



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>A61K 9/107, 31/05</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/10531</b> <b>(43) International Publication Date:</b> 2 March 2000 (02.03.00)
<b>(21) International Application Number:</b> PCT/US99/18801 <b>(22) International Filing Date:</b> 18 August 1999 (18.08.99) <b>(30) Priority Data:</b> 60/097,071 19 August 1998 (19.08.98) US <b>(71) Applicant:</b> RTP PHARMA INC. [CA/US]; 4364 South Alston Avenue, Durham, NC 27713-2280 (US). <b>(72) Inventors:</b> MISHRA, Awadhesh, K.; App. 201, 670 de Gaspé, Iles des Soeurs, Verdun, Québec H3E 1H8 (CA). PACE, Gary, W.; 6913 Hunters Way, Raleigh, NC 27615 (US). <b>(74) Agent:</b> CRAWFORD, Arthur, R.; Nixon & Vanderhye P.C., Suite 800, 1100 North Glebe Road, Arlington, VA 22201-4714 (US).	<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> INJECTABLE AQUEOUS DISPERSIONS OF PROPOFOL		
<b>(57) Abstract</b>		
<p>A stable, sterile, and injectable aqueous dispersion of a water-insoluble microdroplet matrix of mean diameter from about 50 nm to about 1000 nm consisting essentially of between about 1 % to about 15 % of propofol; between about 1 % to about 8 % of a propofol soluble diluent; between about 0.5 % to about 5 % of a surface stabilizing amphiphilic agent; of a pharmaceutically acceptable water-soluble polyhydroxy additive that acts as a tonicity modifier; and provided the ratio of propofol to diluent is about 1:4 to about 1:0.1 and the ratio of propofol to amphiphilic agent is about 1:0.8 to about 1:2.5, and the composition has a viscosity of from about 0.8 to about 15 centipoise.</p>		

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## INJECTABLE AQUEOUS DISPERSIONS OF PROPOFOL

This invention relates to compositions of propofol (2,6-diisopropylphenol) which have a low lipid content and which can be terminally steam sterilized. These formulations can be used as anesthetic agents in which the potential for microbial growth is either very low or eliminated. The low lipid content of these formulations provides for a low or non-existent risk of incidence of hyperlipidemia. In addition these formulations cause little or no irritation around the site of injection.

**BACKGROUND****Prior Art**

Propofol formulations have been used as anesthetic agents. Compositions of propofol and their clinical usage have been described in the scientific literature. In a series of patents Glen and James describe compositions containing propofol suitable for parenteral administration to produce anesthesia in warm-blooded animals as described in US Patent 4,056,635 (1977); US Patent 4,452,817 (1984); and US Patent 4,798,846 (1989).

The compositions described by Glen and James in US Patent 4,056,635 and 4,452,817 are mixtures of propofol with surfactants such as Cremophor-RH40 or Cremophor-EL or Tween-80, in aqueous medium that may also contain ethanol or other pharmaceutically acceptable ingredients.

In a continuation of patent US 4,452,817 Glen and James describe propofol compositions containing 1% to 2% propofol either alone or dissolved in oil such as

arachis oil or ethyl oleate (US Patent 4,798,846). These formulations were claimed to be stabilized with sufficient amount of surfactants selected from polyoxyethylene laurate, stearate, or oleate, a condensation product of ethylene oxide with castor oil, a polyoxyethylene cetyl, lauryl, stearyl or oleyl ether, a polyoxyethylene sorbitan monolaurate, monopalmitate, monostearate, or monooleate, a polyoxyethylene-polyoxypropylene block copolymer, a lecithin and a sorbitan monolaurate, monopalmitate, monostearate, or monooleate.

Based on the above patents a propofol preparation for clinical use (PDR 1996) has been commercially available (Diprivan® 1% Injection) which contains propofol dissolved in soybean oil and is stabilized with egg lecithin. Each milliliter of this formulation consists of 10 mg/mL of propofol, 100 mg/mL of soybean oil, 22.5 mg/mL of glycerol, 12mg/mL of egg lecithin, sodium hydroxide to adjust pH within 7 to 8.5 and sufficient quantity of water. Although clinically useful, this formulation requires the use of strict aseptic techniques during its handling due to the absence of antimicrobial preservatives and concomitant potential of microorganism growth. Indeed, many incidences of serious infection in human subjects have been linked to the use of the commercially available propofol formulation, Diprivan® (Nichols *et al.* (1995), Tessler *et al.* (1992), Ardulno *et al.* (1991), Sosis and Braverman (1993), Sosis *et al.* (1995), Crowther *et al.* (1996)).

In order to minimize the chances of infection arising from the handling of the formulations of propofol during intravenous administration Jones and Platt have recently introduced a new propofol formulation, essentially based on the earlier composition with the added component of an antimicrobial preservative. This product is described by US patents 5,714,520; 5,731,355; and 5,731,356. The antimicrobial preservative that is added

to the new formulation is disodium edetate. In US patent #5,714,520 it is claimed that addition of an amount of edetate limits bacterial growth to no more than a 10-fold increase as determined by the growth of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of said composition at approximately 50 colony forming units (CFU) per mL, at a temperature in the range 20-25°C, whereafter said aliquots are incubated at 20-25°C and are tested for viable counts of said organism after 24 hours, said amount of edetate being no more than 0.1% by weight of said composition.

However, regardless of the presence of edetate as a preservative against growth of microorganisms, the product under US patent 5,714,520 (Diprivan®) is not considered an antimicrobially preserved product under USP standards by some authors, for instance, Sklar (1997). While in the quantity that is present, edetate may be effective against the growth of some types of organisms that are claimed in the said patent, it may not be so effective against a variety of other organisms that may be prevalent in the clinical situations where propofol is administered such as for example, *C. albicans* ATCC 10231 as noted in patent 5,714,520. Indeed, it was noted in patent 5,714,520 that the formulated propofol was not bactericidal against *C. albicans* ATCC 10231 where an approximately 10-fold growth in the inoculum concentration was observed after 48 hours. This result points to the possibility of ineffectiveness of edetate as a preservative against growth of microorganisms in Diprivan® formulation if challenged by other organisms than those cited above or by a higher load of organisms exceeding 100 CFU/mL. Indeed the addition of edetate to the formulation provides little in the way of real improvement. This "improved" formulation continues to be inferior, with respect to antibacterial effectiveness, to the invention described in the Haynes patent (US 5,637,625, see below).

The formulation based on patents US 5,714,520; US 5,731,355; and US 5,731,356 still consists of a high amount of soybean oil (10%) that has been implicated in causing hyperlipidemia in some patients. Apart from the addition of edetate, this formulation is essentially the same as the previously commercialized Diprivan® formulation. In fact it has the same incidence of adverse effects as the previous product as evidenced by the quoted incidence rates for these symptoms in the current PDR, 1999.

#### **Problems in the Clinical Use of Commercial Propofol Formulations**

Many authors have reviewed the clinical usage of propofol formulations. For instance, Smith *et al.* (1994) describe that propofol injection has been used for producing and maintenance of ambulatory anesthesia, neurosurgical and pediatric anesthesia, for monitored anesthesia care, for intensive care sedation, and other clinical situations. Pain after injection of commercial formulations of propofol has been reported to occur in 28-90% of patients e.g., see reports by Mirakhur (1988), Stark *et al.* (1985), Mangar and Holak (1992). Even with low dose propofol administered for sedation, the incidence of pain can be 33-50%. (White and Negus, 1991; Ghouri *et al.* 1994). The mechanism responsible for the venous pain on propofol administration is unknown. The original excipient, Cremophor EL, of the earlier propofol preparation was initially thought to be the causative agent. However, there was no measurable reduction in pain after the change from the Cremophor EL based propofol formulation to the marketed soybean oil and lecithin based formulation (e.g., see Mirakhur (1988), Stark *et al.* (1985), Mangar and Holak (1992). White and Negus, 1991; Ghouri *et al.* 1994). It is believed that the pain is a function of the drug itself, rather than the formulation (Smith *et al.* (1994)).

To decrease the propensity of pain on injection of propofol formulations, Babl *et al.* (1995) have reported the use of 1% and 2% propofol preparations with a mixture of medium-chain triglyceride (MCT) and long-chain triglyceride (LCT) in the dispersed oil phase. Similarly, Doenicke *et al.* (1996, 1997) have demonstrated in human volunteers that use of MCT in the propofol formulation resulted in fewer incidence of severe or moderate pain on injection (9%) compared to that after injection of commercial formulation (59%). These authors have attributed the lower incidence of pain as result of a lower aqueous phase concentration of free propofol that was achieved by increasing the oil concentration in the formulation.

Although increasing the amount of oil may aid in lowering the aqueous propofol concentration and thereby reducing pain on injection, the oil level of as high as 20% used by these authors (Babl *et al.* 1995, and Doenicke *et al.* 1996, and 1997) is likely to further compromise patients requiring prolonged administration of propofol in intensive care units, potentially leading to hyperlipidemia.

While pain on injection may or may not be related to the injection-site tissue-irritation or the thrombogenicity of the administered formulation, these adverse reactions are still prevalent and symptoms continue to be reported in the clinical use of propofol. For instance, in the case of Diprivan<sup>®</sup>, these symptoms span the range of thrombosis and phlebitis and include up to 17.6% incidences of burning/stinging or pain (PDR 1999, p. 3416).

Clearly the need still exists for a clinically acceptable propofol formulation that can satisfy the three most often cited shortcomings of currently marketed and previous experimental formulations; *viz.*,

- growth of microorganisms,
- excessive lipid content, and
- irritation at the site of injection and/or pain on injection.

Alternative propofol formulations, that addressed some of the above-mentioned clinical problems associated with the commercial (Diprivan®) or experimental (*e.g.*, those described by Babl *et al.* 1995, and Doenicke *et al.* 1996, and 1997) propofol injectable products, have been taught by Haynes in the US patent 5,637,625. For instance, Haynes has recognized two problems associated with the use of large quantities of vegetable oil in a commercial formulation consisting of 1% propofol and 10% soybean oil:

- (1) hyperlipidemia in patients undergoing long-term sedation in the intensive care unit (ICU), and
- (2) the risk of bacterial contamination secondary to the high lipid content and lack of antimicrobial preservatives.

Haynes described the formulations of phospholipid coated microdroplets of propofol devoid of fats and triglycerides that provide anesthesia and chronic sedation over extended periods of time without fat overload. Prior to Haynes' teachings no oil based propofol formulations were claimed that contained less than 10% (w/w) oil vehicle. Haynes claimed that these microdroplet formulations are bactericidal (*e.g.* self-sterilizing) because of being free of the material that may support bacterial growth, and thus having extended shelf life.

Considering the observations cited in the clinical literature of propofol, particularly those mentioned above, it appears that Haynes has been able to address two



of the three shortcomings, however, there is still a need for a sterile propofol preparation that can be administered as a bolus intravenous injection or could be given as an infusion, *e.g.*, in the ICU and that possesses particularly all of the following characteristics:

- does not have excessive amount of oils or triglyceride in order to reduce the propensity of a patient to fall victim to hyperlipidemia,
- has sufficient bactericidal or bacteriostatic property so as to provide enhanced patient safety and extended shelf life during use in a clinical setting, and
- causes little or no tissue-irritation at the site of injection.

## DESCRIPTION OF THE INVENTION

### Summary of the Invention

Surprisingly it was found that certain propofol compositions, prepared as an injectable aqueous dispersion of a water-insoluble matrix consisting of propofol and propofol-soluble agents, were capable of substantially limiting or inhibiting the growth of certain microorganisms and did not display the incidence of irritation at the injection site as evidenced by the *in-vivo* experiments.

It was yet another surprising finding that the property of inhibition of microorganism growth in this formulation did not require the addition of any antimicrobial preservative agents.

Even more surprising was the fact that the said aqueous dispersion of propofol could be prepared as a terminally steam sterilizable and stable product containing various

polyhydroxy compounds in its aqueous phase. These polyhydroxy compounds are commonly used in intravenous infusion. It was found that propofol formulations made with the polyhydroxy compounds provided compositions of relatively higher viscosity.

It is also believed that owing to the reduced lipid content, these novel formulations would be much less prone to cause hyperlipidemia in human subjects administered IV formulations of this invention. Additionally, mixtures of LCT and MCT are known to undergo faster metabolic clearance and therefore their use in the propofol formulations of this invention may be clinically advantageous (Cairns *et al.*, 1996; Sandstrom *et al.*, 1995). Accordingly, mixtures of LCT and MCT are one preferred embodiment of the present invention.

Furthermore, the feasibility of formulating very high potency propofol compositions, containing for example 10% w/w propofol, is demonstrated in this invention.

### Composition

The novel compositions described in this invention consist of a nanometer to micrometer size water-insoluble matrix containing up to about 15%, or preferably up to 10%, propofol dispersed in an aqueous phase, comprised as follows:

*The water-insoluble matrix* consists of the anesthetic propofol with lipophilic agents dissolved to adjust the level of anti-microbial activity and the degree of local reaction on injection. Examples of such lipophilic agents include but are not limited to either one or more selected from saturated or unsaturated fatty acid esters such as isopropyl myristate, cholesteryl oleate, ethyl oleate, squalene, squalane, alpha-tocopherol

and/or derivatives of alpha-tocopherol, esters or triglycerides of either medium chain and/or long chain fatty acids of synthetic or natural origin. The natural triglycerides can be selected particularly from the vegetable or animal sources, e.g., pharmaceutically acceptable vegetable oils or fish oils. The latter are also known as omega-3 polyunsaturated oils. The lipophilic agents may also be considered propofol-soluble agents or diluents.

At the surface of the water-insoluble matrix are amphiphilic agents that stabilize the dispersion and are of possible importance in affecting the degree of local reaction on injection. Examples of such amphiphilic agents include charged or uncharged phospholipids of natural sources, e.g., egg or soy lecithin, or hydrogenated lecithin (e.g., phospholipon-90H or phospholipon-100H from Nattermann), or synthetic phospholipids such as phosphatidylcholines or phosphatidylglycerols, pharmaceutically acceptable non-ionic surfactants such as poloxamers (pluronic series of surfactants), poloxamines (tetronic series of surfactants), polyoxyethylene sorbitan esters (e.g., Tween<sup>®</sup> series of surfactants), cholesterol, or other surface modifiers commonly used in pharmaceutical products, or combinations of these surface modifiers.

*The aqueous phase* consists substantially of a mixture of pharmaceutically acceptable polyhydroxy tonicity modifiers such as those commonly used in intravenous infusions, for example sucrose, dextrose, trehalose, mannitol, lactose, glycerol, etc. Preferably, the polyhydroxy compounds are in a quantity sufficient to render the final composition isotonic with blood or suitable for intravenous injection. In case the amount of these polyhydroxy compounds in the formulation is selected such that it is not isotonic with blood, it can be diluted with suitable diluent prior to injection to adjust the tonicity. The aqueous phase may additionally contain some amount of pH adjusting agents such as sodium hydroxide and/or pharmaceutically acceptable acids and/or related salts thereof.

Preferably, the pH is adjusted to be between about 9 to about 4, and more preferably between about 8 to 5. Pharmaceutically acceptable buffer systems may be utilised.

The compositions of the invention may optionally contain other pharmaceutically acceptable agents, for example other antimicrobial agents, local or long acting anesthetics, chelating agents or antioxidants. Examples of which include but are not limited to parabens or sulfite or edetate, lidocaine, or metabisulfite.

Preferably, the compositions of the invention are selected so as to be stable to terminal sterilization under pharmaceutically acceptable conditions.

It was found that propofol formulations made with the polyhydroxy compounds provided compositions of relatively high viscosity. The viscosity of these preparations is from about 1.5 to 8 centipoises and more preferably from about 4 to 6 centipoises. While not adhering to any particular theory, it is believed that such high viscosities may be partly responsible for minimizing the tissue-irritating effect of the formulation.

## METHOD

Propofol is a liquid that is very poorly soluble in water. To manufacture stable injectable propofol formulations with the desired anti-microbial properties, low lipid content and low injection site reactivity and with little or no phase separation of the propofol during mixing or storage, it was found necessary to not only select an appropriate composition of the formulation but also use appropriate processing conditions. Examples of suitable processing conditions are those which provide intense mechanical agitation or high sheer, see for example the procedures described by Haynes (US patent 5,637,625). The formulation is conveniently prepared by the initial preparation of a lipophilic phase and an aqueous phase which are then mixed, however those skilled in the art will appreciate that alternate approaches may be suitable and will

readily be able to determine such approaches. For example, the unit processes as described briefly in the following paragraphs have proven suitable.

### **Premix Preparation**

Propofol, other lipophilic agents, and amphiphilic agents were mixed to prepare the lipophilic phase. The dissolution process was accelerated by heating the mixture while mixing with a high-speed homogenizer. The aqueous phase was usually a mixture of polyhydroxy compounds in water and in some cases also contained well-dispersed phospholipid prepared using a high-speed homogenizer. The premix was prepared by adding the lipophilic phase to the aqueous phase under agitation with a high-speed homogenizer and the pH adjusted. All these operations were performed under a generally inert atmosphere, for example a nitrogen blanket, and the temperature was controlled to minimize oxidation.

### **Homogenization**

The dispersions of the water insoluble matrix in aqueous medium were prepared by either of several homogenization methods. For example, dispersions were prepared by high pressure homogenization of the premix *e.g.*, by utilizing a Rannie MINI-LAB, type 8.30H Homogenizer, APV Homogenizer Division, St. Paul, MN. Alternatively, the dispersions were made by microfluidization of the premix with a Microfluidizer M110EH (Microfluidics, Newton, MA). The temperature of the process-fluid rises rapidly because of homogenization at a high pressure. In some cases high-pressure homogenization at high temperatures (homogenizer inlet temperature above about 30°C) resulted in a dispersion with a tendency to suffer from phase separation. Therefore, the effluent of the homogenizer was cooled to maintain an acceptable temperature at the inlet of the homogenizer.

### **Packaging and Sterilization**

The aqueous dispersion prepared by one of the above processes was filled into glass vials to about 70-90% volume capacity, purged with a generally inert atmosphere, for example nitrogen, and sealed with compatible stoppers and seals. The packaged novel propofol formulations were found generally to be stable pharmaceutically acceptable steam sterilization cycles.

### **Rat Tail Vein Irritation Experiments**

The propofol formulations prepared using the method described above were tested for their ability to cause irritation to the venous tissues by intravenous injection to rats. Female Sprague-Dawley rats, approximately 11 to 12 weeks of age were purchased from Charles River, St. Constant, PQ. Following an acclimation period, rats that appeared healthy, and weighing between 200 and 250 grams, were used.

The formulation to be tested was administered as a single daily bolus injection for 2 days, *i.e.*, on Day 1 and Day 2. The injections were made over a period of approximately 30 seconds, in the caudal vein at a site located approximately 5 cm from the distal end of the tail. The propofol dose of 12.5 mg/kg was given on the basis of body weights determined on Day 1. Rats were observed daily on study Day 1 through Day 3 as follows.

#### **I. *General Observation:***

The animals were checked for general health/mortality and morbidity once daily for three consecutive days. Detailed clinical observations were recorded daily. The animals were observed for overt toxic effects following the intravenous dosing.

## II. *Tail vein irritation:*

The circumference of the rat's tail was measured at approximately 2.5 inches proximal to the animal's body prior to the administration of the test formulation. This measurement served as baseline value for assessing possible swelling of the tail upon intravenous administration of the formulation. On each study day, the treatment site was carefully examined to detect any reactions and the rat's tail circumference measured. Changes in the rat's tail circumference were evaluated by comparing Day 2 and Day 3 measurement to the baseline value obtained before administering the test articles.

### **Pharmacodynamic Indicators**

Each rat of the above experiment was observed during and after the injection. The time required for loss of consciousness (induction time) was recorded. The time to recover (righting response time), indicated by spontaneous attempts to stand up on four feet was also measured. The duration of anesthesia was measured as the difference between the time when righting response occurred minus the time when consciousness was lost.

### **Hemolysis potential**

*In-vitro* evaluation of the hemolytic influence of the preparations of this invention on human whole-blood was determined as a further guide to selecting formulations with a low tendency to produce irritation around the site of injection. The hemolytic potential of the formulation on blood was evaluated by the assay of erythrocyte cytoplasmic marker enzyme, lactate dehydrogenase (LDH). Measurement of erythrocyte cytoplasmic marker

enzyme, LDH, which escapes from the leaky or ruptured erythrocytes into the plasma compartment of the blood, is one of the commonly used quantitative assays described in literature for evaluation of hemolytic potential of injectable formulations (Stenz and Bauer, 1996). The blood was obtained from male or female Caucasian human volunteers of 18 to 65 years age and stabilized with sodium heparin. The test formulation was mixed with an equal volume of human whole blood and incubated at 37°C for about 1 hour. The mixture was then held at ambient temperature for 30 min. followed by centrifugation at 1500 rpm for 10 min. The level of LDH in the supernatant was determined by a standard procedure known to the scientists skilled in this art. As a guide for the present study, a preferred upper limit of acceptability was determined by measuring the LDH levels resulting from applying the hemolysis potential methodology to amiodarone hydrochloride, a compound known to result in vein irritation upon venous injection in clinical settings (PDR 1999, p. 3289). Amiodarone hydrochloride IV solution, tested at 50 mg/mL and after dilution with 5% aqueous dextrose to 1.8 mg/mL as instructed in the product monograph, resulted in LDH values of 8190 IU/L and 673 IU/L respectively.

### **Inhibition of Microorganisms**

The formulations described in the present inventions were tested for their ability to inhibit the growth of microorganisms that are potential source of most likely infections in the clinical situation. Growth of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739 and ATCC 8454), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16403) was measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of a formulation at approximately 1000 colony forming units (CFU) per mL, at a temperature in the range 20-25°C. The inoculated mixtures are incubated at 20-25°C. The viability of



the microorganisms in the inoculated formulation is determined by counting the colonies of said organism after 24 and 48 hours, 7 days and other suitable length of time.

### EXAMPLES

Examples of various formulations including those according to the invention are briefly summarized in the following examples. The *in-vivo* or *in-vitro* behavior of some specific compositions are also presented in these examples.

Unless otherwise specified, all parts and percentages reported herein are weight per unit weight (w/w), in which the weight in the denominator represents the total weight of the formulation. Diameters of dimensions are given in millimeters ( $\text{mm} = 10^{-3}$  meters), micrometers ( $\mu\text{m} = 10^{-6}$  meters), or nanometers ( $\text{nm} = 10^{-9}$  meters). Volumes are given in liters (L), milliliters ( $\text{mL} = 10^{-3}$  L) and microliters ( $\mu\text{L} = 10^{-6}$  L). Dilutions are by volume. All temperatures are reported in degrees Celsius. The compositions of the invention can comprise, consist essentially of or consist of the materials set forth and the process or method can comprise, consist essentially of or consist of the steps set forth with such materials.

The invention is further explained with reference to the following preferred embodiments and the undesirable compositions are also noted. The general procedure used for the examples have been mentioned above; exceptions are noted. The formulations were prepared by the method mentioned above. The raw materials used to prepare the formulations of this invention are summarized below:

Raw Material	Symbol	Source
1,2-Dimristoyl-sn-Glycero-3-Phosphocholine	DMPC	Avanti Polar Lipids Inc., Alabaster, AL, US
1,2-Dimristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)]	DMPG	Avanti Polar Lipids Inc., Alabaster, AL, US
Ethyl Oleate, NF	EO	Croda Leek Ltd, Staffordshire, UK
Glycerin, USP-FCC	GLY	J.T. Baker, Philipsburg, NJ, US
Lipoid E80 (egg lecithin)	E80	Lipoid GmbH, Ludwigshafen
Lipoid EPC (egg phosphatidylcholine)	EPC	Lipoid GmbH, Ludwigshafen
Lipoid SPC (soy phosphatidylcholine)	SPC	Lipoid GmbH, Ludwigshafen
Lipoid SPC-3 (saturated soy phosphatidylcholine)	SSPC	Lipoid GmbH, Ludwigshafen
Mannitol, USP	MAN	J.T. Baker, Philipsburg, NJ, US
Miglyol 810	M810	Hüls America, Piscatway, NJ, US
Propofol	PRO	Albemarle Corporation, Baton Rouge, LA, US
Soybean oil, USP	SO	Spectrum, New Brunswick, NJ, US
(D+) Alpha, alpha-Trehalose	TRE	Pfanzstiehl Laboratories Inc, Waukegan, IL, US

***Example 1: Effect of increasing oil content of the formulation***

Experiments of this example were performed to identify the formulation variables that are factors behind the desirable attributes.

Table I summarizes some examples of the propofol formulations and their attributes with increasing amount of oil. The oil concentration of these formulations was increased by increasing the amount of ethyl oleate from 0.4% to 10%. Propofol

concentration was kept at 1%. Amount of the phospholipid mixture (Lipoid E80 and DMPG) was adjusted with increasing amount of oil to obtain the formulations of good stability.

Rat-tail swelling, an indicator of the tissue-irritation propensity of the formulation (see above) was found to decrease with increasing amount of oil. Formulation #1.4-1.6 with 4-10% ethyl oleate appear to result in unnoticeable rat-tail swelling. This result parallels the reported finding (Babl et al. 1995, and Doenicke *et al.* 1996, and 1997) that the use of higher amounts of oil in propofol preparations reduces the incidence of pain on injection possibly by a reduction of aqueous concentration of propofol. However, these authors have used a much higher amount (20%) of MCT and LCT mixture in their propofol formulations, and such formulations are expected to support the growth of microorganisms.

**Table I: Effect of increasing oil content of the formulation**

Formulation ID	Propofol (% w/w)	Lipoid E80 (% w/w)	DMPG (% w/w)	Ethyl Oleate (% w/w)	Viscosity, cP	Rat Tail Swelling, at 48hr, mm	LDH (IU/L)
1.1	1	0.8	0.15	0.4	0.97	1.39	10918
1.2	1	0.8	0.10	1.0	1.08	0.6	10970
1.3	1	0.8	0.10	2.0	1.06	0.2	10300
1.4	1	1.0	0.25	4.0	1.04	0	3150
1.5	1	1.0	0.25	8.0	1.25	0	1290
1.6	1	1.0	0.25	10.0	1.34	0	770

Hemolytic potential of the formulations of Table I was evaluated as mentioned above, by measuring LDH activity in a sample of human blood mixed with an equivalent

amount of the formulation. The results summarized in Table I demonstrate that the hemolytic potential of the formulation decreases with increasing amount of ethyl oleate.

Although formulation #1.6 with 10% ethyl oleate may possess tolerable hemolytic and injection-site tissue-irritation potential, this formulation is far from satisfactory for the purpose of this invention as it contains a high amount of oil, i.e., ethyl oleate. The problems associated with currently marketed or experimental propofol formulations have been mentioned in the prior art. It has been recognized that a desirable propofol formulation for bolus intravenous injection or for infusion should possess all of the following characteristics simultaneously:

- the formulation does not have excessive amount of oils or triglyceride in order to reduce the propensity of a patient to fall victim to hyperlipidemia,
- the formulation causes little or no irritation at the site of injection, and
- has sufficient bactericidal or bacteriostatic property to provide enhanced patient safety and extended shelf life during use in a clinical setting.

Thus a better suited formulation will have an acceptable level of injection-site tissue-irritation potential but with much lower oil content than in the best formulation (#1.6) of this example. Many such formulations that fulfill these criteria are described in the following examples.

***Example 2: Rat-Tail Vein Irritation and Hemolytic Potential***

In this example are shown a number of formulations that were prepared according to the procedure mentioned above and demonstrate acceptable injection-site tissue-irritation as assessed by the rat-tail vein swelling experiments (see above). These formulations are summarized in Table II. A non-existent irritation potential is displayed by zero increase in the tail circumference upon caudal vein intravenous administration to rats, e.g., of formulation numbers 2.1 to 2.25.

Nevertheless, there were a number of compositions that caused an observable irritation of the tail vein, e.g., formulation numbers 2.26 to 2.29 as well as formulation 2.30 which is reproduced here as described by the Haynes patent (US patent 5,637,625).

In Example 1 it was observed that by increasing the amount of oil from 0.4% to 10% or greater in the formulation, the tissue-irritation potential could be decreased. However, Example 2 indicates that this simplistic notion is not without limitation since in some cases merely increasing the amount of oil in the propofol formulation does not result in a less irritating formula. For instance, in formulation 2.26 the oil level is increased to 6% of ethyl oleate and in 2.27 and 2.28 to 4% of Miglyol-810, but these formulations are still injection-site tissue-irritating, which is evident from the tail swelling values for these formulations.

While the formulations of 2.26-2.30 were irritating, it was surprising that many compositions containing oil level of only up to 4% were non-irritating. For instance, formulation 2.15, which contained as low as only 2% oil, was also a non-irritating preparation. This unexpected result indicates that the preferred compositions of these formulations are not self evident from traditional formulation approaches using linear factorial experimental design not able to reveal possible synergistic effects. Once having

identified an acceptable range for the compositional elements of the formulations which demonstrate acceptable properties, the selection of preferred embodiments is a matter of routine determination using the approaches described above.

An inspection of the data in Table II leads to a surprising finding that many of the compositions which had an acceptable low LDH level while maintaining no evidence of injection-site tissue-irritation, also had either mannitol or trehalose in their aqueous phase. It was further surprising that the viscosity of many of these compositions was greater than 1.2 centipoise and in many cases even greater than 3 centipoise. A high viscosity of these formulations may possibly render them safer with respect to their hemolytic potential.

As established in Example 1 and again here in Example 2, merely increasing the oil level in formulations did not result in decreasing the hemolytic potential, or irritation to the tissues at the site of injection. It appears that below a certain amount of oil (*e.g.*, <10%) the causative factors for improving the hemolytic potential or tissue irritation is a combination of various factors that originate from the specific composition. Thus, the non-irritating formula that also have a low potential of hemolysis are characterized by various formulation components that provide the co-operative effects rendering the preferred formulations less irritating.

### ***Example 3: Inhibition of Microorganisms***

Whether the formulations demonstrated the absence of thrombogenic irritation in rats or caused such irritation, all were examined for the microbicidal or microbistatic effectiveness as mentioned above of which some relevant results are summarized in Table III. Also presented in Table III are the microbicidal effectiveness test results for Diprivan® as a comparison.

There are many compositions that were found to inhibit the growth of microorganisms. Inhibition of microbial growth was determined by a reduction or maintenance in the number of colonies of the inoculated microorganisms. As examples, formulation numbers 2.1, 2.3, and 2.4 of Table II display all the required properties in concert; reduction in irritation potential (no swelling of the rat-tail vein), acceptable hemolytic potential (low LDH values) as well as inhibition of growth of the tested microorganisms (see Table III).

**Table II:** Compositions of some propofol formulations, rat tail vein irritation by these formulations and the hemolytic potential as measured by the lactate dehydrogenase (LDH) levels on incubating with human blood.

Formulation Number	Propofol	Phospholipids						Oil				Tonifier			Attributes	
		E80	EPL	EPC	SPC	SSPC	DMPC	DMPG	EO	SO	M810	Type	Qty	Tail Swelling at 48hr, mm	LDH (IU/L)	Viscosity, cP
2.1	2				2.0	0.5		0.05		4	MAN	5.5	0	179	5.31	
2.2	2				2.2			0.15	4		MAN	5.5	0	287	4.44	
2.3	2				2.0	0.5		0.05		4	MAN	5.5	0	107	5.24	
2.4	2			1.6				0.10		6	TRE	12.5	0	172	4.20	
2.5	2		1.6					0.05		4	MAN	5.5	0	183	1.32	
2.6	2		1.6							4	MAN	5.5	0	168	1.21	
2.7	2	3.0						0.15	4		TRE	20.0	0	185	1.91	
2.8	2				2.0	0.5		0.05	4		MAN	5.5	0	204	3.64	
2.9	2	2.4						0.15	4		MAN	7.5	0	380	1.39	
2.10	2	2.0						0.10		4	GLY	2.5	0	571	1.32	
2.11	2		1.6						4		MAN	7.5	0	604	1.21	
2.12	2	1.6						0.15		4	GLY	2.5	0	668	1.20	
2.13	2	1.6							4		MAN	7.5	0	942	1.20	
2.14	1	1.0						0.25	8		GLY	2.5	0	1290	1.25	
2.15	1						0.80	0.10		2	GLY	2.5	0	2049	1.18	
2.16	2		1.6							4	GLY	2.5	0	2197	1.08	
2.17	2		1.6					0.10		6	GLY	2.5	0	2700	1.23	
2.18	2	1.6		1.6				0.05	4		GLY	2.5	0	2826	1.17	
2.19	2		1.6					0.05		4	GLY	2.5	0	3650	1.17	



Table II: Continued.

Formulation Number	Propofol	Phospholipids						Oil			Tonifier		Attributes		Viscosity, cP		
		E80	EPL	EPC	SPC	SSPC	DMPC	DMPG	EO	SO	M810	Type	Qty	Tail Swelling at 48hr, mm		LDH (IU/L)	
2.20	2	1.6						0.10			4		GLY	2.5	0	5035	1.13
2.21	2	1.6						0.10	6				GLY	2.5	0	7565	1.33
2.22	1					1.0		0.10		6			GLY	2.5	0	7720	1.45
2.23	1					1.5		0.10		10			GLY	2.5	0	7940	2.78
2.24	2	1.6						0.10	4				GLY	2.5	0	8250	1.15
2.25	1					2.0		0.10		8			GLY	2.5	0	8710	2.25
2.26	1	1.0						0.25	6				GLY	2.5	0.2	7020	1.12
2.27	1					1.00		0.10		4			GLY	2.5	0.2	7460	1.49
2.28	2		1.6	1.6				0.05			4		GLY	2.5	0.4	4330	1.53
2.29	2	1.6						0.10			2		GLY	2.5	1.05	8765	1.01
2.30	1	0.8						0.15	0.4				GLY	2.5	0.8	10720	0.95

**Symbols and Note:**

DMPG: dimyristoylphosphatidylcholine; DMPC: dimyristoylphosphatidylglycerol; E80: Lipoid E80; EO: ethyl oleate; EPC: egg phosphatidylcholine; EPL: egg phospholipids; GLY: Glycerin; M810: Miglyol-810; MAN=Mannitol; SO: soybean oil; SPC: soy phosphatidylcholine; SSPC: saturated soy phosphatidylcholine; TRE=Trehalose. Sources of these raw materials are mentioned above.

**Table III:** Log growth of certain microorganisms following an initial inoculation of 10<sup>3</sup> CFU/mL in presence of some propofol formulations.

Formulation	Sample 2	Organism	C. albicans ATCC 10231			P. aeruginosa ATCC 9027			E. coli ATCC 8739			A. niger ATCC 16403			S. aureus ATCC 6538		
			24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day
2.1		91.103	2.8	2.7	2.1	1.5	1.0	1.0	1.7	1.6	1.0	2.8	2.8	2.7	2.4	1.0	1.0
2.3		61.103	2.8	2.3	1.3	1.0	1.0	1.0	1.0	1.0	1.0	2.0	2.1	2.0	1.0	1.0	1.0
2.4		76.103	2.7	2.7	2.5	2.3	1.3	1.0	2.5	2.1	1.0	2.9	2.8	2.7	2.2	1.0	1.0
2.5		81.103	3.0	3.9	6.0	2.4	6.0	6.8	4.8	6.8	6.8	2.8	2.6	2.5	3.3	3.2	1.3
2.6		80.103	2.9	3.4	5.8	1.0	1.0	1.0	4.4	6.8	6.8	2.8	2.6	2.6	3.0	2.5	1.0
2.11		72.103	3.1	4.0	5.8	2.3	5.9	6.8	5.1	6.8	6.8	2.9	2.7	2.6	3.2	3.1	1.0
2.13		50.103-A	4.2	5.0	5.3	2.0	3.7	6.8	1.0	1.0	1.0	2.5	2.4	2.1	2.0	1.0	1.0
		Diprivan	3.2	3.4	3.2	2.8	3.3	6.2	2.2	1.0	1.0	2.9	2.7	2.6	3.2	3.1	1.8

It was surprising to note that these compositions also had either mannitol or trehalose in their aqueous phase. It was further surprising that the viscosity of these compositions was as high as from about 4.2 to about 5.3 centipoise.

As taught by Haynes (US patent # 5,637,625) it may be thought that increasing the amount of lipidic nutrients in the formulation would cause the formulation to support microorganism growth. However, it is surprising to note that by increasing the amount of oil (to up to 4-6%), formulations 2.1, 2.3 or 2.4 do not provide a medium for bacterial growth. It is worth noting that formulations 2.1, 2.3, and 2.4 were neither irritating, nor hemolytic while also inhibiting the growth of microorganisms. These non-irritating, non-hemolytic, and bactericidal or bacteristatic formulations are characterized as non-limiting examples of preferred compositions of this invention.

***Example 4: High Potency Propofol Formulations***

High potency propofol formulations, 4.1-4.3 in Table IV, were prepared by the methods described above. These formulations were found to be terminally steam sterilizable without destabilization.

**Table IV: Propofol formulations of high drug potency**

	<u>Formula 4.1</u>	<u>Formula 4.2</u>	<u>Formula 4.3</u>
Propofol	5.0%	10.0%	10.0%
Cholesterol	0.25%	0.4%	0.5%
Cholesteryl oleate	---	4.0%	3.0%
Phospholipon 90H	1.5%	1.8%	1.5%
DMPG	0.3%	0.3%	0.15%
Glycerol	2.5%	2.5%	2.5%
Sodium hydroxide	qs pH 6.9	qs pH 8.2	qs pH 7.0
Water	qs 100%	qs 100%	qs 100%

These high-potency formulations have been found to be very stable and use pharmaceutically acceptable ingredients without altering the efficacy of the drug. For instance, upon intravenous administration to rats of a dose at 10mg/kg, formulation 4.1 demonstrated acceptable efficacy of general anesthesia.

The formulation 4.2 demonstrates a homogeneous propofol dispersion in aqueous vehicle of 2.5% glycerol. It has as high as 10% propofol, while maintaining a very low fat (cholesterol and cholesteryl oleate) content. It has a volume weighted mean particle size of 82 nm that did not change significantly upon being subjected to various stresses such as freeze/thaw, (128 nm after 3 cycles).

The formula 4.3 is also a very homogeneous dispersion in aqueous vehicle of 2.5% glycerol and consists of 10% propofol while maintaining a very low fat content. It has a volume weighted mean particle size of 80 nm that did not change significantly upon storage at 25°C (71 nm after 70 days).

The high potency formulation (*e.g.*, 10% propofol) would be useful in achieving a much lower volume for intravenous administration while giving the same effective dose. Therefore, the formulations described in this example will allow a relatively smaller contact area of the blood vessel wall with the formulation and may be important with respect to minimizing the incidences of pain or other local adverse reaction on injection.

Such high potency stable formulations of propofol have been prepared and described here for the first time.

**Example 5: Pharmacodynamics**

Propofol formulations of this invention were compared for induction and duration of anesthesia in rats with the reference commercial formulation, Diprivan® (1%) and Disoprivan® (2%). Following 12.5 mg/kg single bolus intravenous injection of these formulations in rats, the time for loss of consciousness and righting response time were measured as mentioned above in the experimental method section. The results are summarized in Table V illustrating the efficacious characteristic of these formulations.

**Table V: Pharmacodynamic Parameters**

Formulation ID	Number of Rats	Average Anesthesia Induction Time (sec)	Average Righting Response Time (min)
2.1	9	24.4	14.9
2.2	4	31.0	16.2
2.3	4	48.0	16.4
2.4	9	32.7	15.8
2.5	9	27.2	19.2
2.6	9	38.4	19.4
2.7	4	24.0	17.3
2.8	4	23.8	16.7
2.9	3	67.3	11.9
2.10	4	34.8	16.3
2.11	8	40.5	18.6
2.12	4	36.3	13.7
Diprivan® (1% with EDTA)	4	20.0	14.6

According to the examples given above, the present invention provides for the identification of propofol formulations that are:

- (a) stable during and after steam sterilization,
- (b) give the required anesthetic effect upon intravenous injection to warm blooded animals,
- (c) inhibit the growth of microorganisms,
- (d) have demonstrated minimum or non-existent incidences of local vein reaction, and
- (e) have a potential of minimum or non-existent incidence of hyperlipidemia.

While the invention and the examples have been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the following claims.

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**What is claimed is:**

1. A stable, sterile, and injectable aqueous dispersion of a water-insoluble microdroplet matrix of mean diameter from about 50 nm to about 1000 nm consisting essentially of
  - (a) between about 1% to about 15% of propofol;
  - (b) between about 1% to about 8% of a propofol soluble diluent;
  - (c) between about 0.5% to about 5% of a surface stabilizing amphiphilic agent;
  - (d) of a pharmaceutically acceptable water-soluble polyhydroxy additive that acts as a tonicity modifier; and
  - (e) provided the ratio of propofol to diluent is about 1:4 to about 1:0.1 and the ratio of propofol to amphiphilic agent is about 1:0.8 to about 1:2.5, and the composition has a viscosity of from about 0.8 to about 15 centipoise, wherein the formulation
    - prevents microbial growth, defined as no more than 0.5 log increase from the initial inoculum, of each of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739 and ATCC 8454), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16403) for at least 7 days as measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of a formulation at approximately 1000 colony forming units (cfu) per mL, at a temperature in the range 20-25°C, whereafter said aliquots are incubated at 20-25°C and are tested for viability of the microorganisms in the inoculated formulation as determined by counting the colonies of

said organism after 24, 48 hours and 7 days or other suitable length of time;

- results in little or no irritation at the site of injection as evidenced by a test wherein said composition is administered as a single daily bolus injection of 12.5 mg/kg, given on the basis of body weight, for 2 or 3 successive days over a period of approximately 30 seconds, in the caudal vein of a rat such that no visual increase in the diameter of the rat tail is noted after 48hrs post injection.
2. The composition of claim 1 wherein the surface stabilizing amphiphilic agent is one or more natural or synthetic surface modifiers selected from ionizable or non-ionizable phospholipids or cholesterol or a mixture of these amphiphilic agents, the total quantity of the amphiphilic agents being such that the ratio of propofol to amphiphilic agent is about 1:0.8 to about 1:2.5 and the types and quantities of the individual amphiphilic agents are selected to provide that:
    - (i) the composition has a non-existent or minimum potential for hemolysis of human or animal blood, and
    - (ii) the irritation to the tissues at the site of injection is either non-existent or minimized, and
    - (iii) the composition elicits an anesthetic effect in warm-blooded animal and human subjects upon intravenous administration.
  3. The composition of claim 1 wherein the propofol-soluble diluent is a synthetic or natural fatty acid, triglyceride thereof or other suitable ester or a mixture thereof.
  4. The composition of claim 1 wherein the ratio of propofol to the amount of propofol-soluble diluent is from about 1:3 to about 1:0.5.

5. The composition of claim 1 wherein the ratio of propofol to the amount of propofol-soluble diluent is from about 1:2 to about 1:1.
6. The composition of claim 1 wherein the propofol-soluble diluent is a mixture of medium-chain triglyceride and vegetable oil.
7. The composition of claim 6 wherein the ratio of medium-chain triglyceride to vegetable oil is from 1:3 to 3:1.
8. The composition of claim 1 wherein the water-insoluble matrix consists of a mixture of the amphiphilic agents of claim 3 and propofol-soluble diluents of claims 4-7 and propofol.
9. The composition of claim 1 wherein the composition contains about 2% to about 10% of propofol.
10. The composition of claim 1 wherein the composition contains a pharmaceutically acceptable water-soluble polyhydroxy additive that provides the propofol containing dispersion with an osmolality of about 250 to about 700 milliosmolal.
11. The composition of claim 1 wherein the osmolality is about 300 to about 500 milliosmolal.
12. The composition of claim 1 wherein the viscosity is from about 2 to about 5 centipoise.

13. The method of reducing or substantially completely eliminating irritation upon injection of formulations containing propofol by administering a stable, sterile, and antimicrobial, aqueous dispersion of water-insoluble microdroplet matrix of mean diameter from about 50 nm to about 1000 nm consisting essentially of about 1% to about 15% of propofol as the active ingredient, up to about 7% of a propofol soluble diluent, and about 0.8% to about 4% of a surface stabilizing amphiphilic agent, and the aqueous phase of the composition consisting of a pharmaceutically acceptable water-soluble polyhydroxy tonicity modifier, the composition being devoid of additional bactericidal or bacteriostatic preservative agents, provided the ratio of propofol to diluent is about 1:4 to about 1:0.1 and the ratio of propofol to amphiphilic agent is about 1:0.8 to about 1:2.5, and the composition has a viscosity of from about 0.8 to about 15 centipoise.
14. The method of inducing anesthesia comprising administering to a subject in need of same an anesthetic inducing amount of a stable, sterile, and antimicrobial injectable aqueous dispersion of a water-insoluble microdroplet matrix of mean diameter from about 50 nm to about 1000 nm consisting essentially of about 1% to about 15% of propofol as the active ingredient, up to about 7% of a propofol soluble diluent, and about 0.8% to about 4% of a surface stabilizing amphiphilic agent, and the aqueous phase of the composition consisting of a pharmaceutically acceptable water-soluble polyhydroxy tonicity modifier, the composition being devoid of additional bactericidal or bacteriostatic preservative agents, provided the ratio of propofol to diluent is about 1:4 to about 1:0.1 and the ratio of propofol to amphiphilic agent is about 1:0.8 to about 1:2.5, and the composition has a viscosity of from about 0.8 to about 15 centipoise.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 99/18801

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K9/107 A61K31/05

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)  
IPC 7 A61K

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 practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 714 520 A (JONES CHRISTOPHER BUCHAN ET AL) 3 February 1998 (1998-02-03) cited in the application column 4, line 38 - line 45 column 5, line 16 - line 53 claims 1-15; example ---	1-14
A	COX EH, ET AL.: "Influence of different fat emulsion-based intravenous formulations on the pharmacokinetics and pharmacodynamics of propofol" PHARMACEUTICAL RESEARCH, vol. 15, no. 3, March 1998 (1998-03), pages 442-448, XP000863268 ISSN: 0724-8741 the whole document --- -/--	1-14

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Patent family members are listed in annex.

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- "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

- "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
- "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
- "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.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 December 1999

Date of mailing of the international search report

22/12/1999

Name and mailing address of the ISA

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Authorized officer

Epskamp, S

# INTERNATIONAL SEARCH REPORT

International Application No  
 PCT/US 99/18801

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 4 056 635 A (GLEN JOHN BAIRD ET AL) 1 November 1977 (1977-11-01) cited in the application column 1, line 51 -column 2, line 25 example 7 ---	1-14
A	US 5 637 625 A (HAYNES DUNCAN H) 10 June 1997 (1997-06-10) cited in the application column 1, line 31 - line 45 claims 1-5; example 1 -----	1,13,14

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/18801

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 13 and 14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/18801

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