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(54) Title: COMPOSITION CONTAINING A RHAMNOLIPID

(57) Abstract: A composition comprising an active ingredient and a rhamnolipid.



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## COMPOSITION CONTAINING A RHAMNOLIPID

The present invention relates to compositions comprising rhamnolipids, methods of preparing and uses of the same.

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The efficacy of a biologically active ingredient (AI), for example an agrochemical, in a composition can often be improved by the addition of further ingredients. The observed efficacy of the combination of ingredients can sometimes be significantly higher than that which would be expected from the individual ingredients used. An adjuvant is a substance which can increase the biological activity of an AI but is itself not significantly biologically active.

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In addition to the effect on biological activity, the physical properties of an adjuvant are of key importance and must be selected with a view to compatibility, stability, toxicity and biodegradability of the formulation concerned.

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An adjuvant is often a surfactant and may be included in a formulation or added separately and is often referred to as being built into formulations or added as tank mix additives. Currently, adjuvant technologies that are used to improve biological activity of agrochemicals are synthetic surfactants often present human safety and environmental issues and are usually sensitive to environmental conditions like temperature, salinity and pH variations.

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In order to address these problems, the present invention therefore provides composition comprising an active ingredient and a rhamnolipid. Preferably the rhamnolipid is an adjuvant.

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Rhamnolipids are a glycolipid biosurfactants (i.e., synthesised by microorganisms) with one or more rhamnose hydrophilic moieties linked to one or more  $\beta$ -hydroxylated fatty acid chains.

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It has surprisingly been found that rhamnolipids, in comparison to known adjuvants, exhibit lower toxicity, higher biodegradability, better environmental capability, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity, and the ability to be synthesized from renewable feed stocks. The application of this type of biosurfactants can therefore increase the potential of adjuvant application under adverse local environments and increase registrability of any final commercial product.

There is thus provided the use of a rhamnolipid to improve the biological performance of an agrochemical.

There is also provide the use of a composition as described herein in the treatment of weeds, pests,  
5 nematodes, molluscs and/or fungi.

Preferably, the active ingredient is an agrochemical. Advantageously, the active ingredient is soluble in water. Preferably the active ingredient has a solubility in water of from 0.1 to 30000 mg/L, such as from 0.55 to 22000 mg/L (20- 25 °C).

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Preferably the rhamnolipid has two rhamnose moieties and/or one or two  $\beta$ -hydroxylated fatty acid chains. Advantageously the fatty acid chains contain from 5 to 20 carbons, such as from 6 to 19, from 7 to 18, or even from 8 to 16 carbons.

15 The rhamnolipid may be obtained from *Pseudomonas aeruginosa*.

There is also provided a method of preparing a composition as defined herein.

The noun “agrochemical” and term “agrochemically active ingredient” are used herein  
20 interchangeably, and include herbicides, insecticides, nematocides, molluscicides, fungicides, plant growth regulators and safeners; preferably herbicides, insecticides and fungicides.

An agrochemical, or a salt of the agrochemical, selected from those given below is suitable for the present invention.

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Suitable herbicides include pinoxaden, bicyclopyrone, mesotrione, fomesafen, tralkoxydim, napropamide, amitraz, propanil, pyrimethanil, dicloran, tecnazene, toclofos methyl, flamprop M, 2,4-D, MCPA, mecoprop, clodinafop-propargyl, cyhalofop-butyl, diclofop methyl, haloxyfop, quizalofop-P, indol-3-ylacetic acid, 1-naphthylacetic acid, isoxaben, tebutam, chlorthal dimethyl, benomyl,  
30 benfuresate, dicamba, dichlobenil, benazolin, triazoxide, fluazuron, teflubenzuron, phenmedipham, acetochlor, alachlor, metolachlor, pretilachlor, thenylchlor, alloxydim, butoxydim, clethodim, cycloxydim, sethoxydim, tepraloxym, pendimethalin, dinoterb, bifenox, oxyfluorfen, acifluorfen, fluazifop, S-metolachlor, glyphosate, glufosinate, paraquat, diquat, fluoroglyphosate-ethyl, bromoxynil, ioxynil, imazamethabenz-methyl, imazapyr, imazaquin, imazethapyr, imazapic, imazamox,  
35 flumioxazin, flumiclorac-pentyl, picloram, amodosulfuron, chlorsulfuron, nicosulfuron, rimsulfuron,

triasulfuron, triallate, pebulate, prosulfocarb, molinate, atrazine, simazine, cyanazine, ametryn, prometryn, terbuthylazine, terbutryn, sulcotrione, isoproturon, linuron, fenuron, chlorotoluron, metoxuron, iodosulfuron, mesosulfuron, diflufenican, flufenacet, fluroxypyr, aminopyralid, pyroxsulam, XDE-848 Rinskor and halauxifen-methyl.

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Suitable fungicides include isopyrazam, mandipropamid, azoxystrobin, trifloxystrobin, kresoxim methyl, mefenoxam, famoxadone, metominostrobin, picoxystrobin, cyprodanil, carbendazim, thiabendazole, dimethomorph, vinclozolin, iprodione, dithiocarbamate, imazalil, prochloraz, fluquinconazole, epoxiconazole, flutriafol, azaconazole, bitertanol, bromuconazole, cyproconazole, difenoconazole, hexaconazole, paclobutrazole, propiconazole, tebuconazole, triadimefon, triticonazole, fenpropimorph, tridemorph, fenpropidin, mancozeb, metiram, chlorothalonil, thiram, ziram, captafol, captan, folpet, fluazinam, flutolanil, carboxin, metalaxyl, bupirimate, ethirimol, dimoxystrobin, fluoxastrobin, orysastrobin, metominostrobin, prothioconazole, adepidyn, bixafen, fluxapyroxad, prothioconazole, pyraclostrobin, revysol, solatenol and xemium.

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Suitable insecticides include thiamethoxam, imidacloprid, acetamiprid, clothianidin, dinotefuran, nitenpyram, fipronil, abamectin, emamectin, tefluthrin, emamectin benzoate, bendiocarb, carbaryl, fenoxycarb, isoprocarb, pirimicarb, propoxur, xylylcarb, asulam, chlorpropham, endosulfan, heptachlor, tebufenozide, bensultap, diethofencarb, pirimiphos methyl, aldicarb, methomyl, cyprmethrin, bioallethrin, deltamethrin, lambda cyhalothrin, cyhalothrin, cyfluthrin, fenvalerate, imiprothrin, permethrin, halfenprox, oxamyl, flupyradifurone, sedaxane, inscalis, rynaxypyr, cyantraniliprole, sulfoxaflor and spinetoram.

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Suitable plant growth regulators include paclobutrazole, trinexapac-ethyl and 1 methylcyclopropene.

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Suitable safeners include benoxacor, cloquintocet-mexyl, cyometrinil, dichlormid, fenchlorazole-ethyl, fencloirim, flurazole, fluxofenim, mefenpyr-diethyl, MG-191, naphthalic anhydride and oxabetrinil.

30

Suitably, the agrochemical is selected from bicyclopypyrone, mesotrione, pinoxaden, fomesafen, tralkoxydim, napropamide, amitraz, propanil, pyrimethanil, dicloran, tecnazene, toclofos methyl, flamprop M, 2,4-D, MCPA, mecoprop, clodinafop-propargyl, cyhalofop-butyl, diclofop methyl, haloxyfop, quizalofop-P, indol-3-ylacetic acid, 1-naphthylacetic acid, isoxaben, tebutam, chlorthal dimethyl, benomyl, benfuresate, dicamba, dichlobenil, benazolin, triazoxide, fluazuron, teflubenzuron, phenmedipham, acetochlor, alachlor, metolachlor, pretilachlor, thenylchlor,

alloxydim, butoxydim, clethodim, cycloxydim, sethoxydim, tepraloxym, pendimethalin, dinoterb, bifenox, oxyfluorfen, acifluorfen, fluoroglycofen-ethyl, bromoxynil, ioxynil, imazamethabenz-methyl, imazapyr, imazaquin, imazethapyr, imazapic, imazamox, flumioxazin, flumiclorac-pentyl, picloram, amodosulfuron, chlosulfuron, nicosulfuron, rimsulfuron, triasulfuron, triallate, pebulate, 5 prosulfocarb, molinate, atrazine, simazine, cyanazine, ametryn, prometryn, terbutylazine, terbutryn, sulcotrione, isoproturon, linuron, fenuron, chlorotoluron, metoxuron, isopyrazam, mandipropamid, azoxystrobin, trifloxystrobin, kresoxim methyl, famoxadone, metominostrobin and picoxystrobin, cyprodanil, carbendazim, thiabendazole, dimethomorph, vinclozolin, iprodione, dithiocarbamate, imazalil, prochloraz, fluquinconazole, epoxiconazole, flutriafol, azaconazole, bitertanol, 10 bromuconazole, cyproconazole, difenoconazole, hexaconazole, paclobutrazole, propiconazole, tebuconazole, triadimefon, triticonazole, fenpropimorph, tridemorph, fenpropidin, mancozeb, metiram, chlorothalonil, thiram, ziram, captan, folpet, fluazinam, flutolanil, carboxin, metalaxyl, bupirimate, ethirimol, dimoxystrobin, fluoxastrobil, orysastrobil, metominostrobin, prothioconazole, thiamethoxam, imidacloprid, acetamiprid, clothianidin, dinotefuran, nitenpyr, 15 fipronil, abamectin, emamectin, bendiocarb, carbaryl, fenoxycarb, isoprocarb, pirimicarb, propoxur, xylylcarb, asulam, chlorpropham, endosulfan, heptachlor, tebufenozide, bensultap, diethofencarb, pirimiphos methyl, aldicarb, methomyl, cypermethrin, bioallethrin, deltamethrin, lambda cyhalothrin, cyhalothrin, cyfluthrin, fenvalerate, imiprothrin, permethrin, halfenprox, paclobutrazole, 1-methylcyclopropene, benoxacor, cloquintocet-mexyl, cyometrinil, dichlormid, fenchlorazole-ethyl, 20 fenclorim, flurazole, fluxofenim, mefenpyr-diethyl, MG-191, naphthalic anhydride and oxabetrinil. Preferred agrochemical active ingredients are selected from fomesafen (suitably as the sodium salt), mesotrione, nicosulfuron, pinoxaden, isopyrazam, epoxiconazole, solatenol and cyantraniliprole.

More preferably, the agrochemical is Nicosulfuron, Mesotrione, isopyrazam or Solatenol.

25 Suitably, compositions of the invention may comprise one or more of the agrochemicals described above.

Generally, any agrochemically active ingredient will be present at a concentration of from about 30 0.000001% to about 90% w/w; preferably from about 0.001% to about 90% w/w.

Agrochemical compositions of the invention may be in the form of a ready-to-use formulation or in concentrate form suitable for further dilution by the end user, and the concentration of agrochemical and rhamnolipid will be adjusted accordingly. In concentrated form, compositions of the invention

typically contain an agrochemical at from 5 to 90% w/w, more preferably from 5 to 75% w/w, even more preferably from 10 to 50% w/w, of the total composition. Ready-to-use compositions of the invention will typically contain an agrochemical at from 0.000001% to 1% w/w, more preferably from 0.000001% to 0.5% w/w, and more preferably still from 0.001% to 0.1% w/w, of the total composition.

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Typically, a rhamnolipid will have a concentration of from about 0.0005% to about 90% w/w of the total composition; preferably from about 0.01% to about 90% w/w. When in concentrated form, compositions of the invention typically contain a rhamnolipid at from 1% to 80% w/w, preferably from 5% to 60% w/w, more preferably from 5% w/w to 40% w/w and even more preferably from 5% w/w to 20% w/w of the total composition. Ready to use compositions of the invention typically contain a rhamnolipid at from about 0.0005% to about 2% w/w of the total composition, more preferably from about 0.01% to about 1% w/w and even more preferably from 0.05% w/w to 0.5% w/w of the total composition.

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15 The compositions of the present invention may relate to concentrates designed to be added to a farmer's spray tank of water or they may be applied directly without further dilution.

Preferably compositions are selected from an SC (suspension concentrate); an SL (soluble liquid); an EC (emulsifiable concentrate); a DC (dispersible concentrate); a WG (water dispersible granule); a SG (soluble granule); an EW (emulsion in water); a SE (suspension-emulsion); a CS (capsule suspension); and an OD (oil dispersion).

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Furthermore, an adjuvant system as herein described may be designed to be added to a formulation of an agrochemical (for example by mixing with water in a farmer's spray tank).

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The compositions of the present invention may include other ingredients such as a dispersing agent, a surfactant, an emulsifier, a solvent, a polymer, an anti-foam agent, an anti-bacterial agent, a colourant and a perfume, which are well known to the man skilled in the art. Standard formulation publications disclose such formulation components suitable for use with the present invention (for example, Chemistry and Technology of Agrochemical Formulations, Ed. Alan Knowles, published by Kluwer Academic Publishers, The Netherlands in 1998; and Adjuvants and Additives: 2006 Edition by Alan Knowles, Agrow Report DS256, published by Informa UK Ltd, December 2006). The compositions may also comprise other ingredients for improving formulation compatibility; such as hydrotropes and

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viscosity reducing aids, which may be suitable for use with the alcohol alkoxylate adjuvants of the present invention.

The compositions of the present invention may include other adjuvants. Examples are surfactants (e.g. non-ionic, anionic, cationic or amphoteric), wetting agents, spreading agents, sticking agents, humectants and penetration agents. Further examples of suitable adjuvants are mineral oils, vegetable oils, fatty acid esters, esters of aliphatic or aromatic dicarboxylic acids, alcohol ethoxylates, alkylphenol ethoxylates, alkylamine ethoxylates, ethoxylates of triglycerides, ethoxylates of fatty acids, ethoxylates of fatty acid esters, ethoxylates of sorbitan fatty acid esters, alkyl polyglycosides and silicone-based adjuvants. Preferred suitable adjuvants are surfactants which provide improved wetting or improved spray retention properties.

Unless otherwise stated are percentages are given as percentages by total weight and all embodiments and preferred features may be combined in any combination.

The invention is described by the following non-limiting Examples.

### Examples

The following examples demonstrate the effect on biological performance of the present invention.

#### *Herbicides*

##### Nicosulfuron

The efficacy of rhamnolipids as an adjuvant for the herbicide nicosulfuron was tested in a glasshouse against two weed species. Nicosulfuron was added to the spray tank as a standard WG (water dispersible granule) formulation. All spray solutions also contained 12.5% v/v of iso-Propanol as an inert non-adjuvant additive to provide spray retention.

The plants were sprayed with nicosulfuron (in the absence of an adjuvant) at rates of 15 and 60 grams of pesticide per hectare using a laboratory track sprayer which delivered the aqueous spray composition at a rate of 200 litres per hectare. The spray tests were also carried out using nicosulfuron in conjunction with the rhamnolipids at a rate of 0.2% v/v. The weed species and their growth stage

at spraying were *Abutilon theophrasti* (ABUTH; growth stage 13), *Chenopodium album* (CHEAL; growth stage 14-15), *Digitaria sanguinalis* (DIGSA; growth stage 14) and *Setaria viridis* (SETVI; growth stage 13-14). Each spray test was replicated three times.

- 5 The efficacy of the herbicide was assessed visually and expressed as a percentage of the leaf area killed. Samples were assessed at a time period of 21 days following application. The results shown in Tables 1- 4 below are mean averages over the three replicates at the two rates of nicosulfuron.

Table 1 – *Abutilon theophrasti*

Adjuvant	15g/ha	60g/ha
No Adjuvant	15	42
0.2% rhamnolipids	35	68

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Table 2- *Chenopodium album*

Adjuvant	15g/ha	60g/ha
No Adjuvant	12	22
0.2% rhamnolipids	55	70

Table 3 – *Digitaria sanguinalis*

Adjuvant	15g/ha	60g/ha
No Adjuvant	8	17
0.2% rhamnolipids	75	87

15 Table 4- *Setaria viridis*

Adjuvant	15g/ha	60g/ha
No Adjuvant	47	73
0.2% rhamnolipids	78	87

### Mesotrione

- 20 The efficacy of rhamnolipids as an adjuvant for the herbicide mesotrione was tested in a glasshouse against four weed species. Mesotrione was added to the spray tank as a standard SC (suspension concentrate) formulation. All spray solutions also contained 12.5% v/v of iso-Propanol as an inert non-adjuvant additive to provide spray retention



The plants were sprayed with mesotrione (in the absence of an adjuvant) at rates of 30 and 60 grams of pesticide per hectare using a laboratory track sprayer which delivered the aqueous spray composition at a rate of 200 litres per hectare. The rhamnolipids were added to the spray solution at a rate of 0.2% v/v. The four weed species were *Polygonum convolvulus* (POLCO; growth stage 13-15),  
 5 *Brachiaria platyphylla* (BRAPL; growth stage 13-14), *Euphorbia heterophylla* (EPHHL; growth stage 11-12) and *Digitaria sanguinalis* (DIGSA; growth stage 14). Each spray test was replicated three times.

The efficacy of the herbicide was assessed visually and expressed as a percentage of the leaf area killed. Samples were assessed at a time period of 21 days following application. The results shown in  
 10 the tables below are mean averages over the three replicates at the two rates of mesotrione.

Table 5 - *Brachiaria platyphylla*

Adjuvant	30g/ha	60g/ha
No Adjuvant	18	27
0.2% rhamnolipids	60	65

Table 6 – *Digitaria sanguinalis*

Adjuvant	30g/ha	60g/ha
No Adjuvant	25	68
0.2% rhamnolipids	85	93

Table 7 – *Euphorbia heterophylla*

Adjuvant	30g/ha	60g/ha
No Adjuvant	37	48
0.2% rhamnolipids	67	72

Table 8 – *Polygonum convolvulus*

Adjuvant	30g/ha	60g/ha
No Adjuvant	55	62
0.2% rhamnolipids	82	96

## 20 Fungicide

The test data relate to curative testing against *Septoria tritici* on wheat. The wheat plants in pots are inoculated 3 to 5 days prior to spraying as indicated.

The isopyrazam is formulated as an aqueous suspension concentrate formulation containing 250 g/L of isopyrazam. The suspension concentrate formulation is diluted to the concentrations of 1.3, 3.2, 8 and 20 grams of pesticide per hectare with water prior to spraying.

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The Solatenol™ is formulated as a wettable granule (WG) containing 150 g/L of fungicide. The wettable granule formulation is diluted to the concentrations of 0.3, 1, 3 and 9 grams of pesticide per hectare with water prior to spraying.

10 In both fungicides, when adjuvant is included it is used at 0.1 %w/v in the spray dilution unless otherwise indicated. All spray solutions also contained 10.0% v/v of iso-Propanol as an inert non-adjuvant additive to provide spray retention. The spray application is 200l L/ha. Three assessments are made per pot of wheat with 4 replicate pots fully randomised. The three replicate pots are visually assessed and given a score of disease severity. The percent efficacy corresponds to the reduction in

15 average disease severity in the treated pots compared to the untreated pots as a percentage of the average disease severity of the untreated pots. The results are shown in Table 9 below and represent side-by-side experiments.

Table 9

AI	Rate [g/ha]	Adjuvant	Efficacy
Isopyrazam	20	-	32.1
Isopyrazam	8	-	16.6
Isopyrazam	20	Rhamnolipids	44.1
Isopyrazam	8	Rhamnolipids	50.6
Isopyrazam	3.2	Rhamnolipids	41.1
Isopyrazam	1.3	Rhamnolipids	17.6
Solatenol	9		14.0
Solatenol	3		-4.1
Solatenol	9	Rhamnolipids	75.6
Solatenol	3	Rhamnolipids	55.9
Solatenol	1.0	Rhamnolipids	1.0
Solatenol	0.3	Rhamnolipids	30.6

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It can be seen that rhamnolipids surprisingly act as extremely effective adjuvant for a range of active ingredients and compositions.

The invention is defined by the claims.

**Claims**

1. A composition comprising an active ingredient and a rhamnolipid.
- 5 2. A composition according to claim 1, wherein the rhamnolipid is an adjuvant.
3. A composition according to claim 1 or 2, wherein the active ingredient is an agrochemical.
4. A composition according to any of the preceding claims, wherein the active ingredient is  
10 soluble in water.
5. A composition according to any of the preceding claims, wherein the rhamnolipid has two rhamnose moieties.
- 15 6. A composition according to any of the preceding claims, wherein the rhamnolipid has one or two  $\beta$ -hydroxylated fatty acid chains.
7. A composition according to claim 6, wherein the fatty acid chains contain from 8 to 16  
20 carbons.
8. A composition according to any of the preceding claims, wherein the rhamnolipid is obtained from *Pseudomonas aeruginosa*.
9. A composition according to any of the preceding claims, wherein the composition is an SC  
25 (suspension concentrate); an SL (soluble liquid); an EC (emulsifiable concentrate); a DC (dispersible concentrate); a WG (water dispersible granule); a SG (soluble granule); an EW (emulsion in water); a SE (suspension-emulsion); a CS (capsule suspension); or an OD (oil dispersion).
- 30 10. A method of preparing a composition as defined in any of the preceding claims.
11. Use of a rhamnolipid to improve the biological performance of an agrochemical.
12. Use of a composition according to any of claims 1 to 9 in treatment of weeds, pests,  
35 nematodes, molluscs and/or fungi.

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/084029

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A01N25/30 A01N43/16 A01P3/00 A01P13/00  
ADD.

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)

A01N A01P

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

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

EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 111 134 125 A (UNIV ZHEJIANG) 12 May 2020 (2020-05-12) examples 5-6 -----	1-12
X	US 2007/191292 A1 (GANDHI N R [US] ET AL) 16 August 2007 (2007-08-16) examples 3B-3D -----	1-12
X	CN 102 090 394 A (ZHEJIANG SHENGHUA BIOK BIOLOGY) 15 June 2011 (2011-06-15) the whole document -----	1-12
X	CN 113 575 608 A (GUANGDONG ZHONGXUN AGRI SCIENCE CORP) 2 November 2021 (2021-11-02) examples ----- -/--	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

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"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

"&amp;" document member of the same patent family

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## INTERNATIONAL SEARCH REPORT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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