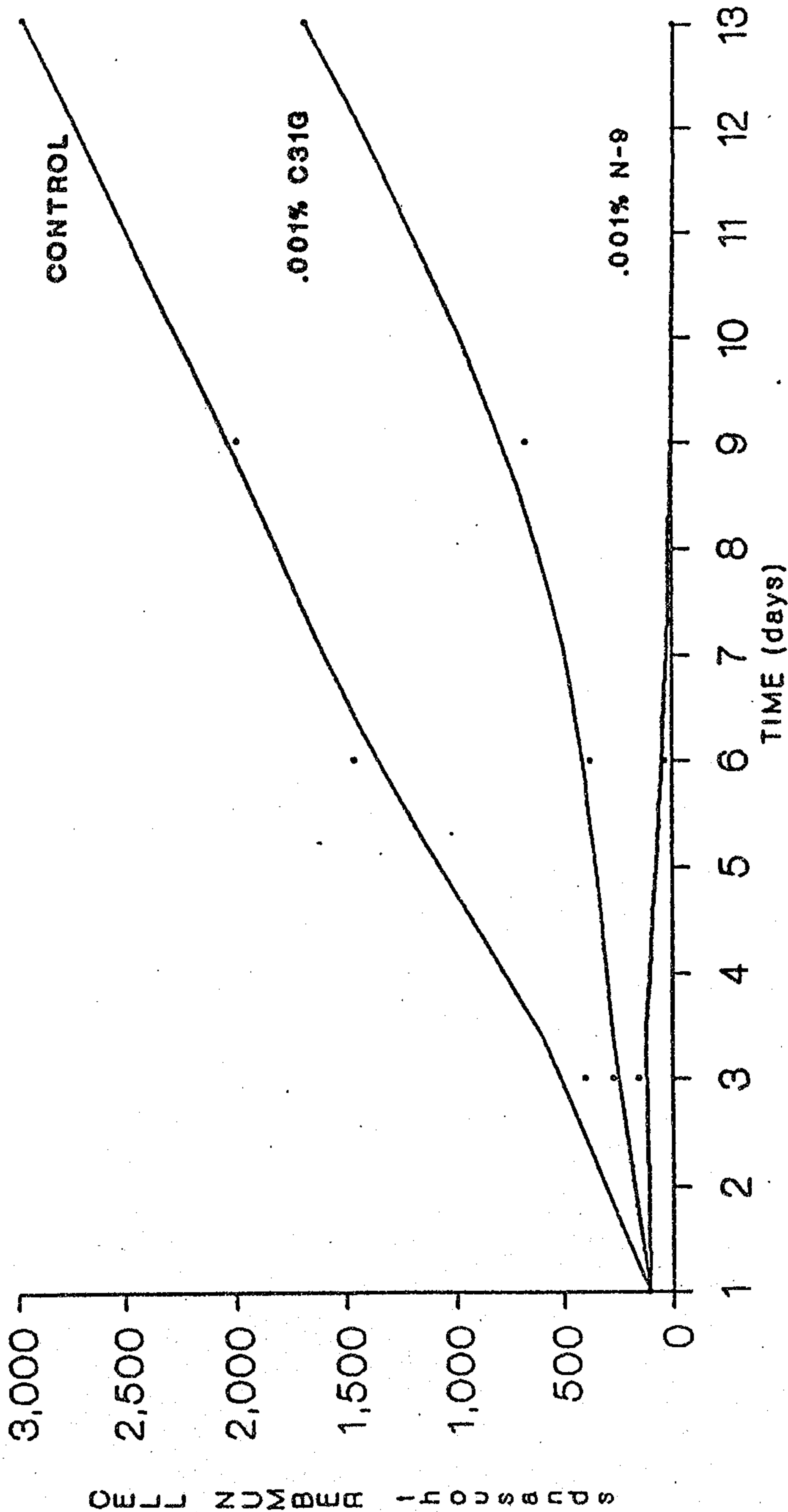


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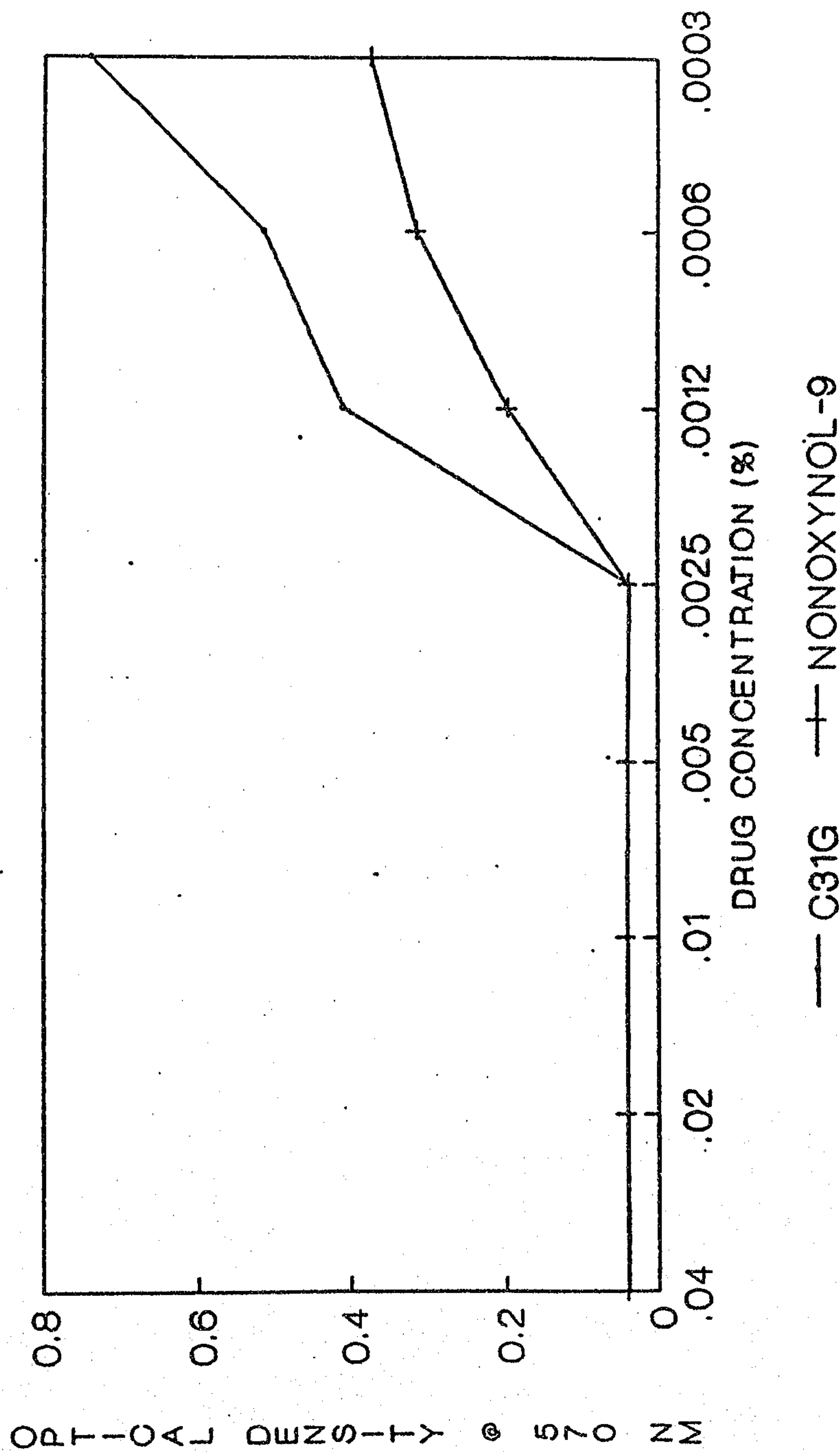
FIG. 4
IN VITRO TOXICITY OF C31G VS N-9



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FIG. 5
FIVE DAY TOXICITY STUDY: MTT

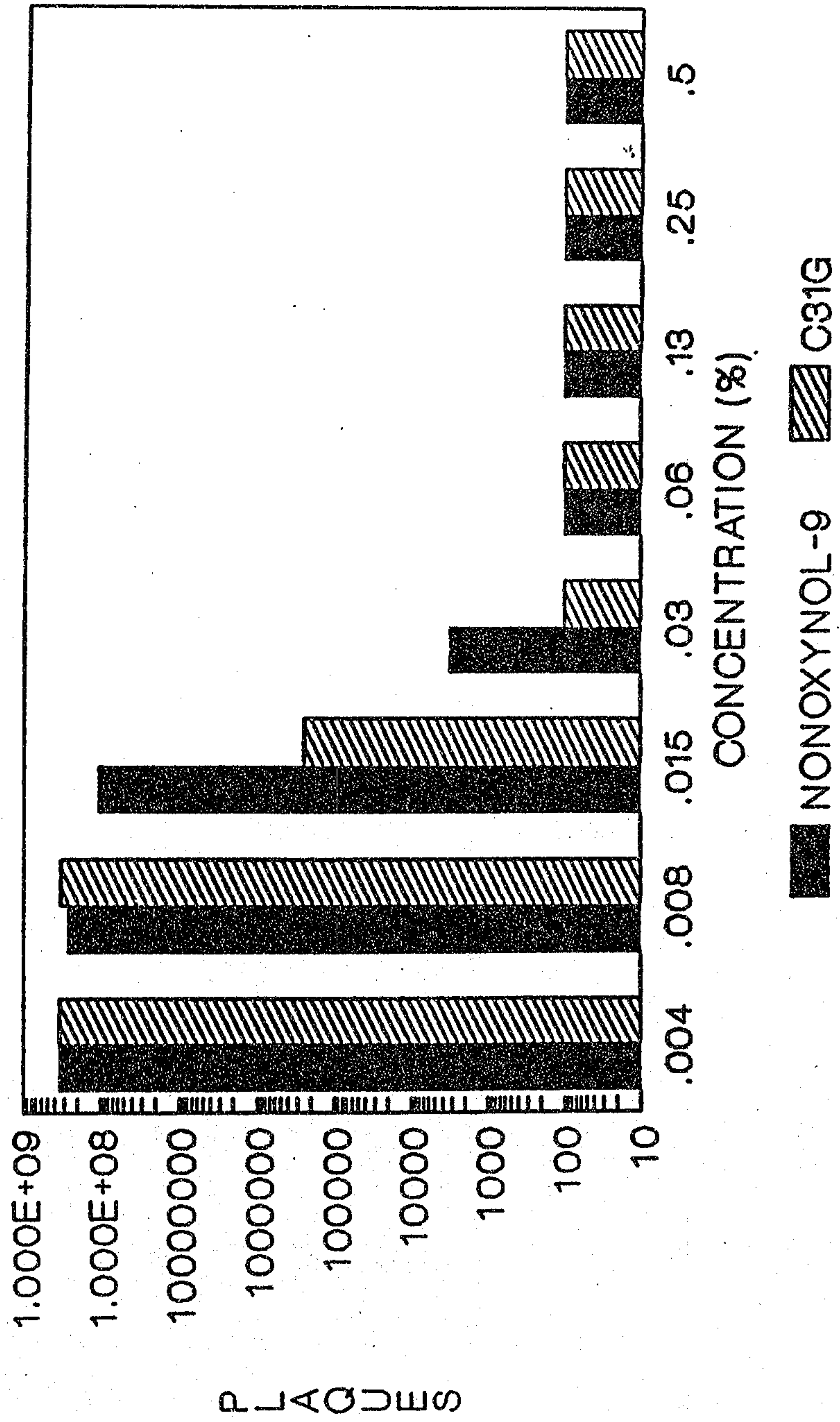


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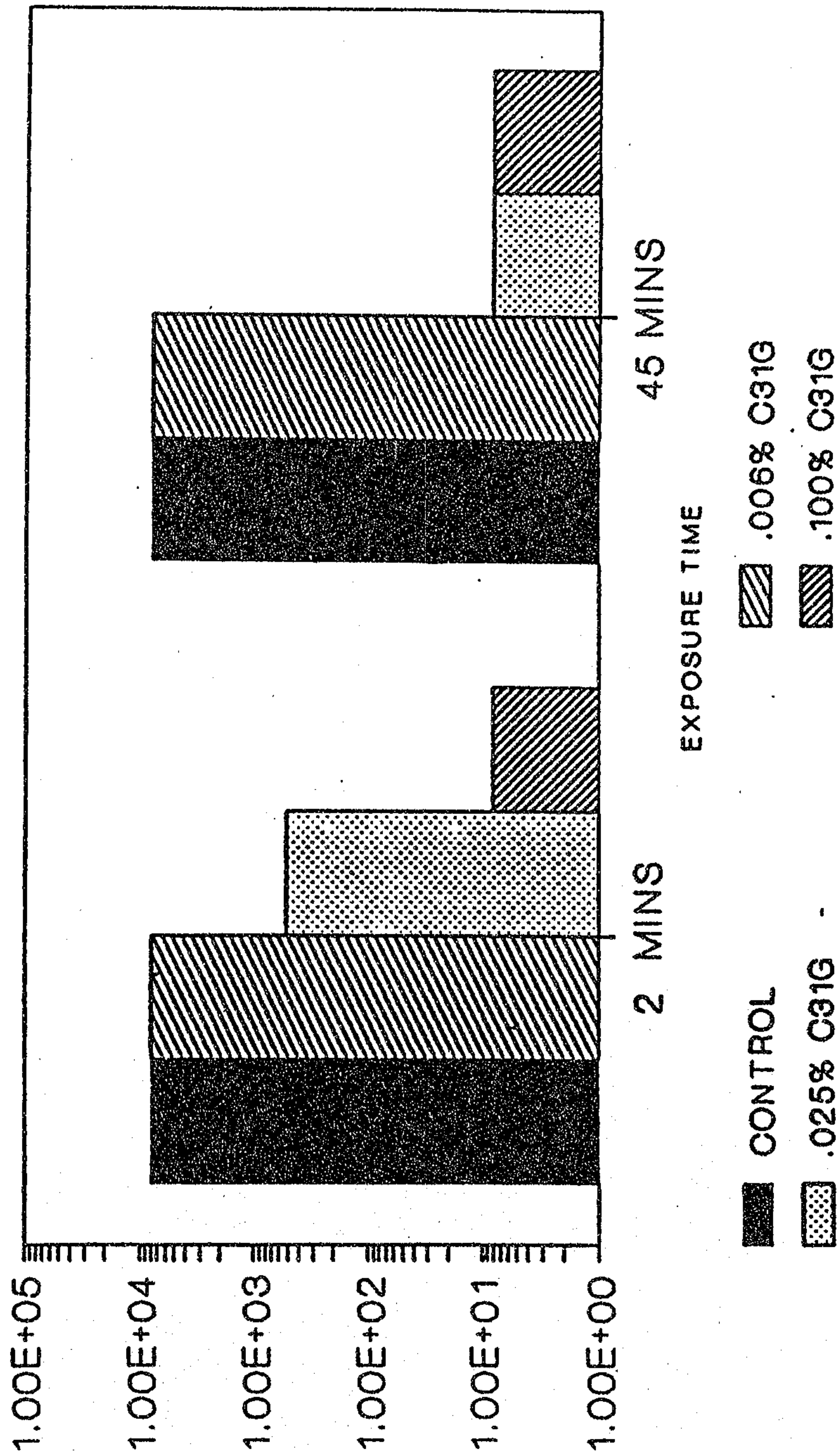
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FIG. 6
EFFECT OF C31G ON HSV



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FIG. 7
EFFECT OF C31G ON HIV

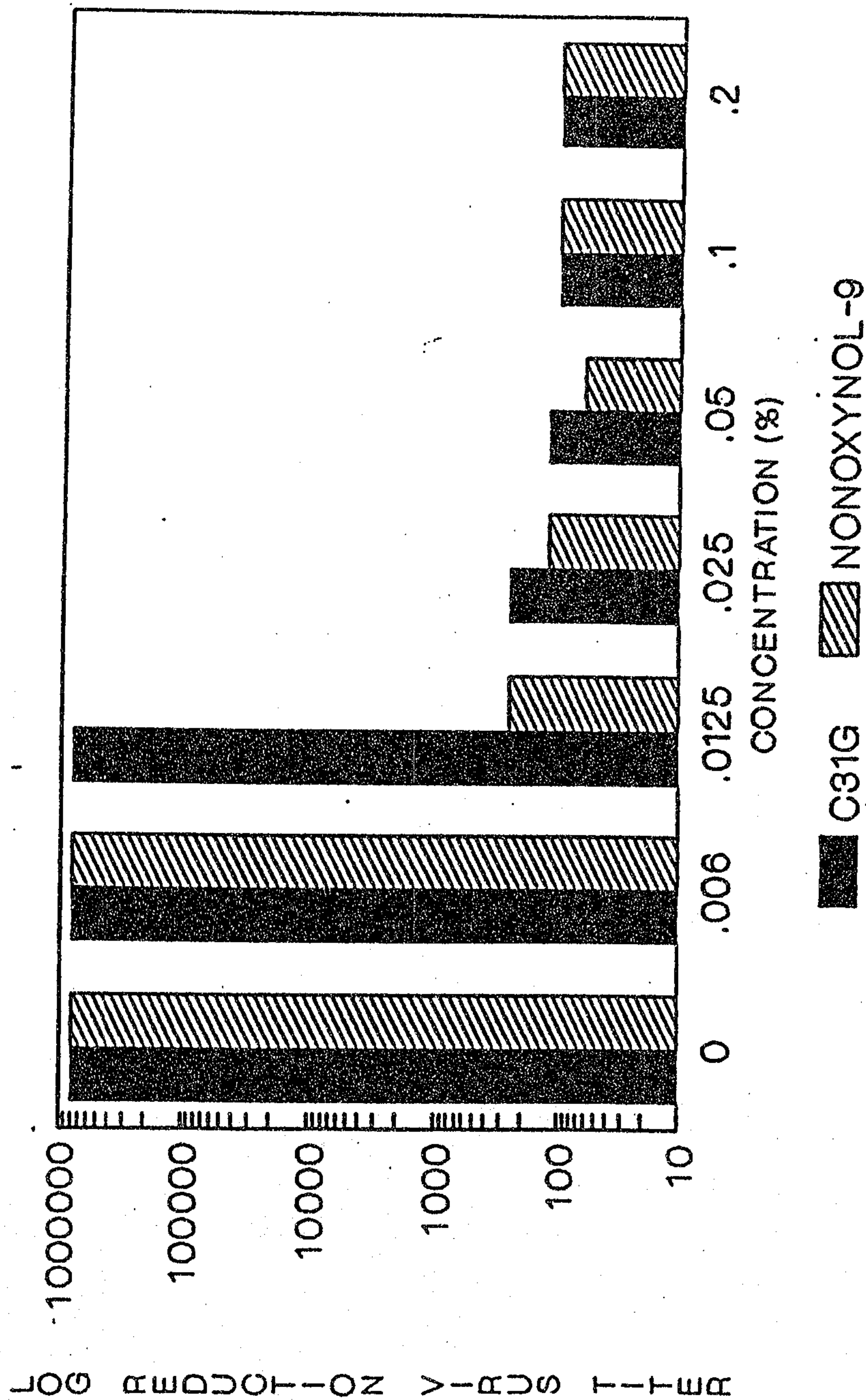


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FIG. 8
INACTIVATION OF HIV BY C31G OR N-9



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FIG. 9
INACTIVATION OF HIV BY C31G OR N-9

<u>CONCENTRATION (%)</u>	<u>P24 % REDUCTION</u>	
	C31G	N-9
.006	0	0
.012	20	85
.025	99	98
.05	99	98
.1	95	97
.2	100	100